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ANIMAL

Effects of multi-enzyme supplementation in a corn and soybean meal-based diet on growth performance, apparent digestibility, blood characteristics, fecal microbes and noxious gas emission in growing pigs

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Abstract

The objective of this study was to determine the effect of multi-enzyme supplementation in a corn and soybean meal-based diet on the growth performance, apparent nutrient digestibility, blood profile, fecal microbes and noxious gas emission in growing pigs. A total of 80 crossbred [(Landrace × Yorkshire) × Duroc] growing pigs with an average body weight (BW) of 25.04 ± 1.44 kg were used in a 6-week experiment. The experimental treatments were as follows: CON, basal diet and; T1, basal diet + 100 mg/kg multi-enzyme. During the experiment, the pigs fed the diet with multi-enzyme supplementation had a higher gain to feed ratio (G/F) ($p < 0.05$) than the pigs fed the diet without multi-enzyme supplementation. On day 42, the pigs fed the diet with multi-enzyme supplementation had decreased H_2S and NH_3 emissions ($p < 0.05$) than the pigs fed the diet without multi-enzyme supplementation. However, no effect was observed on nutrient digestibility, blood profiles and fecal microbes among the treatments ($p > 0.05$). In conclusion, it is suggested that multi-enzyme supplementation in a corn and soybean meal based diet can partly improve the growth performance and noxious gas emission of growing pigs.

Keywords: growing pigs, growth performance, multi-enzyme, noxious gas emission

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Introduction

Enzyme products have been extensively evaluated in poultry, cattle and swine diets focusing on enzyme preparations, the physiological status of the animal and feed ingredient. Beneficial effects of addition of single or multiple enzyme preparations, such as cellulase, β-glucanase, α-amylase, β-mannanase, xylanase, and protease to diets fed to swine have been reported (Ngoc et al., 2011; O'Shea et al., 2014; Passos et al., 2015; Upadhaya et al., 2016a; Upadhaya et al., 2016b). Corn has been considered as the best quality grain especially for pig. In addition, the response and its degree due to dietary enzyme supplementation have been negligible or relatively less to other grains. Since

the primary non-starch polysaccharides (NSP) in corn is cellulose, a structural insoluble fiber, enzymes hydrolyzing soluble fiber did not respond well upon supplementation. Therefore, cellulase and protease supplementation to swine diet was proven more effective compared to other enzymes without cellulase (de Souza et al., 2007). In addition, Willamil et al. (2012) showed that multi-enzyme (xylanase and β-glucanase as main activities) supplementation to wheat-based diet improved nutrient utilization and growth performance in growing pigs. However, mannanase-only supplementation to corn based diet was failed to improve feed to gain ratio (F/G), but the F/G was improved by multi-enzyme supplementation including cellulase as well as mannanase in nursery pig (Ragland et al., 2008). On the other hand, mannanase supplementation to distillers dried grains with solubles (DDGS) included pig diet was effective to improve average daily gain (ADG) (Yoon et al., 2010), probably due to relatively higher mannan in DDGS than corn grain. Owing to decreased fiber content and high nutrient digestibility, it has been more challenging to obtain beneficial effects for corn and soybean meal (SBM) diets through the exogenous enzymes supplementation.

Most of the available information on the application of multi-enzyme has been generated from younger pig studies. In addition, older pigs appear less effective because older pigs are more able to digest fiber than younger pigs (Ao et al., 2010). The objective of the current study was to examine the effects of multi-enzyme (protase, cellulase, β-glucanase, β-mannanase, xylanase, and amylase) supplementation on growth performance, apparent nutrient digestibility, blood characteristics, fecal microbial, and noxious gas emission in growing pigs fed corn and SBM diet.

Materials and Methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University. The multi-enzyme was provided by commercial company (Da-Sion Product Co., Ltd., Busan, Korean). The multi-enzyme contained 180 units/g protase, 5,826 units/g cellulase, 2,677 units/g β-glucanase, 518 units/g β-mannanase, 6,299 units/g xylanase, and 1,624 units/g amylase.

Experimental design, animal, and housing

A total of 80 crossbred [(Landrace × Yorkshire) × Duroc] growing pigs with an average body weight (BW) of 25.04 ± 1.44 kg were used in a 6-week experiment. Pigs were randomly assigned into one of the two experimental diets according to BW. Pigs were housed in groups of five barrows per pen with eight replicates per treatment. Pigs were housed in an environmentally controlled facility and room temperature was maintained at approximately 24℃. Each pen (1.2 × 1.6) was equipped with a self-feeder and nipple waterer to allow *ad libitum* access to feed and water throughout the experimental period. The experimental treatments included: CON, basal diet; T1, basal diet + 100 mg/kg multi-enzyme. All diets were provided in mash form and formulated to meet or exceed the NRC (2012) recommendation for all nutrients and regardless of treatments (Table 1).

Sampling and measurements

BW and feed intake were recorded initially and week 6 of the experiment period. Feed consumption was recorded on a pen basis during the experiment to calculate the ADG, average daily feed intake (ADFI) and gain to feed ratio (G/F). Chromic oxide (Cr_2O_3) was added to the diet as an indigestible marker at 2 g/kg of the diet for 7 days prior to fecal collection at 6th

week to calculate dry matter (DM) and nitrogen (N) digestibility. Fecal samples were collected randomly from 2 pigs in each pen, mixed and pooled. All fecal samples, as well as feed samples, were stored at - 20℃ until analysis.

Before chemical analysis, the fecal samples were thawed and dried at 60℃ for 72 h, after which they were ground to pass through a 1-mm screen. The procedures used for determination of DM and N digestibility were in accordance with the methods established by AOAC (2005). N was determined using a Kjeltec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Crude protein was calculated as N × 6.25. Dietary DM (method 930.15), crude protein (method 968.06), calcium (method 984.01), phosphorus (method 965.17), crude ash (method 942.05), ether extract (method 920.39), and crude fiber (method 962.09) were analyzed according to the procedures described by AOAC (2005). Individual amino acid composition was measured using an Amino Acid Analyzer (Beckman 6300, Beckman Coulter Inc., Fullerton, USA) after a 24 h for 6 N-HCl hydrolysis at 110℃. Chromium was analyzed by ultraviolet absorption spectrophotometry (UV-1201; Shimadzu, Tokyo, Japan) according to the methods of Williams et al. (1962). The digestibility was then calculated using the following formula: Digestibility (%) = [1 − {(N_f × C_d)/ (N_d × C_f)}] × 100, where N_f = nutrient concentration in feces (%DM), C_d = chromium concentration in diets (%DM), N_d = nutrient concentration in diets (%DM), and C_f = chromium

Table 1. Composition of the basal growing diets (as-fed basis; g kg^{-1}).

Items	Basal diet
Ingredients	
Corn	493.7
Wheat	150.0
Soybean meal 45% CP	224.6
DDGS	15.0
Palm kernel meal	20.0
Yellow grease	39.0
Molasses	30.0
Limestone	12.7
Salt	3.0
DL-Methionine	0.3
L-Lysine H2SO4	2.8
Phytase	0.5
Vitamin premix[y]	5.0
Mineral premix[z]	3.4
Calculated composition	
Metabolisable energy (kcal kg^{-1})	3,350
Analysed composition	
Crude protein	167.0
Ether extract	64.3
Crude fiber	28.2
Ash	51.4
Calcium	7.1
Total phosphorus	4.2
Available lysine	9.6

CP, crude protein; DDGS, distillers dried grains with solubles.
[y]Provided per kg of complete diet: vitamin A, 4000 IU; vitamin D_3, 800 IU; vitamin E, 17 IU; vitamin K, 2 mg; vitamin B_2, 4 mg; vitamin B_6, 1 mg; vitamin B_{12}, 16 μg; pantothenic acid, 11 mg; niacin, 20 mg; biotin, 0.02 mg.
[z]Provided per kg of complete diet: Cu, 220 mg; Fe, 175 mg; Zn, 191 mg; Mn, 89 mg; I, 0.3 mg; Co, 0.5 mg; Se, 0.15 mg.

concentration in feces (%DM).

For the blood profile, four pigs were randomly selected from each treatment and bled via jugular venipuncture and added to heparinized tubes for blood urea nitrogen (BUN) analysis, and non-heparinized tubes for serum creatinine analysis at the 6th week of the experiment, respectively. After collection, BUN concentration was analyzed using the Abbott Spectrum urea nitrogen test (Series II, Abbot Laboratories, Dallas, USA). Creatinine concentrations were determined using an Astra-8 Analyzer (Beckman Instruments, Inc., Brea, USA).

For fecal microbial, fecal samples were collected directly by massaging the rectum of 2 pigs randomly selected from each pen on day 42, and transported to the laboratory. The obtained fecal sample (1 g) from each pen was diluted with 9 mL of 10 g L^{-1} peptone broth (Becton, Dickinson and Co., Franklin Lakes, USA) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 10 g L^{-1} peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The MacConkey agar plates and lactobacilli medium III agar plates were then incubated for 24 h at 37℃ and 48 h at 39℃, respectively. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

Feces and urine were collected on d 42 from 4 pigs per treatment. The urine was collected in a bucket via a funnel below the cage. Samples were kept in sealed containers and were immediately stored at - 4℃ for the duration of the period. After the collection period, feces and urine samples were pooled and each mixed well for each pen. The subsamples of slurry (150 g feces and 150 g of urine were mixed well; 1 : 1 on the wet weight basis) were taken and stored in 2.61 plastic boxes in duplicate as described by Cho et al. (2008). Each box had a small hole in the middle of one side wall, which was sealed with adhesive plaster so as to maintain anaerobic condition. The samples were permitted to ferment for 7 d at room temperature (25℃). After the fermentation period, a Gastec (model GV-100) gas-sampling pump was utilized for gas detection (Gastec Corp., Gastec detector tube No. 3M and 3La for NH_3 and H_2S; No. 70 and 70L for R.SH (total mercaptan), Gastec Corp., detector tube, Japan). The adhesive plasters were punctured, and 100 mL of headspace air was sampled approximately 2.0 cm above the feces surface.

Statistical analysis

All data were subjected to the general linear model (GLM) procedures of SAS as a randomized complete block design (SAS institute, 2001). Pen was used as experimental unit for growth performance, digestibility and fecal microbial, whereas pig was used as experimental unit for blood profile and noxious gas emission. Variability in data was expressed as standard error of means (SEM). Differences among all treatments were separated by using the Tukey's test. A probability level of $p < 0.05$ was considered to be statistically significant.

Results

The effects of dietary multi-enzyme on growth performance, nutrient digestibility, blood profile, fecal microbial, and noxious gas emission were summarized in Table 2 - Table 6. However, there were no differences ($p > 0.05$) in ADFI, ADG, DM and N digestibility, blood profile and fecal microbial among all the treatments. During the experiment, pigs fed the diet containing multi-enzyme had higher G/F ($p < 0.05$) compared with pigs fed the diet without multi-enzyme supplementation.

On day 42, pigs fed the diet containing multi-enzyme had decreased H2S and NH3 emission ($p < 0.05$) compared with pigs fed the diet without multi-enzyme supplementation.

Table 2. Effect of dietary multi-enzyme on growth performance in growing pigs.

Items	CON	T1	SEM	p-value
Body weight (kg)				
Initial	25.04	25.05	0.01	0.80
Day 42	54.92	55.80	0.40	0.16
Day 0 - 42				
ADG (g)	711	733	9.44	0.15
ADFI (g)	1714	1723	22.40	0.78
G/F	0.4138a	0.4252b	0.003	0.02

ADG, average daily gain; ADFI, average daily feed intake; CON, basal diet; T1, CON + 100 mg/kg multi-enzyme; SEM, standard error of means.
a, b: Means in the same row with different superscript differ significantly ($p < 0.05$).

Table 3. Effect of dietary multi-enzyme on apparent digestibility in growing pigs.

Items (%)	CON	T1	SEM	p-value
Dry matter	77.11	78.06	0.60	0.30
Nitrogen	76.45	77.44	0.59	0.28

CON, basal diet; T1, CON + 100 mg/kg multi-enzyme; SEM, standard error of means.

Table 4. Effect of dietary multi-enzyme on blood characteristics in growing pigs.

Items (mg/dL)	CON	T1	SEM	p-value
BUN	11.3	10.0	0.84	0.36
Creatinine	0.85	0.64	0.14	0.37

BUN, blood urea nitrogen; CON, basal diet; T1, CON + 100 mg/kg multi-enzyme; SEM, standard error of means.

Table 5. Effect of dietary multi-enzyme on fecal microbial in growing pigs.

Items (log_{10}cfu/g)	CON	T1	SEM	p-value
Lactobacillus	7.23	7.51	0.07	0.08
E. coli	5.91	5.79	0.05	0.20

CON, basal diet; T1, CON + 100 mg/kg multi-enzyme; SEM, standard error of means.

Table 6. Effect of dietary multi-enzyme on noxious gas emission in growing pigs.

Items (ppm)	CON	T1	SEM	p-value
NH_3	4.6a	4.0b	0.11	0.03
Total mercaptans	3.7	3.6	0.31	0.80
H_2S	3.1a	2.4b	0.13	0.04

CON, basal diet; T1, CON + 100 mg/kg multi-enzyme; SEM, standard error of means.
a, b: Means in the same row with different superscript differ significantly ($p < 0.05$).

Discussion

Growth performance

The results of the current study indicated that multi-enzyme supplementation affected on G/F but had no influence on ADFI and ADG among all treatment groups. Our findings are similarly with a study conducted by Omogbenigun et al. (2004), who reported that an improvement in ADG and G/F had been observed in piglets fed diets based on corn and wheat supplemented with an enzyme cocktail containing cellulase, galactanase, mannanase and pectinase. Besides, Ao et al. (2010) observed that ADG and G/F was increased with 100 mg/kg enzyme complex (α-1,6-β-galactosidase, β-1,4-mannanase, and β-1,4-mannosidase) supplementation to corn and SBM based diets for growing pigs. However, the results are not always consistent. In a study with rape seed meal and DDGS-based diets supplemented with xylanase and β-glucanase enzymes, Mc Alpine et al. (2012) found no improvement in ADFI, ADG and feed conversion efficiency of grower-finisher pigs. The contradictions in the impact of multi-enzyme supplementation on growth performance may be attributed to the differences in the diets composition, and the age of pigs used. In addition, the enzyme source, the situations under which the specific ingredient was grown, the storage and process of feed, the interactions among dietary compositions and health status may also exert a significant effect on growth performance (Kim et al., 2003; Willamil et al., 2012).

Nutrient digestibility

Previous reports indicate that different exogenous enzyme supplementation in swine diet can enhance the digestibility, leading to improved growth performance and nutrient digestibility (Nyachoti et al., 2006; Kiarie et al., 2013). A study by Omogbenigun et al. (2004) demonstrated that multi-enzyme (cellulase, galactase, mananase, and pectinase) having activity when supplemented to corn and SBM diet on the digestibility of nutrients in both ileum and total tract in piglets. In contrast, Wubben et al. (2000) found that multiple enzymes (cellulase, hemicullulase, amylase, xylanase, alpha-galactosidase, and protease) supplementation in corn and SBM diets did not exert benefits on nutrient digestibility of growing pigs. In addition, the ileal digestibility of DM and N were not affected in growing pigs fed corn and SBM diet supplemented or not with α-galactosidase (Smiricky et al., 2002). In the current experiment, there were no differences in DM and N digestibility among all the treatments.

The apparent contradictions in the effectiveness of enzyme supplementation among studies are mainly attributable to differences in age of the pigs and the composition of diets used (Omogbenigun et al., 2004). In general, the impact of enzymes supplementation on nutrient digestion declines with age of the pig particularly, because digestive capacity in pigs improves with age as the enzyme system matures and gut microbial population increases (Lindemann et al., 1986). Older pigs have a more mature gastrointestinal system, increasing the ability of the gut to digest cereal components of the ration through the effects of both pancreatic enzyme section and bacterial fermentation. Also, the extent to which enzymes supplementation improve nutrient digestibility tend to be low when using diets containing highly digestible ingredients (Johnson et al., 1993). From a practical swine nutrition perspective, it would appear that supplementing enzymes to pig diets will be more beneficial when using diets based on ingredients that are of lower quality and poorly digested.

Blood profile

The BUN and creatinine values generally help to assess renal damage in animals and humans. Creatinine and urea both are metabolic wastes that enter into the bloodstream and are discharged out by kidneys. When kidney filtration rate declines, creatinine and urea levels in the blood increase spontaneously (Kaneko et al., 2008). Previous studies showed that addition of multi-enzymes to the diets had no significant effect on blood constituents (Wang et al., 2009; Ao et al., 2010), which is in agreement with the result of the current study. Moreover, Wang et al. (2009) reported that BUN was not affected by the addition of the enzyme cocktail (α-1,6-β-galactosidse, β-1,4-mannanase and β-1,4-mannosidase) to corn-SBM diets. Considering the above results, in our study, it indicates that the addition of multi-enzyme to corn-SBM diet results in a diet with the same protein quality as the control diet. To our best knowledge, a few experiments have been conducted to compare the BUN or creatinine coefficients of multi-enzyme containing corn-SBM diets in pigs. However, the results of this study are not adequate to conclude that corn-SBM diets supplemented with multi-enzyme have inferior nitrogen balances when compared to non-multi-enzyme diets. Therefore, further studies are necessary to investigate the carcass trait amino acid profile of pigs provided with the experimental diets used in this study.

Noxious gas emission

When dietary protein is not fully utilized by growing pigs can result in a build-up of N-rich compounds in the resulting manure stimulating NH_3 and odor emissions (Nahm, 2003; Dourmad and Jondreville, 2007). In the current study, the fecal NH_3 and H_2S concentration was significantly decreased by multi-enzyme supplemented diet. Similarly, Mc Alpine et al. (2012) reported that finisher pigs offered protease-xylanase supplemented diet had reduced NH_3 emissions. In contrast, Atakora et al. (2011) founded that growing finishing pigs fed phytase-xylanase supplementation of wheat grain based diets had no effect on gas emission. The large differences in age and diet might be the reasons for the differences in results across these inconsistent reports. Ferket et al. (2002) indicated that nitrogen gaseous emissions are related to intestinal microbial ecosystem and nutrient utilization. From our study, reduction of NH_3 and H_2S emission could possibly be due to a reduction of the pathogenic bacterial population in the gastrointestinal tract or due to enhancement of beneficial microbial activity (Dibner and Buttin, 2002; Upadhaya et al., 2016a). Microbial fermentation of undigested proteins and amino acids in the hindgut produces NH_3 and contributes to the NH_3 output in the manure (Gaskins, 2000). Therefore, the lower fecal NH_3 and H_2S concentration in the multi-enzyme supplementation on pigs may indicate better digestion of dietary proteins and amino acids. This is through limiting the availability of non-digested proteins, which then serve as substrate for NH_3 production in the large intestine (Tactacan et al., 2016). However, no comparisons could be made with other studies because there was a scarcity of information on the effects of multi-enzyme supplementation on fecal noxious gas emission in pigs. Further research is necessary to elucidate the important factors involved.

Conclusion

Multi-enzyme supplementation was effective in enhancing G/F. Besides, fecal NH_3 and H_2S emission were reduced by enzymes supplementation. This suggested that multi-enzyme be utilized in corn-SBM diets had positive effects on growing pig performance.

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