Original Research Article

# Comparative standardized ileal amino acid digestibility and metabolizable energy contents of main feed ingredients for growing pigs when adding dietary β-mannanase

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## ARTICLE INFO

## ABSTRACT

The present study was conducted to test whether the dietary supplementation of β-mannanase affects amino acids (AA) digestibility, metabolizable energy (ME) contents of corn, wheat, soybean meal, distillers dried grains with solubles, and palm kernel meal (PKM), nutrient digestibility, and growth performance of pigs. In Exp. 1, 22 cannulated pigs were used for 10 dietary treatments including 5 feed ingredients and 2 β-mannanase concentrations (0 and 0.5 g/kg of the diet) in 6 periods in an incomplete Latin square design to determine the AA and energy digestibility. In Exp. 2, 200 growing pigs were randomly allotted to 4 treatments with 2 nutrient levels (high and low) and 2 concentrations of β-mannanase ($2 \times 2$ factorial arrangement) in 2 phases (phase 1, d 0 to 21; and phase 2, d 22 to 42). In Exp. 1, β-mannanase increased the mean standardized ileal digestibility (SID) of AA in all feed ingredients. The amount of digestible energy was increased ($P < 0.05$) in β-mannanase-treated PKM. Pigs fed β-mannanase showed a greater ($P < 0.05$) digestibility of gross energy (GE). The feed-to-gain (F:G) ratio was improved ($P < 0.01$) in pigs fed high-nutrient diets. Pigs fed β-mannanase in the diets had greater ($P < 0.05$) average daily gain and F:G. In phase 2, the concentration of fecal ammonia was decreased ($P < 0.05$) in pigs fed β-mannanase. Considering the 2 experiments, it can be concluded that β-mannanase increases the SID of AA, which has to be considered in balancing the rations.

## 1. Introduction

Supplementation of the diets with exogenous enzymes in swine nutrition has attracted a great deal of attention to improve performance, nutrient requirement, and potentially reduce environmental pollution from manure in the last decade. Beta-mannan alone makes up approximately 15% to 37% of the total non-starch polysaccharides (NSP) content of pig diets (Centraal Veevoeder Bureaux [CVB], 1998). Pigs are not able to degrade β-1,4-mannosyl and α-1,6-galactosyl bonds due to the lack of endogenous β-mannanase enzyme (Kim et al., 2017). The potential of exogenous enzymes on the digestibility of nutrient has been well documented (Mohammadi Gheisar et al., 2016; Roofchaei et al., 2019; Zhang et al., 2018), however there is a general lack of knowledge on the extent of influence. The addition of exogenous enzymes to the diet improves the digestibility of energy (Cadogan and Choct, 2015) and dry matter (DM) (Cadogan and Choct, 2015; Zdunczyk et al., 2013). Considering the content of β-mannan in the individual feedstuffs, a change in digestibility of nutrients would be expected when exogenous β-mannanase is added to the

diet. Thus, the effects of enzyme supplementation depend, to some extent, on the chemical and physical characteristics of feeds.

Dietary amino acids (AA) account as the most costly ingredients in feed, and more attention should be given to correctly formulating the diets in order to minimize nitrogen excretion and feed cost. The exogenous dietary enzymes have been shown to decrease the digestibility of crude protein (CP) in non-ruminants (Kim et al., 2017; Ryu et al., 2017). The total amount of AA in the diets, as well as the excretion of nitrogen from pigs, may be decreased if diets are formulated based on corrected values for the standardized ileal digestibility (SID) of AA when β-mannanase is supplemented to the diet. In addition, previous studies have indicated that the supplementation of exogenous β-mannanase could affect the digestibility of energy (Kim et al., 2018a). Imbalance dietary protein and energy ingestion by pigs can cause poor performance and impaired intestinal microbiota (Kong et al., 2016; Ji et al., 2017). Thus, an accurate estimation of metabolizable energy (ME) is crucial to allow the diet to be formulated based on the least cost to save expensive nutrients such as AA. There is a lack of published information on SID of AA, digestible energy (DE), or ME values in corn, wheat, soybean meal (SBM), distillers dried grains with solubles (DDGS), and palm kernel meal (PKM) when the diet is supplemented with β-mannanase for growing pigs. The objectives of these experiments were to determine the values for the SID of AA, DE, and ME of corn, wheat, SBM, DDGS and PKM with and without β-mannanase, and to test the estimated values in the diet of growing pigs.

## 2. Materials and methods

The experiments were conducted at the Kangwon National University farm facility and approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, South Korea. The commercial β-mannanase (patent 100477456-0000, CTC Bio, Inc.) was produced by using *Bacillus subtilis* (WL-7) grown in Luria broth and contained 800,000 U of β-mannanase/kg. One unit of β-mannanase is the amount of enzyme, which liberates 1 μmol of total reducing sugar (glucose equivalence) per minute at pH 4.0 and 30 °C.

### 2.1. Experiment 1

Twenty two barrows were surgically equipped with a T-cannula in the distal ileum. Ten barrows with an average initial body weight (BW) of $22.2 \pm 2.4$ kg were used randomly in 6 periods to determine DE and ME contents of 5 feed sources (corn, wheat, SBM, DDGS, and PKM; Table 1) and 2 β-mannanase concentrations (totally 10 treatments) in a $6 \times 10$ incomplete Latin square design. In addition, 12 barrows with an average initial body weight (BW) of $24.9 \pm 2.0$ kg were used randomly in 6 periods to determine the SID of AA in 5 feed sources (corn, wheat, SBM, DDGS, and PKM) and 2 β-mannanase concentrations, and 2 pigs of the 12 pigs were offered a N-free diet to determine basal endogenous ileal AA flow in a $6 \times 12$ incomplete Latin square design. Each experimental period lasted 7 d (4 d adaptation period to experimental diets followed by a 3-d total collection of samples).

The SID of AA was calculated according to the following equation described by Stein et al. (2007):

$$AID\ (\%) = [1 - (AA_{digesta}/AA_{diet}) \times (Cr_{diet}/Cr_{digesta})] \times 100,$$

$$IAA_{end} = (AA_{digesta}) \times (Cr_{diet}/Cr_{digesta}),$$

$$SID\ (\%) = AID + (IAA_{end}/AA_{diet}) \times 100,$$

**Table 1**
Analyzed nutrient composition of corn, wheat, soybean meal (SBM) distillers dried grains with soluble (DDGS), and palm kernel meal (PKM) (%, as-fed basis).

| Item | Feed sources | | | | |
|---|---|---|---|---|---|
| | Corn | Wheat | SBM | DDGS | PKM |
| DM | 86.79 | 86.48 | 88.48 | 88.27 | 91.40 |
| CP | 7.42 | 12.42 | 46.86 | 27.51 | 15.46 |
| Ether extract | 3.35 | 1.87 | 1.67 | 9.73 | 6.45 |
| Ash | 1.28 | 1.76 | 6.15 | 3.83 | 4.12 |
| Calcium | 0.02 | 0.08 | 0.34 | 0.06 | 0.25 |
| Phosphorus | 0.25 | 0.38 | 0.61 | 0.72 | 0.64 |
| GE, kcal/kg | 3,954 | 3,982 | 4,274 | 4,786 | 4,237 |
| Indispensable AA | | | | | |
| Arg | 0.36 | 0.54 | 3.28 | 1.27 | 1.34 |
| His | 0.21 | 0.27 | 1.28 | 0.76 | 0.19 |
| Ile | 0.26 | 0.38 | 2.08 | 1.08 | 0.46 |
| Leu | 0.86 | 0.74 | 3.51 | 3.35 | 0.77 |
| Lys | 0.24 | 0.37 | 2.88 | 0.83 | 0.35 |
| Met | 0.16 | 0.19 | 0.69 | 0.66 | 0.20 |
| Phe | 0.35 | 0.50 | 2.30 | 1.42 | 0.51 |
| Thr | 0.26 | 0.33 | 1.77 | 1.13 | 0.37 |
| Val | 0.34 | 0.50 | 2.14 | 1.37 | 0.63 |
| Dispensable AA | | | | | |
| Ala | 0.55 | 0.41 | 1.97 | 2.10 | 0.53 |
| Asp | 0.50 | 0.57 | 5.11 | 1.96 | 0.96 |
| Cys | 0.16 | 0.28 | 0.70 | 0.46 | 0.16 |
| Glu | 1.30 | 2.89 | 7.96 | 5.17 | 2.32 |
| Gly | 0.29 | 0.47 | 1.90 | 1.11 | 0.53 |
| Pro | 0.58 | 1.06 | 2.31 | 2.24 | 0.36 |
| Ser | 0.33 | 0.45 | 2.11 | 1.29 | 0.53 |
| Tyr | 0.23 | 0.28 | 1.63 | 1.18 | 0.28 |

DM = dry matter; CP = crude protein; GE = gross energy; AA = amino acids.

where AID is apparent ileal digestibility; $AA_{diet}$ and $AA_{digesta}$ are AA concentrations of the diet and ileal digesta, respectively (g/kg of DM); $Cr_{diet}$ and $Cr_{digesta}$ are chromium concentrations of the diet and ileal output, respectively (g/kg of DM); and $IAA_{end}$ refers to the basal ileal endogenous loss of an AA (g/kg of DM intake).

The pigs were individually housed in metabolism cages that measured 1.2 m × 1 m and equipped with a feeder, fully slatted floors, and urinary trays, which allowed the separate collection of urine and fecal materials from each pig. The temperature of the rooms housing the pigs was maintained at 21 °C, and the lights were kept on 24 h a day. The experimental diets were specially formulated as shown in Tables 2 and 3. Vitamins and minerals were added to all diets according to requirement estimates (NRC, 2012). The feed was provided at daily amounts of 2.5 times the estimated maintenance requirement for energy ($2.5 \times 197$ kcal of ME/kg of $BW^{0.60}$; NRC, 2012). The daily feed allowance was divided into 2 equal meals and provided to pigs at 09:00 and 17:00.

In Exp. 1, the initial 3 d of the experiment were considered an adaptation period to the diet. On d 4, a marker (0.5% chromic oxide) was mixed into the morning meal. Fecal samples were collected as the marker appeared in the feces. On d 7, a second marker (0.5% ferric oxide) was included in the morning meal. The fecal collection was quantitatively continued until the second marker appeared in the feces, according to the marker-to-marker approach (Adeola, 2001). Urine collection started at 09:00 on d 8 and ceased at 09:00 on d 13. Urine was collected in a urine bucket over 50 mL of 6 mol/L HCl. The total quantities of feces and 20% of the collected urine were stored at −20 °C immediately after collection. The DE and ME of each experimental ingredient were calculated using the difference method with the chromium oxide (Cr; 0.25%) concentration of feed, digesta, and feces (Adeola, 2001). Fecal samples were dried in an air-forced drying oven at 60 °C and ground before analysis, and urine samples were dried in a freeze drier before analysis. Diet, fecal, and urine samples were analyzed for gross

**Table 2**
Ingredients and composition of experimental diets to evaluate amino acids digestibility in Exp. 1 (%, as-fed basis).

| Item | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Corn | Wheat | SBM | DDGS | PKM | N-free |
| Ingredients | | | | | | |
| Corn | 96.27 | – | – | – | – | – |
| Wheat | – | 96.75 | – | – | – | – |
| SBM | – | – | 46.76 | – | – | – |
| DDGS | – | – | – | 71.85 | – | – |
| PKM | – | – | – | – | 56.87 | – |
| Cornstarch | – | – | 40 | 15 | 30 | 80.58 |
| Glucose | – | – | 10 | 10 | 10 | 10 |
| Cellulose | – | – | – | – | – | 5 |
| Celite[1] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – |
| Limestone | 1.38 | 1.47 | 1.08 | 1.75 | 1.28 | 0.92 |
| Mono calcium phosphate | 0.95 | 0.38 | 0.76 | – | 0.45 | 2.05 |
| Choline chloride (50%) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Mineral premix[2] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Vitamin premix[3] | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Chromic oxide | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated composition | | | | | | |
| CP | 7.1 | 12.1 | 21.8 | 19.7 | 8.8 | 0.0 |
| Calcium | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Phosphorus | 0.45 | 0.45 | 0.45 | 0.51 | 0.45 | 0.45 |

SBM = soybean meal; DDGS = distillers dried grains with solubles; PKM = palm kernel meal; CP = crude protein.
[1] β-mannanase (800,000 U/kg) was added by replacing Celite in the diets.
[2] Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin $D_3$, 40 IU vitamin E, 5.0 mg vitamin $K_3$, 5.0 mg vitamin $B_1$, 20 mg vitamin $B_2$, 4.0 mg vitamin $B_6$, 0.08 mg vitamin $B_{12}$, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.
[3] Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

**Table 3**
Ingredients and composition of experimental diets to evaluate energy digestibility in Exp. 1 (%, as-fed basis).

| Item | Experimental diets | | | | |
|---|---|---|---|---|---|
| | Corn | Wheat | SBM | DDGS | PKM |
| Ingredients | | | | | |
| Corn | 96.53 | – | 68.1 | 48.1 | 68.1 |
| Wheat | – | 96.79 | – | – | – |
| SBM | – | – | 30 | – | – |
| DDGS | – | – | – | 50 | – |
| PKM | – | – | – | – | 30 |
| Celite[1] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Limestone | 1.38 | 1.47 | 1.35 | 1.74 | 1.43 |
| Mono calcium phosphate | 0.95 | 0.40 | 0.44 | – | 0.41 |
| Choline chloride (50%) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Mineral premix[2] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Vitamin premix[3] | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Calculated composition | | | | | |
| ME, kcal/kg | 3,090 | 3,060 | 3,141 | 3,208 | 2,898 |
| CP | 7.15 | 12.01 | 19.01 | 17.24 | 9.62 |
| Calcium | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Phosphorus | 0.45 | 0.45 | 0.45 | 0.47 | 0.45 |

SBM = soybean meal; DDGS = distillers dried grains with solubles; PKM = palm kernel meal; ME = metalizable energy; CP = crude protein.
[1] β-mannanase (800,000 U/kg) was added by replacing Celite in the diets.
[2] Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin $D_3$, 40 IU vitamin E, 5.0 mg vitamin $K_3$, 5.0 mg vitamin $B_1$, 20 mg vitamin $B_2$, 4.0 mg vitamin $B_6$, 0.08 mg vitamin $B_{12}$, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.
[3] Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

energy (GE) using a bomb calorimeter (Model 1241, Parr Instrument Co., Molin, IL, US).

### 2.2. Experiment 2

A total of 200 growing pigs (Yorkshire × Landrace × Duroc) with an average initial BW of 49.9 ± 0.8 kg were randomly allotted to 4 treatments in a 2 × 2 factorial arrangement with 2 concentrations of nutrient including a standard diet (3,300 kcal ME; 17% and 16% protein in phases 1 and 2 respectively) and a low nutrient diet (3,260 kcal ME; 16.51% and 15.63% protein in phases 1 and 2 respectively), and 2 concentrations of β-mannanase (0 and 0.5 g/kg of the diet). There were 5 pens in each treatment, with 10 pigs per pen. Each 1.5 m × 5 m pen had a 2-hole dry self-feeder and a nipple waterer to allow ad libitum access to feed and water. The experimental diets were fed for 42 d in 2 phases: phase 1 (d 0 to 21) and phase 2 (d 22 to 42). For a feeding trial, pigs were housed in partially slatted, concrete floor pens.

The ME and AA values of ingredients (corn, wheat, SBM, DDGS, and PKM) in this feeding trial were calculated with or without dietary β-mannanase (Exp. 1). As calculated in the following equation, the predicted ME or AA for the low nutrient diet (3,260 kcal g/kg of ME; Exp. 2) supplemented with β-mannanase was predicted to be 40 kcal higher than energy values evaluated for non-β-mannanase-supplemented diets based on NRC (2012). The diets were formulated to meet or exceed the requirement of NRC (2012). Ingredients and chemical composition of experimental diet are presented in Table 4.

$$DL = [Corn \times (ME_{2Corn} - ME_{1Corn})] + [Wheat \times (ME_{2Wheat} - ME_{1Wheat})] + [SBM \times (ME_{2SBM} - ME_{1SBM})] + [DDGS \times (ME_{2DDGS} - ME_{1DDGS})] + [PKM \times (ME_{2PKM} - ME_{1PKM})],$$

where DL is the predicted energy or AA difference between β-mannanase and without β-mannanase in diet, Corn is the corn ratio in the diet, Wheat is the wheat ratio in the diet, SBM is the soybean ratio in the diet, DDGS is the DDGS ratio in the diet, PKM is the PKM ratio in the diet, $ME_1$ is the predicted ME or AA in Exp. 1 without β-mannanase, $ME_2$ is the predicted ME or AA in Exp. 1 with β-mannanase.

For the analysis of nutrient digestibility, Cr was used as an indigestible marker in each phase diet to calculate digestibility coefficients. All pigs in all pens were fed diets mixed with chromic oxide from d 14 to 21 and d 35 to 42. Fecal grab samples were collected from the floor of each pen during the last 4 d of each phase. The feces collected were pooled to represent one pen and dried in an air-forced drying oven at 60 °C for 72 h and ground in a 1-mm screen Wiley mill for chemical analysis. Fecal samples were dried in an air-forced drying oven at 60 °C and ground before analysis. Diet and fecal samples were analyzed for GE using a bomb calorimeter (Parr Adiabatic Calorimeter 1241; Parr Instrument Co., Molin, IL, US).

To measure the concentration of volatile fatty acids (VFA), fecal samples were collected (d 14 and 28) directly from the anus of one randomly selected pig in each pen to minimize the air contact. These samples were immediately sealed in vinyl bags and placed on ice. Fecal VFA concentrations were measured by gas chromatography (HP 6890 Plus; Hewlett Packard, Houston, TX) using the method in Choi et al. (2018) study, and ammonia-N concentration was analyzed as per Chaney and Marbach (1962).

On d 21 and 42 of Exp. 2, a 10-mL blood sample was collected by jugular vein puncture from 2 randomly selected pigs in each pen

using a disposable vacutainer tube containing sodium heparin as an anticoagulant (Becton Dickinson, Franklin, NJ, US) at 9:00. A serum automatic biochemical analyzer (Fuji Dri-chem 3500i; Fujifilm, Tokyo, Japan) was applied to measure the concentrations of total protein (TP), blood urea nitrogen (BUN), total cholesterol (TCHO), glucose (GLU), triglycerides (TG), albumin, and globulin. After centrifugation (3,000 × g for 20 min at 4 °C), plasma samples were separated and stored at −20 °C and later analyzed for concentrations of blood parameters.

Additionally, the proximate analysis in experimental diets and fecal samples in 6 periods (n = 6 samples/treatment) was conducted using the method of AOAC (2007). The GE of ingredients and diets was measured using a bomb calorimeter (Parr Instrument Co). The experimental diets and fecal samples were analyzed for Cr concentration (Fenton and Fenton, 1979) using a spectrophotometer (Jasco V-550; Jasco Corp, Tokyo, Japan).

### 2.3. Statistical analysis

All data were analyzed using SAS (SAS Inst. Inc., Cary, NC, US). In Exp. 1, in incomplete Latin square designs, orthogonal contrasts were conducted to verify if the addition of β-mannanase to each feed material increases energy or AA digestibility using the GLM procedure with each pig as the experimental unit. In Exp. 2, data were analyzed as a 2 × 2 factorial arrangement of treatments in a completely randomized design. The main effects of dietary energy level, β-mannanase, and their interaction were determined by the GLM procedure of SAS (SAS Inst. Inc.). The pen was used as the experimental unit for the analysis of growth performance and nutrient digestibility data. For the analysis of blood metabolites and VFA, the mean of 2 selected pigs from each pen was used as the experimental unit. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

## 3. Results

In Exp. 1, the effects of β-mannanase on SID of AA are shown in Table 5. The SID of His, Leu, Met, Ala, Cys, and Gly were greater ($P < 0.05$) for pigs fed the diet containing corn with β-mannanase than for pigs fed corn without β-mannanase. The SID values of Lys, Met, Phe, Thr, Val, Ala, Cys, Pro, and Tyr for pigs fed β-mannanase-supplemented wheat are higher ($P < 0.05$) than those pigs fed wheat without β-mannanase. A greater ($P < 0.05$) SID of His, Lys, Asp, Glu, and Gly were observed in pigs fed SBM with β-mannanase than in pigs fed SBM without β-mannanase. The SID for Arg, Lys, Phe, Val, Glu, Gly, Pro, and Ser were greater ($P < 0.05$) in pigs fed DDGS with β-mannanase than in pigs fed DDGS without β-mannanase. The differences between the measured SID increased ($P < 0.05$) for His, Lys, Met, Phe, Cys, Gly, Pro, and Tyr in pigs fed β-mannanase-supplemented PKM compared with pigs fed PKM without β-mannanase. The overall SID result of the diets with β-mannanase showed that the mean SID of both indispensable and dispensable AA were increased ($P < 0.01$) in β-mannanase-supplemented diets compared with untreated treatments in an individual comparison manner for corn, wheat, SBM, DDGS, and PKM. The supplementation of β-mannanase to the diets numerically increased the available DE of corn, wheat, SBM, DDGS, and PKM around 33, 35, 33, 43, and 72 kcal, respectively (Table 4). There was no difference between the measured values for DE and ME in pigs fed corn, wheat, SBM, and DDGS. However, the DE was increased ($P < 0.05$) in pigs fed the diet containing PKM with β-mannanase compared with pigs fed PKM without β-mannanase. The ME content of corn, wheat, SBM, DDGS, and PKM were numerically increased around 33, 39, 46, 51, and 77 kcal respectively.

In Exp. 2, there were no interactions between nutrient level and β-mannanase in any of the parameters. The apparent total tract digestibility (ATTD) of DM in phase 1 tended to be increased ($P = 0.09$) in pigs fed β-mannanase (Table 6). In both phases, pigs fed β-mannanase showed a greater ($P < 0.05$) ATTD of GE, however, the ATTD of CP was not affected. No variations were observed in the ATTD of DM, GE, and CP in pigs fed high nutrient level diets.

In phase 1, the average daily gain (ADG) of pigs fed high-nutrient diets was higher (Table 7; $P < 0.05$) than that for those fed the low-nutrient diets, moreover, ADG in β-mannanase-supplemented diet was tended to be greater ($P = 0.096$) than that for the untreated diets. However, pigs fed diets supplemented with β-mannanase or high-nutrient diets exhibited similar average daily feed intake (ADFI) and feed-to-gain ratio (F:G) at d 21. During the second phase, no effect was observed on ADG and ADFI in nutrient-level diets, but F:G ratio was tended to improve ($P = 0.089$). Overall, there was a tendency for a greater ADG ($P = 0.064$) and ADFI ($P = 0.083$) in pigs fed high-nutrient diets. In addition, the F:G ratio was improved in pigs fed high-nutrient diets. Pigs fed the β-mannanase in the diets had greater ($P < 0.05$) ADG and F:G ratio.

The high-nutrient and β-mannanase-supplemented diets did not show any changes in the concentration of TCHO, TG, GLU, BUN, TP, albumin, and globulin in plasma of pigs in both phases 1 and 2 (data not shown).

No difference was observed in the concentration of acetic acid, propionic acid, butyric acid, and ammonia (Table 8). In phase 1, a tendency ($P = 0.051$) for a lower fecal ammonia was observed in pigs offered β-mannanase-supplemented diets. In phase 2, the

**Table 4**
Ingredients and composition of experimental diets in Exp. 2 (%, as-fed basis).

| Item | Experimental diets | | | |
| --- | --- | --- | --- | --- |
| | Phase 1 | | Phase 2 | |
| | β-mannanase[1] | | β-mannanase[1] | |
| | 0 | 0.05% | 0 | 0.05% |
| Ingredients | | | | |
| Corn | 53.37 | 55.67 | 51.10 | 53.22 |
| SBM | 18.66 | 17.18 | 12.85 | 11.72 |
| Wheat | 5.00 | 5.00 | 5.00 | 5.00 |
| DDGS | 10.00 | 10.00 | 15.00 | 15.00 |
| Palm kernel meal | 7.00 | 7.00 | 10.00 | 10.00 |
| Animal fat | 2.89 | 2.02 | 3.14 | 2.10 |
| Celite[1] | 0.10 | 0.10 | 0.10 | 0.10 |
| L-lysine·HCl (78%) | 0.37 | 0.37 | 0.34 | 0.34 |
| DL-Met (50%) | 0.04 | 0.04 | 0.13 | 0.13 |
| Choline chloride (50%) | 0.05 | 0.05 | 0.05 | 0.05 |
| Limestone | 1.20 | 1.20 | 1.09 | 1.09 |
| Mono-dicalcium phosphate | 0.52 | 0.52 | 0.40 | 0.40 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 |
| Mineral premix[2] | 0.20 | 0.20 | 0.20 | 0.20 |
| Vitamin premix[3] | 0.30 | 0.30 | 0.30 | 0.30 |
| Total | 100 | 100 | 100 | 100 |
| Calculated composition | | | | |
| ME, kcal/kg | 3,300 | 3,260 | 3,300 | 3,260 |
| CP | 17.00 | 16.51 | 16.00 | 15.63 |
| Calcium | 0.59 | 0.59 | 0.52 | 0.52 |
| Av. phosphorus | 0.23 | 0.23 | 0.21 | 0.21 |
| Lys | 0.85 | 0.82 | 0.73 | 0.70 |
| Met + Cys | 0.48 | 0.46 | 0.44 | 0.42 |

SBM = soybean meal; DDGS = distillers dried grains with solubles; ME = metabolizable energy; CP = crude protein.
[1] β-mannanase (800,000 U/kg) was added by replacing Celite in the diets.
[2] Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin D₃, 40 IU vitamin E, 5.0 mg vitamin K₃, 5.0 mg vitamin B₁, 20 mg vitamin B₂, 4.0 mg vitamin B₆, 0.08 mg vitamin B₁₂, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.
[3] Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

**Table 5**
Standardized ileal digestibility (SID) of amino acids (AA) of corn, wheat, soybean meal (SBM), distillers dried grains with solubles (DDGS), and palm kernel meal (PKM) in growing pigs (%).

| Item | Diets | | | | | | | | | | SEM | P-value for contrasts [1] | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Corn | | Wheat | | SBM | | DDGS | | PKM | | | Corn | Wheat | SBM | DDGS | PKM |
| | β-mannanase | | β-mannanase | | β-mannanase | | β-mannanase | | β-mannanase | | | | | | | |
| | 0 | 0.05% | 0 | 0.05% | 0 | 0.05% | 0 | 0.05% | 0 | 0.05% | | | | | | |
| Indispensable AA | | | | | | | | | | | | | | | | |
| Arg | 84.3 | 86.7 | 83.0 | 85.9 | 89.7 | 92.3 | 78.1 | 81.5 | 83.6 | 86.2 | 1.03 | 0.111 | 0.055 | 0.075 | 0.023 | 0.075 |
| His | 81.4 | 84.9 | 84.7 | 86.2 | 83.3 | 86.1 | 77.2 | 79.4 | 80.2 | 83.8 | 0.76 | <0.01 | 0.17 | 0.012 | 0.053 | <0.01 |
| Ile | 83.0 | 84.8 | 79.4 | 81.5 | 79.4 | 81.5 | 74.8 | 76.1 | 79.3 | 81.2 | 0.97 | 0.206 | 0.149 | 0.137 | 0.362 | 0.178 |
| Leu | 84.3 | 87.1 | 85.5 | 87.7 | 80.4 | 82.6 | 84.5 | 86.0 | 78.6 | 80.4 | 0.86 | 0.024 | 0.073 | 0.075 | 0.24 | 0.157 |
| Lys | 73.5 | 74.1 | 63.7 | 66.4 | 85.8 | 88.0 | 58.7 | 61.6 | 72.4 | 74.8 | 0.68 | 0.536 | <0.01 | 0.029 | <0.01 | 0.017 |
| Met | 84.0 | 86.1 | 85.6 | 88.6 | 85.1 | 86.6 | 81.6 | 82.8 | 82.7 | 85.0 | 0.71 | 0.047 | <0.01 | 0.142 | 0.225 | 0.025 |
| Phe | 82.2 | 84.7 | 82.3 | 84.8 | 92.4 | 93.5 | 79.4 | 82.4 | 79.3 | 83.1 | 0.88 | 0.058 | 0.049 | 0.405 | 0.019 | <0.01 |
| Thr | 75.0 | 76.7 | 70.2 | 73.3 | 80.3 | 82.0 | 68.3 | 70.3 | 72.6 | 74.6 | 0.82 | 0.145 | 0.01 | 0.142 | 0.094 | 0.084 |
| Val | 78.6 | 81.6 | 78.1 | 81.7 | 83.8 | 85.1 | 72.2 | 75.9 | 82.6 | 84.7 | 1.14 | 0.067 | 0.031 | 0.436 | 0.027 | 0.196 |
| Sub-mean | 80.8 | 82.9 | 79.7 | 82.1 | 84.5 | 86.4 | 75.0 | 77.3 | 79.0 | 81.5 | 0.38 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Dispensable AA | | | | | | | | | | | | | | | | |
| Ala | 80.3 | 82.9 | 68.2 | 71.1 | 83.4 | 84.0 | 77.6 | 79.2 | 67.6 | 69.4 | 0.76 | 0.021 | <0.01 | 0.582 | 0.159 | 0.106 |
| Asp | 77.2 | 78.9 | 73.4 | 75.8 | 81.2 | 84.9 | 66.6 | 68.9 | 70.9 | 73.1 | 0.93 | 0.205 | 0.078 | <0.01 | 0.082 | 0.116 |
| Cys | 82.4 | 85.2 | 85.3 | 87.9 | 89.8 | 90.6 | 72.4 | 74.2 | 75.4 | 78.7 | 0.78 | 0.013 | 0.026 | 0.451 | 0.111 | <0.01 |
| Glu | 83.9 | 84.5 | 92.4 | 94.1 | 90.7 | 93.5 | 78.3 | 81.6 | 82.6 | 84.2 | 0.84 | 0.577 | 0.151 | 0.022 | <0.01 | 0.198 |
| Gly | 60.4 | 64.3 | 70.2 | 71.2 | 82.6 | 85.3 | 60.3 | 62.8 | 58.4 | 61.6 | 0.68 | <0.01 | 0.329 | <0.01 | 0.011 | <0.01 |
| Pro | 61.4 | 62.2 | 71.7 | 73.9 | 78.5 | 79.4 | 66.5 | 68.7 | 51.3 | 53.7 | 0.65 | 0.4 | 0.018 | 0.299 | 0.022 | 0.014 |
| Ser | 79.7 | 81.3 | 82.9 | 83.8 | 79.2 | 81.0 | 73.8 | 76.5 | 77.9 | 79.7 | 0.80 | 0.171 | 0.454 | 0.113 | 0.021 | 0.113 |
| Tyr | 77.8 | 79.6 | 79.6 | 82.8 | 82.3 | 84.0 | 78.6 | 79.7 | 79.6 | 82.4 | 0.90 | 0.182 | 0.016 | 0.186 | 0.423 | 0.034 |
| Sub-mean | 75.6 | 77.4 | 77.8 | 79.3 | 83.4 | 85.3 | 71.8 | 73.9 | 70.5 | 72.8 | 0.40 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Total-mean | 78.2 | 80.3 | 78.6 | 81.0 | 84.0 | 85.9 | 73.5 | 75.7 | 75.0 | 77.4 | 0.39 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Energy retention (DM basis), kcal/kg | | | | | | | | | | | | | | | | |
| DE | 3,496 | 3,529 | 3,456 | 3,491 | 3,643 | 3,676 | 3,624 | 3,667 | 3,046 | 3,118 | 19.3 | 0.233 | 0.206 | 0.233 | 0.122 | 0.023 |
| ME | 3,412 | 3,445 | 3,353 | 3,392 | 3,347 | 3,393 | 3,425 | 3,476 | 2,911 | 2,988 | 24.4 | 0.344 | 0.265 | 0.189 | 0.146 | 0.056 |

DE = digestible energy; ME, metabolizable energy; SEM = Standard error of means.
[1] The contrast between with and without β-mannanase in individual feed materials.

## 4. Discussion

The effects of β-mannanase on SID of AA among the 5 feed materials (corn, wheat, SBM, DDGS, and PKM) were tested, and we found that β-mannanase improved the overall SID of AA in corn, wheat, SBM, DDGS, and PKM. The concentrations of AA in corn, wheat, and SBM determined in growing pigs was within the range of that were previously reported (NRC, 2012). Several studies showed that NSP has an adverse effect on AA losses (Passos et al., 2015; Cadogan and Choct, 2015; Kim et al., 2018b). Increased AID

concentration of fecal ammonia was decreased in pigs fed β-mannanase.

of AA in diets containing β-mannanase is likely caused by the fact that supplemented β-mannanase decreases the digesta viscosity by hydrolyzing the bonds between mannan units (Shim et al., 2017). In addition, it is well documented that β-mannanase supplementation can have led to an increased release of encapsulated nutrients in the complex structure of cell walls (Cadogan and Choct, 2015; Kim et al., 2017). As expected, the greatest effect of β-mannanase on GE digestibility was shown in PKM because of the highest β-mannan content. Beta-mannanase has been successful in increasing apparent energy digestibility in pigs (Kim et al., 2018a); however,

**Table 6**
Effect of nutrient level (NL) and β-mannanase supplementation on apparent total tract digestibility of nutrients in growing pigs (%).[1]

| Item | NL1 | | NL2 | | SEM | P-value | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | β-mannanase | | β-mannanase | | | NL | BM | NL × β-mannanase |
| | 0 | 0.05% | 0 | 0.05% | | | | |
| Phase 1 (d 21) | | | | | | | | |
| DM | 81.4 | 82.6 | 82.1 | 83.1 | 0.55 | 0.29 | 0.087 | 0.971 |
| GE | 77.0 | 78.0 | 77.2 | 78.6 | 0.54 | 0.492 | 0.036 | 0.744 |
| CP | 81.0 | 81.7 | 81.3 | 82.2 | 0.70 | 0.551 | 0.322 | 0.889 |
| Phase 2 (d 42) | | | | | | | | |
| DM | 80.0 | 81.3 | 80.2 | 81.3 | 0.85 | 0.917 | 0.179 | 0.807 |
| GE | 79.3 | 80.5 | 79.3 | 81.2 | 0.69 | 0.533 | 0.033 | 0.609 |
| CP | 80.1 | 80.7 | 80.3 | 81.3 | 1.00 | 0.689 | 0.422 | 0.822 |

SEM = standard error of means; DM = dry matter; GE = gross energy; CP = crude protein.
[1] NL1, 3,260 kcal ME, 16.51% and 15.63% protein in phases 1 and 2, respectively; NL2, 3,300 kcal ME, 17% and 16% protein in phases 1 and 2, respectively.

**Table 7**
Effects of nutrient level (NL) and β-mannanase supplementation on growth performance in pigs.[1]

| Item | NL1 | | NL2 | | SEM | P-value | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | β-mannanase | | β-mannanase | | | NL | BM | NL × β-mannanase |
| | 0 | 0.05% | 0 | 0.05% | | | | |
| Phase 1 (d 0 to 21) | | | | | | | | |
| ADG, g | 781 | 803 | 809 | 835 | 13.5 | 0.042 | 0.096 | 0.717 |
| ADFI, g | 2,366 | 2,350 | 2,347 | 2,331 | 17.9 | 0.303 | 0.382 | 0.912 |
| F:G | 3.02 | 2.94 | | | 0.05 | 0.015 | 0.041 | 0.808 |
| Phase 2 (d 22 to 42) | | | | | | | | |
| ADG, g | 843 | 863 | 853 | 893 | 15.3 | 0.221 | 0.064 | 0.503 |
| ADFI, g | 2,864 | 2,860 | 2,857 | 2,849 | 24.8 | 0.707 | 0.827 | 0.797 |
| F:G | 3.4 | 3.34 | 3.37 | 3.19 | 0.05 | 0.089 | 0.02 | 0.363 |
| Overall (d 0 to 42) | | | | | | | | |
| ADG, g | 813 | 833 | 830 | 864 | 12.4 | 0.064 | 0.043 | 0.538 |
| ADFI, g | 2,614 | 2,618 | 2,568 | 2,578 | 23.3 | 0.083 | 0.761 | 0.381 |
| F:G | 3.23 | 3.13 | 3.07 | 2.99 | 0.04 | <0.01 | 0.048 | 0.963 |

SEM = standard error of means; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed-to-gain ratio.
[1] NL1, 3,260 kcal ME, 16.51% and 15.63% protein in phases 1 and 2, respectively; NL2, 3,300 kcal ME, 17% and 16% protein in phases 1 and 2, respectively.

**Table 8**
Effects of nutrient level (NL) and β-mannanase supplementation on fecal volatile fatty acids (VFA) and NH₃-N in pigs.[1]

| Item | NL1 | | NL2 | | SEM | P-value | | |
|------|-----|-----|-----|-----|-----|---------|---|---|
| | β-mannanase | | β-mannanase | | | NL | β-mannanase | NL × β-mannanase |
| | 0 | 0.05% | 0 | 0.05% | | | | |
| Phase 1 (d 21) | | | | | | | | |
| VFA, mg/kg | | | | | | | | |
| Acetic acid | 355 | 351 | 353 | 349 | 9.3 | 0.857 | 0.695 | 0.958 |
| Propionic acid | 224 | 222 | 224 | 222 | 12.3 | 0.955 | 0.879 | 0.917 |
| Butyric acid | 212 | 214 | 214 | 216 | 14.3 | 0.901 | 0.912 | 0.945 |
| NH₃-N | 242 | 218 | 250 | 224 | 12.4 | 0.602 | 0.051 | 0.937 |
| Phase 2 (d 42) | | | | | | | | |
| VFA, mg/kg | | | | | | | | |
| Acetic acid | 363 | 361 | 361 | 359 | 14.9 | 0.904 | 0.873 | 0.957 |
| Propionic acid | 234 | 228 | 231 | 227 | 7.2 | 0.805 | 0.531 | 1 |
| Butyric acid | 225 | 225 | 219 | 221 | 9.3 | 0.613 | 0.882 | 0.736 |
| NH₃-N | 270 | 248 | 264 | 240 | 7.6 | 0.345 | 0.011 | 0.473 |

SEM = standard error of means.

[1] NL1, 3,260 kcal ME, 16.51% and 15.63% protein in phases 1 and 2, respectively; NL2, 3,300 kcal ME, 17% and 16% protein in phases 1 and 2, respectively.

the feed materials such as corn contains lower NSP than DDGS, PKM, or SBM, which is a factor believed to contribute to inconsistent efficiency of β-mannanase in improving GE digestibility in different feed materials. The importance of the measured GE content in the β-mannanase-treated diet is more likely that the extra energy contents can compromise the other nutrients balance. The main reason for the significant effect of β-mannanase on the digestibility of energy in PKM refers to the enzymatic effects of β-mannanase on cleaving the bonds (Kim et al., 2017, 2018b), while the NSP content in corn and wheat are negligible.

In Exp. 1, the ATTD of DM tended to be greater in pigs fed β-mannanase in phase 1. Previous research conducted by Kim et al. (2013) reported that the addition of β-mannanase increased DM and GE digestibility. Physical factors, including difficulties in the incorporation of fiber in the enzymatic phase in the small intestine of the weaning pigs, may limit total energy digestibility, which highlights the role of enzymes in increasing the digestion rate (Kim et al., 2017; Ryu et al., 2017). Several studies have confirmed the positive response of NSP degrading enzymes when a high concentration of fiber included in the diet (Omogbenigun et al., 2004; Zhang and Adeola, 2017). Changing the digestibility of GE can contribute to the NSP digestibility since NSP normally does not count as a digestible part of the diet. In a related study, Yoon et al. (2010) reported that β-mannanase supplementation increased the ATTD of DM and CP but showed no effect on ATTD of GE in growing pigs offered corn-SBM-based diets included high DDGS content.

The CP digestibility results of this assay did not reflect the results obtained from the in vivo study on SID of AA in feed materials. The reason why the CP digestibility did not differ among the treatments remains unclear; however, it is likely due to increasing in undigested protein flow to the large intestine in diets without β-mannanase enzyme, as the concentration of ammonia in the feces increased in pigs fed the diets without β-mannanase enzyme.

The chemical composition of both PKM and DDGS revealed a potential for use in commercial pig diets as low-cost feed materials. The result of the present study showed a greater ADG in pigs fed β-mannanase in the diet containing PKM and DDGS. The results support the data of Kim et al., (2017) showing that, compared with control diet, the ADG of growing pigs linearly improved by 8.8% in a two-phase feeding regime when β-mannanase supplemented to the diet. Kim et al. (2018b) reported a linear increase in ADG of weaned pigs fed corn-SBM-based feeds formulated with different levels of β-mannanase. The present study demonstrated that pigs in the β-mannanase group showed a greater F:G ratio. Dietary mannan is considered as an important contributor to the increase in viscosity of digesta (Passos et al., 2015). The inconsistent effects

of β-mannanase enzyme on growth performance observed on the growth performance of pigs might be attributed to differences in the level of dietary mannan and the kind or amount of β-mannanase in the diet. From a productive perspective, the present results revealed that the growth performance was affected by dietary β-mannanase. Our results also showed that the ATTD of GE and SID of AA improved in pigs fed β-mannanase. Surprisingly, no difference was shown in the ADFI of growing pigs fed the diets with or without β-mannanase, as was observed by the previous work (Kim et al., 2013). The above result is not without contradiction because the ADG depends on the energy density of the diet (Tiwari and Jha, 2016; Chen et al., 2017). However, other reports conflict with the rule that the level of energy affects ADFI at different energy levels in pigs. For example, Kim et al. (2018a) and Choi et al. (2017) reported no change in feed intake with different dietary energy density in lactating sows. This might be correlated to the fact that the inconsiderable energy difference between high and low nutrient diets in the current study (only 40 kcal) cannot properly affect the ADFI in an ad libitum trial.

In the swine gut, there are interactions between microbiota and diet (Kong et al., 2016; Ji et al., 2017). Not surprisingly, diets containing high NSP content may change the fermentation types and rates regarding the change in the flow of nutrients to the distal sections of intestine. The observed tendency of lower fecal ammonia in piglets fed β-mannanase diets in the first phase, together with the observed effects of β-mannanase on digestibility of GE, may be due to a higher absorption in distal sections of the intestine and a lower nutrient flow to the proximal section to increase the fermentation ratio. In contrast to results of the presented study, Zdunczyk et al. (2013) have reported that the concentration of acetic acid, propionic acid, isobutyric acid, and butyric acid can be decreased by the dietary supplementation of exogenous enzyme containing β-mannanase. The focus of the present study was exclusively on proper balancing the diets in order to decrease the rate of wasted nutrients. The viscous nature of NSPs in gastrointestinal tract gives them the ability to encompass the nutrients in their complex structure to bring them to the proximal sections of the intestine (Cadogan and Choct, 2015; Passos et al., 2015). We report that there was a decreased NH₃—N in the feces of piglets in response to dietary β-mannanase supplementation, showing that the dietary AA may be highly utilized.

## 5. Conclusion

In conclusion, the present study showed that β-mannanase improved the digestibility of GE and AA. Increasing the digestibility

of AA allows the AA content in the diet to be lowered while maintaining the same level of pig performance, which may consequently lead to lower feed cost.

## Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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