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Original Research Article

Phosphorus utilization response of pigs and broiler chickens to diets supplemented with antimicrobials and phytase

Katherine McCormick [a, b], Carrie L. Walk [c], Craig L. Wyatt [c], Olayiwola Adeola [a, *]

[a] Department of Animal Sciences, Purdue University, West Lafayette 47907-2054, USA
[b] JBS United, Sheridan 46069, USA
[c] AB Vista Feed Ingredients, Marlborough, Wiltshire SN8 4AN, UK

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ABSTRACT

Three experiments were conducted to evaluate the phosphorus (P) utilization responses of pigs and broiler chickens to dietary supplementation with antimicrobials and phytase and to determine if P digestibility response to phytase is affected by supplementation with antimicrobials. Experiment 1 used 4 diets (a basal negative control formulated to contain 0.41% total P and 0.71% calcium [Ca] without added antimicrobials, basal negative control with added carbadox, basal negative control with added tylosin, or basal negative control with added virginiamycin) and six 18-kg barrows in individual metabolism crates per diet. There was no effect of antimicrobials on P and Ca digestibility or retention. Carbadox supplementation increased ($P < 0.05$) digestibility and retention of gross energy (GE) and supplementation with tylosin increased ($P < 0.05$) N retention relative to the basal negative control diet. Experiment 2 used eight 19-kg barrows in individual metabolism crates per treatment and 9 dietary treatments arranged in a 3 × 3 factorial of antimicrobials (none, tylosin, or virginiamycin) and phytase (0, 500, or 1,500 FTU/kg). Phytase addition to the diets linearly increased ($P < 0.05$) apparent total tract digestibility or retention of P, Ca, nitrogen (N) and GE. Supplementation with antimicrobials did not affect apparent total tract digestibility or retention of P, Ca, N or GE. There were linear effects ($P < 0.01$) of phytase on Ca utilization in diets that were not supplemented with antimicrobials but only tendencies ($P < 0.10$) in diets supplemented with tylosin or virginiamycin. Phytase linearly improved ($P < 0.05$) N utilization in diets supplemented with tylosin or virginiamycin but not in diets without added antimicrobials. Experiment 3 was a broiler chicken experiment with the same experimental design as Exp. 2 but feeding 8 birds per cage and 10 replicate cages per diet. Antimicrobial supplementation improved ($P < 0.05$) feed efficiency and adding tylosin improved ($P < 0.05$) tibia ash but did not affect nutrient utilization. Dietary phytase improved ($P < 0.01$) growth performance, tibia ash and apparent ileal digestibility and retention of P regardless of antimicrobial supplementation. Overall, phytase supplementation improved growth performance and nutrient digestibility and retention, regardless of supplementation of diets with antimicrobials. Supplementation of diets with antimicrobials did not affect P digestibility or retention because of a lack of interaction between antimicrobials and phytase, there was no evidence that P digestibility response to phytase is affected by supplementation with antimicrobials.

1. Introduction

Supplementation of antimicrobials to livestock and poultry diets is intended to prevent diseases and reduce morbidity in the production environment. Research has demonstrated that antimicrobial supplementation can improve growth performance by increasing nutrients utilization (Cromwell, 2001). Orally-fed antimicrobials directly influence the microflora within the gastrointestinal tract by reducing competition for nutrients and microbial

* Corresponding author.
E-mail address: ladeola@purdue.edu (O. Adeola).
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metabolites that can negatively affect growth performance of the host (Visek, 1978; Hedde, 1981; Anderson et al., 1999). Supplementation of tylosin, an antimicrobial, to a P-deficient corn-soybean meal diet had no effect on apparent digestibility of dry matter (DM), gross energy (GE), nitrogen (N), calcium (Ca), or phosphorus (P) in swine (Lindemann et al., 2010). However, Agudelo et al. (2007) and Stewart et al. (2010) have shown that supplementation of virginiamycin to swine diets improved P utilization and apparent ileal digestibility (AID) of amino acids, DM and GE. Furthermore, cyadox, increased growth performance and P digestibility in swine (Wang et al., 2005). Pigs and chickens do not efficiently utilize P predominantly stored in cereal grains and oilseed meals as phytin due to low endogenous phytase production (Maenz and Classen, 1998), necessitating supplementation of diets with exogenous phytase, which allows for a decrease of inorganic P supplementation thus reducing P excretion into the manure and environment.

Diets may be supplemented with both phytase and antimicrobials. Because supplementation of P-deficient diet with phytase is known to improve P digestibility and some studies have shown that some antimicrobials may also improve P digestibility, it is of interest to determine if P digestibility response to phytase is affected by supplementation with antimicrobials. The current experiments evaluated responses of pigs and chickens to diets supplemented with 3 antimicrobials (tylosin, virginiamycin, or carbadox) alone or in combination with phytase.

2. Materials and methods

The Purdue Animal Care and Use Committee (Purdue University, West Lafayette, IN 47907) approved all animal procedures used in the 3 studies.

2.1. Experiments one and two

Crossbred (Yorkshire × Landrace × Duroc) barrows ($n = 24$ in Exp. 1, BW = 17.5 ± 0.48 kg; $n = 72$ in Exp. 2, BW = 19.1 ± 0.23 kg) were housed individually in stainless steel metabolism crates (0.83 m × 0.71 m) that allowed for separate collection of feces and urine. Pigs were weighed and allocated to 6 (Exp. 1) or 8 (Exp. 2) blocks based on BW and randomly assigned to diets within block.

In Exp. 1, a basal negative control (NC) corn-soybean meal diet, deficient in digestible P but met or exceeded other nutrient recommendations (NRC, 2012) for 20- to 30-kg pigs was formulated. Three other diets consisted of antimicrobial premixes, prepared using corn as a carrier and added to the NC diet to supply 55 mg carbadox (Mecadox 10, Phibro Animal Health, Teaneck, NJ) per kg diet, 44 mg tylosin (Tylan 40, Elanco Animal Health, Greenfield, IN, USA) per kg diet, or 11 mg virginiamycin (Stafac 20, Phibro Animal Health, Teaneck, NJ, USA) per diet (Table 1). Diets in Exp. 2 consisted of a basal corn-soybean meal NC similar to that in Exp. 1. Phytase premix was prepared using corn as a carrier to supply 0, 500, or 1,500 phytase units (FTU)/kg (Table 2). The phytase used was an *Escherichia coli* 6-phytase expressed in *Trichoderma reesei* (Quantum Blue, AB Vista Feed Ingredients Marlborough, UK). Antimicrobial premixes were prepared to supply 0, 44 mg/kg tylosin, or 28 mg/kg virginiamycin (Table 2). The diets were arranged in a 3 × 3 factorial of phytase (0, 500, or 1,500 FTU/kg) and antimicrobials (none, tylosin, or virginiamycin). All diets in Exp. 1 and 2 were fed in mash form.

Nutrient balance protocols followed procedures described by Adeola and Kong (2014). Pigs were fed at 4% of BW with the feed divided into 2 daily meals fed at 09:00 and 18:00. The initiation and the termination of the collection period was marked by the addition of chromic oxide to the morning meal and observation of its appearance in the feces. During the collection period total amount of feces was collected twice daily and stored at −20 °C until the end of the collection period. The urine volume was measured and recorded daily and a 30% subsample was taken and stored at −20 °C until further processing. Ten milliliters of 30% formaldehyde solution was added to urine collection buckets daily to minimize nitrogen volatilization and bacteria growth. Collection of urine began at the feeding of the initiation marker and ended at the feeding of the termination marker. Any leftover feed and waste was collected daily and dried to accurately determine feed intake. There was a 5-d adaptation followed by a 5-d collection period in Exp. 1 but a 7-d adaptation followed by a 7-d collection period in Exp. 2.

Table 1
Ingredient and analyzed composition of negative control diets (NC) and NC with added antimicrobials used in Exp. 1 (DM basis).

Item	Dietary treatments			
	NC	NC + carbadox	NC + tylosin	NC + virginiamycin
Ingredients, g/kg				
Corn	643.7	623.7	623.7	623.7
Soybean meal	300.0	300.0	300.0	300.0
Soybean oil	25.0	25.0	25.0	25.0
NaCl	3.3	3.3	3.3	3.3
Limestone (38% Ca)	16.0	16.0	16.0	16.0
Monocalcium phosphate[1]	1.5	1.5	1.5	1.5
Lysine·HCl	4.5	4.5	4.5	4.5
DL-Methionine	1.5	1.5	1.5	1.5
L-Threonine	1.5	1.5	1.5	1.5
Selenium premix[2]	0.5	0.5	0.5	0.5
Vitamin premix[3]	1.5	1.5	1.5	1.5
Mineral premix[4]	1.0	1.0	1.0	1.0
Carbadox premix[5]	0.0	20.0	0.0	0.0
Tylosin premix[5]	0.0	0.0	20.0	0.0
Virginiamycin premix[5]	0.0	0.0	0.0	20.0
Total	1,000.0	1,000.0	1,000.0	1,000.0
Analyzed composition, g/kg				
Dry matter	874	881	883	881
Gross energy, kcal/kg	3,972	4,001	3,993	4,010
Metabolizable energy, kcal/kg	3,764	3,862	3,766	3,849
Nitrogen	3.51	3.32	3.33	3.43
Calcium	10.0	11.3	10.4	10.3
Phosphorus	3.7	3.7	3.8	3.8
STTD phosphorus[6]	1.9	1.9	1.9	1.9
Carbadox, mg/kg	0.0	48.9	0.0	0.0
Tylosin, mg/kg	0.0	0.0	38.1	0.0
Virginiamycin, mg/kg	0.0	0.0	0.0	10.2

STTD = standardized total tract digestible.
[1] Contained 16% Ca, 21% P.
[2] Selenium premix supplied 300 μg of selenium per kilogram of diet.
[3] Vitamin premix supplied per kilogram of diet: vitamin A, 3,630 IU; vitamin D_3, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg, vitamin B_{12}, 23.1 μg; riboflavin, 5.28 mg; D-pantothenic acid, 13.1 mg; niacin, 19.8 mg.
[4] Mineral premix supplied per kilogram of diet: Cu (as $CuCl_2$), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as $FeCO_3$), 121 mg; Mn (as MnO), 15 mg; and Zn (as ZnO), 121 mg.
[5] Carbadox premix supplied 55 mg carbadox (active drug)/kg diet (Mecadox 10, Phibro Animal Health, Teaneck, NJ, USA); tylosin premix supplied 44 mg tylosin (active drug)/kg diet (Tylan 40, Elanco Animal Health, Greenfield, IN, USA); virginiamycin premix supplied 11 mg virginiamycin (active drug)/kg diet (Stafac 20, Phibro Animal Health, Teaneck, NJ, USA); ground corn used as a carrier in all premixes.
[6] Calculated STTD phosphorus.

2.2. Experiment three

Male Ross 708 day-old broiler chickens were fed a standard broiler starter diet from d 1 to 5 post hatch (Adeola and Walk,

Table 2
Ingredient and analyzed compositions of experimental diets used in Exp. 2 (DM basis).

Item	Antimicrobial								
	None	None	None	Tylosin	Tylosin	Tylosin	Virginiamycin	Virginiamycin	Virginiamycin
	Phytase, FTU/kg								
	0	500	1,500	0	500	1,500	0	500	1,500
Ingredients, g/kg									
Corn	648.2	628.2	588.2	628.2	608.2	568.2	628.2	608.2	568.2
Soybean meal	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Soybean oil	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
NaCl	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Vitamin premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Selenium premix[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Limestone (38% Ca)	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Lysine·HCl	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
DL-Methionine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Threonine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Phytase premix[4]	0.0	20.0	60.0	0.0	20.0	60.0	0.0	20.0	60.0
Tylosin premix[5]	0.0	0.0	0.0	20.0	20.0	20.0	0.0	0.0	0.0
Virginiamycin premix[5]	0.0	0.0	0.0	0.0	0.0	0.0	20.0	20.0	20.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Analyzed composition, g/kg									
Dry matter	938	939	941	936	935	939	938	945	939
Gross energy, kcal/kg	4,072	4,021	4,087	4,020	3,968	4,040	4,056	4,100	4,024
Metabolizable energy, kcal/kg	3,713	3,627	3,701	3,633	3,615	3,649	3,659	3,668	3,748
Nitrogen	3.14	3.02	3.16	3.23	3.15	3.31	3.18	3.11	3.18
Calcium	7.7	9.8	9.6	9.4	8.9	8.0	8.3	8.7	8.0
Phosphorus	3.5	3.7	3.6	3.7	3.8	3.6	3.7	4.1	3.7
STTD of phosphorus[6]	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Phytase, FTU/kg	50	610	1,660	<50	527	1,370	<50	511	1,560
Tylosin, mg/kg	0	0	0	40.2	34.1	38.7	0	0	0
Virginiamycin, mg/kg	0	0	0	0	0	0	24.9	15.3	14.9

[1] Vitamin premix supplied per kilogram of diet: Vitamin A, 3,630 IU; vitamin D_3, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg, vitamin B_{12}, 23.1 μg; riboflavin, 5.28 mg; D-pantothenic acid, 13.1 mg; niacin, 19.8 mg.
[2] Mineral premix supplied per kilogram of diet: Cu (as $CuCl_2$), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as $FeCO_3$), 121 mg; Mn (as MnO). 15 mg; and Zn (as ZnO), 121 mg.
[3] Selenium premix supplied 300 μg of selenium per kilogram of diet.
[4] Phytase premix was prepared with ground corn to supply 25 FTU/g of premix.
[5] Tylosin premix supplied 44 mg tylosin (active drug)/kg diet (Tylan 40, Elanco Animal Health, Greenfield, IN, USA); virginiamycin premix supplied 28 mg virginiamycin (active drug)/kg diet (Stafac 20, Phibro Animal Health, Teaneck, NJ, USA); ground corn used as a carrier in all premixes.
[6] Calculated standardized total tract digestibility of phosphorus.

2013). All broilers were reared in stainless-steal, electrically heated battery cages (Alternative Design Manufacturing and Supply Inc., Shalom Springs, AR) and followed a step down of temperatures to 35°C from d 1 to d 7 post hatch, 32°C from d 7 to d 14 post hatch, and 27°C from d 14 to d 23 post hatch. Birds were weighed and allocated to 10 blocks based on initial BW (84.7 ± 1.2 g), and randomly assigned to a dietary treatment within block. Diets in Exp. 3 consisted of a basal corn-soybean meal NC that is deficient in digestible P, but met or exceeded NRC (1994) recommendations for all other nutrients. Phytase premix was prepared using corn as a carrier to supply 0, 500, or 1,500 FTU/kg (Table 3). The phytase used was an *E. coli* 6-phytase expressed in *T. reesei* (Quantum Blue, AB Vista Feed Ingredients Marlborough, UK). Antimicrobial premixes were prepared to supply 0, 44 mg/kg tylosin, or 11 mg/kg virginiamycin (Table 3). The diets were also arranged in a 3 × 3 factorial of phytase (0, 500, or 1,500 FTU/kg) and antimicrobials (none, tylosin, or virginiamycin). Diets were fed in mash form. The addition of 5 g of TiO_2/kg was a digestible marker for estimation of nutrients digestibility.

On d 5 post-hatching, 720 male birds were weighed, sorted and allotted to 10 blocks based on BW and within blocks to 9 diets. There were 8 birds per cage in each of 10 blocks per diet in a randomized complete block design (RCBD). Birds were provided unrestricted access to feed and water for 18 d. Birds and feeders were weighed at the start and end of the experiment to calculate BW gain, feed intake and G:F ratio. Bird mortality was monitored and any bird that was removed was weighed, feed intake and G:F ratio was adjusted based on a model to estimate individual feed intake (Lindemann and Kim, 2007). On d 23 post-hatching, birds were asphyxiated with CO_2 and ileal digesta was collected from the distal two-thirds of the ileum. Ileal contents from birds were flushed with distilled water into plastic containers, pooled by cage, and stored in a freezer (−20 °C) until dried and ground. The left tibia was collected from the 4 heaviest birds in each cage for bone ash determination. Excreta collected was pooled by cage for 3 d prior to the end of the experiment and stored at −20 °C until dried and ground.

2.3. Chemical analyses

At the conclusion of Exp. 1 and 2, urine was thawed and filtered using glass wool, and feces, orts, and urine were weighed and dried in a forced-draft oven at 55 °C. Diets and dried feces were ground using a grinding mill (Retsch ZM 100, Retsch GmbH and Co. K. G., Hann, Germany) and sub-sampled for analysis. In Exp. 3, ileal digesta and excreta were dried in a forced-draft oven at 55 °C. Diets, dried ileal digesta, and excreta were ground as described above. Dry matter of diets, feces, ileal digesta, and excreta were determined by drying samples at 105 °C for 24 h. Gross energy of diets, feces, urine, ileal digesta, and excreta were determined using adiabatic bomb calorimetry (Model 1261, Parr Instrument Co., Moline, IL, USA) and benzoic acid as an internal

Table 3
Ingredient and analyzed compositions of experimental diets used in Exp. 3 (DM basis).

Item	Antimicrobial								
	None	None	None	Tylosin	Tylosin	Tylosin	Virginiamycin	Virginiamycin	Virginiamycin
	Phytase, FTU/kg								
	0	500	1,500	0	500	1,500	0	500	1,500
Ingredients, g/kg									
Corn	544.1	524.1	484.1	524.1	504.1	464.1	524.1	504.1	464.1
Soybean meal	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0
Soybean oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
NaCl	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Vitamin-mineral premix[1]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Titanium dioxide premix[2]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Monocalcium phosphate	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Limestone (38% Ca)	15.8	15.8	15.8	15.8	15.8	15.8	15.8	15.8	15.8
Lysine·HCl	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
DL-Methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Threonine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Phytase premix[3]	0.0	20.0	60.0	0.0	20.0	60.0	0.0	20.0	60.0
Tylosin premix[4]	0.0	0.0	0.0	20.0	20.0	20.0	0.0	0.0	0.0
Virginiamycin premix[4]	0.0	0.0	0.0	0.0	0.0	0.0	20.0	20.0	20.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Analyzed composition, g/kg									
Dry matter	887	887	886	887	895	888	894	901	892
Gross energy, kcal/kg	4,525	4,442	4,539	4,582	4,571	4,557	4,569	4,566	4,544
Metabolizable energy, kcal/kg	3,407	3,207	3,362	3,391	3,391	3,400	3,351	3,350	3,426
Nitrogen	3.41	3.51	3.32	3.44	3.47	3.24	3.46	3.51	3.54
Calcium	10.0	9.3	9.6	9.3	9.9	9.7	9.3	8.8	9.7
Phosphorus	4.1	4.0	4.2	4.3	3.8	4.0	4.0	4.1	4.1
Phytase FTU/kg	<50	660	2,060	<50	827	2,350	<50	846	2.370
Phytic acid, g/100 g	0.94	0.92	0.91	0.92	0.92	0.82	0.93	0.97	0.99
Tylosin, mg/kg	0.0	0.0	0.0	45.1	33.3	41.3	0.0	0.0	0.0
Virginiamycin, mg/kg	0.0	0.0	0.0	0.0	0.0	0.0	7.8	8.2	17.9

[1] Supplied the following per kg of diet: Vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg, choline chloride, 771 mg; vitamin B_{12}, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate, 2.2 mg; folic acid, 990 ug; pyridoxine hydrochloride, 2.2 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μg.
[2] Titanium premix was prepared as 1 g of TiO_2 mixed with 4 g of corn.
[3] Phytase premix was prepared with ground corn to supply 25 FTU/g of premix.
[4] Tylosin premix supplied 44 mg tylosin (active drug)/kg diet (Tylan 40, Elanco Animal Health, Greenfield, IN, USA); virginiamycin premix supplied 11 mg virginiamycin (active drug)/kg diet (Stafac 20, Phibro Animal Health, Teaneck, NJ, USA); ground corn used as a carrier in all premixes.

standard. Diets, feces, and excreta were prepared by a nitric-perchloric acid wet ash before Ca and P determination. Phosphorus determination used a colorimetric assay with the addition of acid molybdate and Fiske's Subbarow reducer solution added to the wet-ash samples. Color intensity was proportional to P concentration estimated by spectrophotometry and absorbance read at 630 nm using Dynex plate reader (Bynex Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using flame atomic absorption spectroscopy method (Varian Spectr. AA 220FS, Varian Australia Pty Ltd., Victoria, Australia). Nitrogen determination of diets, feces, urine, ileal digesta, and excreta was analyzed by the combustion method using a LECO Model FP-2,000 Nitrogen Analyzer (LECO, St. Joseph, MI, USA). Phytase and phytic acid analysis of diets and phytase premix was performed by ESC (Tredomen Park, Ystrad Mynach, UK) by the ELISA and Quantum method for phytase levels, and Megazyme method for total phytic acid in diets. Tylosin activity was analyzed by Covance Laboratories (Princeton, NJ, USA) using Elanco 1996 method B00925; carbadox and virginiamycin activities were analyzed by Eurofins Lancaster Laboratories (Portage, MI, USA) using the method of agar diffusion (method PHB-104 version 02). Tibia bones collected in Exp. 3 were defatted in a Soxhlet extractor, dried, weighed, and ashed in a muffle furnace at 600 °C for bone ash determination. The analysis of Ti for diets and excreta was conducted by digesting samples with 60% sulfuric acid, and then adding 30% H_2O_2 to the mixture. The mixture was then prepared and read using spectroscopy at 410 nm (Myers, 2004).

2.4. Calculations and statistical analyses

In Exp. 1 and Exp. 2, apparent total tract digestibility and retention of nutrients was determined by the equations: Digestibility (%) = 100 × [(Intake - Fecal output)/Intake]; Retention (%) = 100 × [(Intake - Fecal output - Urine output)/Intake].

In Exp. 3, the index method was used to determine AID and retention with the equation: AID or retention (%) = $[1 - (T_i/T_o) \times (N_o/N_i)] \times 100$, where T_i is the concentration of titanium in the diet, T_o is the concentration of titanium in the ileal digesta or excreta, N_o is the concentration of nutrient or GE in the ileal digesta or excreta, and N_i is the concentration of nutrient or GE in the diet.

Data from Exp. 1 were analyzed as RCBD with 5 degrees of freedom (df) for blocks and 3 df for diets. Single-degree of freedom contrasts were used to compare the effects of each antimicrobial to the negative control diet devoid of antimicrobials. Data from Exp. 2 and Exp. 3 were analyzed as 3 × 3 factorial in RCBD with 7 or 9 df for blocks in Exp. 2 or 3, respectively, 2 df for antimicrobial, 2 df for phytase, and 4 df for antimicrobial by phytase interaction. Contrasts for phytase within antimicrobial and contrasts for each antimicrobial to the negative control diet devoid of antimicrobials were used to separate means. Model assumptions of normality were validated using the Shapiro-Wilkes test (Proc univariate) and the assumption of equal variance was validated using the Brown and Forsythe's test. The individual pig or the cage of broilers was the experimental unit. Single degree of freedom contrasts were used to compare effects of each antimicrobial to the negative control diet in Exp. 1. Single-degree-of-freedom contrasts were used to determine

the linear and quadratic effect of phytase supplementation. Because phytase treatments were unequally spaced (0, 500, or 1,500 FTU/kg), Interactive Matrix Language Procedure was used to generate contrast coefficients for structured unequally spaced levels. An α level of 0.05 was used for determination of significance among means and between 0.05 and 0.10 was considered a trend.

3. Results and discussion

The analyzed inclusion level of carbadox was 48.9 mg/kg, tylosin was 38.1 mg/kg, and virginiamycin was 10.2 mg/kg (Table 1). Pigs were deemed healthy and free of any clinical signs of disease at the start of the experiment. The initial BW of pigs across treatment was 17.5 ± 0.48 kg and final BW (18.4, 18.7, 18.1, and 17.8 ± 0.69 kg, respectively) was not influenced by diet ($P > 0.10$). Supplementation of diets with antimicrobials did not impact DM, N, or GE digestibility ($P > 0.10$) or DM retention ($P > 0.10$; Table 4). Supplementation with carbadox improved GE retention ($P = 0.05$) from 83.4% to 84.9% and N-corrected GE retention ($P < 0.01$) from 79.2% to 81% when compared with the NC diet. Supplementation of tylosin to the NC diet significantly decreased ($P < 0.05$) N retention from 63.3% to 56.3%. Carbadox supplementation improved ($P < 0.05$) GE digestibility and retention, and virginiamycin supplementation tended ($P < 0.1$) to improve GE digestibility and retention relative to the NC diet. Supplementation with antimicrobials did not significantly affect digestibility or retention of P or Ca (Table 4).

Analyzed phytase concentrations were approximately <50, 610, or 1,660 FTU/kg in the NC diet, <50, 527, or 1,370 FTU/kg in diets with tylosin, and <50, 511, or 1,560 FTU/kg in diets with virginiamycin (Table 2). The analyzed concentrations of tylosin in the diets with 0, 500, or 1,500 FTU/kg of phytase were 40.2, 34.1, or 38.7 mg/kg, respectively. The analyzed virginiamycin concentrations in the diets with 0, 500, or 1,500 FTU/kg of phytase were 24.9, 14.88, or 15.32 mg/kg, respectively (Table 2). Supplementation with antimicrobials did not affect digestibility or retention of P, Ca, N, or GE. There was no effect of antimicrobial supplementation on Ca digestibility or retention (Table 5). However, supplementation with phytase linearly improved ($P < 0.01$) digestibility and retention of P, Ca, N, and GE (Table 5). There were linear effects ($P < 0.01$) of phytase on Ca utilization in diets that were not supplemented with antimicrobials but only tendencies ($P < 0.10$) in diets supplemented with tylosin or virginiamycin resulting in an interaction between antimicrobials and phytase on Ca utilization. Phytase linearly improved ($P < 0.05$) N utilization in diets supplemented with tylosin or virginiamycin but not in diets without added antimicrobials resulting in an interaction between antimicrobials and phytase on N utilization (Table 5).

Diets supplemented with 500 or 1,500 FTU/kg of phytase were analyzed to contain 660 or 2,060 FTU/kg for NC diets, 827 or 2,350 FTU/kg for diets supplemented with tylosin, and 846 or 2,370 FTU/kg for diets supplemented with virginiamycin (Table 3). Analyzed tylosin activity for 0, 500, or 1,500 FTU/kg was 45.1, 33.3, and 41.3 mg/kg, respectively. Analyzed virginiamycin activity for 0, 500, or 1,500 FTU/kg was 7.8, 8.2, and 17.9 mg/kg, respectively. Feeding antimicrobials from d 5 to 23 had no influence on final BW, BW gain or feed intake. However, there was an effect ($P < 0.05$) of antimicrobials on G:F ratio and tibia ash (Table 6). Tylosin or virginiamycin improved ($P < 0.05$) G:F ratio whereas only tylosin improved ($P < 0.05$) tibia ash compared with NC diet. Phytase supplementation improved ($P < 0.01$) BW gain, feed intake and G: F ratio from d 5 to 23 (Table 6). Phytase supplementation linearly increased ($P < 0.05$) weight gain, G:F ratio, and tibia ash percent regardless of antimicrobial supplementation (Table 6).

Apparent ileal digestibilities and retention of P, N and GE were not affected by supplementation of diets with antimicrobials (Table 7). Addition of phytase to diets linearly improved ($P < 0.01$) AID and retention of P in broiler chickens regardless of supplementation with antimicrobials but had no effect on N and GE utilization. There were interaction effects ($P < 0.05$) of antimicrobial and phytase supplementation on AID of N with a tendency for a decrease in N digestibility with added phytase in diets supplemented with tylosin. Phytase supplementation linearly improved ($P < 0.05$) AID of GE in broiler chickens fed diets containing virginiamycin but had no effect on diets without antimicrobials or diets containing tylosin resulting in antimicrobial by phytase interaction (Table 7).

The addition of antimicrobials to diets changes bacterial populations in the gastrointestinal tract (Henderickx et al., 1983; Anderson et al., 1999; and Dibner and Richards, 2005). The changes in the microflora population can affect nutrient availability, reduce harmful microbes, and trigger an immune and inflammatory response (Henderickx et al., 1983; Cromwell, 2001; Gaskins, 2001; Kelly and King, 2001; and Dibner and Richards, 2005). By reducing or changing the presence of the microflora, there is a potential for the host to utilize amino acids as an energy, along with reduced ammonia excretion when competition between the host and microflora is reduced (Veraeke et al., 1979; Henderickx et al.,

Table 4
Antimicrobial effect on apparent total tract digestibility and retention of P, Ca, N, and energy for pigs in Exp. 1 (%).[1]

Item	P digestibility	P retention	Ca digestibility	Ca retention	N digestibility	N retention	Gross energy digestibility	Gross energy retention
Diet 1[2]	35.1	34.8	49.1	39.7	87.4	63.3	86.5	83.4
Diet 2[3]	34.0	33.7	55.2	47.0	88.6	64.2	88.2	84.9
Diet 3[4]	31.5	31.1	50.3	40.9	86.0	56.3	86.6	83.0
Diet 4[5]	38.3	38.0	44.6	36.3	87.5	64.9	87.3	84.5
SEM[6]	2.39	2.36	4.84	4.60	0.71	2.15	0.46	0.46
P-value[7]								
Diet 1 vs. 2	0.75	0.74	0.39	0.28	0.23	0.77	<0.05	<0.05
Diet 1 vs. 3	0.30	0.29	0.86	0.87	0.19	<0.05	0.92	0.55
Diet 1 vs. 4	0.35	0.36	0.52	0.60	0.86	0.62	0.21	0.10

[1] Values are means of 6 pigs per diet.
[2] Negative control (NC) corn-soybean meal diet, deficient in digestible P but met or exceeded other nutrient recommendations (NRC, 2012).
[3] NC + Carbadox.
[4] NC + Tylosin.
[5] NC + Virginiamycin.
[6] Pooled standard error of the mean.
[7] Single-degree-of-freedom contrasts for indicated diet numbers.

Table 5
Dietary exogenous phytase and antimicrobials supplementation effects on apparent total tract digestibility (dig) and retention (ret) of P, Ca, N, and energy in pigs in Exp. 2 (%).[1]

Item	Added antimicrobial	Added phytase, FTU/kg	P dig	P ret	Ca dig	Ca ret	N dig	N ret	Gross energy dig	Gross energy ret
Diet 1	0	0	44.3	44.0	62.4	62.3	88.5	60.5	88.8	85.6
Diet 2	0	500	60.0	59.7	78.8	78.8	86.6	63.7	87.4	84.6
Diet 3	0	1,500	71.3	71.0	80.3	80.2	87.7	63.4	88.3	85.4
Diet 4	Tylosin	0	41.6	41.3	66.8	66.7	86.5	59.6	87.6	84.3
Diet 5	Tylosin	500	64.5	64.2	75.2	75.1	87.4	65.6	87.7	85.0
Diet 6	Tylosin	1,500	73.7	73.5	75.5	75.4	89.4	67.1	88.7	85.8
Diet 7	Virginiamycin	0	46.8	46.5	70.8	70.6	85.7	61.5	88.6	85.0
Diet 8	Virginiamycin	500	60.0	59.7	70.2	70.1	86.6	64.1	87.5	84.8
Diet 9	Virginiamycin	1,500	75.0	74.7	77.8	77.6	88.6	66.9	88.5	85.8
SEM[2]			1.96	1.96	2.83	2.83	0.65	1.71	0.40	0.42
	0		58.5	58.2	73.8	73.8	87.6	62.5	88.2	85.2
	Tylosin		59.9	59.7	72.5	72.4	87.8	64.1	88.0	85.0
	Virginiamycin		60.6	60.3	72.9	72.8	86.8	64.2	88.2	85.2
		0	44.2	43.9	66.7	66.5	86.9	60.6	88.3	85.0
		500	61.5	61.2	74.7	74.7	86.9	64.5	87.5	84.8
		1,500	73.3	73.1	77.9	77.7	88.6	65.8	88.5	85.7
SEM[3]			1.13	1.13	1.62	1.62	0.37	1.05	0.23	0.24
P-value										
Antimicrobial			0.42	0.43	0.84	0.84	0.92	0.45	0.83	0.87
Phytase			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
Linear effect of phytase			<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	0.24	<0.05
Antimicrobial × Phytase			0.15	0.15	<0.10	<0.10	<0.10	<0.10	0.24	0.23
Linear effect of phytase in 0 Antimicrobial (diets 1 to 3)			<0.01	<0.01	<0.01	<0.01	0.64	0.32	0.71	0.93
Linear effect of phytase in Tylosin (diets 4 to 6)			<0.01	<0.01	<0.10	<0.10	<0.01	<0.01	0.15	0.15
Linear effect of phytase in Virginiamycin (diets 7 to 9)			<0.01	<0.01	<0.10	<0.10	<0.01	<0.05	0.17	0.11

[1] Values are means of 8 pigs for simple effects and 24 pigs for main effects.
[2] Pooled standard error of the mean for simple effects.
[3] Pooled standard error of the mean for main effects.

Table 6
Dietary exogenous phytase and antimicrobials supplementation effects on growth performance and bone mineralization in broiler chickens in Exp. 3.[1]

Item	Added antimicrobial	Added phytase, FTU/kg	Final weight, g	Weight gain, g	Feed intake, g	Gain:feed. g/kg	Tibia ash, %
Diet 1	0	0	528	443	792	568	35.4
Diet 2	0	500	680	595	847	702	42.7
Diet 3	0	1,500	777	692	946	731	47.4
Diet 4	Tylosin	0	551	466	787	592	37.5
Diet 5	Tylosin	500	695	610	841	725	43.3
Diet 6	Tylosin	1,500	800	715	930	769	49.0
Diet 7	Virginiamycin	0	538	453	776	588	36.2
Diet 8	Virginiamycin	500	678	594	830	715	42.1
Diet 9	Virginiamycin	1,500	814	730	950	768	46.2
SEM[2]			13.9	13.9	24.9	12.3	0.83
	0		661	577	862	667	41.8
	Tylosin		682	597	853	695	43.2
	Virginiamycin		677	592	852	691	41.5
		0	539	454	785	583	36.4
		500	684	600	840	714	42.7
		1,500	797	712	942	756	47.5
SEM[3]			8.0	8.0	14.4	7.1	0.48
P-value							
Antimicrobial			0.18	0.18	0.86	<0.05	<0.05
Phytase			<0.01	<0.01	<0.01	<0.01	<0.01
Linear effect of phytase			<0.01	<0.01	<0.01	<0.01	<0.01
Antimicrobial × Phytase			0.64	0.64	0.97	0.89	0.69
Linear effect of phytase in 0 antimicrobial (diets 1 to 3)			<0.01	<0.01	<0.01	<0.01	<0.01
Linear effect of phytase in tylosin (diets 4 to 6)			<0.01	<0.01	<0.01	<0.01	<0.01
Linear effect of phytase in virginiamycin (diets 7 to 9)			<0.01	<0.01	<0.01	<0.01	<0.01

[1] Values are means of 10 replicate cages for simple effects and 30 replicate cages for main effects.
[2] Pooled standard error of the mean for simple effects.
[3] Pooled standard error of the mean for main effects.

1983; Cromwell, 2001). Some means in which antimicrobials potentially improve growth are through reduced fermentation, decreased nutrient use by microflora, reduced thickness of the intestinal lining, improved nutrient absorption, reduced toxin production by bacteria, and reduced sub-clinical intestinal infections (Feighner and Dashkevicz, 1987; Butaye et al., 2003). Disease-causing pathogens are common in commercial livestock and poultry operations, therefore the potential to contract disease

Table 7
Dietary exogenous phytase and antimicrobials supplementation effects on apparent ileal digestibility (dig) and total tract retention (ret) of P, N, and energy in broiler chickens in Exp. 3 (%).[1]

Item	Added antimicrobial	Added phytase, FTU/kg	Ileal P dig	P ret	Ileal N dig	N ret	Ileal energy dig	Energy ret
Diet 1	0	0	48.7	63.1	82.6	69.1	74.2	75.3
Diet 2	0	500	51.7	65.0	81.9	67.4	70.9	72.2
Diet 3	0	1,500	70.6	78.5	84.0	67.0	73.1	74.1
Diet 4	Tylosin	0	52.9	57.4	84.1	67.2	72.1	74.0
Diet 5	Tylosin	500	50.6	66.5	85.7	68.4	73.2	74.2
Diet 6	Tylosin	1,500	71.1	77.3	82.3	65.7	72.3	74.6
Diet 7	Virginiamycin	0	44.1	56.4	82.8	66.0	72.1	73.3
Diet 8	Virginiamycin	500	52.2	62.3	82.9	66.1	72.6	73.4
Diet 9	Virginiamycin	1,500	70.1	78.6	84.8	69.1	75.1	75.4
SEM[2]			2.22	2.17	0.71	1.27	0.91	0.95
	0		57.0	68.9	82.8	67.8	72.7	73.9
	Tylosin		58.2	67.1	84.0	67.1	72.6	74.3
	Virginiamycin		55.5	65.8	83.5	67.1	73.3	74.0
		0	48.6	59.0	83.2	60.6	72.8	74.2
		500	51.5	64.6	83.5	64.5	72.2	73.3
		1,500	70.6	78.2	83.7	65.8	73.5	74.7
SEM[3]			1.28	1.25	0.41	0.74	0.52	0.55
P-value								
Antimicrobial			0.33	0.22	0.17	0.74	0.61	0.87
Phytase			<0.01	<0.01	0.70	0.98	0.23	0.18
Linear effect of phytase			<0.01	<0.01	0.41	0.88	0.22	0.36
Antimicrobial × Phytase			0.25	0.35	<0.01	0.12	<0.05	0.23
Linear effect of phytase in 0 Antimicrobial (diets 1 to 3)			<0.01	<0.01	<0.10	0.31	0.76	0.63
Linear effect of phytase in tylosin (diets 4 to 6)			<0.01	<0.01	<0.10	0.28	0.97	0.64
Linear effect of phytase in virginiamycin (diets 7 to 9)			<0.01	<0.01	<0.10	<0.10	<0.05	<0.10

[1] Values are means of 10 replicate cages for simple effects and 30 replicate cages for main effects.
[2] Pooled standard error of the mean for simple effects.
[3] Pooled standard error of the mean for main effects.

is high, and thus the most accepted method in which antimicrobials influence growth promotion is through disease control (Cromwell, 2001).

In Exp. 1, the supplementation of carbadox increased GE utilization when compared with NC diets. Carbadox is a strong chemotherapeutic and thus has the potential to decrease the microflora and therefore allow for greater nutrient availability to the host. Carbadox targets gram negative bacteria that have membranes with lipopolysaccharide, which stimulates an immune and inflammatory response that utilizes GE and nutrients (Bhunia, 2008). Therefore the addition of carbadox has the potential to reduce this response, due to a decrease in gram negative bacteria, and allow for nutrients and energy to be available to the host.

Tylosin is a 16-member ring macrolide that targets mainly gram-positive bacteria; however, it can also target some gram-negative bacteria (Brisson-Noel et al., 1988; Butaye et al., 2003). Tylosin is used in the swine industry to treat and control swine dysentery, and in the poultry industry it treats *Mycoplasma gallisepticum*, a chronic respiratory disease. Tylosin is a bacteriostatic antimicrobial that disrupts protein synthesis through the dissociation of peptidyl-tRNA from the 50S subunit of the ribosome (Brisson-Noel et al., 1988). Peptidyl-tRNA disruption affects peptide bond formation, thus amino acids are unable to be linked together to form proteins. In Exp. 1, N utilization was negatively impacted with tylosin supplementation, which could be due to the competition between the host and microflora for uptake of amino acids or tylosin negatively affecting the hosts' metabolic processes (Veraeke et al., 1979).

Virginiamycin is a streptogramin that targets mainly gram positive bacteria and treats swine dysentery and is used in the poultry industry to prevent and control *Clostridium perfringens*. Virginiamycin inhibits protein synthesis by 2 components (M and S) working synergistically to release an incomplete protein from the ribosome. The M component inhibits protein elongation that leads to a conformational change of the ribosome, thus increasing the affinity of the S component to bind to the ribosome. Binding of the S component leads to the release of the incomplete protein (Bouanchaud, 1997; Butaye et al., 2003; Page, 2003). Results of Exp. 1 showed that carbadox increased GE utilization, and tylosin increased N retention of 18 to 20 kg pigs, which are consistent with direct influence on microflora within the gastrointestinal tract by reducing competition for nutrients and microbial metabolites that can negatively the host (Anderson et al., 1999; Dibner and Richards, 2005). In the broiler chicken study, the addition of virginiamycin improved G:F ratio when compared with diets devoid of antimicrobials with the same phytase levels. Orally-fed virginiamycin is not absorbed through the gastrointestinal wall of the animal, therefore alterations in nutrient utilization must be caused by changes in the microflora of the gastrointestinal tract. Overall, virginiamycin supplementation did not improve final BW, DM, N, Ca, and P digestibility and retention regardless of species. However, several pig experiments have been conducted that have shown supplementation with virginiamycin improved P digestibility and retention, which may be due to differences in age of pigs, diets, feeding level, length of adaptation period, and sanitation situations (Agudelo et al., 2007; Lindemann et al., 2010; Stewart et al., 2010). Phytase is an enzyme that can be fed to non-ruminant animals to reduce the amount of inorganic phosphorus fed, and therefore decrease the amount of phytate P that is excreted into manure and the environment. Supplementation of diets fed to swine and broiler chickens with phytase improves growth performance, Ca, P, DM, E, and N digestibility and retention, and this has been previously reported (Sands et al., 2001, 2009; Adeola et al., 2004; Jendza et al., 2006). Because supplementation of P-deficient diet with phytase is known to improve P digestibility and some studies have shown

that some antimicrobials may also improve P digestibility, one of the objectives of the current studies was to determine if P digestibility response to phytase is affected by supplementation with antimicrobials. In the pig study (Exp. 1), there were tendencies for interactions between antimicrobial and phytase supplementation in Ca and N utilization. For Ca, this was due to a less pronounced effect of phytase in diets supplemented with tylosin or virginiamycin than those diets that were not supplemented with antimicrobials. Nitrogen retention was not affected by phytase supplementation in diets without added antimicrobials in Exp. 2; however, phytase improved N retention in pigs fed diets supplemented in tylosin or virginiamycin. There were no interactions between antimicrobial and phytase supplementation for any of the growth performance response in the broiler chicken study (Exp. 3). There were interactions between supplementation of diets with antimicrobial and phytase for ileal nitrogen and GE digestibility. Some of these interaction responses may be due to virginiamycin the microflora present in the gastrointestinal tract. It is possible that the microflora in the small intestine utilize phytate, thus liberating more phytate bound nutrients. Overall, the addition of phytase is able to improve growth performance and P utilization, which is in agreement with other literature (Cromwell et al., 1993; Wodzinski and Ullah, 1996; Harper et al., 1997). Further investigation is needed to understand how antimicrobials can influence nutrient utilization and potential interactions with other feed additives.

4. Conclusions

Overall, phytase supplementation improved growth performance, nutrient digestibility and retention, regardless of supplementation of diets with antimicrobials. Supplementation of diets with antimicrobials did not affect P digestibility or retention because of a lack of interaction between antimicrobials and phytase. There was no evidence that P digestibility response to phytase is affected by supplementation with antimicrobials.

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