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

Article history:
Received 30 July 2016
Received in revised form 21 October 2016
Accepted 5 November 2016
Available online 10 November 2016

Keywords:
Antimicrobials
Chickens
Phosphorus
Phytase
Pigs

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.

© 2017, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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). Oral-fed antimicrobials directly influence the microflora within the gastrointestinal tract by reducing competition for nutrients and microbial
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, Águedo 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 oil-seed 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 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). 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

---

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

| Item                  | 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 phosphate¹ | 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 premix²      | 0.5         | 0.5           | 0.5          | 0.5                |
| Vitamin premix³        | 1.5         | 1.5           | 1.5          | 1.5                |
| Mineral premix⁴        | 1.0         | 1.0           | 1.0          | 1.0                |
| Carbadox premix⁵       | 0.0         | 20.0          | 0.0          | 0.0                |
| Tylosin premix⁶        | 0.0         | 0.0           | 20.0         | 0.0                |
| Virginiamycin premix⁷  | 0.0         | 0.0           | 0.0          | 20.0               |
| Total phosphorus, g/kg | 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 |

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 D₃, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg; vitamin B₁₂, 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 CuCl₂), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as FeCO₃), 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.

---

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.

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, 2014).
2013). All broilers were reared in stainless-steel, 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 de- asphyxiated with CO2 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.

1. 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, Hann, Germany) and benzoic acid as an internal standard. Gross energy of diets, dried feces, and dried ileal digesta were determined using adiabatic bomb calorimetry (Model 1261, Parr Instrument Co., Moline, IL, USA) and benzoic acid as an internal standard. Gross energy of diets, dried ileal digesta, and excreta were determined using adiabatic bomb calorimetry (Model 1261, Parr Instrument Co., Moline, IL, USA) and benzoic acid as an internal standard.

Table 2

| Item                  | None | None | Tylosin | Tylosin | Virginiamycin | Virginiamycin | Virginiamycin |
|-----------------------|------|------|---------|---------|---------------|---------------|---------------|
| Antimicrobial         | Phytase, FTU/kg | Phytase, FTU/kg | Phytase, FTU/kg | Phytase, FTU/kg | Phytase, FTU/kg | Phytase, FTU/kg | Phytase, FTU/kg |
| Phytase, FTU/kg       | 0    | 50   | 527     | 1,370   | 0             | 20.0          | 60.0          |
| Tylosin, mg/kg        | 0    | 0    | 20.0    | 20.0    | 0             | 20.0          | 20.0          |
| Virginiamycin, mg/kg  | 0    | 0    | 0       | 0       | 0             | 20.0          | 60.0          |

1. Vitamin premix supplied per kilogram of diet: Vitamin A, 3,630 IU; vitamin D3, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg; vitamin B12, 23.1 µg; riboflavin, 5.28 mg; D-pantothenic acid, 13.1 mg; nacinc, 19.8 mg.

2. Mineral premix supplied per kilogram of diet: Cu (as CuCl2), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as FeCO3), 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/kg 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.

K. McCormick et al. / Animal Nutrition 3 (2017) 77–84

79
and read using spectroscopy at 410 nm (Myers, 2004).

I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 mg.

Adding 30% H2O2 to the mixture. The mixture was then prepared for ash determination. The analysis of Ti for diets and excreta was conducted by digesting samples with 60% sulfuric acid, and then combustion method using a LECO Model FP-2,000 Nitrogen Analyzer (LECO, St. Joseph, MI, USA). Phosphorus determination used a colorimetric assay with the addition of acid molybdate and Fiske’s Subbarow reducer solution.

Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.

Phytase and phytic acid in diets. Tylosin activity was analyzed by Covance Technologies Inc., Chantilly, VA, USA). Concentration of Ca in diets, feces, and excreta was determined using an atomic absorption method for Ca and P determination. Phosphorus and phytic acid levels, and Megazyme method for total phytase levels, and Megazyme method for total phytase levels.
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 $\alpha$ 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 $17.25 \pm 0.48$ kg and final BW ($18.4, 18.7, 18.1, and 17.7 \pm 0.69$ 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).

Diet 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 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; 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$^2$   | 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$^4$   | 38.3            | 38.0        | 44.6             | 36.3         | 87.5            | 73.4        | 87.3                      | 84.5                   |
| SEM$^5$      | 2.39            | 2.36        | 4.84             | 4.60         | 0.71            | 2.15        | 0.46                      | 0.46                   |

$^1$ 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.
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

| 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^1         | 0                   | 1.96                  | 1.96  | 2.83  | 2.83   | 0.65   | 1.71  | 0.40  | 0.42             |                  |
| Tylosin       | 500                 | 58.5                  | 58.2  | 73.8  | 73.8   | 88.6  | 62.5  | 88.2  | 85.2             |                  |
| Virginiamycin | 1,500               | 59.9                  | 59.7  | 72.5  | 72.4   | 87.8  | 64.1  | 88.0  | 85.0             |                  |
| SEM^1         | 0                   | 58.5                  | 58.2  | 73.8  | 73.8   | 88.6  | 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             |                  |
| SEM^2         | 0                   | 44.2                  | 43.9  | 66.7  | 66.5   | 86.9  | 60.6  | 86.3  | 85.0             |                  |
| Virginiamycin | 61.5                | 61.2                  | 74.7  | 74.7   | 86.9  | 64.5  | 87.5  | 84.8             |                  |
| SEM^2         | 1.13                | 1.13                  | 1.62  | 0.37  | 1.05   | 0.23  | 0.24  |       |                  |
| 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             |                  |
| SEM^3         | 0                   | 44.2                  | 43.9  | 66.7  | 66.5   | 86.9  | 60.6  | 86.3  | 85.0             |                  |
| 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             |                  |
| SEM^3         | 1.13                | 1.13                  | 1.62  | 0.37  | 1.05   | 0.23  | 0.24  |       |                  |

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.

| 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^1         | 0                   | 13.9                  | 13.9            | 243            | 123            | 0.83           |              |
| Tylosin       | 13.9                | 13.9                  | 243             | 123            | 0.83           |              |              |
| Virginiamycin | 677                 | 592                   | 853             | 695            | 43.2           |              |              |
| SEM^2         | 0                   | 539                   | 454             | 785            | 583            | 36.4           |              |
| Tylosin       | 684                 | 600                   | 840             | 714            | 42.7           |              |              |
| Virginiamycin | 797                 | 712                   | 942             | 756            | 47.5           |              |              |
| SEM^2         | 8.0                 | 8.0                   | 14.4            | 7.1            | 0.48           |              |              |

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.
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; 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 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.

Acknowledgements

The authors gratefully acknowledge Pat Jaynes for her competent technical assistance.

References

Adeola O, Konc K. Energy value of dried distillers grains with solubles and oilseed meals for pigs. J Anim Sci 2014;92:164–70.
Adeola O, Walk CL. Linking ideal digestible phosphorus and bone mineralization in broiler chickens fed diets supplemented with phytase and highly soluble calcium. Poult Sci 2013;92:2109–17.
Adeola O, Sands JS, Simms PN, Schulze H. The efficacy of an E. coli-derived phytase preparation. J Anim Sci 2004;82:2657–66.
Agudelo JH, Lindemann MD, Cromwell GL, Newman MC, Nimmo RD. Virginiamycin improves phosphorus digestibility and utilization by growing-finishing pigs fed a phosphorus-deficient, corn-soybean meal diet. J Anim Sci 2007;85:2173–82.
Anderson DB, McCracken VJ, Aminov RI, Simpson JM, Mackie RI, Vestegen MWA, et al. Gut microbiology and growth-promoting antibiotics in swine. Pig News Inf 1999;20:115N–22N.
Bhunia AK. Biology of microbes associated with food. In: Feldman DR, editor. Foodborne microbial pathogens: mechanisms and pathogenesis. New York. N.Y.: Springer Science + Business Media, LLC; 2008. p. 17–22.
Bouanchaud D, Strepptomycins from parenteral to oral. In: Zimmer SN, Young LS, editors. Expanding indications for the new macrolides, azalides, and streptomycins. New York, N.Y.: Marcel Dekker, Inc.; 1997. p. 51–66.
Brison-Noel A, Trieu-Cuot P, Courvalin P. Mechanism of action of spiramycin and other macrolides. J Antimicrob Chemother 1988;22(Suppl. B):13–23.
Butaye P, Devrieses LA, Haesebrock F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clin Microbiol Rev 2002;16:174–88.
Cromwell GL, Stahlly TS, Coffey RD, Monegue HJ, Randolph JH. Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J Anim Sci 1993;71:1831–40.
Cromwell GL, Antimicrobial and promicrobial agents. In: Lewis AJ, Southern LL, editors. Swine nutrition. 2nd ed. Boca Raton, FL: CRC Press; 2001. p. 401–21.
Dibner J, Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 2005;84:634–43.
Feighner SD, Daskalovich MP. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial choleyltaurine hydrolytic activity. Appl Environ Microbiol 1987;53:311–6.
Gaskins HR. Intestinal bacteria and their influence on swine growth. In: Lewis AJ, Southern LL, editors. Swine nutrition. 2nd ed. Boca Raton, FL: CRC Press; 2001. p. 585–608.
Harper AF, Kornegay ET, Schell TC. Phytase supplementation of low-phosphorus growing-finishing pig diets improves phosphorus digestibility, and bone mineralization, and reduces phosphorus excretion. J Anim Sci 1997;75:3174–86.
Hedde RD. Intestinal fermentation in the pig and how it is influenced by age and virginiamycin. In: Proceedings of the growth promotion mode of action symposium. Philadelphia: SmithKline Corp.; 1981. p. 10–20.
Hendericx HK, Vervaekte I, Decuyper J, Dierick N. The nutritional mode of action of some feed additives in pigs. Vet Res Commun 1983;7:9.
Jendza JA, Dilger RN, Sands JS, Adeola O. Efficacy and equivalency of an Escherichia coli-derived phytase for replacing inorganic phosphorus in the diets of broiler chickens and young pigs. J Anim Sci 2006;84:3364–74.
Kelly D, King TP. Luminal bacteria: regulation of gut function and immunity. In: Piva A, Bach Knudsen KE, Lindberg JE, editors. Gut environment of pigs. Nottingham, UK: Nottingham University Press; 2001. p. 113–31.
Lindemann MD, Kim BG. Technical note: a model to estimate individual feed intake of swine in group feeding. J Anim Sci 2007;85:972–5.
Lindemann MD, Quant AD, Monegue JS, Wang M, Cromwell GL, Newman MC. Evaluation of antibiotic effects on phosphorus digestibility and utilization by growing-finishing pigs fed a phosphorus-deficient, corn-soybean meal diet. J Anim Sci 2010;88:1752–8.
Maerz DD, Classen HL. Phytase activity in the small intestinal brush border membrane of the chicken. Poult Sci 1998;78:1577–86.
Myers WD. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci 2004;82:179–83.
NRC. Nutrient requirements of poultry. 9th ed. Washington, DC: Natl. Acad. Press; 1994.
NRC. Nutrient requirements of swine. 11th ed. Washington, DC: Natl. Acad. Press; 2012.
Sands JS, Ragland D, Baxter C, Joern BC, Sauber TE, Adeola O. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J Anim Sci 2001;79:2134–42.
Sands JS, Ragland D, Dilger RN, Adeola O. Responses of pigs to Apergillus niger phytase supplementation of low-protein or high-phytin diets. J Anim Sci 2009;87:2581–9.
Stewart LL, Kim BG, Gramm BR, Nimmo RD, Stein HH. Effect of virginiamycin on the apparent ideal digestibility of amino acids by growing pigs. J Anim Sci 2010;88:1718–24.
Veraeeke I, Decuyper J, Dierick NA, Hendericx HK. Quantitative in vitro evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. J Anim Sci 1979;49:846–56.
Visek WJ. The mode of growth promotion by antibiotics. J Anim Sci 1978;46:1447–69.
Wang Y, Yuan Z, Zhu H, Ding M, Fan S. Effect of cyadox on growth and nutrient digestibility in weanling pigs. South Afr J Anim Sci 2005;35:117–25.
Wodzinski RJ, Ullah AHJ. Phytase Adv Appl Microbiol 1996;42:263–302.