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Effects of Beta Mannanase and Xylanase Supplementation in Low Energy Density Diets on Performances, Nutrient Digestibility, Blood Profiles and Meat Quality in Finishing Pigs

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ABSTRACT

This study was conducted to evaluate the effects of mannanase and xylanase supplementation in low energy density diets on performances, nutrient digestibility, blood profiles and meat quality in finishing pigs. Mannanase and xylanase improves the nutrient utilization by hydrolyzing Non-starch Polysaccharide (NSP) that prevents the nutrient digestion and absorption. A total of ninety-six pigs (Landrace× Yorkshire)×Duroc, 69.1±1.4 kg average initial b.wt.) were used in a 8 week study. Pigs were allotted into four treatments with six pens/replicates (2 barrows and 2 gilts per pen) per treatment in completely randomized design according to its sex and bodyweight. Dietary treatments were: PC (positive control; basal diet), NC (negative control; 120 kcal kg⁻¹ lower energy diet), NM (NC+0.05% mannanase) and NMX (NC+0.025% mannanase and 0.025% xylanase complex). The present results suggested that the inclusion of NMX led to a higher Average Daily Growth (ADG) than NC treatment (p<0.05). The Average Daily Feed Intake (ADFI) was depressed by NMX treatment compared with NM treatment (p<0.05). Pig fed NMX treatment led to a higher growth efficiency (G:F) than NC and NM treatments (p<0.05). Dietary NMX treatment increased Apparent Total Tract Digestibility (ATTD) for gross energy in 4 week and nitrogen in 8 week (p<0.05) compared with NC treatment. Significantly higher (p<0.05) blood glucose concentration was observed in NMX treatment (83.83 mg dL⁻¹) than NC treatment (77.17 mg dL⁻¹) in 8 week. Dietary NC, NM and NMX treatment decreased the 10th-rib backfat thickness compared with PC treatment (p<0.05). In conclusion, the inclusion of β-mannanase and xylanase could improve the growth performance, nutrient digestibility and reduce the backfat thickness with low density diet comparable to the high nutrient density diet.

Key words: Mannanase, xylanase, growth performance, meat quality, finishing pigs

INTRODUCTION

Plant sources contain a considerable amount of NSP (non-starch polysaccharide) such as β-glucan, mannan, cellulose and pectin, which cannot be digested by poultry and swine (McCracken *et al.*, 2001). The main NSP in cell wall of plant are xylose and mannan, which could increase the viscosity to prevent the nutrients utilization by the animals (Burnett, 1966; White, 1981) and subsequently decrease the growth performance (Blackburn and Johnson, 1981; Rainbird *et al.*, 1984; Edwards *et al.*, 1988). Studies had suggested that NSP in cell wall could prevent the nutrients digestion and absorption (Omogbenigun *et al.*, 2004; Chesson, 1987). Therefore, an increasing attention is paid on enzyme utilization in livestock nutrition.

Several studies suggested that exogenous enzymes can hydrolyze carbohydrates into smaller units (Diebold *et al.*, 2004, 2005) and subsequently improve the growth performance and nutrient digestibility of pigs (Kwon *et al.*, 2003; Shim *et al.*, 2003; Kim *et al.*, 2006a). Pettey *et al.* (2002) reported that the β -mannanase 0.05% supplementation improved the growth performance and feed efficiency in growing-finishing pigs. Nortey *et al.* (2007) suggested energy digestibility was increased in growing pigs fed diets supplemented with xylanase at 167 mg kg⁻¹. Chesson (1987) also suggested that xylanase and mannanase combination improved the nutrient absorption. Present previous study also suggested that the inclusion of enzyme improved the growth performance of the low nutrient diet comparable to the high nutrient diet (Wang *et al.*, 2009). Therefore, we hypothesized that enzyme supplementation could improve the nutrient utilization of low nutrient density diet comparable to the high nutrient density.

Collectively, study was conducted to investigate the effects of mannanase and xylanase addition in low-energy density diet on growth performance, nutrient digestibility, blood profiles and meat quality in finishing pigs.

MATERIALS AND METHODS

Experimental animals, housing and diets: A total of ninety six (Landrace×Yorkshire)×Duroc) pigs (average initial BW of 69.1±1.44 kg; 48 gilts and 48 barrows) were used in this 8 week growth trial. At the beginning of the experiment, pigs were allotted on the basis of initial BW to four dietary treatments by a randomized complete block design. There were 6 replicate pens (1.8×1.8 m²) per treatment with 4 pigs (2 barrows and 2 gilts) per pen. Dietary treatments included: 1) PC (positive control; basal diet), 2) NC (negative control; -120 kcal kg⁻¹ energy of PC), 3) NM (NC+0.05%mannanase) and 4) NMX (NC+0.025% mannanase and 0.025% xylanase complex). The enzymes (CTC bio, Co. Ltd, Seoul, Korea) used in this study are mannanase and xylanase. The guaranteed activities of enzymes are 800 and 700 unit g⁻¹ for mannanase and xylanase, respectively. All the diets were formulated to meet or exceed NRC (1988) recommendations (Table 1). Pigs were allowed *ad libitum* access to feed and water through a self-feeder and nipple water.

Experimental procedures, sampling and analysis: Body weight of pigs and feed consumption were measured at the end of 1th, 4th and 8th week to calculate the Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and gain/feed ratio (G/F).

Chromium oxide (Cr₂O₃) was added to the diet at 0.20% as an indigestible marker at the beginning of 4th and 8th week to calculate the digestibility coefficient. Fecal grab samples were then collected randomly from at least two pigs in each pen. Feed and fecal samples were dried and were finely ground to pass through a 1 mm screen and to determine the Dry Matter (DM) and Nitrogen (N) concentrations according to the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan). The apparent digestibility of DM and N were calculated using indirect ratio methods. The gross energy was determined by measuring the heat of combustion in the samples using a bomb calorimeter (Mode 1231, Parr instrument Co., USA). The ATTD of DM, N and energy were calculated using indirect methods described by Williams *et al.* (1962).

For the serum profile, 2 pigs (1 gilt and 1 barrow) from each pen were randomly selected and blood samples were collected via anterior vena cavapuncture at the beginning of 1th (initial), 4th

Table 1: Diet composition¹ (as-fed basis)

Ingredients (%)	PC	NC	NM	NMX
Corn	54.87	38.80	38.80	38.75
Soybean meal (%)	31.77	23.40	23.35	23.40
Wheat	-	3.00	3.00	3.00
Corn DDGS ²	-	6.00	6.00	6.00
Tapioca	-	5.00	5.00	5.00
Soybean hull	-	2.00	2.00	2.00
Wheat bran	-	2.00	2.00	2.00
Rapeseed meal	1.60	3.80	3.80	3.80
Rice bran	1.50	5.00	5.00	5.00
Tallow	4.35	4.35	4.35	4.35
Molasses	3.10	4.00	4.00	4.00
Dicalcium phosphate	1.18	1.01	1.01	1.01
Limestone	0.79	0.85	0.85	0.85
Salt	0.20	0.20	0.20	0.20
L-lysine (74%)	0.34	0.34	0.34	0.34
Vitamin premix ³	0.20	0.15	0.15	0.15
Trace mineral premix ⁴	0.10	0.10	0.10	0.10
Mannanase			0.05	0.25
Xylanase				0.25
Calculated chemical composition²				
DE (kcal kg ⁻¹)	3,400	3,280	3,280	3,280
Crude protein (%)	17.00	14.90	15.10	15.00
Lys (%)	0.98	0.96	0.96	0.96
Ca (%)	0.75	0.72	0.72	0.72
P (%)	0.62	0.65	0.65	0.65

¹PC: Basal diet, NC: 120 kcal lower energy diet, NM: NC+0.05%mannanase, NMX: NC+0.05% β-mannanase and xylanase complex. ²Corn distiller's dried grains with solubles: analyzed composition (as fed basis): 92% DM: 27.0% crude protein: 9.0% crude fat: 8.5% crude fiber: 0.75% lys.: 0.47% met: 0.51% thr.: 0.24% trp.: 0.14% calcium and 0.89% phosphorus, ³Provided per kg of complete diet: 6: 500 IU vitamin A: 950 IU vitamin D₃: 27 IU vitamin E: 2.0 mg vitamin K₃: 3.6 mg vitamin B₆: 1.3 mg vitamin B₁₂: 15 mg pantothenic acid: 26.0 mg niacin and 0.03 mg biotin. ⁴Provided per kg of complete diet: 50 mg Mn (as manganese oxide): 70 mg Zn (as zinc oxide): 54 mg Cu (as copper sulfate): 0.5 mg I (as calcium iodate): 0.5 mg Co and 0.25 mg Se

and 8th week of experiment. Blood samples were collect and then centrifuged (3,000×g) for 15 min at 4°C. Glucose and blood urea nitrogen (BUN) were determined by the automatic biochemistry analyzer (HITACHI 747, Japan).

Meat quality: At the end of the experiment, all pigs were slaughtered at a local commercial slaughter house. After chilling at 2°C for 24 h, a piece of the right loin sample was removed between the 10th and 11th ribs. Sensory evaluation (Color, marbling and firmness scores) was conducted according to the National Pork Producers Council Standards (NPPC, 1991) at ambient temperature. Immediately after the subjective tests were conducted, the lightness (L*), redness (a*) and yellowness (b*) values were measured at 3 locations on the surface of each sample using a Model CR-410 Chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). At the same time, duplicate pH values of each sample were directly measured using a pH meter (Pittsburgh, PA, USA). The water holding capacity (WHC) was measured in accordance with the methods described by Kauffman *et al.* (1986). Briefly, a 0.3 g sample was pressed at 3,000 psi for 3 min on a 125 mm

diameter piece of filter paper. The areas of the pressed sample and the expressed moisture were delineated and then determined using a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water: Meat area was then calculated, giving a measure of WHC (a smaller ratio indicates a higher WHC). The loin muscle area (LMA) was measured by tracing the longissimus muscle surface at the 10th rib, which was also conducted using the aforementioned digitizing area-line sensor. Drip loss was measured using approximately 2 g of meat sample according to the plastic bag method described by Honikel (1998).

The 2-Thiobarbituric acid reactive substances (TBARS) were measured using the method described by Witte *et al.* (1970). The TBARS values were expressed in terms of milligrams of malonaldehyde (MDA) per kilogram of muscle. Trichloroacetic acid solution (TCA, 20% wt/vol) was utilized for the extraction. UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) was employed for the spectrophotometric analyses.

Statistical analyses: All data were analyzed using a randomized complete block design following GLM procedures of SAS (SAS, 1996 Inst. Inc., Cary, NC), with each pen being used as the experimental unit. The means of the treatments were also compared by Duncan (1955) multiple range test. Variability in the data was expressed as the SE of mean and the selected level of significance was 0.05.

RESULTS

Growth performance: In phase 1 (0-4 weeks), the ADG was increased ($p < 0.05$) by NMX treatment compared with PC and NC treatments (Table 2). Dietary NMX and NM treatments increased ADFI compared with PC treatment ($p < 0.05$). The G:F was greater ($p < 0.05$) in NMX (0.357) and PC treatments (0.335) than in NC treatment (0.304). In phase 2 (4 to 8 weeks), ADG was significantly higher ($p < 0.05$) in NC treatment (0.888 kg) which was followed by NMX, NM and PC treatment (0.872, 0.838 and 0.820 kg), respectively. The G:F was higher in NMX and NM treatments than that in NC treatment ($p < 0.05$). Overall, dietary NMX led to a higher ADG than that in NC treatment ($p < 0.05$), while G:F was greater in NMX and PC treatments than in NC treatment ($p < 0.05$).

Table 2: Effect of mannanase and xylanase supplementation of low-energy density diets on growth performance in finishing pigs¹

Items	PC	NC	NM	NMX	SE ²
0-4 weeks					
ADG (kg)	0.759 ^b	0.729 ^b	0.764 ^{ab}	0.800 ^a	0.013
ADFI (kg)	2.270 ^b	2.405 ^a	2.435 ^a	2.244 ^b	0.038
G:F	0.335 ^b	0.304 ^c	0.314 ^c	0.357 ^a	0.006
5- 8 weeks					
ADG (kg)	0.888 ^a	0.820 ^c	0.838 ^{bc}	0.872 ^{ab}	0.015
ADFI (kg)	2.981	2.861	2.890	2.841	0.047
G:F	0.298 ^{ab}	0.287 ^b	0.290 ^b	0.307 ^a	0.005
Overall					
ADG (kg)	0.824 ^{ab}	0.775 ^c	0.801 ^{bc}	0.836 ^a	0.011
ADFI (kg)	2.626 ^{ab}	2.633 ^{ab}	2.663 ^a	2.542 ^b	0.030
G:F	0.314 ^b	0.294 ^c	0.301 ^c	0.329 ^a	0.004

¹PC: Basal diet, NC: 120 kcal lower energy diet; NM, NC+0.05%mannanase, NMX: NC+0.025% β -mannanase and 0.025% xylanase complex, ²Standard error, ^{a,b,c}Means in the same row with difference superscripts differ ($p < 0.05$)

Table 3: Effect of mannanase and xylanase supplementation in low-energy density diets on apparent total tract nutrient digestibility (ATTD) in finishing pigs¹

Items (%)	PC	NC	NM	NMX	SE ²
4 weeks					
Dry matter	76.38	74.31	75.31	77.76	1.32
Nitrogen	76.10	74.33	75.20	77.25	1.30
Gross energy	74.50 ^{ab}	69.99 ^b	73.34 ^{ab}	76.10 ^a	1.61
8 weeks					
Dry matter	78.52 ^a	75.83 ^b	77.39 ^{ab}	77.69 ^{ab}	0.62
Nitrogen	76.32 ^{ab}	74.46 ^b	77.37 ^{ab}	78.07 ^a	0.95
Gross energy	77.37 ^a	73.99 ^b	75.10 ^{ab}	76.28 ^{ab}	0.98

¹PC: Basal diet, NC: 120 kcal lower energy diet, NM: NC+0.05% mannanase, NMX: NC+0.025% β -mannanase and 0.025% xylanase complex, ²Standard error, ^{a,b}Means in the same row with difference superscripts differ (p<0.05)

Table 4: Effect of mannanase and xylanase supplementation in low-energy density diets on blood profiles in finishing pigs¹

Items (mg dL ⁻¹)	PC	NC	NM	NMX	SE ²
Glucose					
Initial	73.29	73.17	74.83	74.33	3.19
4 weeks	77.83	75.83	78.33	78.67	3.41
8 weeks	79.17 ^{ab}	77.17 ^b	81.00 ^{ab}	83.83 ^a	2.02
Blood urea nitrogen					
Initial	15.82	16.23	15.92	15.68	1.00
4 weeks	16.13	14.37	15.82	15.97	1.49
8 weeks	15.68	14.92	15.07	15.97	1.23

¹PC: Basal diet, NC: 120 kcal lower energy diet, NM: NC+0.05% mannanase, NMX: NC+0.025% β -mannanase and 0.025% xylanase complex, ²Standard error, ^{a,b}Means in the same row with difference superscripts differ (p<0.05)

Nutrient digestibility: No significant effect was observed in N digestibility (Table 3). At the end of 4 week, energy digestibility was higher in NMX treatment than that in NC treatment (p<0.05). At the end of 8 week, DM digestibility was higher (p<0.05) in PC treatment (78.52%) than that in NC treatment (75.83%). The N digestibility value (%) was 78.07, 77.37, 76.32 and 74.46 in NMX, NM, PC and NC treatments (p<0.05) respectively at the end of 56 day. Gross energy digestibility was greater in NMX and PC treatments than in NC treatment (p<0.05).

Blood glucose and urea nitrogen: The glucose level did not differ at the beginning of 1 week and 4 week of this trial (Table 4). At the end of the experiment, glucose level in blood was higher (p<0.05) in NMX treatment (83.83 mg dL⁻¹) than that in NM, PC and NC treatment (81.00, 79.17 and 77.17 mg dL⁻¹) respectively. There were no effects of dietary treatments on BUN throughout the experiment.

Meat quality: The effects of β -mannanase and xylanase supplementation on meat quality were presented in Table 5. NC, NM and NMX treatments decreased (p<0.05) the 10th-rib backfat thickness (23.3, 23.8 and 24.1 mm) compared with PC treatment (26.5 mm). No difference was observed on the other characteristics investigated in the current study (p>0.05).

Table 5: Effect of mannanase and xylanase supplementation in low-energy density diets on meat quality in finishing pigs¹

Items	PC	NC	NM	NMX	SE ²
Drip loss (1 day) (%)	5.87	6.03	5.84	5.58	0.11
Cooking loss (1 day) (%)	25.81	26.66	26.12	24.74	1.90
Ultimate pH	6.07	5.93	5.90	5.97	0.07
Loin muscle area (cm ²)	42.55	41.18	42.85	43.28	2.40
Meat color					
Lightness (L*)	54.87	55.61	54.43	54.21	0.99
Redness (a*)	17.01	16.81	16.91	16.30	0.36
Yellowness (b*)	6.36	6.50	6.55	6.58	0.39
Backfat thickness (mm)	26.5 ^a	23.3 ^b	23.8 ^b	24.1 ^b	0.1
Water holding capacity (%)	43.06	42.23	42.92	43.40	0.69
TBARS (mg.MA kg ⁻¹)	0.023	0.026	0.025	0.024	0.002

¹PC: Basal diet, NC: 120 kcal lower energy diet, NM: NC+0.05% mannanase; NMX: NC+0.025% β -mannanase and 0.025% xylanase complex, ²Standard error, ^{a,b}Means in the same row with difference superscripts differ (p<0.05)

DISCUSSION

In current study, we found that the enzyme mixture improved the ADG and G/F ratio. These results were in agreement with Kim *et al.* (2006b), who suggested that supplementation of 0.05% enzyme mixture (1,6- β -galactosidase, β -1,4-mannanase) improved the ileal nutrient and energy digestibility of lower ME diet comparable to the 3% higher ME diets. Min *et al.* (1992) and Kwon *et al.* (2003) had previously suggested that growing-finishing pig fed enzyme complex supplemented diets had better growth performance and suggested that the improved nutrient digestibility may explain the increased ADG in that study. Similarly, Barrera *et al.* (2004) reported that CP and ileal amino acid digestibility was increased with the increasing levels of xylanase in growing pigs. Nortey *et al.* (2008) reported that 167 mg kg⁻¹ xylanase supplementation increased ileal digestibility of energy and DM. Yoon *et al.* (2010) also demonstrated a linear improvement in ADG and apparent total tract digestibility of GE and CP with increasing (200, 400 and 600 unit kg⁻¹) β -mannanase levels in finisher pigs. In this study, we found that β -mannanase and xylanase complex improved the energy and N digestibility, although it did not significantly increase the digestibility of DM. Previous studies have demonstrated that the positive effect of single or multi-carbohydrases is primarily a result of successful degradation of NSP (Meng and Slominski, 2005). Shim *et al.* (2003) also indicated that carbohydrase enzyme complex addition improved the energy digestibility. It is well known that feed stuffs are heterogeneous in composition and a single enzyme only targets a portion of the NSP and cannot significantly improve the whole digestibility (Kim *et al.*, 2003; Olukosi *et al.*, 2007). For example, Pettey *et al.* (2002) reported that the inclusion of single β -mannanase failed to influence the apparent digestibility of energy, nitrogen, phosphorus or dry matter in pigs. Other studies also failed to find a positive effect on growth and nutrient digestibility in response to carbohydrases supplementation (Officer, 1995; Barrera *et al.*, 2004; Olukosi *et al.*, 2007). But interestingly, the addition of β -mannanase alone has been found to improve growth performance and nutrient digestibility (Jackson *et al.*, 1999, 2004; Daskiran *et al.*, 2004). However, these studies were conducted in poultry; the reason for the difference may be due to the different animal used in different study. Collectively, our results indicated that the inclusion of enzyme complex could improve the nutrient digestibility and subsequently the growth performance of the low energy diet comparable to the high energy diet.

In terms of the blood characteristic, we did not observed any effect on the BUN content throughout the experiment, which is inconsistent with the results of Kim *et al.* (2006b), who indicated that finishing pigs fed diets with protease had higher BUN content. The reason is likely to be the different enzymes used in different studies. Moreover, it was previously suggested that the blood levels of glucose, insulin and IGF-I may be decreased by the mannan and galactomannan in the feed (Rainbird *et al.*, 1984; Nunes and Malmlof, 1992). Yoon *et al.* (2010) had previously suggested that the inclusion of β -mannanase increased the glucose level in growing-finishing pig. Pettey *et al.* (2002) also indicated that 0.05% β -mannanase addition improved blood IGF-I concentration in growing-finishing pigs. In the present study, the inclusion of enzyme complex increased the glucose level of the low energy diets, which to some extent indicate the enzyme successfully hydrolyzed the NSP in the diet and reflected that the growth performance and nutrient digestibility in this study.

In terms of the meat quality, we found that the inclusion of the enzyme and low energy diet did not affect the meat quality except the backfat thickness, wherein the inclusion of high nutrient density diet led to a higher backfat thickness compared with other treatment. Similarly, our previous study (Wang *et al.*, 2009) also demonstrated that high-nutrient-density diet could increase the backfat thickness compared with those with low nutrient density diet. Interestingly in that study, they also suggested that the inclusion of enzyme did not affect the backfat thickness in that study, which is also confirmed in our study. Therefore, our study indicated that the inclusion of the enzyme in the low energy density diet could reduce the backfat thickness of the finishing pig compared with those with high energy diet.

CONCLUSION

In conclusion, the inclusion of β -mannanase and xylanase could improve the growth performance, nutrient digestibility and reduce the back fat thickness in low density diet comparable to the high nutrient density diet.

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