



Effects of dietary mixture of protease and probiotics on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs

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Abstract

This study was conducted to evaluate the effects of dietary mixture of protease and probiotics on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. A total of 48 growing pigs were randomly allotted into 2 dietary (6 pigs/pen; 4 replicates/treatment). The treatments were a diet based on corn and soybean meal (CON) and CON supplemented with 0.01% of dietary mixture of protease and probiotics (MULTI). No differences were found on growth performance (average daily gain, ADG; overall, 874.06 vs. 881.14 g/d; $p > 0.05$), blood constituents (white blood cell, WBC; phase I, 17.51 vs. $19.96 \times 10^3 /\mu\text{L}$; phase II, 19.65 vs. $21.95 \times 10^3 /\mu\text{L}$; $p > 0.05$), and carcass characteristics during overall experimental period between CON and MULTI. In conclusion, the addition of dietary mixture of protease and probiotics in growing-finishing pig diet did not have any beneficial effects.

Keywords: Blood constituents, Carcass characteristics, Growing-finishing pigs, Probiotics, Protease

Background

Addition of protease in pig diets is a way to improve protein utilization. The main mechanism of dietary protease is to increase the hydrolysis of proteins in the small intestine [1], resulting in liberating amino acids and peptides for utilization and absorption by pigs [2]. Previous studies showed that dietary protease in pig diets decreased the nitrogen (N) pollution by enhancing N digestibility of

pigs and reducing N excretion of pigs [3–5]. In addition, enzyme cocktails including protease and other nutrient enzymes are commonly used in the swine industry to improve growth performance and nutrient utilization for pigs [6–14].

Probiotics are live microorganisms which have beneficial effects on the gut health of the host [15]. Moreover, probiotics for farm animals have positive effects on growth, gastrointestinal ecosystem, efficiency of feed utilization, immune system, or gastrointestinal

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tract diseases [16]. Probiotics may act directly on the host to enhance anti-pathogen defense line and to secrete certain probiotics agents that can adversely affect the survival of deleterious bacteria [17]. Most microorganisms used as probiotics are common intestinal microbes such as *Bacillus*, *Lactobacillus*, or *Bifidobacterium* species [18]. Previous studies reported that probiotics have beneficial effects on growth performance, nutrient digestibility, and etc. for pigs [19–22].

Recently, the swine industry has been trying to make some dietary mixtures using enzymes and/or probiotics to have their beneficial effects all together. There are some studies to verify the effects of dietary mixtures of enzymes and probiotics [2,10,23,24], but the experimental evidence for their positive or negative effects in pig diets is limited. Therefore, the objective of this study was to investigate effects of dietary mixture of protease and probiotics on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs.

Materials and Methods

The experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use committee at the National Institute of Animal Science. This experiment was conducted at the facility of National Institute of Animal Science Farm.

Animal and experimental design

A total of 48 pigs (Landrace × Yorkshire × Duroc; 35.3 ± 0.69 kg of average initial body weight (BW); all barrows) were randomly assigned to 2 dietary treatments (6 pigs/pen; 4 replicates/treatment) in a completely randomized design. The treatments were a diet based on corn and soybean meal (CON) and CON supplemented with 0.01% of dietary mixture of protease and probiotics (MULTI). The MULTI used in this study was a commercial product (Syncra® SWI 201, Dupont feed enzyme and probiotics system, United Kingdom). The basal diet was formulated to meet or exceed the nutrient requirements of NRC (2012) for growing-finishing pigs (Table 1). The pigs were fed respective dietary treatments with a 2-phase feeding program for 12 weeks. Pigs were housed in conventional facilities with an all-slatted concrete floor (3.2 × 3.6 m²) and allowed *ad libitum* access to diets and water during the overall experimental period.

Data and sample collection and measurement

The pig BW and average daily feed intake (ADFI) were weighed and recorded at the starting day and end of each phase. The gain to feed ratio (G:F) was calculated using average daily gain (ADG) and ADFI. Blood samples were collected from the jugular vein of 2 pigs per replicate using 8 mL EDTA tubes. The blood samples

Table 1. Composition of basal diets for phase I and II (as-fed basis)

Items	Phase I (grower)	Phase II (finisher)
Ingredient (%)	100.00	100.00
Corn (7.2%)	62.83	62.66
Soft wheat (11.5%)	–	11.00
Soybean meal (45%)	28.35	19.72
Animal fat	3.07	1.84
Molasses	3.00	3.00
Mono-dicalcium phosphate	0.62	0.27
Lime stone	1.04	0.86
Salt	0.30	0.30
L-Lysine (98%)	0.25	0.02
DL-Methionine (98%)	0.06	–
L-Tryptophan (20%)	0.15	–
Choline-chloride (50%)	0.05	0.05
Phytase	0.05	0.05
Vitamin-mineral premix ¹⁾	0.23	0.23
Calculated chemical composition		
Metabolizable energy (kcal/kg)	3,300.00	3,300.00
Crude protein (%)	18.00	15.00
Total calcium (%)	0.59	0.45
Total phosphorus (%)	0.50	0.41
SID Lysine (%)	0.89	0.66
SID Methionine (%)	0.34	0.25
SID Methionine + Cysteine (%)	0.65	0.53
SID Tryptophan (%)	0.19	0.13

¹⁾The vitamin-mineral premix provided the following quantities of vitamins and minerals per kilogram of diets: vitamin A, 10,000 IU; vitamin D₃, 2,000 IU; vitamin E, 250 IU; vitamin K₃, 0.5 mg; vitamin B₁, 0.49 mg as mononitrate; thiamin, 0.49 mg as thiamin mononitrate; riboflavin, 1.50 mg; pyridoxine, 1 mg as pyridoxine hydrochloride; vitamin B₁₂, 0.01 mg; niacin, 10 mg as nicotinic acid; pantothenic acid, 5 mg as calcium pantothenate; folic acid, 1 mg; biotin as d-biotin, 0.1 mg; choline, 125 mg as choline chloride; Mn, 60 mg as manganese sulfate; Zn, 75 mg as zinc sulfate; Fe, 20 mg as ferrous sulfate; Cu, 3 mg as cupric sulfate; I, 1.25 mg as calcium iodate; Co, 0.5 mg as cobaltous carbonate; and Mg, 10 mg as magnesium oxide.

were analyzed to measure number of white blood cells and proportions of their differentiation using a multi-parameter automated hematology analyzer calibrated for porcine blood (Hemavet 950FS, Drew Scientific, UK).

Slaughter and carcass evaluation

After completion of the finishing period, pigs fed experimental diets until slaughtered. Approximately four hours before transport, feed was withdrawn. The live BW of finishing pigs used in the experiments was recorded before slaughter. To reduce stress, the pigs were showered with water, and water was freely available for drinking during lairage. The pigs were rested for about eight hours. Pigs were slaughtered according to industry accepted procedures (Korea Institute for Animal Products Quality Evaluation). The final live

BW of pigs was recorded, and then they were slaughtered through electrical stunning and scalding-singeing. The hot carcass weight (HCW) was recorded, and dressing percentage was calculated by comparing final live BW and HCW. After splitting, the back fat depth at the 11th and 12th thoracic vertebra and between the last thoracic vertebra and the 1st lumbar vertebra was measured.

Statistical analysis

Data were analyzed with the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA) in the completely randomized design. The experimental unit was the pen. The statistical model for growth performance, blood constituents, and carcass characteristics included dietary treatments as the fixed effect and initial BW as the covariate. Results are presented as a mean ± SEM. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

Results and Discussion

The MULTI did not affect growth performance of growing-finishing pigs during the overall experimental period (Table 2). Most previous studies showed that pigs fed diets with dietary protease or probiotics had higher growth rate of pigs than those fed diets without dietary protease or probiotics [2,7,9,25–28]. However,

some research reported that addition of dietary protease or probiotics in pig diets did not affect growth rate of pigs [29,30]. The reason for the no beneficial effect of dietary MULTI on growth rate may be related to the basal diet with sufficient nutrients because some studies showed positive effect of enzyme supplementation in low-nutrient or low-CP content diets [12,25]. In addition, low concentration of the protein enzyme and/or probiotics may contribute to no beneficial effect on growth rate of pigs because some studies showed a dietary mixture of protease and probiotics with higher concentrations.

Addition of MULTI in the pig diet did not modulate blood constituent of pigs (Table 3). These results were similar to the results reported by Chen et al. [22], Tactacan et al. [31], and Yan et al. [32]. In addition, the blood constituent of all pigs in this experiment showed a normal range of values maybe because of normal conditions without any diseases. Generally, blood indicators for immunity of pigs do not change well in normal pig, feed, and environment conditions [33]. Moreover, there were no differences on carcass characteristics among dietary treatment (Table 4). These results were similar to the results of previous studies reported by O’Shea et al. [26], Zamora et al. [29], and Burnham et al. [34]. However, some research reported that addition of dietary protease or probiotics in pig diets influenced carcass characteristics of pigs [7,33,35,36].

Overall, further investigation is needed to verify nutrient digest-

Table 2. Effects of dietary mixture of protease and probiotics on growth performance of growing-finishing pigs¹⁾

Items ²⁾	CON	MULTI	SEM	p-value
Phase I (1–60 d)				
Initial BW (kg)	35.19	35.32	0.69	0.893
Final BW (kg)	85.60	85.37	0.99	0.869
ADG (g/d)	840.25	834.22	9.80	0.669
ADFI (g/d)	2,428.09	2,410.25	26.11	0.761
G:F (g/g)	0.353	0.354	0.04	0.922
Phase II (61–88 d)				
Final BW (kg)	112.73	112.23	1.48	0.816
ADG (g/d)	968.77	959.45	27.71	0.813
ADFI (g/d)	3,108.87	3,151.76	143.48	0.894
G:F (g/g)	0.313	0.314	0.15	0.689
Overall (1–88 d)				
Initial BW (kg)	35.19	35.32	0.68	0.893
Final BW (kg)	112.73	112.23	1.48	0.816
ADG (g/d)	881.14	874.06	13.19	0.707
ADFI (g/d)	2,768.48	2,781.01	83.06	0.947
G:F (g/g)	0.321	0.321	0.08	0.704

¹⁾Each value is the mean value of 4 replicates (6 pigs/pen).

²⁾CON, basal diet; MULTI, CON + 0.01% protease and probiotics; SEM, standard error of means; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 3. Effects of dietary mixture of protease and probiotics on complete blood count of growing-finishing pigs¹⁾

Items ²⁾	CON	MULTI	SEM	p-value
Phase I (60 d)				
RBC (× 10 ⁶ /μL)	7.67	7.35	0.18	0.235
WBC (× 10 ³ /μL)	19.96	17.51	1.55	0.335
NE (%)	33.71	31.15	2.85	0.540
LY (%)	57.89	59.38	2.39	0.665
MO (%)	5.96	6.83	0.53	0.364
EO (%)	2.33	2.58	0.27	0.580
BA (%)	0.11	0.08	0.05	0.638
Phase II (88 d)				
RBC (× 10 ⁶ /μL)	7.27	7.08	0.24	0.584
WBC (× 10 ³ /μL)	21.95	19.65	1.57	0.340
NE (%)	27.47	25.70	1.47	0.425
LY (%)	58.19	63.21	2.67	0.211
MO (%)	7.31	7.50	0.72	0.859
EO (%)	3.79	3.56	0.31	0.621
BA (%)	0.09	0.03	0.03	0.167

¹⁾Each value is the mean value of 4 replicates (6 pigs/pen).

²⁾CON, basal diet; MULTI, CON + 0.01% protease and probiotics; SEM, standard error of means; RBC, red blood cell; WBC, white blood cell; NE, neutrophil; LY, lymphocyte; MO, monocyte; BO, eosinophil; BA, basophil.

Table 4. Effects of dietary mixture of protease and probiotics on carcass characteristics of growing-finishing pigs¹⁾

Items ²⁾	CON	MULTI	SEM	p-value
Live weight (kg)	117.60	117.16	1.71	0.857
Hot carcass weight (kg)	85.29	84.87	1.34	0.827
Dressing percentage (%)	72.52	72.43	0.32	0.845
Backfat thickness (mm)	27.50	26.07	1.44	0.489
Carcass grade	1.50	1.73	0.22	0.454

¹⁾Each value is the mean value of 4 replicates (6 pigs/pen).

²⁾CON, basal diet; MULTI, CON + 0.01% protease and probiotics; SEM, standard error of means; Based on a scale with 1, grade 2; 2, grade 1; 3, grade 1*.

ibility, growth performance, carcass characteristics, or etc. of pigs by increasing the concentrations of protease and probiotics or controlling the nutrient levels of basal diets.

Conclusion

The present study showed addition of dietary mixture of protease and probiotics in the pig diet did not affect growth rate, blood constituents, and carcass characteristics of growing-finishing pigs.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study- can be available from the corresponding author.

Authors' contributions

Conceptualization: Kim YH, Jeong YD.

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Formal analysis: Choi YH.

Methodology: Jeong YD.

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Ethics approval and consent to participate

This study was approved by IACUC of Rural Development Administration (No. NIAS-2019-374).

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