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#### **Original Research Article**

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

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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.

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#### 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

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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 cornsoybean 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  $3 \times 3$  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

#### 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	1.5	1.5	1.5	1.5
phosphate <sup>1</sup>				
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 <sup>2</sup>	0.5	0.5	0.5	0.5
Vitamin premix <sup>3</sup>	1.5	1.5	1.5	1.5
Mineral premix <sup>4</sup>	1.0	1.0	1.0	1.0
Carbadox premix <sup>5</sup>	0.0	20.0	0.0	0.0
Tylosin premix <sup>5</sup>	0.0	0.0	20.0	0.0
Virginiamycin premix <sup>5</sup>	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,	3,972	4,001	3,993	4,010
kcal/kg				
Metabolizable	3,764	3,862	3,766	3,849
energy, kcal/kg 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 <sup>6</sup>	5.7 1.9	1.9	5.8 1.9	5.8 1.9
Carbadox, mg/kg	0.0	48.9	0.0	0.0
Tylosin, mg/kg	0.0	48.9	0.0 38.1	0.0
Virginiamycin,	0.0	0.0	38.1 0.0	10.2
mg/kg	0.0	0.0	0.0	10.2

STTD = standardized total tract digestible.

<sup>1</sup> Contained 16% Ca, 21% P.

 $^2\,$  Selenium premix supplied 300  $\mu g$  of selenium per kilogram of diet.

 $^3$  Vitamin premix supplied per kilogram of diet: vitamin A, 3,630 IU; vitamin D<sub>3</sub>, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg, vitamin B<sub>12</sub>, 23.1 µg; riboflavin, 5.28 mg; D-pantothenic acid, 13.1 mg; niacin, 19.8 mg.

<sup>4</sup> Mineral premix supplied per kilogram of diet: Cu (as CuCl<sub>2</sub>), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as FeCO<sub>3</sub>), 121 mg; Mn (as MnO), 15 mg; and Zn (as ZnO), 121 mg.
<sup>5</sup> Carbadox premix supplied 55 mg carbadox (active drug)/kg diet (Mecadox 10,

<sup>5</sup> 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.

<sup>6</sup> Calculated STTD phosphorus.

appearance in the feces. During the collection period total amount of feces was collected twice daily and stored at  $-20^{\circ}$ 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  $-20^{\circ}$ 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 5d adaptation followed by a 5-d collection period in Exp. 1 but a 7d adaptation followed by a 7-d collection period in Exp. 2.

#### 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,

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