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The Effect of Various Inclusion Levels of β-Mannanase on Nutrient Digestibility in Diets Consisting of Corn, Soybean Meal and Palm Kernel Expellers Fed to Growing Pigs

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Abstract: An experiment was conducted to determine the effects of various inclusion levels of β-mannanase on the energy and mannan digestibility in diets containing corn, Soybean Meal (SBM) and Palm Kernel Expellers (PKE) fed to growing pigs. The PKE contained 92.6% dry matter, 4,417 kcal gross energy kg^{-1}, 4.02% ash and 31.3% mannan on an as-fed basis. Six barrows with an initial body weight of 41.4±1.7 kg were individually housed in metabolism crates equipped with a feeder and a nipple drinker. Pigs were randomly allotted to a 6×6 Latin square design with 6 dietary treatments and 6 periods. An experimental period consisted of a 4-d adaptation period and a 5-d collection period. Pigs were fed corn-SBM-based diets containing 15% PKE with 0, 400, 800, 1,600, 2,400 or 3,200 U kg^{-1} of β-mannanase. A chromic oxide was added as an indigestible marker for fecal collection according to a marker-to-marker procedure. Urine was also collected from each period. Each of the 6 diets contained 3.60, 3.91, 4.06, 2.57, 3.57 and 2.59% of mannan, respectively. No linear and quadratic effects of β-mannanase supplementation on apparent total tract digestibility of energy were observed. The digestibility of mannan was also not affected by the increasing level of β-mannanase supplementation. In conclusion, exogenous β-mannanase did not affect the energy and mannan digestibility in corn-SBM-based diets containing 15% of PKE fed to growing pigs.

Keywords: β-Mannanase, Palm Kernel Expellers, Pigs

Introduction

Dietary mannan, one of Non-Starch Polysaccharides (NSP), has negative effects on swine performance (Rainbird *et al*., 1984). Studies have been reported that β-mannanase supplementation to corn-Soybean Meal (SBM)-based diets affects nutrient digestibility and growth performance in pigs (Pettey *et al*., 2002; Lv *et al*., 2013). Palm Kernel Expellers (PKE) has recently received great attention because of the low price compared with other ingredients (Kim *et al*., 2001; Fang *et al*., 2007; Son *et al*., 2012). However, the content of mannan in PKE was much greater than that in other ingredients widely used for swine diets (Table 1; Mok *et al*., 2013).

Table 1. Nutrient composition of corn, Soybean Meal (SBM) and Palm Kernel Expellers (PKE; as-fed basis)

	Ingredient		
Item (%)	Corn	SBM	PKE
Dry matter	86.30	87.30	92.60
Crude protein	6.85	46.90	16.90
Ether extract	3.45	1.14	6.74
Ash	1.43	6.12	4.02
Neutral detergent fiber	19.70	31.40	68.30
Acid detergent fiber	2.92	6.52	37.80
Calcium	0.04	0.23	0.28
Phosphorus	0.28	0.63	0.66
Mannan	0.29	1.20	31.30

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There is a possibility that the β-mannanase can be used more efficiently in the PKE-based diets due to abundant substrates. Enzyme complex of mannanase and cellulase effectively hydrolyzed the fiber components of PKE and the pre-treating PKE with the enzyme complex resulted in improvement in the true metabolizable energy value of pre-treated PKE compared with the raw PKE in diets fed to broilers (Saenphoom *et al*., 2013). In pigs, β-mannanase improved apparent ileal and total tract digestibilities of Dry Matter (DM), organic matter and energy in diets containing 10% PKE fed to pigs (Mok *et al*., 2013). However, data on the dose-dependent effects of β-mannanase on energy and mannan digestibility of PKE-containing diets are rare.

Therefore, the objective of present study was to determine the effects of various inclusion levels of β-mannanase on nutrient digestibility in diets consisting of corn, SBM and PKE fed to growing pigs.

Materials and Methods

Animals and Experimental Design

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Konkuk University. Six crossbred barrows with an average initial body weight of 41.4 kg (standard deviation = 1.7) were used to determine the effect of supplemental β-mannanase. Animals were allotted to a 6×6 Latin square design with 6 diets and 6 periods (Kim and Kim, 2010). Pigs were individually housed in metabolism crates that were equipped with a feeder and a nipple drinker.

Diets and Feeding

Six experimental diets were corn-SBM-based diets containing 15% PKE with 6 concentrations of supplemental β-mannanase at 0, 400, 800, 1,600, 2,400 or 3,200 U kg^{-1} of β-mannanase (Table 2). All diets were prepared in mash form. The exogenous carbohydrase, β-mannanase, was produced by *Bacillus subtilis* grown on Luria broth. The amount of feed provided daily per pig was calculated as approximately 3 times the estimated energy requirement for maintenance (197 kcal kg^{-1} body $weight^{0.60}$; Kil *et al*., 2013).

Daily feed allowance was divided into 2 equal meals and fed to the pigs at 0900 and 1700. Water was freely accessible at all times. Vitamins and minerals were included in all diets to meet or exceed nutrient requirement estimates (NRC, 2012).

Sample Collection and Chemical Analysis

An experimental period consisted of 9 d. Feces were collected according to the marker-to-marker procedure (Kong and Adeola, 2014) during 5 d of the collection period after 4 d of the adaptation period. Chromic oxide was added as an indigestible marker to the morning diets on d 5 and 10. Urine was collected from 1400 on d 5 to 1400 on d 10. The feces and urine was immediately stored at -20°C after collection.

Table 2. Ingredient composition and mannan content of experimental diets[a] (as-fed basis)

	β-Mannanase (U kg^{-1})					
Item	0	400	800	1,600	2,400	3,200
Ingredient (%)						
Ground corn	56.40	56.40	56.40	56.40	56.40	56.40
Soybean meal, 48% crude protein	25.00	25.00	25.00	25.00	25.00	25.00
Palm kernel expellers	15.00	15.00	15.00	15.00	15.00	15.00
L-Lysine·HCl	0.50	0.50	0.50	0.50	0.50	0.50
β-Mannanase	-	0.05	0.10	0.20	0.30	0.40
β-Mannanase carrier	0.40	0.35	0.30	0.20	0.10	-
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[b]	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition (%)						
Mannan	3.60	3.91	4.06	2.57	3.57	2.59

[a]All diets were formulated to contain 3,213 kcal metabolizable energy kg^{-1}, 19.6% crude protein, 1.18% Standardized Ileal Digestible (SID) lysine, 0.51% SID methionine + cysteine, 0.56% threonine, 0.19% tryptophan, 0.69% calcium and 0.31% standardized total tract digestible phosphorus; [b]Provided per kg of diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; Zn, 100 mg as zinc oxide

Table 3. Effects of various inclusion levels of β-mannanase on energy digestibility in corn-soybean meal-palm kernel expeller-based diets fed to growing pigs[a,b] (as-fed basis)

	β-Mannanase (U kg^{-1})							P-value	
Item[c]	0	400	800	1,600	2,400	3,200	SEM[d]	Linear	Quadratic
Diet intake (kg 5 $days^{-1}$)	9.71	9.64	9.60	9.72	9.52	9.47	0.58	0.07	0.55
GE intake (Mcal 5 $days^{-1}$)	38.7	38.8	38.8	39.3	38.4	38.0	2.35	0.12	0.09
Fecal GE output (Mcal 5 $days^{-1}$)	6.71	6.53	7.00	6.74	6.72	6.47	0.32	0.36	0.14
ATTD of energy (%)	82.5	83.0	81.8	82.8	82.3	82.9	0.67	0.79	0.49
DE in diet (kcal kg^{-1})	3,290	3,345	3,312	3,345	3,325	3,323	27.00	0.50	0.21
Urinary GE output (Mcal 5 $days^{-1}$)	1.24	1.23	1.14	1.11	0.97	1.07	0.13	0.05	0.38
ME in diet (kcal kg^{-1})	3,163	3,217	3,192	3,232	3,221	3,208	28.00	0.19	0.14

[a]Each least squares mean represents 6 observations except a diet containing 2,400 U kg^{-1} of β-mannanase (5 observations); [b]Diet intake, GE intake, fecal GE output and urinary GE output were based on 5 d of collection; [c]GE = Gross Energy; DE = Digestible Energy; ATTD = Apparent Total Tract Digestibility; ME = Metabolizable Energy; [d]SEM = Standard Error of the Means

Table 4. Effects of various inclusion levels of β-mannanase on the mannan digestibility in corn-soybean meal-palm kernel expeller-based diets fed to pigs [a,b](as-fed basis)

	β-Mannanase (U kg^{-1})							P-value	
Item	0	400	800	1,600	2,400	3,200	SEM[b]	Linear	Quadratic
Mannan intake (g 5 $days^{-1}$)	329.0	326.0	325.0	329.0	322.0	320.0	20.0	0.07	0.55
Mannan output (g 5 $days^{-1}$)	21.8	22.1	27.2	29.5	21.0	31.0	7.3	0.08	0.86
Mannan digestibility (%)	93.8	93.6	92.0	91.5	94.1	90.9	1.8	0.08	0.88

[a]Each least squares mean represents 6 observations except a diet containing 2,400 U kg^{-1} of β-mannanase (5 observations); [b]Mannan intake and output were based on 5 d of collection; [c]SEM = Standard Error of the Means

Fecal samples were dried in a forced-air drying oven at 55°C and ground before analysis. The diets, feces and urine were analyzed for gross energy using a bomb calorimeter (Parr 1261; Parr Instruments Co., Moline, IL, USA). Ingredient samples were analyzed for DM, crude protein, ether extract, crude fiber, ash, calcium, phosphorus, neutral detergent fiber and acid detergent fiber (AOAC, 2005). Diets and ingredient samples were also analyzed for the mannan concentration. The samples were hydrolyzed using 72% (w/w) H_2SO_4 for 1 h. Then, the samples were diluted with distilled water to H_2SO_4 concentration to 1 *N* and incubated at 121°C for 45 min. The mannan contents in hydrolysates were determined using an evaporative light scattering detector and a Shodex sugar column SP0810 (8.0×300 mm; Mok *et al*., 2013).

Calculations and Statistical Analysis

The contents of digestible energy and metabolizable energy in experimental diets were calculated by the procedures described by Kong and Adeola (2014). The mannan digestibility was calculated based on the mannan intake and output:

$$\text{Mannan digestibility (\%)} = (Md-Mf)/Md \times 100$$

where, Md is the total mannan intake (g) during collection period (from d 5 to 9) and Mf is the total fecal mannan output (g) during collection period (from d 5 to 9).

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The model included dietary treatment as a fixed variable and animal and period as random variables. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of the supplemental β-mannanase concentrations.

The experimental unit was a pig and significance was determined at an α of 0.05.

Results

During the fifth period, one pig fed the diet containing 2,400 U kg^{-1} β-mannanase had diarrhea and was excluded from the experiment. All other pigs were healthy throughout the experimental period.

The effects of various inclusion levels of β-mannanase on energy digestibility in corn-SBM-PKE-based diets fed to growing pigs are shown in Table 3.

There was no linear and quadratic effect on the Apparent Total Tract Digestibility (ATTD) of energy among the experimental treatments. However, the urinary GE output linearly decreased ($p<0.05$) by the increasing level of β-mannanase supplementation.

No diffferences were observed for the mannan digestibility among the various inclusion levels of β-mannanase in the experimental diets (Table 4).

Discussion

The PKE is a rich source of NSP components such as mannan and it contains about 31% of mannan which is greater than that of the corn and SBM used in this experiment (Table 1). Generally, the PKE contained 40 to 45% of NSP and 28 to 32% of mannan (Sundu *et al*., 2006; Mok *et al*., 2013). The analyzed value of mannan content in the PKE used in the present study is in agreement with previous studies.

In the present study, 15% of PKE was included in all experimental diets. Despite the experimental diets had enough substrates for the β-mannanase, no differences were observed for the energy digestibility of corn-SBM-PKE-based diets with β-mannanase supplementation.

Mok *et al*. (2013) showed that the apparent ileal digestibility and ATTD of the DM and energy increased when 1,600 U kg^{-1} of β-mannnanase supplemented in the corn-SBM-based diets including 10% of PKE. These results are consistent with the study of Radcliffe *et al*. (1999) that reported increased the apparent ileal digestibility of DM and ATTD of energy for pigs fed corn-SBM-based diets with 0.5% of β-mannnanase addition. However, Pettey *et al*. (2002) found no effects of β-mannnanase on the DM and energy digestibility in pigs fed corn-SBM-based diets.

This inconsistency may be explained by the method for estimating digestibility. Mok *et al*. (2013) and Radcliffe *et al*. (1999) used the index method using the chromic oxide as an indigestible index for the estimation of digestibility, but Pettey *et al*. (2002) and this study used the total collection method for the feces and urine collections. The lack of response may be associated with potentially large variability in the index method.

An activity of exogenous carbohydrase may also be an issue on the energy digestibility study. Lower enzyme activity of exogenous carbohydrase in the gastrointestinal tract could become the reason for the lack of beneficial effect (Kim *et al*., 2004).

Mannan-endo-1,4-β-mannosidase, known as β-mannnanase is an “endo” acting enzyme that hydrolyzes mannan components of feedstuffs such as β-galactomannan and linear β-mannan (McCleary, 1988). The mannan can be degraded to mannan-oligosaccharide or a small unit of sugar monomer such as mannose by the action of β-mannnanase.

However, only monosaccharides can be absorbed in the small intestine and the other metabolites such as mannan-oligosaccharide, mannotriose and mannobiose are not readily absorbed (Kong *et al*., 2011; NRC, 2012). In this study, it is possible that a relatively small quantity of mannose supplied energy to pigs than other experiments. For this reason, we could have found no effect of β-mannnanase supplementation regardless of the supplemental concentrations of β-mannanase.

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Further research is required to clarify the action of β-mannanase on substrates in energy utilization and to determine the optimum inclusion level of β-mannanase when the mannan-rich ingredients are used in swine diets.

Conclusion

In this study, no effects of β-mannanase supplementation on energy or mannan digestibility of corn-SBM-based diet with 15% of PKE were observed.

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Author’s Contributions

W.B. Kwon: Conducted the animal experiment, summarized the data, and wrote most of the manuscript.

S.K. Park: Contributed to the animal experiment, the chemical analysis and manuscript preparation.

C. Kong: Reviewed statistical analysis procedures and provided important input in writing the manuscript.

B.G. Kim: Supervised the project and the preparation of the manuscript.

Ethics

The authors declare no ethical issues.

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