Open Access
Asian Australas. J. Anim. Sci.
Vol. 28, No. 7 : 1014-1019 July 2015
http://dx.doi.org/10.5713/ajas.15.0275
www.ajas.info
pISSN 1011-2367 eISSN 1976-5517

Effects of Supplemental Beta-mannanase on Digestible Energy and Metabolizable Energy Contents of Copra Expellers and Palm Kernel Expellers Fed to Pigs

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ABSTRACT: The purpose of this study was to determine the effect of β-mannanase supplementation on digestible energy (DE) and metabolizable energy (ME) contents of copra expellers (CE) and palm kernel expellers (PKE) fed to pigs. Six barrows with an initial body weight of 38.0 kg (standard deviation = 1.5) were randomly allotted to a 6×6 Latin square design with 6 dietary treatments and 6 periods. Six experimental diets were prepared in a 3×2 factorial treatment arrangement with 3 diets of a corn-soybean meal-based diet, a CE 30% diet, and a PKE 30% diet and with 2 concentrations of supplemental β-mannanase at 0 or 2,400 U/kg. All diets had the same proportion of corn:soybean meal ratio at 2.88:1. The marker-to-marker procedure was used for fecal and urine collection with 4-d adaptation and 5-d collection periods. No interactive effects were observed between diet and β-mannanase on energy digestibility and DE and ME contents of experimental diets. However, diets containing CE or PKE had less ($p<0.05$) DE and ME contents compared with the corn-soybean meal-based diet. The DE and ME contents in CE and PKE were not affected by supplemental β-mannanase. Taken together, we failed to find the effect of β-mannanase supplementation on energy utilization in CE and PKE fed to pigs. (**Key Words:** Energy Utilization, β-Mannanase, Copra Expellers, Palm Kernel Expellers, Swine)

INTRODUCTION

Sharp increases in the prices of traditional feedstuffs such as corn and soybean meal (SBM) have feed producers seeking alternatives to traditional feed ingredients in swine diets. Copra expellers (CE) and palm kernel expellers (PKE), co-products from vegetable oil industry, are produced by the mechanical oil extraction from dried coconut kernels and palm nut kernels, respectively (Son et al., 2012; Sulabo et al., 2013). These co-products can be good candidates for alternative feedstuffs because of the relatively low price and fairly good nutrient composition compared with corn and SBM (Agunbiade et al., 1999; Kim et al., 2001). However, the high concentration of non-starch polysaccharides (NSP) such as mannan in CE and PKE (Table 1) can limit the use of these ingredients in swine diets. Previous studies have shown that utilization of carbohydrates and protein retention were hindered by mannan in swine diets (Kratzer et al., 1964; Rainbird et al., 1984).

Supplemental NSP-degrading enzymes have been reported to improve nutrient digestibility in swine diets (Yin et al., 2000; 2001). Beta-mannanase is one of NSP-degrading enzymes that hydrolyze mannan in feedstuffs (McCleary, 1988). A recent study indicated that supplemental β-mannanase improves dry matter (DM), organic matter, and energy digestibility in PKE-containing diets fed to growing pigs (Mok et al., 2013). A previous study also reported an increase in the ME content and a nutrient digestibility improvement of copra meal treated by β-mannanase in broiler diets (Khanongnuch et al., 2006). The results of these studies indicate that the efficiency of nutrient digestibility will be increased when β-mannanase is added to CE- or PKE-containing swine diets. However, data on the effect of β-mannanase supplementation on energy digestibility of CE- or PKE-containing diets are rare. There

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Submitted Mar. 28, 2015; Revised Apr. 20, 2015; Accepted Apr. 25, 2015

Table 1. Energy and nutrient composition of corn, soybean meal (SBM), copra expellers (CE), and palm kernel expellers (PKE), as-is basis

Item	Ingredient			
	Corn	SBM	CE	PKE
Dry matter (%)	86.3	87.3	88.7	92.6
Gross energy (kcal/kg)	3,958	4,251	4,214	4,417
Crude protein (%)	6.9	46.9	21.0	16.9
Ether extract (%)	3.45	1.14	7.46	6.74
Crude fiber (%)	2.27	4.19	9.22	12.5
Ash (%)	1.43	6.12	6.71	4.02
Calcium (%)	0.04	0.23	0.11	0.28
Phosphorus (%)	0.28	0.63	0.55	0.66
Neutral detergent fiber (%)	19.7	31.4	54.5	68.3
Acid detergent fiber (%)	2.92	6.52	27.4	37.7
Mannan (%)	0.29	1.20	24.6	31.3

is also a lack of information on the effect of β-mannanase supplementation on digestible energy (DE) and metabolizable energy (ME) contents of CE and PKE. Therefore, the objective of present study was to determine the effect of supplemental β-mannanase on the DE and ME contents of CE and PKE fed to pigs.

MATERIALS AND METHODS

Animal care

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Konkuk University.

Animal, diet and feeding

Six crossbred barrows with an initial body weight of 38.0 kg (standard deviation = 1.5) were used to measure the effect of supplemental β-mannanase on the DE and ME contents of CE and PKE fed to pigs. Animals were allotted to a 6×6 Latin square design with 6 dietary treatments and 6 periods (Kim and Kim, 2010). Pigs were individually housed in metabolism crates that were equipped with a feeder and a nipple drinker.

Six experimental diets were prepared in a 3×2 factorial treatment arrangement. There were 3 types of diets (corn-SBM-based diet, CE 30% diet, and PKE 30% diet) and 2 concentrations of supplemental β-mannanase (0 and 2,400 U/kg). The energy and nutrient composition of corn, SBM, CE, and PKE are presented in Table 1. A basal diet based on corn and SBM was formulated (Table 2). Two additional diets were formulated by including 30% of CE and PKE to the basal diet at the expense of corn and SBM. All diets contained the same proportion of corn:SBM ratio at 2.88:1. A supplemental enzyme, β-mannanase, was added to the 3 different diets at the expense of β-mannanase carrier. Vitamins and minerals were included in all diets to meet or exceed nutrient requirement estimates (NRC, 2012).

The amount of feed provided daily per pig was approximately 3 times the estimated requirement for maintenance (i.e., 197 kcal of ME/kg of body weight$^{0.60}$; Kil et al., 2013). The daily feed allowance divided into 2 equal meals, and fed to pigs at 0800 and 1600. The feed allowance for each pig was adjusted based on the body weight of pigs at the beginning of each period. Water was

Table 2. Ingredient composition of experimental diets, as-fed basis

Items	β-Mannanase (U/kg)					
	Corn-SBM		CE		PKE	
	0	2,400	0	2,400	0	2,400
Ingredient (%)						
Ground corn	72.00	72.00	49.73	49.73	49.73	49.73
Soybean meal, 48% crude protein	25.00	25.00	17.27	17.27	17.27	17.27
Copra expellers	-	-	30.00	30.00	-	-
Palm kernel expellers	-	-	-	-	30.00	30.00
β-Mannanase	-	0.30	-	0.30	-	0.30
β-Mannanase carrier	0.30	-	0.30	-	0.30	-
Ground 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[1]	0.50	0.50	0.50	0.50	0.50	0.50
Analyzed composition						
Gross energy (kcal/kg)	3,895	3,946	3,958	3.969	4,043	4,049
Mannan (%)	0.44	0.83	3.90	3.61	2.14	3.39

Corn-SBM, corn-soybean meal-based diet; CE, copra expellers 30% diet; PKE, palm kernel expellers 30% diet.

[1] 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.

freely accessible at all times.

Sample collection

An experimental period consisted of 4-d adaptation and 5-d collection periods. Feces were collected according to the marker-to-marker procedure (Kong and Adeola, 2014) using chromic oxide (Cr_2O_3) as an indigestible marker. Chromic oxide was added to the morning diets on d 5 and 10. Urine was collected from 1400 on d 5 to 1400 on d 10 with 200 mL of 3 *N* HCl for reducing ammonia loss and limiting microbial activity. The total quantity of feces and approximately 200 mL of sub-sampled urine were immediately stored at –20℃ after collection.

Chemical analysis

Fecal samples were dried in a forced-air drying oven at 55℃ and ground before analysis. The diets, feces and urine were analyzed for gross energy (GE) using a bomb calorimeter (Parr 1261; Parr Instruments Co., Moline, IL, USA). To analyze DM in the ingredients, diets, and feces, all samples were dried in the forced-air drying oven at 135℃ for 2 h (method 930.15; AOAC, 2005). Ingredient samples were also analyzed for crude protein (method 984.13; AOAC, 2005), ether extract (method 920.39; AOAC, 2005), and crude fiber (method 978.10; AOAC, 2005). Ingredient samples were analyzed for ash (method 942.05; AOAC, 2005), calcium (method 968.08; AOAC, 2005), phosphorus (method 964.06; AOAC, 2005) neutral detergent fiber (method 2002.04; AOAC, 2005), and acid detergent fiber (method 973.18; AOAC, 2005). Diets and ingredient samples were also analyzed for the mannan concentration. Briefly, 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 1 *N* and incubated at 121℃ for 45 min. The mannan contents in hydrolysates were determined using an evaporative light scattering detector and a Shodex sugar column SP0810 (8.0 mm×300 mm; Showa Denko K.K., Tokyo, Japan).

Calculations and statistical analysis

The DE and ME values in experimental diets and ingredients were calculated by the method for difference procedure described by Kong and Adeola (2014). Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The model included diet, β-mannanase and the interaction between diet and β-mannanase as fixed variables and animal and period as random variables. The experimental unit was a pig, and the statistical significance was determined at an alpha of 0.05.

RESULTS

All animals were healthy and easily consumed the provided feed throughout the experimental period. The interactive effect between diet and β-mannanase was not observed in energy digestibility, DE and ME values of the experimental diets (Table 3). There was also no effect of β-mannanase supplementation, but there was a diet effect on energy digestibility, DE and ME contents of the experimental diets ($p<0.001$). The energy digestibility was greater ($p<0.05$) in the basal diet than in the CE- or PKE-containing diet. The basal diet also had a greater ($p<0.05$) DE content than a CE 30% diet or a PKE 30% diet (3,365 vs 3,187 and 3,188 kcal/kg on an as-fed basis). As the urinary energy output did not differ among the diets, the ME content in the basal diet was also greater ($p<0.05$) than in a CE 30% or a PKE 30% diet (3,251 vs 3,082 and 3,068 kcal/kg on an as-fed basis).

No effect of β-mannanase supplementation in DE and ME values of CE and PKE were observed (Table 4). When calculated on an as-fed basis, the DE and ME contents in CE (2,876 and 2,787 kcal/kg, respectively) were

Table 3. Effects of supplemental β-mannanase on digestibility of corn-soybean meal-, copra expellers-, or palm kernel expellers-based diets fed to pig[1,2]

Items	β-Mannanase (U/kg)						RMSE	p-value		
	Corn-SBM		CE		PKE					
	0	2,400	0	2,400	0	2,400		Diet	Enzyme	Diet×enzyme
Feed intake (kg/5 d)	8.77	8.84	8.94	9.02	8.89	8.89	1.35	0.949	0.911	1.000
GE intake (Mcal/5 d)	34.1	34.9	35.4	35.8	35.9	36.0	5.4	0.790	0.824	0.988
Fecal GE output (Mcal/5 d)	4.77	4.93	6.95	6.89	7.59	7.59	0.81	<0.001	0.891	0.945
Energy digestibility (%)	86.0	85.7	80.2	80.6	78.7	78.8	1.7	<0.001	0.858	0.900
DE in diet (kcal/kg)	3,347	3,383	3,174	3,200	3,184	3,192	70	<0.001	0.321	0.889
Urinary GE output (Mcal/5 d)	0.96	1.01	0.95	0.94	1.03	1.09	0.28	0.609	0.724	0.938
ME in diet (kcal/kg)	3,235	3,267	3,066	3,097	3,069	3,067	79	<0.001	0.436	0.836

Corn-SBM, corn-soybean meal-based diet; CE, copra expellers 30% diet; PKE, palm kernel expellers 30% diet, RMSE, root mean square of error; GE, gross energy; DE, digestible energy; ME, metabolizable energy.

[1] Each least squares mean represents 6 observations.

[2] Feed intake, GE intake, fecal GE output, and urinary GE output were based on 5 d of collection.

Table 4. Energy values for copra expellers (CE) and palm kernel expellers (PKE) fed to pigs[1]

Ingredient	β-Mannanase (U/kg)				RMSE	p-value		
	CE		PKE					
	0	2,400	0	2,400		Ingredient	Enzyme	Ingredient×enzyme
As-fed basis								
DE (kcal/kg)	2,873	2,879	2,905	2,851	264	0.984	0.828	0.782
ME (kcal/kg)	2,772	2,802	2,781	2,701	287	0.697	0.835	0.645
Dry matter basis								
DE (kcal/kg)	3,238	3,246	3,137	3,079	292	0.274	0.833	0.786
ME (kcal/kg)	3,125	3,159	3,003	2,917	318	0.176	0.844	0.649

RMSE, root mean square of error; DE, digestible energy; ME, metabolizable energy.
[1] Each least squares mean represents 6 observations.

comparable to those in PKE (2,878 and 2,741 kcal/kg, respectively) regardless of β-mannanase supplementation.

DISCUSSION

There are many different factors involved in inhibiting energy and nutrient utilization of feedstuffs by animals. Non-starch polysaccharides are one of the main inhibitors that have negative effects on the digestibility and utilization of nutrients in non-ruminant diets (Choct et al., 2010). Beta-mannan is a component of NSP and exists as 4 different forms (pure mannan, galactomannan, glucoamannan, and galactoglucomannan) in the cell wall structure of feed ingredients (Sundu et al., 2006). The CE and PKE are rich sources of NSP, and contain relatively high concentration of mannan compared with corn and SBM (Table 1). In the present study, the experimental diets containing 30% of CE or PKE were used to test the effect of β-mannanase supplementation on energy digestibility. Despite that the experimental diets had a large quantity of substrates for the β-mannanase and considerably high dose of enzyme supplementation (2,400 U/kg), we failed to find the effects of β-mannanase supplementation on the energy digestibility of experimental diets. In terms of the corn-SBM basal diet, our results were consistent with a previous study reported by Petty et al. (2002), who demonstrated that there was no difference on DM and energy digestibility in corn-SBM-based diets with β-mannanase addition. In both studies, a total fecal collection method was used to measure the digestibility of energy and nutrients. On the other hand, results from a recent study suggested that 1,600 U/kg of β-mannanase supplementation increased the digestibility of DM, organic matter and energy in a 15% PKE-containing diet (Mok et al., 2013). Kim et al. (2013) also reported increased DM and energy digestibility in a swine diet containing 5% of palm kernel meal by 400 U/kg of β-mannanase supplementation. Similarly, results of several experiments indicated positive effects of supplemental β-mannanase on nutrient digestibility in corn-SBM-based diets (Radcliffe et al., 1999; Lv et al., 2013). All these studies that showed positive responses to supplemental β-mannanase used an index method with chromic oxide as an indigestible index. There is potentially larger variability when the index method is used for digestibility determination compared with a total collection method. A variation in analyzed index concentrations may also cause inaccurate determination of nutrient digestibility. As none of the aforementioned studies reported the analyzed index concentrations, the variability in analyzed index concentrations that may have caused false positive effects of supplemental enzyme on nutrient digestibility is uncertain.

Lower enzyme activity of exogenous carbohydrases in the gastrointestinal tract may be a reason for the lack of effects of the supplemental enzyme on the energy digestibility (Kim et al., 2004). The β-mannanase (mannan-endo-1,4-β-D-mannosidase, EC 3.2.1.78) product used in this study was an endo-acting carbohydrase, attacking the 1,4-β-D-mannan main chain of the mannan compounds and releasing mannan-oligosaccharides or a small amount of mannose (McCleary, 1988; Kong et al., 2011). However, only mannose belongs to the energy-yielding fraction of mannan metabolites which can be absorbed in the small intestine. In the present study, it is possible that a relatively small quantity of energy-yielding monosaccharide supplied energy to pigs than other experiments. For this reason, although 30% of mannan-rich ingredients and considerably high dose of enzyme (2,400 U/kg) were used, we found no effect of β-mannanase supplementation in this experiment.

The differences in fecal GE output and energy digestibility among diets were mainly due to the 30% inclusion of PKE or CE which contains greater concentrations of less digestible nutrients such as neutral detergent fiber and acid detergent fiber. Our observations coincide with the data reported by Son et al. (2012). The intake of high-fiber diets has been known to increase passage rate of digesta, which cause reduced digestibility of energy-yielding nutrients (Ravindran et al., 1984; Kim et al., 2007).

The DE and ME contents in CE estimated in the present

study were less than published data (NRC, 2012). This difference appears to be mainly due to the lower DE:GE ratio measured in the present study compared with NRC (2012) as the GE concentration in CE were comparable to the value in NRC (2012). The DE and ME contents for PKE obtained in this experiment were slightly less than the value reported by NRC (2012), and the DE:GE ratio in PKE was less than that in NRC (2012). However, the ME:DE ratio in PKE in our study was in good agreement with the value in NRC (2012). A recent study reported that the DE values of PKE from Indonesia and PKE from Costa Rica were 3,000 and 2,785 kcal/kg, respectively, and the ME values of PKE from Indonesia and PKE from Costa Rica were 2,891 and 2,681 kcal/kg, respectively (Sulabo et al., 2013). These values are close to our observations.

Clear reasons for the large variation in the DE:GE ratio among sources of CE and PKE are not known; however, it is possible that the different inclusion level of test ingredients may partly contribute to these variations because a low inclusion rate of a test ingredient generally causes a large variability in different procedures. It has been reported that energy digestibility in CE is largely variable due to the variability in crude protein digestibility (Sulabo et al., 2013). It is also likely that the difference of pigs' body weight may affect energy digestibility in CE and PKE because an improved utilization of fiber with increasing body weight increases the energy digestibility, especially when high-fiber ingredients are used (Noblet, 2007). Moreover, dietary fiber may lower the utilization of other energy-containing nutrients such as crude protein and fat (Noblet and Perez, 1993; Le Goff and Noblet, 2001).

CONCLUSION

The results obtained in the present study indicate that there was no effect of β-mannanase supplementation on the DE and ME values of CE and PKE fed to pigs.

ACKNOWLEDGMENTS

The authors are grateful for the support by CTCBIO Inc. (Seoul, Republic of Korea) and National Institute of Animal Science (Rural Development Administration, Republic of Korea; PJ 009340). A part of the present work resulted from the Konkuk University research support program.

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