The microbial pH-stable exogenous multienzyme improved growth performance and intestinal morphology of weaned pigs fed a corn–soybean-based diet

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ABSTRACT

The present study investigated the effects of dietary supplementation of multienzyme preparation on growth performance, apparent total tract digestibility (ATTD) of nutrients, and gut health of weaned pigs. A total of 240 weaned pigs (initial body weight: 7.3 ± 0.7 kg) were randomly allotted to five treatments on the basis of body weight. The dietary treatments included a corn–soybean meal (SBM)-based diet supplemented with 0 (control), 0.025, 0.050, 0.075, or 0.10% multienzyme preparation. The experimental diets were fed in a meal form for two phases (d 0–14, phase I and d 15–28, phase II). Increasing multienzyme supplementation linearly improved overall (d 0–28) average daily gain, gain:feed, and ATTD of dry matter, gross energy, and crude protein. At d 21, pigs fed dietary increasing levels of multienzyme preparation had linearly greater Lactobacillus spp. (ileum and caecum and faeces) and fewer Clostridium spp. (ileum and caecum) and Escherichia coli (ileum and faeces) populations. On d 28, villus height of the jejunum and ileum were linearly increased with dietary increase in multienzyme preparation. In conclusion, this multienzyme has potential to improve the growth performance, ATTD of nutrients, and gut health of weaned pigs fed a corn–SBM-based diet in the absence of antibiotics growth promoters.

INTRODUCTION

Exogenous enzymes have been successfully used in the pigs industry to minimize or eliminate the negative effect of antinutritional factors, to stimulate nutrient digestibility and improve nutritive values of high non-starch polysaccharides (NSP) containing feedstuffs such as wheat, oats, barley, and rye (Willamil et al. 2012; Kianfar et al. 2013; Karimi & Zahedi 2015). However, it is thought that feedstuff like corn and SBM may not benefit from exogenous enzyme supplementation due to lower level of soluble NSP and lesser viscosity (Kim et al. 2004; Willamil et al. 2012). Corn contains 10–11% NSP, which are mainly arabinoxylan and β-galactomannan, and SBM contains 22.7% NSP such as α-galactosides (e.g. stachyose (fructose, glucose, and two galactoses), raffinose (fructose, glucose, and galactose) and β-galactomannan that cannot be metabolized by monogastric animals because they lack endogenous enzymes targeting these NSP or antinutritional factors (CVB 1998). This indicates that there is some room for the improvement of their nutritional value with exogenous enzyme supplementation (Thacker 2005; Jo et al. 2012). It has been reported that with appropriate enzyme preparations, these antinutritional effects can be alleviated with a potential to improve the nutritional value of corn–SBM-based diets for pigs (Ji et al. 2008; Li et al. 2010; Jo et al. 2012).

Studies on the use of exogenous enzymes to degrade indigestible dietary components have yielded inconsistent results mainly because of the presence of complex substrates in feedstuffs and the use of enzyme activities often not suitable for effective hydrolysis of such substrate (Masey O’Neill et al. 2014). It has been reported that a combination of different enzyme activities is required for complete degradation of complex NSP and to improve nutrient utilization in pigs (Ji et al. 2008;Jo et al. 2012). Results of several experiments have indicated that dietary supplementation of multienzyme preparations can improve the growth performance and nutrient digestibility in pigs (Omogbenigun et al. 2004; Yi et al. 2013). Enzymes are beneficial to digest NSP in the diet if they can pass the stomach acidic environment. Therefore, a novel pH-stable multienzyme preparation containing amylase, protease, mannanase, xylanase, and phytase has been developed to improve the performance and gut health of weaned pigs fed corn–SBM-based diet. The objectives of the present study were to investigate the effects of dietary supplementation of multienzyme preparation on growth performance, ATTD of nutrients and energy, intestinal and faecal microflora, and intestinal morphology of weaned pigs fed a corn–SBM-based diet in the absence of antibiotics growth promoters.

MATERIALS AND METHODS

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Republic of Korea.

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A multienzyme preparation used in the present study was provided by Advanced Enzyme Technologies Ltd. (Thane, India). In short, the multienzyme preparation containing amylase, protease, mannanase, xylanase, and phytase was produced by controlled fermentation of Bacillus amyloliquefaciens, Bacillus licheniformis, Trichoderma citrinoviride, and Pichia pastoris, respectively. Pure enzyme product provided 800,000 BAU/kg of amylase, 1,000,000 PCU/kg of protease, 2,500,000 MNU/kg of β-mannanase, 600,000 XU/kg of xylanase, and 1,000,000 FTU/kg of phytase. One unit of amylase is the amount of enzyme, which liberates 1 µmol of glucosidic linkage from a dextrinized starch substrate per minute at 6.6 pH and 30°C. One unit of protease is the amount of enzyme, which liberates 1 µg of phenolic compound (tyrosine equivalent) from casein substrate per minute at 7.0 pH and 37°C. One unit of β-mannanase is the amount of enzyme, which liberates 1 µmol of total reducing sugar (glucose equivalent) from mannan substrate (Locust bean gum) per minute at 5.3 pH and 50°C. One unit of xylanase is defined as the amount of enzyme, which liberates 1 µmol of reducing sugar (xylose equivalent) from xylan substrate (Beech-wood xylan) per minute at 5.3 pH and 50°C. One unit of phytase is defined as the amount of enzyme, which liberates 1 µmol of inorganic phosphate from sodium phytate substrate per minute at 5.0 pH and 50°C. The enzymes intrinsically are able to tolerate pH = 2.5 and temperature of 85°C.

**Animals and experimental design**

A total of 240 weaned pigs (Landrace × Yorkshire × Duroc, initial body weight: 7.3 ± 0.7 kg) were randomly allotted to five treatments on the basis of body weight (4 pens per treatment with 12 pigs in each pen). All the piglets were clinically healthy at the start of the trial and originated from 30 sows in their third parity. The dietary treatments included a corn–SBM-based basal diet (control) and control diet supplemented with 0.025%, 0.050%, 0.075%, or 0.10% multienzyme preparation. The experimental diets were formulated to contain 3350 kcal/kg ME and 1.23% SID lysine. Diets for phase I were formulated to contain 3400 kcal/kg ME and 1.35% SID lysine. Diets for phase II were formulated to contain 3350 kcal/kg ME and 1.23% SID lysine. All diets met or exceeded the nutrient requirements recommended by NRC 2012 (Table 1).

The piglets were housed in partially slatted concrete floor pens (3.0 × 3.0 m²). The temperature in the barn was 30°C at the beginning of the experiment and was slowly decreased to 25°C on d 8 and then kept constant until the end of the trial. The humidity ranged between 60% and 70%. All pens were equipped with an infrared heating lamp, a self-feeder, and low pressure nipple drinker to allow ad libitum access to feed and water.

**Experimental procedure and sampling**

The pigs were individually weighed at the start of the trial and on d 14 and 28. Feed consumption was recorded at the end of each phase and the average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were calculated. The ADG and the ADFI were calculated by dividing total pen weight gain and total pen feed consumption respectively by the number of animal days (including body weight gain and feed intake of all dead piglets in the pen). For the digestibility trials, diets containing 2.5 g/kg chromium oxide as indigestible marker were fed to pigs during the last seven days of each phase and then faecal grab samples were collected randomly from four pigs from each pen during the last three days of each phase. The faeces were pooled and dried in an air forced drying oven at 60°C for 72 h and ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas scientific, Swedesboro, NJ, USA) using a 1-mm screen and used for chemical analysis. Additionally, fresh faecal samples were collected from two random piglets per pen at d 21 and used for analysis of faecal microflora counts. The samples collected for microbial analysis were immediately placed on ice until the analyses were conducted later on the corresponding day.

To study the effect of dietary treatments on small intestinal morphology and microflora, representative pigs from each treatment (two per replicate) reflecting average body weight were selected and sacrificed by electrocution at the end of the experiment. The ileum and caecum contents were collected in sterilized plastic bottles and stored at 7°C for bacterial analysis later on the same day. The samples of intestinal segment from the region of the duodenum, jejunum, and ileum after removal of its contents were flushed with physiological saline and submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 30 g/l glutaraldehyde, 20 g/l paraformaldehyde, and 15 g/l acrolein and then brought to the laboratory to study morphological changes.

**Table 1. Ingredient and chemical composition of the basal diets (on DM-basis).**

| Item                | Phase I (d 0–14) | Phase II (d 15–28) |
|---------------------|-----------------|-------------------|
| Ingredient, %       |                 |                   |
| Corn                | 23.05           | 35.08             |
| Extruded corn       | –               | 16.65             |
| SBM, 45%            | 33.80           | 15.00             |
| Dehulled SBM, 48%   | –               | 16.45             |
| Fish meal, 60%      | 5.00            | 5.00              |
| Whey powder         | 20.00           | –                 |
| Lactose             | 12.00           | 5.00              |
| Sucrose             | –               | 2.20              |
| Soybean oil         | 3.83            | 2.20              |
| Limestone           | 0.70            | 0.60              |
| MDCP                | 0.42            | 0.65              |
| L-lysine (78%)      | 0.01            | 0.02              |
| DL-Methionine (100%)| 0.04            | –                 |
| Choline 30%         | 0.05            | 0.05              |
| Salt                | 0.20            | 0.20              |
| Mineral premixa     | 0.30            | 0.30              |
| Vitamin premixb     | 0.30            | 0.30              |
| ZnO                 | 0.30            | 0.30              |
| Chemical composition, calculatedc |              |                   |
| ME, kcal/kg         | 3400            | 3350              |
| Crude protein       | 22.00           | 20.85             |
| Calcium             | 0.80            | 0.70              |
| Available phosphorous| 0.40           | 0.33              |
| Lysine              | 1.35            | 1.23              |
| Methionine + Cystine| 0.74            | 0.68              |

Note: SBM: soybean meal; MDCP: mono- and dicalcium phosphate.

aSupplied per kg diet: 162 mg Fe (ferrous sulphate), 96 mg Cu (copper sulphate), 46.49 mg Mn (manganese sulphate), 0.9 mg I (calcium iodate), 0.9 mg Co (cobalt sulphate), 0.3 mg Se (sodium selenite).
bSupplied per kg diet: 9600 IU vitamin A, 1800 IU vitamin D3, 24 mg vitamin E, 1.5 mg vitamin B1, 12 mg vitamin B6, 2.4 mg vitamin B12, 0.045 mg vitamin B12, 1.5 mg vitamin K3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid, 18 mg ethoxyquin.
cBased on NRC (2012) values.
Chemical and microbial analyses

Experimental diets and excreta samples were analysed in triplicate for dry matter (DM, method 930.15; AOAC 2007), crude protein (CP, method 990.03; AOAC 2007), ash (method 942.05; AOAC 2007), and calcium and phosphorus (method 985.01; AOAC 2007). The gross energy (GE) of diets and faeces was measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL, USA), and chromium concentration was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979).

The microbiological assay of intestinal digesta and faecal samples was carried out by the procedure suggested by Choi et al. (2011). In short, 1 g of mixed content was diluted with 9 ml of Butterfield’s phosphate buffer solution, followed by further serial dilutions in Butterfield’s phosphate buffer dilution solution. Duplicate plates were then inoculated with 0.1 ml sample and incubated. The microbial groups enumerated were Lactobacillus spp. (MRS agar + 0.02% NaNO3 + 0.05% l-cystine hydrochloride monohydrate), Clostridium spp. (Triptose sulphite cycloserine agar, Oxoid, Hampshire, UK), and coliforms (violet red bile agar, Difco Laboratories, Detroit, MI, USA). The anaerobic conditions during the assay of Clostridium spp. were created by using a gas-pak anaerobic system (BBL, No. 260678, Difco, Detroit, MI, USA). The microbial populations were log transformed before statistical analysis.

Small intestinal morphology

Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Jin et al. 2008). A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height (VH) was measured from the tip of the villi to the villus crypt junction, crypt depth (CD) was defined as the depth of the invagination between adjacent villi, and villus width was measured at the mid of the villus. All morphological measurements (VH or CD) were made in 10-μm increments by using an image processing and analysis system (Optimus software version 6.5, Media Cybergenetics, North Reading, MA).

Table 2. Effects of dietary supplementation of multienzyme preparation on growth performance of weaned pigs.a,b

| Multienzyme | 0 (Control) | 0.025 | 0.050 | 0.075 | 0.10 | Linear | Quadratic |
|-------------|-------------|-------|-------|-------|------|--------|----------|
| Phase 1 (d 0–14) | | | | | | | |
| ADG g/d | 251 ± 19.2 | 266 ± 8.1 | 275 ± 8.8 | 282 ± 10.4 | 287 ± 7.0 | .029 | .657 |
| ADFI g/d | 408 ± 8.8 | 413 ± 3.0 | 417 ± 3.0 | 420 ± 2.1 | 423 ± 1.2 | .059 | .798 |
| G:F, g/kg | 617 ± 41.6 | 643 ± 22.0 | 659 ± 24.2 | 673 ± 32.1 | 679 ± 20.6 | .097 | .705 |
| Phase 2 (d 15–28) | | | | | | | |
| ADG g/d | 421 ± 15.9 | 433 ± 14.4 | 448 ± 18.2 | 458 ± 20.7 | 476 ± 16.5 | .026 | .918 |
| ADFI g/d | 619 ± 14.9 | 635 ± 7.7 | 639 ± 16.8 | 645 ± 18.6 | 647 ± 16.8 | .216 | .664 |
| G:F, g/kg | 684 ± 52.4 | 682 ± 20.1 | 701 ± 27.3 | 710 ± 20.8 | 738 ± 38.8 | .218 | .695 |
| Overall (d 0–28) | | | | | | | |
| ADG g/d | 336 ± 4.5 | 349 ± 4.2 | 361 ± 7.4 | 370 ± 5.9 | 382 ± 5.5 | .001 | .778 |
| ADFI g/d | 513 ± 10.6 | 524 ± 4.5 | 528 ± 8.5 | 532 ± 10.2 | 535 ± 8.8 | .106 | .657 |
| G:F, g/kg | 655 ± 20.3 | 666 ± 13.9 | 684 ± 22.9 | 696 ± 13.1 | 713 ± 19.6 | .007 | .862 |

Data represent means based on four replicates per treatment
The dietary treatments were a corn–SBM-based diet supplemented with 0% (control), 0.025%, 0.050%, 0.075%, or 0.10% multienzyme preparation containing amylase, protease, mannanase, xylanase, and phytase.

Statistical analyses

Data generated in the present study were subjected to statistical analysis using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a randomized complete block design. The linear and quadratic contrasts were used to compare effects of increasing dietary multienzyme preparation levels (0%, 0.025%, 0.050%, 0.075%, and 0.10%). The pen was used as the experimental unit for the analysis of growth performance and digestibility coefficient. Individual piglet was used as experimental unit for analysis of faecal and intestinal microflora and intestinal morphology. Probability values of ≤0.05 were considered significant.

Results

Growth performance

There was a linear increase (P < .05) in ADG during all studied periods and G:F during overall study period as dietary multienzyme preparation level increased (Table 2). ADFI and G:F tended to increase (linear, P < .1), as dietary multienzyme preparation level increased during phase I. However, during phase II and overall period, dietary multienzyme preparation had no effects (linear, P > .05) on ADFI of pigs. Furthermore, increasing multienzyme levels increased (linear, P > .05) the overall G:F.

Apparent total tract digestibility

Increasing dietary supplementation of multienzyme preparation in weaned pigs linearly increased (P < .05) the ATTD of DM, GE, and CP during phase I and phase II (Table 3). Digestibility of calcium and phosphorous tended to improve in both phases (P < .1).

Microbial population

A linear increase in Lactobacillus spp. population (ileum and caecum and faeces) with increasing dietary levels of multienzyme preparation was observed (P < .05; Table 4). The colonization of Clostridium spp. increased (linear, P < .05) in ileum and caecum. The increase in multienzyme levels resulted in decreased (linear, P < .05) E. coli colonization in ileum and
However, Omogbenigun et al. (2004) suggested that a combination of various enzyme activities is required to degrade complex NSP and improve nutrient utilization in pigs. In the present study, dietary supplementation of a multienzyme preparation containing amylase, protease, β-mannanase, xylanase, and phytase improved the growth performance, nutrient digestibility, and gut health of weaned pigs fed a corn–SBM-based diet.

The improved growth performance is in agreement with data reported by Yi et al. (2013), who observed an improvement in ADG and G:F of weaned pigs fed a corn–SBM-based diet supplemented with increasing levels of a multienzyme preparation containing amylase, protease, and xylanase. Similarly, it was reported that pigs fed a corn–SBM-based diet complemented by a multienzyme preparation (α-galactosidase, galactomannanase, xylanase, and β-glucanase) had increased ADG and G:F than pigs fed the diet without the enzyme supplement (Ao et al. 2010). Omogbenigun et al. (2004) also reported an improvement in ADG and G:F of weaned pigs fed a complex diet (corn, wheat, oat screenings, oat groats, barley, SBM, canola meal, and peas) supplemented with a multienzyme preparation containing amylase, protease, cellulase, xylanase, gluconase, galactanase, mannanase, pectinase, and phytase. The lack of influence on the ADFI by supplementing diets with an exogenous multienzyme preparation is in agreement with previous experiments (Thacker 2005; Ao et al. 2010; Yi et al. 2012; Yi et al. 2013). However, Omogbenigun et al. (2004) suggested that a combination of various enzyme activities is required to degrade complex NSP and improve nutrient utilization in pigs. In the present study, dietary supplementation of a multienzyme preparation containing amylase, protease, β-mannanase, xylanase, and phytase improved the growth performance, nutrient digestibility, and gut health of weaned pigs fed a corn–SBM-based diet.

### Intestinal morphology

An increase in VH of the jejunum and ileum with increasing dietary levels of multienzyme preparation was observed (linear, P < .05; Table 5). In addition, CD of the duodenum and ileum tended to decrease (linear, P < .10) and villus height: crypt depth (VH:CD) of the duodenum, jejunum, and ileum tended to increase (linear; P < .10) with increase in dietary multienzyme preparation.

### Discussion

All plant feeds invariably have some antinutritional factors, such as NSP and phytate. According to CVB (1998), corn and SBM contain 10.0% and 22.7% NSP, respectively. Therefore, it may be beneficial to use a multienzyme preparation containing carbohydrates, protease, and phytase. Thus far, the use of exogenous enzymes to degrade NSP in corn–SBM-based diets has yielded inconsistent results, mainly because enzyme activities do not effectively hydrolyse the NSP present in corn and SBM (Willamil et al. 2012; Yi et al. 2013).

### Table 3. Effects of dietary supplementation of multienzyme preparation on ATTD (%) of weaned pigs.a,b

| Multienzyme   | 0 (Control) | 0.025 | 0.050 | 0.075 | 0.10 | Linear | Quadratic |
|---------------|-------------|-------|-------|-------|------|--------|-----------|
| Phase 1 (d 0–14) |             |       |       |       |      |        |           |
| Dry matter    | 82.75 ± 0.55 | 83.30 ± 0.36 | 84.15 ± 0.48 | 84.82 ± 0.71 | 85.30 ± 0.81 | 0.006 | .896     |
| Gross energy  | 80.60 ± 0.36 | 81.26 ± 0.71 | 81.85 ± 0.63 | 82.44 ± 1.01 | 83.04 ± 0.93 | 0.24 | .970     |
| Crude protein | 78.69 ± 0.86 | 79.20 ± 0.76 | 80.00 ± 0.58 | 80.47 ± 0.75 | 81.01 ± 0.70 | 0.23 | .920     |
| Calcium       | 48.67 ± 1.72 | 49.96 ± 1.35 | 50.69 ± 0.92 | 51.28 ± 1.45 | 51.91 ± 1.38 | 0.096 | .783     |
| Phosphorus    | 40.86 ± 1.71 | 41.63 ± 0.76 | 42.56 ± 0.46 | 42.81 ± 0.84 | 43.16 ± 0.73 | 0.086 | .687     |
| Phase 1 (d 15–28) |    |       |       |       |      |        |           |
| Dry matter    | 82.09 ± 0.23 | 82.68 ± 0.61 | 83.19 ± 0.63 | 83.90 ± 0.86 | 84.62 ± 0.86 | 0.014 | .860     |
| Gross energy  | 79.69 ± 0.26 | 80.78 ± 0.74 | 81.18 ± 0.63 | 81.57 ± 0.69 | 82.13 ± 0.71 | 0.017 | .699     |
| Crude protein | 77.17 ± 0.79 | 78.09 ± 0.81 | 78.80 ± 0.90 | 79.33 ± 0.77 | 79.68 ± 0.78 | 0.028 | .669     |
| Calcium       | 47.72 ± 0.96 | 48.25 ± 1.33 | 49.04 ± 1.45 | 49.90 ± 1.21 | 50.84 ± 1.20 | 0.063 | .849     |
| Phosphorus    | 39.98 ± 0.60 | 40.87 ± 0.94 | 41.20 ± 1.52 | 42.04 ± 0.63 | 42.31 ± 0.55 | 0.064 | .836     |

### Table 4. Effects of dietary supplementation of multienzyme preparation on ileal, caecal, and faecal (d 21) microflora of weanling pig.a,b,c

| Lactobacillus spp. (log10 cfu/g) | 0 (Control) | 0.025 | 0.050 | 0.075 | 0.10 | Linear | Quadratic |
|---------------------------------|-------------|-------|-------|-------|------|--------|-----------|
| Ileum                           | 9.19 ± 0.21 | 9.39 ± 0.19 | 9.56 ± 0.13 | 9.61 ± 0.14 | 9.69 ± 0.13 | 0.32 | .570     |
| Caecum                          | 9.25 ± 0.21 | 9.45 ± 0.18 | 9.58 ± 0.12 | 9.67 ± 0.12 | 9.73 ± 0.12 | 0.29 | .873     |
| Faeces                          | 8.54 ± 0.15 | 8.78 ± 0.14 | 8.95 ± 0.10 | 8.97 ± 0.12 | 9.00 ± 0.14 | 0.16 | .271     |
| E. coli (log10 cfu/g)            |             |       |       |       |      |        |           |
| Ileum                           | 6.48 ± 0.22 | 6.29 ± 0.26 | 6.18 ± 0.21 | 5.96 ± 0.21 | 5.85 ± 0.23 | 0.042 | .965     |
| Caecum                          | 6.61 ± 0.21 | 6.44 ± 0.27 | 6.26 ± 0.31 | 5.98 ± 0.31 | 5.92 ± 0.31 | 0.057 | .913     |
| Faeces                          | 6.88 ± 0.07 | 6.73 ± 0.12 | 6.63 ± 0.16 | 6.46 ± 0.21 | 6.34 ± 0.22 | 0.21 | .958     |
| Clostridium spp. (log10 cfu/g)   |             |       |       |       |      |        |           |
| Ileum                           | 6.38 ± 0.22 | 6.23 ± 0.25 | 6.14 ± 0.20 | 5.95 ± 0.20 | 5.81 ± 0.16 | 0.047 | .921     |
| Caecum                          | 6.65 ± 0.20 | 6.46 ± 0.26 | 6.33 ± 0.28 | 6.07 ± 0.30 | 5.94 ± 0.19 | 0.036 | .977     |
| Faeces                          | 5.45 ± 0.01 | 5.42 ± 0.02 | 5.41 ± 0.02 | 5.39 ± 0.02 | 5.40 ± 0.03 | 0.091 | .463     |

*Data represent means based on four replicates per treatment.

The dietary treatments were a corn–SBM-based diet supplemented with 0 (control), 0.025%, 0.050%, 0.075%, or 0.10% multienzyme preparation containing amylase, protease, mannanase, xylanase, and phytase.

The microbial populations were log transformed before statistical analysis.
and energy digestibility in weaned pigs. In the present study, dietary supplementation with the multienzyme preparation observed differences in nutrient digestibility among studies may be due to variability in the age, diet compositions, and NSP level in the diets. The greater digestibility of nutrients, increase in beneficial intestinal microflora, and improvements of gut morphology in this study could be attributed to successful degradation of non-digestible feed ingredients by dietary enzymes.

In the present study, dietary supplementation with the multienzyme preparation increased the numbers of *Lactobacillus* spp. and decreased the numbers of potential pathogens, such as *E. coli* and *Clostridium* spp. in the ileum and caecum of pigs. In agreement, Yi et al. (2013) observed increased *Lactobacillus* and decreased *E. coli* numbers in the caecum and colon of weaning pigs fed a corn–SBM-based diet supplemented with a multienzyme preparation. Similarly, it was reported that supplementation of exogenous xylanase increased the numbers of *Lactobacillus* and decreased the numbers of coliforms and *Salmonella* in the ileum of broilers (Nian et al. 2011). The increased *Lactobacillus* spp. populations in the ileum and caecum might have increased the amount of sugar released by enzymatic action and thereby stimulated the growth of *Lactobacillus*, which produces lactic acid. It has been reported that an increased proportion of lactic acid promotes gut health by decreasing the pH, which is considered an effective means of preventing the growth of potential pathogens, like *E. coli*, *Clostridium* spp., and *Salmonella* (Bjerrum et al. 2005). The increased population of beneficial microflora and decreased population of potential pathogens observed in the present study might explain the improved ATTD of the nutrients and the enhanced growth performance of weaned pigs fed multienzyme-supplemented diets.

The intestinal morphology, including VH, CD, and VH:CD of the duodenum, jejunum, and ileum, is indicative of the gut health in pigs. Increasing the VH suggests an increased surface area capable of greater absorption of available nutrients (Caspar 1992). Increased VH and VH:CD are directly correlated with an increased epithelial turnover (Fan et al. 1997), and longer villi correlate to activation of cell mitosis (Samanya et al. 2013). Contrary to results of the present investigation, Officer (1995) found no improvement in either ADG or feed efficiency of weaned pigs fed a wheat–SBM-based diet supplemented with a multienzyme preparation containing β-glucanase, cellulase, hemicellulase, and pentosanase. Similarly, Thacker (2005) and Willamil et al. (2012) did not observe any significant improvement in ADG and feed efficiency of growing pigs fed a corn–SBM-based diet supplemented with a multienzyme preparation. However, differences in the age of the pigs, diet composition, types, and concentrations of enzymes used, the NSP fraction of the diets, and the mode of action of the exogenous enzymes in the gastrointestinal tract may account for the contradictory findings. It has been reported that the beneficial effects of exogenous enzymes depend on the substrate type, physiological status of the animal, and the dietary NSP content (Ji et al. 2008; Li et al. 2010). The improved growth performance with dietary supplementation of a multienzyme preparation observed in the present study is suggested to be due to the enhanced energy and nutrient digestibility, resulting from an increased endogenous enzyme accessibility to the nutrients.

Previously it has been reported that exogenous enzymes hydrolyse the cell walls, increase cell wall permeability, or degrade the long-chain polysaccharides and the phytates (Grandhi 2001), consequently improving nutrient digestibility and the energy content in pig diets (Ji et al. 2008; Li et al. 2010; Jo et al. 2012). Corn and SBM both contain NSP as well as phytates. Hence, it is expected that a combination of carbohydrases, protease, and phytase would improve the nutrient and energy digestibility in weaned pigs. In the present study, weaned pigs fed increasing dietary levels of multienzyme preparation increased the ATTD of DM, GE, and CP. These results are consistent with the findings of Omogbenigun et al. (2004), who reported improved digestibility of DM, CP, and GE in weaned pigs fed a corn–SBM-based diet supplemented with a multienzyme preparation containing amylase, glucanase, invertase, xylanase, and phytase. Increased digestibility of DM, GE, and CP was also reported by long-term (49 days) supplementation of xylanase and phytase to a corn–SBM-based diet fed to growing pigs (Kim et al. 2008). In contrast to previous reports (Omogbenigun et al. 2004; Kim et al. 2008) and the present results, Willamil et al. (2012) indicated that there was no effect on digestibility of DM, CP, and energy in growing pigs fed corn–SBM-based or wheat–barley–rye–SBM-based diets supplemented with a multienzyme preparation. Observed differences in nutrient digestibility among studies may be due to the variability in the age, diet compositions, and NSP level in the diets. The greater digestibility of nutrients, increase in beneficial intestinal microflora, and improvements of gut morphology in this study could be attributed to successful degradation of non-digestible feed ingredients by dietary enzymes.

| Table 5. Effects of dietary supplementation of multienzyme preparation on intestinal morphology of weanling pig (d 28).a,b |
|-----------------------------------------------|
| Multienzyme | 0 (Control) | 0.025 | 0.050 | 0.075 | 0.10 | Linear | Quadratic |
| Duodenum | | | | | | | |
| VH (μm) | 404.7 ± 1.8 | 408.8 ± 6.2 | 414.3 ± 5.2 | 415.1 ± 3.8 | 417.7 ± 6.7 | .136 | .757 |
| CD (μm) | 239.8 ± 5.3 | 236.5 ± 7.2 | 231.2 ± 5.8 | 228.4 ± 5.2 | 225.5 ± 5.3 | .065 | .885 |
| VH/CD | 1.69 ± 0.07 | 1.74 ± 0.08 | 1.80 ± 0.06 | 1.82 ± 0.06 | 1.86 ± 0.08 | .087 | .855 |
| Jejunum | | | | | | | |
| VH (μm) | 404.5 ± 5.7 | 406.8 ± 6.9 | 412.3 ± 8.7 | 416.9 ± 4.1 | 419.9 ± 3.4 | .050 | .983 |
| CD (μm) | 238.1 ± 3.9 | 237.7 ± 2.5 | 235.7 ± 3.5 | 233.3 ± 3.9 | 231.8 ± 4.2 | .161 | .856 |
| VH/CD | 1.70 ± 0.05 | 1.71 ± 0.05 | 1.75 ± 0.06 | 1.79 ± 0.05 | 1.81 ± 0.05 | .082 | .907 |
| Ileum | | | | | | | |
| VH (μm) | 336.9 ± 7.7 | 344.2 ± 6.2 | 347.2 ± 5.3 | 352.1 ± 10.0 | 357.7 ± 6.2 | .048 | .964 |
| CD (μm) | 228.3 ± 3.8 | 226.1 ± 3.9 | 222.3 ± 3.1 | 222.0 ± 2.3 | 221.0 ± 3.6 | .100 | .649 |
| VH/CD | 1.48 ± 0.06 | 1.53 ± 0.05 | 1.56 ± 0.04 | 1.59 ± 0.06 | 1.62 ± 0.06 | .059 | .849 |

VH: villus height; CD: crypt depth; VH/CD: villus height to crypt depth ratio.

aData represent means based on four replicates per treatment and two samples per replicate (n = 8).

bThe dietary treatments were a corn–SBM-based diet supplemented with 0 (control), 0.025%, 0.050%, 0.075%, or 0.10% multienzyme preparation containing amylase, protease, mannanase, xylanase, and phytase.
Yamauchi 2002). Montagne et al. (2003) reported that the NSP content and composition of the diet influence the epithelial morphology and cell turnover of the gut mucosa. The inclusion of soluble NSP in the diet can stimulate the growth of commensal gut microbes, leading to increased production of organic acids and a lower pH in the large intestine (Bach Knudsen et al. 1991). Insoluble NSP decreases the transit time, provides a substrate that is slowly degraded by the microbiota in the distal large intestine, and modulates gut morphology by increasing villus length (Hedemann et al. 2006). In the current study, we observed an increased VH in the jejunum and ileum with supplementation of the multienzyme preparation. Also, CD of the duodenum and ileum tended to decrease, and VH:CD of the duodenum, jejunum, and ileum tended to increase with an increase in the dietary multienzyme preparation. These results concur with data reported by Willamil et al. (2012), who observed an improvement in VH and VH:CD, and decrease in CD of the ileum of growing pigs fed wheat-based diets supplemented with a carbohydrase complex. Contrary to the present results, however, Kim et al. (2004) reported that there were no effects of multienzyme preparation on the VH and CD in the duodenum, jejunum, and ileum of weaned pigs fed a corn–SBM-based diet. Willamil et al. (2012) also observed no effects of a carbohydrase complex on ileal VH and CD of pigs fed a corn–SBM diet. The increased VH and VH:CD, and decreased CD observed in the current study, indicate improved absorption capacity and decreased nutrient and growth ‘cost’ of intestinal maintenance, which in turn may have contributed to the increased nutrient digestibility and growth performance.

Conclusions

Overall, data from this experiment suggest that increasing multienzyme inclusion can yield equal or greater performance and digestibility of DM, GE, and crude protein. Dietary supplementation of multienzyme preparation has the potential to linearly decrease the ileal, caecal, and faecal colonization of E. coli and Clostridium spp. Furthermore, a general improvement in the gut morphological health and the number of Lactobacillus spp. in ileum, caecum, and faeces were observed.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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