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NON RUMINANT NUTRITION

# Growth performance, nutrient digestibility, and fecal microbial composition of weaned pigs fed multi-enzyme supplemented diets

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## Abstract

A study determined the effects of supplementing corn-based diets for weaned pigs with multi-enzymes on growth performance, apparent total tract digestibility (ATTD) of nutrients, fecal score, and fecal microbial composition. A total of 132 pigs (initial body weight = 7.23 kg) that had been weaned at 21 d of age and fed a drug-free nursery diet for 7 d were housed in 33 pens of 4 barrows or gilts, blocked by body weight and gender, and fed 3 experimental diets at 11 pens per diet. The diets were corn-based diet without or with multi-enzyme A or B. Multi-enzyme A supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 1,500 U of amylase per kilogram of diet. Multi-enzyme B was the same as multi-enzyme A except that it supplied amylase at 150 U/kg, and that its source of amylase was different from that of multi-enzyme A. All diets contained phytase at 1,000 U/kg. The diets were fed for 35 d in 2 phases; phase 1 for the first 14 d and phase 2 for the last 21 d of the trial. Fecal score was determined daily during the first 7 d of the trial. Fecal samples were collected from rectum of 1 pig per pen on days 2, 7, 14, and 35 of the trial for determining bacterial composition. Also, fresh fecal samples were collected from each pen on days 41 and 42 to determine ATTD of nutrients. Multi-enzyme B increased ($P < 0.05$) average daily gain (**ADG**) for phases 1 and 2. For the overall study period, multi-enzyme B increased ($P < 0.05$) ADG from 262 to 313 g, and average daily feed intake (**ADFI**) from 419 to 504 g. Multi-enzyme A increased ($P < 0.05$) overall ADG from 262 to 290 g, but did not affect ADFI. Multi-enzyme A or B did not affect ATTD of gross energy, but increased ($P < 0.05$) the ATTD of ether extract from 30% to 36% or 37%, respectively. Multi-enzyme A did not affect fecal score; however, multi-enzyme B tended to decrease ($P = 0.09$) fecal score, implying that it tended to decrease diarrhea. Firmicutes were the most abundant phylum of fecal bacteria (its relative abundance ranged from 58% to 72%). Bacteroidetes and Actinobacteria were the 2nd and 3rd most abundant phyla of fecal bacteria. Neither multi-enzyme affected fecal bacterial composition. In conclusion, the addition of multi-enzyme A or B to phytase-supplemented corn-based diet for weaned pigs can improve their growth performance and fat digestibility. However, multi-enzyme B was more effective than multi-enzyme A in terms of improving the growth performance of weaned pigs fed corn-based diet.

**Key words:** fecal microbial composition, growth performance, multi-enzyme, nutrient digestibility, weaned pigs

**Abbreviation**

| | |
|---|---|
| ADFI | average daily feed intake |
| ADG | average daily gain |
| ATTD | apparent total tract digestibility |
| CP | crude protein |
| DM | dry matter |
| EE | ether extract |
| G:F | gain to feed ratio |
| GE | gross energy |
| NSP | non-starch polysaccharides |
| OM | organic matter |
| OTU | operational taxonomic units |
| PCA | principal component analysis |

## Introduction

Cereal grains contain phytate and nonstarch polysaccharides (**NSP**) that reduce nutrient digestibility in pigs (Woyengo et al., 2009; Adeola and Cowieson, 2011; Woyengo and Nyachoti, 2013). Also, NSP can induce inflammatory response in pigs (Ferrandis Vila et al., 2018), which in turn, lead to increased utilization of energy and nutrients for maintenance at the expense of growth (Huntley et al., 2018). Furthermore, starch granules that are embedded in the proteins matrix in cereal grains are less accessible by starch-degrading enzymes than starch that is not embedded in the proteins matrix (Zaefarian et al., 2015).

Supplementation of cereal grain-based diets for pigs with NSP-degrading enzymes (that target the most abundant NSP in the cereal grains) and phytase may alleviate the negative effects of NSP and phytate, respectively. Also, supplementation of cereal grain-based diets for pigs with protease may increase the degradation of protein that interacts with NSP and starch, thereby increasing nutrient utilization. Supplemental amylase may also improve starch digestibility. Weaned pigs have poorly developed gastrointestinal tract and immune system compared with growing-finishing pigs (Heo et al., 2013), and may, thus, benefit more than growing-finishing pigs from dietary supplementation with enzymes. The effects of supplementing cereal grain-based diets for weaned pigs with enzyme products that contain one, or various combinations of the NSP-degrading enzymes on nutrient utilization by pigs have been determined in several studies and reviewed (Adeola and Cowieson, 2011). However, there is limited information on the effects of adding a combination of NSP-degrading enzymes, protease, and different sources of amylases to phytase-supplemented diets for weaned pigs on nutrient utilization and indicators of gut health. Efficacy of supplemental enzymes may vary depending on source (type of microorganism that is used to produce it; McCleary et al., 2015). Thus, it is hypothesized that addition of enzyme products containing NSP-degrading enzymes, protease, and amylase to phytase-supplemented diets for weaned pigs can improve growth performance, gut health, and nutrient digestibility; and that the magnitude of improvement in growth performance, gut health, and nutrient digestibility of weaned pigs vary depending on the sources of the enzymes in the enzyme products. The objective of the current study was to determine the effects of adding 2 multi-enzyme products that contained xylanase, β-glucanase, protease, and 1 of 2 novel amylase products to phytase-supplemented corn–soybean meal-based diets for weaned pigs on growth performance, apparent total tract digestibility (**ATTD**) of energy and nutrients, fecal score, and fecal bacterial composition.

## Materials and Methods

The experimental animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (#17-093A).

### Animals and housing

A total of 132 pigs (60 barrows and 72 gilts; initial body weight = 7.23 ± 1.03 kg; Large White-Landrace female × Duroc male; Pig Improvement Company, Austin, MN), weaned at 21 d of age were obtained from the Swine Education and Research Facility, South Dakota State University (Brookings, SD), and used in this study. The pigs were group housed in pens and fed a drug-free nursery diet for 1 wk. Pigs were then weighed, blocked by weight, and housed in 33 pens (15 pens of 4 barrows and 18 pens of 4 gilts) in an environment-controlled room. The pens (1.8 × 2.4 m) had fully slated-concrete floors, metal spindle walls (1.0 m high), and solid polyvinyl chloride gates. Each pen was equipped with a cup drinker, a double-spaced dry feeder, and a heat lamp. Room temperature was maintained at 28 ± 1 °C during the first week. Thereafter, room temperature was maintained at 27 ± 2 °C throughout the experimental period.

### Experimental diets and procedure

Three diets were fed to 33 pens to give 11 replicates per diet (5 pens of barrows and 6 pens of gilts per diet) in a randomized complete block design. The diets were corn–soybean meal-based diet without or with multi-enzyme A or multi-enzyme B (Table 1). Multi-enzyme A supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 1,500 U of amylase per kilogram of diet. Multi-enzyme B was the same as multi-enzyme A with regard to supply of enzyme activities except for amylase, which supplied 150 U/kg and whose source was different from that of multi-enzyme A. All diets contained phytase at 1,000 FTU/kg, and were fed in mash form. The enzymes were supplied by DuPont Nutrition & Biosciences (Wilmington, DE). The basal diet was formulated to meet or exceed the NRC (2012) net energy and nutrient requirement estimates for weaned pigs. The diets were fed for 5 wk in 2 growth phases based on age; phase 1 for the first 2 wk of the trial and phase 2 for the last 3 wk of the trial. Acid-insoluble ash (Celite 281; World Minerals, Santa Barbara, CA) was included in phase 2 diets as an indigestible marker (at 1%). Pigs were allowed ad libitum access to experimental diets and water throughout the experiment. The body weight of pigs and feed intake were determined by phase for calculation of average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**). The occurrence and severity of diarrhea was assessed daily during the first 7 d of the trial on a pen basis by using a fecal consistency scoring system (1 = firm feces, 2 = semi-solid feces, 3 = normal feces, 4 = mild diarrhea, and 5 = severe diarrhea). On day 2 of the study, fecal samples were collected by rectal palpation from 1 representative pig (based on body weight) from each pen. Also, on days 7, 14, and 35 of the study, fecal samples were collected by rectal palpation from the same pen representative pigs selected prior to the beginning of the feeding trial. The collected fecal samples were snap-frozen in liquid N, and stored at −80 °C for determination of microbial composition. Fecal samples were also collected from each pen during the last 2 d of the experiment and stored at −20 °C for later calculation of ATTD of energy and nutrients.

**Table 1.** Ingredient and calculated nutrient composition of the basal diets, as-fed basis[1]

| Item | Basal diets | |
|---|---|---|
| | Phase 1 | Phase 2 |
| Ingredient, % | | |
| Corn | 56.83 | 59.70 |
| Soybean meal | 21.95 | 24.00 |
| Barley | 6.00 | 7.50 |
| Whey powder | 8.00 | 2.50 |
| Fish meal | 4.00 | 2.00 |
| Soybean oil | 0.00 | 0.30 |
| Monoclacium phosphate | 0.35 | 0.44 |
| Limestone | 1.14 | 1.15 |
| Salt | 0.50 | 0.60 |
| $_{L}$-Lys HCl | 0.43 | 0.34 |
| $_{DL}$-Met | 0.09 | 0.08 |
| $_{L}$-Thr | 0.18 | 0.18 |
| $_{L}$-Trp | 0.03 | 0.01 |
| Vitamin premix[2] | 0.05 | 0.05 |
| Mineral premix[3] | 0.15 | 0.15 |
| Zinc oxide | 0.30 | 0.00 |
| Celite[4] | 0.00 | 1.00 |
| Calculated composition | | |
| Digestible energy, kcal/kg | 3,250 | 3,380 |
| Metabolizable energy, kcal/kg | 3,280 | 3,250 |
| Net energy, kcal/kg | 2,448 | 2,412 |
| Crude protein, % | 20.20 | 20.08 |
| Ether extract, % | 4.60 | 4.78 |
| Neutral detergent fiber, % | 9.14 | 10.27 |
| Ca, % | 0.80 | 0.70 |
| Total P, % | 0.71 | 0.76 |
| Standardized total tract digestible P, % | 0.40 | 0.33 |
| Standardized ileal digestible Lys, % | 1.35 | 1.23 |
| Standardized ileal digestible Met, % | 0.39 | 0.36 |
| Standardized ileal digestible Thr, % | 0.79 | 0.73 |

[1]Phase 1 diets were fed from days 1 to 14, whereas phase 2 diets were fed from days 14 to 35.
[2]Provided per kilogram of diet: 2226 IU vitamin A, 340 IU vitamin D3, 11.3 IU vitamin E, 0.01 mg vitamin B12, 0.91 mg menadione, 2.04 mg riboflavin, 12.5 mg pantothenic acid, 11.3 mg niacin, 0.23 mg folic acid, 0.68 mg pyridoxine, 0.68 mg thiamine, and 0.04 mg biotin.
[3]Provided per kilogram of diet: 75 mg Zn as $ZnSO_4$, 75 mg Fe as $FeSO_4$; 7 mg Cu as $CuSO_4$, and 20 mg Mn as $MnSO_4$.
[4]Celite 281 (World Minerals Inc., Santa Barbara, CA) used as acid insoluble ash.

## Preparation and analysis of samples for nutrient and enzyme activities

### *Sample preparation*

Fecal samples were thawed, and dried in an oven at 60 °C for 4 d (Woyengo et al., 2008). Diet and dried fecal samples were ground through a 0.75-mm screen using a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany) before chemical analysis. All samples were analyzed for dry matter (**DM**), gross energy (**GE**), ether extract (**EE**), crude protein (**CP**), organic matter (**OM**), and acid-insoluble ash. Diet samples were additionally analyzed for enzyme activities.

### *Sample analysis*

Energy was determined using an adiabatic bomb calorimeter (model 1261, Parr Instrument Co., Moline, IL), using benzoic acid as a calibration standard. The samples were analyzed for DM (method 930.15), EE (method 920.39), and CP (method 990.03) according to the Association of Official Analytical Chemists (AOAC; 2007) procedures. The acid-insoluble ash contents of the diet and fecal samples were determined according to the method of McCarthy et al. (1974). Phytase activity in diets was determined by ISO method (method 30024:2009[E]; ISO, 2009). Xylanase and β-glucanase activities in diets were determined at DuPont Nutrition & Biosciences Innovation Laboratories (Brabrand, Denmark). One xylanase unit is the amount of enzyme that releases 0.5 µmol of reducing sugar equivalents (as xylose by the Dinitrosalicylic acid-reducing sugar method) from an oat-spelled-xylan substrate per min at pH 5.3 and 50 °C. One β-glucanase unit is the amount of enzyme that releases 2.4 µmol of reducing sugar equivalents (as glucose by the Dinitrosalicylic acid-reducing sugar method) from barley glucan per min at pH 5.0 and 50 °C.

## Fecal microbial analysis

Microbial genomic DNA was extracted from 50 to 100 mg of fecal samples from the 33 pigs (11 pigs per treatment) using the Qiagen MagAttract PowerSoil DNA KF Kit. DNA extractions were performed using the automated Thermo KingFisher Flex instrument. The extraction was performed as per the kit instructions and the resultant metagenomic DNA was utilized for 16S Bacterial Community Sequencing. Metagenomic DNA was processed prior to sequencing as follows: 35 cycles of PCR (95 °C 10 min + 35× 95 °C 15 s + 55 °C 30 s + 72 °C 2 min) were used to amplify the 16S V4 region. The PCR reactions consisted of 0.2 µM final concentration of each of the following 16S V4 PCR primers with added Illumina sequencing adapters; 515F: 5′ TCG TCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGC GGTAA and 806R: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAGGGACTACHVGGGTWTCTAAT, 25 µL ABI Universal TaqMan Master Mix, 23.8 µL of Molecular Biology Grade Water and 1 µL of metagenomic DNA for a total 50 µL PCR reaction. The resultant PCR products were purified using Agencourt AmPure Magentic Beads on the Agilent Bravo Liquid Robotic Workstation as per manufacturer's instructions. The purified PCR products were then uniquely indexed using Illumina Nextera v2 PCR primers. The index PCR was run for 10 cycles (95 °C 10 min + 10× 95 °C 30 s + 55 °C 30 s + 72 °C 2 min). Each index PCR reaction included 5 µL of each Illumina Index primer + 25 µL ABI Universal TaqMan Master Mix + 14 µL Molecular Biology Grade Water + 1 µL Purified V4 16S PCR product. The indexed PCR products were pooled and purified using Agencourt AmPure Magentic Beads on the Agilent Bravo Liquid Robotic Workstation as per manufacturer's instructions. The purified samples were then quantified using the KAPA Illumina Library qPCR Quantifiaction kit run on an ABI Quantstudio 7 qPCR instrument as per the manufacturer's instructions. The quantified pool was then run on the Illumina MiSeq at a final concentration of 8 pM using version 3 MiSeq reagents for 2 × 250 Paired End run conditions. The resultant sequences were then demultiplexed using the MiSeq Reporter software and the resultant FASTQ files were utilized for analysis.

The 16S Amplicon data from Illumina Miseq sequencing were processed by an in-house pipeline as described below. Paired-end reads were first merged by Flash (Magoč and Salzberg, 2011) with parameters "-O -M 800". The forward and reverse primers were removed from the merged reads, and reads with an overall quality score <20 were discarded by RDP Initial Process tool (Fish et al., 2013) with parameters "-F 2 -R 1 -m 220 -x 270 -n 0". The trimmed reads were assigned to bacterial and archaeal taxonomy by RDP Classifier with training set no. 16 with bootstrap cutoff of 50% (Wang et al., 2007). Any sample with <5,000 sequences was excluded from downstream

analysis. The reads that passed the above quality processing steps were clustered at 99% by CD-HIT (Li and Godzik, 2006) to obtain operational taxonomic units (**OTU**). To focus on relatively high abundant OTUs, only OTUs with abundance above 0.1% in at least 1 sample were kept. The representative sequence from each OTU was assigned to the closest species by RDP pairwise alignment tool (Fish et al., 2013) against a vetted 16S reference database containing mostly 16S genes from type strains and public genomes.

### Calculations and statistical analysis of growth performance and ATTD of nutrients data

The ATTD of GE, DM, OM, EE, and CP was calculated using the indicator method (Eq. [2]; Stein et al., 2007). Data were analyzed using the MIXED procedure of SAS (SAS 9.3, SAS Institute Inc., Cary, NC) in a randomized complete block design. The pen was used as the experimental unit and block was random factor. Phase was the repeated term in models involving time. Means were separated by probability of difference. Statistical differences were considered to be significant at $P \leq 0.05$, and tendencies were observed at $0.05 < P \leq 0.10$.

### Calculations and statistical analysis of fecal microbial composition data

#### *Alpha and β diversity*

The number of unique OTU and relative abundance of phyla, genera, and OTUs were obtained based on the classification and clustering results as described above. The relative abundance matrices were compared and visualized using principal component analysis (**PCA**). The R vegan package was used to perform PCA. PERMANOVA was used to test if there was significant difference in bacterial taxonomic composition between groups of microbiome samples.

#### *Differentially abundant taxa*

The differentially abundant taxa between treatments were detected by DESeq2 (Love et al., 2014) using the OTU assignment from the above described clustering analysis. The *P*-values were adjusted for false discovery rate using Benjamini–Hochberg correction. Only taxa with adjusted *P*-value < 0.05 were considered significant.

## Results

The analyzed CP values for the diets in Table 2 were close to the calculated values in Table 1. The effects of multi-enzyme supplementation on body weight, ADG, ADFI, G:F, and fecal score are shown in Table 3. Supplementation of the basal diet with multi-enzyme A or B increased ($P < 0.05$) body weight of pigs at end the phase 1 (day 14) of feeding. Supplementation of the basal diet with multi-enzyme B increased ($P < 0.05$) final body weight. Also, supplementation of the basal diet with multi-enzyme A tended to increase ($P = 0.10$) final body weight of pigs. In phase 1, pigs fed the multi-enzyme B-supplemented diet had a greater ($P < 0.05$) ADG than those fed the basal diet. Also, in phase 1, pigs fed multi-enzyme A-supplemented diet tended to have a greater ($P = 0.052$) ADG than those fed the basal diet. During phase 2, pigs fed the multi-enzyme B-supplemented diet had greater ($P < 0.05$) ADG than those fed the basal diet, and pigs fed the multi-enzyme A-supplemented diet had ADG that did not differ from that of pigs fed the basal diet. Overall (days 0 to 35), pigs fed a multi-enzyme A- or multi-enzyme B-supplemented diet had greater ($P < 0.05$) ADG than those fed the basal diet. The overall ADG for multi-enzyme B-supplemented diets tended to be greater ($P = 0.10$) than that for multi-enzyme B-supplemented diet. The ADFI for pigs fed the diet with multi-enzyme B tended to be greater ($P = 0.070$) than that of pigs fed the basal diet during phase 1 of feeding. The ADFI for pigs fed the diet with multi-enzyme B was greater ($P < 0.05$) than that of pigs fed the basal diet during phase 2. However, the ADFI for pigs fed the diet with multi-enzyme A did not differ from that of pigs fed the basal diet during phases 1 and 2 of feeding. Overall, the ADFI for diets with multi-enzyme B was greater ($P < 0.05$) than for the basal diet, whereas ADFI for diets with multi-enzyme A did not differ from that of pigs fed the basal diet. The G:F was not affected by dietary treatment during phase 1 or 2 or during the entire study period. Supplementation of the basal diet with multi-enzyme B tended

**Table 2.** Analyzed composition and enzyme activity in diets, as-fed basis

| | Diet[2] | | |
|---|---|---|---|
| Item[1] | Basal | Multi-enzyme A | Multi-enzyme B |
| Dry matter, % | | | |
| Phase 1 | 90.12 | 90.21 | 90.06 |
| Phase 2 | 90.18 | 90.39 | 90.11 |
| GE, kcal/kg | | | |
| Phase 1 | 4,060 | 4,026 | 4,054 |
| Phase 2 | 4,084 | 4,045 | 4,035 |
| Starch, % | | | |
| Phase 1 | 46.62 | 48.70 | 45.90 |
| Phase 2 | 47.01 | 47.77 | 48.08 |
| Crude protein, % | | | |
| Phase 1 | 21.16 | 21.55 | 21.57 |
| Phase 2 | 21.24 | 20.99 | 21.22 |
| Ether extract, % | | | |
| Phase 1 | 2.61 | 2.44 | 2.18 |
| Phase 2 | 2.72 | 2.82 | 2.74 |
| Ash, % | | | |
| Phase 1 | 6.26 | 5.51 | 5.96 |
| Phase 2 | 5.97 | 5.71 | 6.37 |
| Ca, % | | | |
| Phase 1 | 1.18 | 0.91 | 1.15 |
| Phase 2 | 0.94 | 0.95 | 1.08 |
| P, % | | | |
| Phase 1 | 0.70 | 0.60 | 0.66 |
| Phase 2 | 0.60 | 0.62 | 0.66 |
| Phytase activity, FTU/kg | | | |
| Phase 1 | 1,087 | 954 | 838 |
| Phase 2 | 880 | 941 | 826 |
| Xylanase, UX/kg | | | |
| Phase 1 | BDL[3] | 4,572 | 3,947 |
| Phase 2 | BDL | 4,066 | 3,822 |
| β-Glucanase, BGU/kg | | | |
| Phase 1 | BDL | 186 | 240 |
| Phase 2 | BDL | 236 | 225 |

[1]Phase 1 diets were fed from days 1 to 14, whereas phase 2 diets were fed from days 14 to 35.
[2]Basal, phytase-supplemented corn–barley–soybean meal-based diets; multi-enzyme A, basal diet supplemented with multi-enzyme A product that supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 1,500 U of amylase per kilogram of diet; and multi-enzyme B, basal diet supplemented with multi-enzyme B product that supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 150 U of amylase per kilogram of diet.
[3]BDL, below detectable limit.

**Table 3.** Growth performance and fecal score of nursery pigs fed experimental diets[1]

| | Diets[2] | | | | |
|---|---|---|---|---|---|
| Item | Basal | Multi-enzyme A | Multi-enzyme B | SEM | P-value |
| Body weight, kg | | | | | |
| Day 0 | 7.37 | 7.36 | 7.36 | 0.058 | 0.998 |
| Day 14 | 9.81[b] | 10.34[a] | 10.56[a] | 0.164 | 0.004 |
| Day 21 | 17.19[b] | 18.15[ab] | 18.95[a] | 0.425 | 0.016 |
| ADG, g | | | | | |
| Days 0 to 14 | 174.31[b] | 212.54[ab] | 228.78[a] | 13.37 | 0.022 |
| Days 14 to 35 | 349.68[b] | 368.42[ab] | 397.07[a] | 13.33 | 0.054 |
| Days 0 to 35 | 262.00[b] | 290.48[a] | 312.92[a] | 9.60 | 0.002 |
| ADFI, g | | | | | |
| Days 0 to 14 | 160.61 | 197.78 | 204.11 | 16.44 | 0.145 |
| Days 14 to 35 | 677.80 [b] | 730.15[ab] | 803.08[a] | 36.11 | 0.062 |
| Days 0 to 35 | 419.20[b] | 463.96[ab] | 503.60[a] | 19.58 | 0.012 |
| G:F, g/g | | | | | |
| Days 0 to 14 | 1.11 | 1.15 | 1.12 | 0.076 | 0.923 |
| Days 14 to 35 | 0.51 | 0.50 | 0.50 | 0.014 | 0.831 |
| Days 0 to 35 | 0.81 | 0.83 | 0.81 | 0.037 | 0.949 |
| Fecal score[3] | | | | | |
| Days 0 to 7 | 4.34 | 4.31 | 4.09 | 0.102 | 0.183 |

[ab]Within a row, means without a common superscript differ ($P < 0.05$).
[1]Values are LS means of 11 pens of pigs with 4 pigs per pen.
[2]Basal, phytase-supplemented corn-barley-soybean meal-based diets; Multi-enzyme A, basal diet supplemented with multi-enzyme A product that supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 1,500 U of amylase per kilogram of diet; and Multi-enzyme B, basal diet supplemented with multi-enzyme B product that supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 150 U of amylase per kilogram of diet.
[3]Fecal score: 1 to 5, where 1, firm feces; 2, semi-solid feces; 3, normal feces; 4, mild diarrhea; 5, severe diarrhea.

to reduce ($P = 0.09$) fecal score; however, supplementation of the basal diet with multi-enzyme A did not affect fecal score.

The effects of multi-enzyme supplementation on ATTD of energy and nutrients in nursery pigs are presented in Table 4. Supplementation of the basal diet with multi-enzyme B tended to increase ($P = 0.052$) the ATTD of CP. Supplementation of the basal diet with multi-enzyme A or B increased ($P < 0.05$) the ATTD of EE. There were no effect of enzyme supplementation on ATTD of GE. Sequence reads affiliated to Firmicutes were overall the most abundant at the phylum level, with mean relative abundances ranging from 58% to 72% across treatments (Figure 1). The 2nd and the 3rd most abundant were Bacteroidetes and Actinobacteria, respectively. Accordingly, *Lactobacillus* was the most highly represented genus, with group means between 11% and 27% (Figure 2). While differences in bacterial composition ($P < 0.05$) were observed by PCA and PERMANOVA between different time points (Figure 3 and Table 5). No differences in composition were observed among dietary treatments at any time point investigated (Figure 4; Table 6). Pair-wise comparative analyses between treatments at each time point revealed differences in abundance for 33 low abundance OTU.

**Table 4.** ATTD of energy and nutrients in nursery pigs fed experimental diets[1]

| | Diets[2] | | | | |
|---|---|---|---|---|---|
| ATTD, % | Basal | Multi-enzyme A | Multi-enzyme B | SEM | P-value |
| Dry matter | 82.24 | 82.04 | 82.69 | 0.451 | 0.574 |
| Organ matter | 83.95 | 83.80 | 84.43 | 0.415 | 0.529 |
| Gross energy | 82.71 | 82.34 | 83.03 | 0.438 | 0.535 |
| Crude protein | 75.88 | 77.10 | 77.99 | 0.740 | 0.143 |
| Ether extract | 29.95[b] | 35.95[a] | 37.06[a] | 1.573 | 0.007 |

[1]Values are means of 11 replicates.
[2]Control, phytase-supplemented corn–barley–soybean meal-based diets; multi-enzyme A, supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 1,500 U of amylase per kilogram of diet; multi-enzyme B, supplied 4,000 U of xylanase, 150 U of β-glucanase, 3,500 U of protease, and 150 U of amylase per kilogram of diet.
[ab]Within a row, means without a common superscript differ ($P < 0.05$).

## Discussion

In this study, the effects of adding multi-enzyme to phytase-supplemented corn–soybean meal-based diet on growth performance, ATTD, fecal score, and fecal microbial composition of weaned pigs were determined. The ADG of pigs fed corn–soybean meal-based diet was improved by supplementation with multi-enzyme A or B despite the nonsignificant effect of the multi-enzymes on ATTD of OM and GE. Li et al. (2018) also reported improved growth performance of weaned pigs fed high fiber diets without a change detected in ATTD of GE due to supplemental fiber-degrading enzymes. There are 2 possible mechanisms (other than improvement in total tract nutrient digestibility) by which NSP-degrading enzymes may improve growth performance of pigs. First, the NSP-degrading enzymes can increase ileal nutrient digestibility without affecting total tract nutrient digestibility, implying that the NSP-degrading enzymes can shift part of hindgut fermentation towards small intestine enzymatic digestion. For instance, Woyengo et al. (2015) observed increased in vitro digestion of wheat by porcine pepsin and pancreatin and reduced in vitro fermentation of

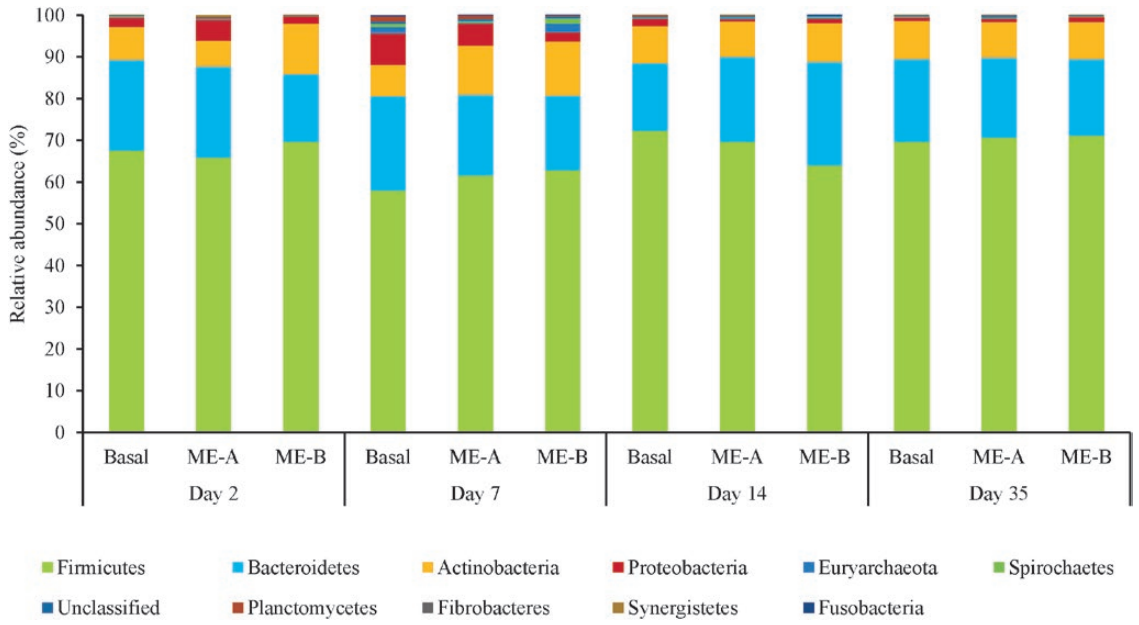


**Figure 1.** Taxonomic composition (at phylum level) of fecal bacterial communities from 3 dietary treatments at 4 different time points[1]. [1]Values are represented as the mean abundances of taxonomic groups from each treatment-time point group. Basal = basal diet; ME-A = basal diet + multi-enzyme A; and ME-B = basal diet + multi-enzyme B.

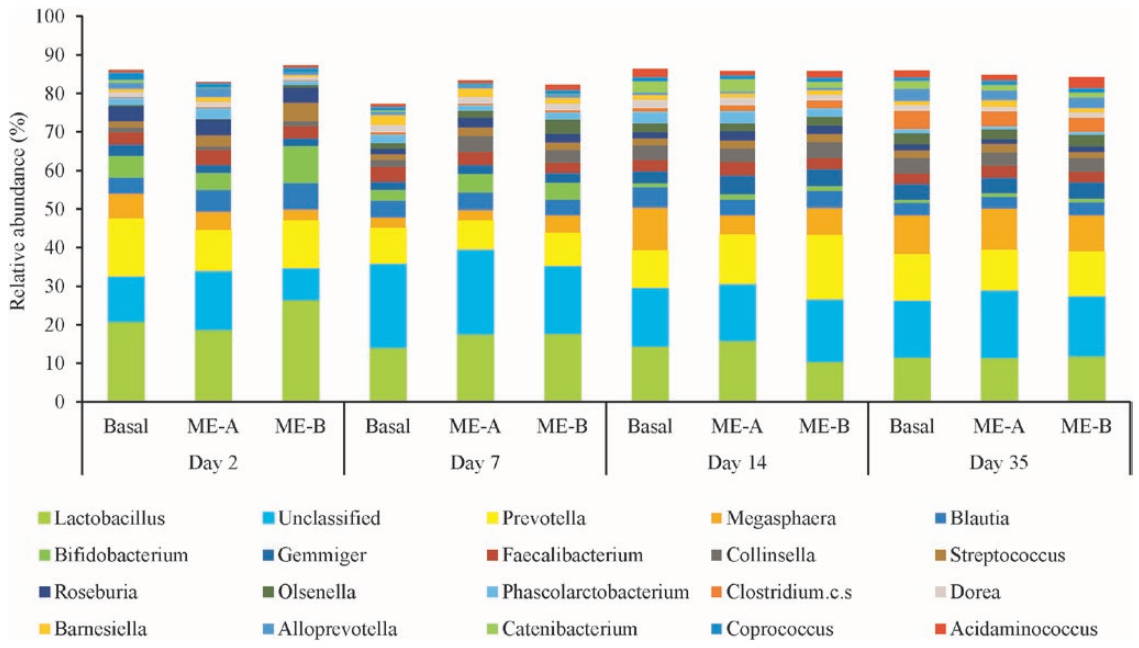


**Figure 2.** Taxonomic composition (at genus level) of fecal bacterial communities from 3 dietary treatments at 4 different time points[1]. [1]Values are represented as the mean abundances of taxonomic groups from each treatment-time point group. Basal = basal diet, ME-A = basal diet + multi-enzyme A, and ME-B = basal diet + multi-enzyme B.

the resulting undigested nutrients due to supplemental NSP-degrading enzymes; the overall in vitro digestibility (in vitro digestion plus in vitro fermentation) of the wheat millrun was not affected by supplemental NSP-degrading enzymes. Lee et al. (2018) also observed increased in vitro digestion of canola co-products by porcine pepsin and pancreatin and reduced in vitro fermentation of the resulting undigested nutrients due to supplemental NSP-degrading enzymes; the overall in vitro digestibility (in vitro digestion plus in vitro fermentation) of the canola co-products was not affected by supplemental NSP-degrading enzymes. Glucose and other hexose sugars that are the main end products of digestion of carbohydrates by digestive enzymes in the small intestine are more efficient sources of energy than volatile fatty acids that are the main end products of carbohydrate fermentation (Coles et al., 2013). Amino acids and peptides, which are end products of protein digestion in the

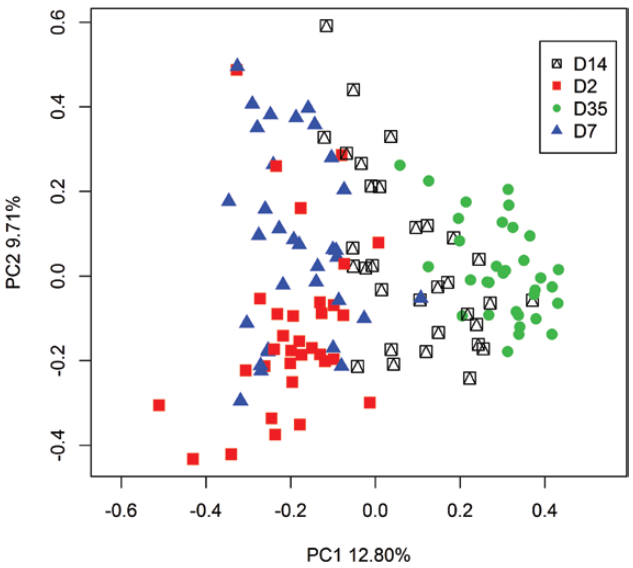


**Figure 3.** Comparative analysis of bacterial composition at 4 different time points[1] by PCA. The *x* and *y* axes correspond to principal components 1 (PC1) and 2 (PC2), which explained the highest level of variation. Bacterial communities were found to be significantly different between time points by PERMANOVA ($P < 0.05$). [1]D2 = day 2 of the experiment; D7 = day 7 of the experiment; D14 = day 14 of the experiment; and D35 = day 35 of the experiment.

**Table 5.** Comparative analysis of fecal bacterial composition at 4 different days of fecal collection by PERMANOVA

| Comparisons | | | |
|---|---|---|---|
| 1 | 2 | P-value | Effect size |
| Day 2 | Day 7 | 0.001 | 0.06 |
| Day 2 | Day 14 | 0.001 | 0.10 |
| Day 2 | Day 35 | 0.001 | 0.22 |
| Day 7 | Day 14 | 0.001 | 0.07 |
| Day 7 | Day 35 | 0.001 | 0.18 |
| Day 14 | Day 35 | 0.001 | 0.10 |

small intestine, can be utilized for protein synthesis, whereas ammonia, which is the end product of protein fermentation in the large intestine can be absorbed, but it is excreted via urine as urea. Thus, the NSP-degrading enzymes-induced shifting of nutrient digestibility from the large intestine fermentation toward small intestine digestion can result in improved growth performance of pigs without significant effect on ATTD of OM or GE. Secondly, dietary NSP increases endogenous secretion of nutrients in small intestine (Nyachoti et al., 1997; Agyekum and Nyachoti, 2017). These endogenously secreted nutrients can be reabsorbed from gastrointestinal tract (Nyachoti et al., 1997), leading to insignificant change in ATTD of OM or GE. Energy is spent during the synthesis and reabsorption of endogenous nutrients, implying that an increase in endogenous secretion of nutrients in pigs results in an increase in utilization of dietary energy for maintanance at the expense of growth (Nyachoti et al., 1997; Agyekum and Nyachoti, 2017). Thus, the NSP-degrading enzymes can reduce endogenous nutrient secretion in the small intestine of pigs, leading to increased growth performance of pigs without significant effect on ATTD of OM or GE. Thirdly, dietary fiber induced mucin secretion in the small intestine of pigs (Zaefarian et al., 2015). Synthesis of mucin was induced by pro-inflammation cytokines (Iwashita et al., 2003; Blanchard et al., 2004), implying that fiber induces mucin secretion by inducing a immune response. Immune stimulation results in increased utilization of dietary energy and nutrients for maintanance at the expense of growth (Huntley et al., 2018). Thus, NSP-degrading enzymes that hydrolyze NSP reduce imflammation as a result. Indeed, supplementation of diets for turkeys (Ayoola et al., 2015) and broilers (Yaghobfar and Kalantar, 2017) with NSP-degrading enzymes reduce small intestine mucin production. In the current study, multi-enzyme B reduced fecal score, implying that it may have improved fluid balance. In addition, Li et al. (2018) observed reduced small intestinal permeability and reduced immune activation in weaned pigs due to supplementation of high fiber diets with fiber degrading enzymes. Results of other studies (Omogbenigun et al., 2004; Olukosi et al., 2007; Zhang et al., 2014; Li et al., 2018) also reported improved growth performance of pigs due to supplementation of NSP-degrading enzymes to diets of weaned pigs regardless of whether or not the supplemental enzymes improved nutrient digestibility. However, some studies did not report improved growth performance of pigs due to supplementation of NSP-degrading enzymes to diets for weaned pigs (Officer, 1995; Mavromichalis et al., 2000; Kim et al., 2004). Effect of NSP-degrading enzymes on growth performance for weaned pigs can be affected by ingredient composition of the basal diet and composition of the enzyme product used. For instance, the basal diets fed in the studies of Omogbenigun et al. (2004) and Olukosi et al. (2007), in which enzyme supplementation improved growth performance of weaned pigs, contained cereal grains and some cereal milling co-products as the major sources of energy, and soybean meal as the major source of protein; the basal diets contained very small amounts (if any) of feedstuffs of animal origin. Basal diets fed in the studies of Officer (1995) and Mavromichalis et al. (2000), in which enzyme supplementation did not improve growth performance of weaned pigs, did not contain cereal milling co-products, and contained feedstuffs of animal origin as the major sources of protein.

The magnitude of improvement in growth performance of pigs was greater in phase 1 than in phase 2, which could be attributed to the fact that newly weaned pigs have poorly developed digestive system for fibrous diets and are more susceptible to gut infections during the first 2 wk, and hence they can benefit more from enzyme supplementation during the first 2 or 3 wk after weaning than during weeks 4 to 6 after weaning. Multi-enzyme B was more effective in improving growth performance of pigs than multi-enzyme A, which may partly be explained by the tendency of multi-enzyme B (but not multi-enzyme A) to reduce fecal score (implying reduced diarrhea), and to increase ATTD of CP. Multi-enzymes A and B were not different in enzyme activities except for amylase; multi-enzyme A supplied 1,500 units amylase per kilogram, whereas multi-enzyme B supplied 150 units amylase per kilogram. The effectiveness of a feed enzyme is expected to increase with an increasein its level in the feed. Thus, the difference between multi-enzyme A and multi-enzyme B with regard to growth performance was due to difference in the source of amylase between the multi-enzymes because the dose of amylase in multi-enzyme A was greater than that in multi-enzyme B. Amylase activity in small intestine of pigs is low at weaning (at 21 d of age) and increases with age up to 6 wk of age (Hudman et al, 1957; Lindemann et al., 1986; Torres-Pitarch et al., 2017). Thus, the addition of amylase in diets of weaned pigs can increase starch digestibility in the small intestine, leading to increased efficiency of utilization of

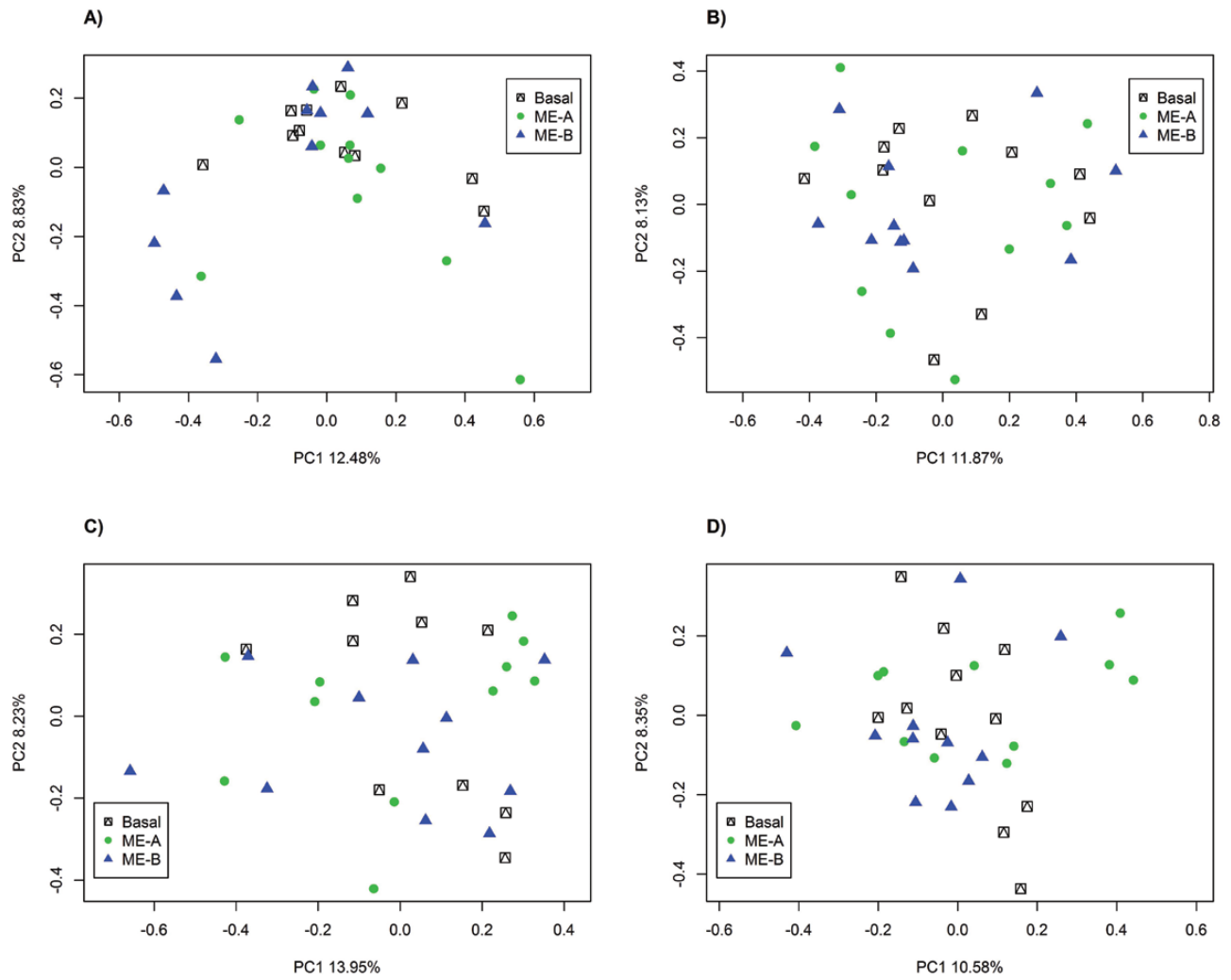


**Figure 4.** Comparative analysis of bacterial composition among 3 dietary treatments (basal, ME-A, and ME-B)[1] at 4 time points by PCA. Panels displayed are: (A) day 2 of the study, (B) day 7 of the study, (C) day 14 of the study, and (D) day 35 of the study. No significant differences were found by PERMANOVA ($P > 0.05$) among bacterial communities from different treatments at the same time point. [1]Basal = basal diet, ME-A = basal diet plus multi-enzyme A, and ME-B = basal diet plus multi-enzyme B.

starch-derived energy. Also, supplemental amylase can increase the efficacy of other supplemental enzymes by digesting starch (that otherwise physically block supplemental enzymes from their substrates), thereby increasing the accessibility of the supplemental enzymes to their substrates (Woyengo et al., 2019). An increase in digestibility of nutrients within the small intestine of weaned pigs can result in improved gut integrity due to increased luminal nutrient supply, leading to reduced diarrhea (Wijtten et al., 2011).

Multi-enzymes that contain NSP-degrading enzymes are expected to increase digestibility of NSP and of nutrients that are bound to NSP. Multi-enzymes that contain starch-digesting enzymes are also expected to completement the amylase level which was low in newly weaned piglet (Lindemann 1986). Indeed, supplemental multi-enzymes increased ATTD of GE, NSP, and CP in weaned pigs fed corn- or wheat-based diets (Omogbenigun et al., 2004). Supplemental multi-enzymes also increased ATTD of GE and EE in weaned pigs fed corn–wheat-based diets (Koo et al., 2017). Similarly, multi-enzyme A or B supplementation increased ATTD of EE in the current study. Also, multi-enzyme B supplementation tended to increase ATTD of CP in the current study. However, multi-enzyme A or B supplementation did not affect ATTD of GE, which was contrary to expectations. Li et al. (2018) also reported non-significant effect of xylanase on ATTD of GE in weaned pigs fed corn-based diets. Weiland (2017) reported reduced ATTD of ADF in weaned pigs fed low-fiber (corn–soybean meal-based) diets, but increased ATTD of ADF in weaned pigs fed high-fiber (corn–corn DDGS-soybean meal based) diets due to xylanase supplementation, implying that the change in ATTD of ADF due to fiber degrading enzymes is partly dependent on amount of fiber in the basal diet. However, it is not clear how low amount of fiber in the basal diet can result in reduced ATTD of fiber due to supplemental NSP-degrading enzymes.

Under the conditions tested, no significant differences in fecal bacterial composition were observed as a result of multi-enzyme supplementation. These results indicate that the addition of exogenous enzymes to the basal diet did not sufficiently change the conditions of the gastrointestinal environment to affect fecal bacterial composition. As there was no difference in formulation between the basal diet and enzyme-supplemented diets other than supplementation with multi-enzymes, the presence of exogenous enzymes presumably increased the availability of nutrients without dramatically altering other gut parameters that influence fecal bacterial composition. However, because only fecal bacterial communities were investigated in this study,

**Table 6.** Comparative analysis of bacterial composition amongst dietary treatments at 4 different time points by PERMANOVA

| Day | Comparisons[1] | | P-value | Effect size |
|---|---|---|---|---|
| | 1 | 2 | | |
| 2 | Basal | Multi-enzyme A | | 0.04 |
| 2 | Basal | Multi-enzyme B | 0.151 | 0.07 |
| 2 | Multi-enzyme A | Multi-enzyme B | 0.353 | 0.05 |
| 7 | Basal | Multi-enzyme A | 0.954 | 0.03 |
| 7 | Basal | Multi-enzyme B | 0.489 | 0.05 |
| 7 | Multi-enzyme A | Multi-enzyme B | 0.841 | 0.04 |
| 14 | Basal | Multi-enzyme A | 0.529 | 0.05 |
| 14 | Basal | Multi-enzyme B | 0.534 | 0.05 |
| 14 | Multi-enzyme A | Multi-enzyme B | 0.914 | 0.03 |
| 35 | Basal | Multi-enzyme A | 0.942 | 0.02 |
| 35 | Basal | Multi-enzyme B | 0.881 | 0.03 |
| 35 | Multi-enzyme A | Multi-enzyme B | 0.818 | 0.03 |

[1]Basal, basal diet; B, basal diet + multi-enzyme A; and ME-B, basal diet + multi-enzyme B.

it remains to be determined whether the presence of the test multi-enzyme products in corn–soybean meal-based diets for weaned pigs affect the composition of symbionts in upstream gut compartments.

In conclusion, addition of a multi-enzyme complex to a phytase-supplemented corn–soybean meal-based diet for weaned pigs improved their growth performance and ATTD of fat. However, multi-enzyme did not affect the ATTD of GE, implying that the multi-enzyme complex improved growth performance through mechanisms other than improvement in ATTD of GE. The multi-enzyme products used in the current study may be added to a phytase-supplemented corn–soybean meal-based diet for weaned pigs to improve growth performance. However, multi-enzyme B product may be slightly more effective in improving growth performance and reducing diarrhea of weaned pigs than multi-enzyme product A.

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## Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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