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# Effects of protease supplementation on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs

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**Abstract**

This experiment was conducted to evaluate the effects of dietary protease on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. A total of 48 growing pigs (initial body weight, 34.8 ± 0.62 kg) were randomly assigned to 2 dietary treatments (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 protease (PRO). Pigs were fed respective dietary treatments with a 2-phase feeding program for 12 weeks. Pigs fed PRO had higher average daily gain (ADG; phase I, 866.38 vs. 821.75 g/d; overall, 910.96 vs. 866.30 g/d; $p < 0.05$) and gain to feed ratio (G:F; phase I, 0.345 vs. 0.363 g/g; $p < 0.05$) than those fed CON. However, there were no differences on blood constituents and carcass characteristics between CON and PRO of growing-finishing pigs. In conclusion, dietary protease supplementation in the typical diet for growing-finishing pigs improved growth rate.

**Keywords:** Blood constituents, Carcass characteristics, Growing-finishing pigs, Growth performance, Protease

## Background

Soybean meal is one of major ingredients for pigs as a protein source, but it contains several anti-nutritional factors (ANF), such as lectins, oligosaccharides, haemagglutinin, goitrogenic factors, trypsin inhibitors, and antigenic proteins, that cause reduction of protein utilization of pigs by inhibiting the secretion of pancreatic digestive enzymes and increasing the loss of endogenous secretions of pigs [1–4]. These ANFs can be reduced by heat treatment, fermentation process, addition of enzymes, or etc., resulting in improvement of protein utilization of pigs [4,5]. Futhermore, there are two issues in the swine production, such as the increase of feed cost and environmental pollution from undigested protein by pigs. The protein sources including soybean meal in pig diets are relatively expensive compared with other ingredients and thus the unavailable protein by pigs cause the increase of feed cost. The undigested protein by pigs is also related to the environmental pollution by nitrogen emission from pigs [6,7]. Therefore, the swine industry has been looking for the ways to solve these issues.

Addition of dietary protease in pig diets may be one of strat-

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egies to improve nutrient availability and reduce the feed cost in the swine industry. Dietary protease is generally used to reduce the undigested protein and contribute to improvement of nutrient utilization of pigs [8]. However, dietary protease in pig diets has been commonly used as one of enzyme sources for commercially available multi-enzyme products [9–13]. Recently, several studies showed beneficial effects on growth performance, nutrient digestibility, or etc. of pigs fed diets containg dietary protease alone, not multi-enzyme types [14–16], but there was limited evidence for its effects and modes of action. Therefore, the objective of this study was to investigate the effects of dietary protease in on the 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 growing pigs [(Landrace × Yorkshire) × Duroc; 34.8 ± 0.62 kg of 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 protease (PRO). The protease used in this study was a commercial product (Ronozyme® ProAct, DSM nutrition products, Kaiseraugst, Switzerland) containing 75,000 protease units/g derived from *Bacillus licheniformis*. The diets were formulated to meet or exceed the nutrient requirements of growing and finishing pigs, estimated by the NRC (2012) (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 $m^2$) and allowed access to diets and water during the overall experimental period.

### Data and sample collection and measurement

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

**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 $D_3$, 2,000 IU; vitamin E, 250 IU; vitamin $K_3$, 0.5 mg; vitamin $B_1$, 0.49 mg as mononitrate; thiamin, 0.49 mg as thiamin mononitrate; riboflavin, 1.50 mg; pyridoxine, 1 mg as pyridoxine hydrochloride; vitamin $B_{12}$, 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.

### 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 $0.05 \le p < 0.10$, respectively.

## Results and Discussion

Pigs fed PRO had higher ADG ($p < 0.05$) during phase Ⅰ and overall experimental period and gain to feed ratio ($p < 0.05$) during phase Ⅰ than those fed CON, but there was no difference on ADFI of pigs between CON and PRO (Table 2). Most previous studies also showed that pigs fed diets with dietary protease had higher growth rate of pigs than those fed diets without dietary protease [14–17]. However, other research reported that addition of dietary protease in pig diets did not improved growth rate [18–20]. The reason for the positive effect of dietary protease on growth rate may be related to the improvement of nutrient digestibility and utilization from more hydrolysis of protein by addition of dietary protease in pig diets [21–24].

Addition of dietary protease in the pig diet did not modulate blood constituents of pigs (Table 3). These results were similar to the results reported by Tactacan et al. [24]. The blood constituents measured in this experiment were the total number of white blood cell and its differentiation, such as neutrophils, lymphocytes, monocytes, eosinophils, and basophils, that can be used as indicators for inflammation or immunity [25–27]. The modification of indicators for immunity by some changes of pig diet formula may not be easy in normal conditions of pigs and environment. During the whole experimental period of this experiment, pigs and environmental conditions were normal and thus the blood constituents of pigs may not be changed by addition of dietary protease in pig diets in this experiment. In addition, there were no differences on carcass characteristics among dietary treatments (Table 4). These results were similar to the results of previous studies [16,28,29], but Wang et al. [30] showed addition of dietary protease in the pig diet changed carcass characteristics of pigs. However, further investigation is needed to clarify the effects of dietary protease on the blood constituents and carcass characteristics of growing-finishing pigs.

**Table 2. Effects of dietary protease on growth performance of growing-finishing pigs[1)]**

| Items[2)] | CON | PRO | SEM | *p*-value |
|---|---|---|---|---|
| Phase Ⅰ (1–60 d) | | | | |
| Initial BW (kg) | 34.93 | 34.67 | 0.62 | 0.770 |
| Final BW (kg) | 84.23 | 86.65 | 1.07 | 0.117 |
| ADG (g/d) | 821.75 | 866.38 | 12.01 | 0.012 |
| ADFI (g/d) | 2,380.48 | 2,388.60 | 20.79 | 0.834 |
| G:F (g/g) | 0.345 | 0.363 | 0.01 | 0.020 |
| Phase Ⅱ (61–88 d) | | | | |
| Final BW (kg) | 111.16 | 114.83 | 1.59 | 0.108 |
| ADG (g/d) | 961.77 | 1,006.49 | 27.55 | 0.257 |
| ADFI (g/d) | 3,110.12 | 3,216.52 | 77.21 | 0.417 |
| G:F (g/g) | 0.311 | 0.313 | 0.01 | 0.873 |
| Overall (1–88 d) | | | | |
| Initial BW (kg) | 34.93 | 34.67 | 0.62 | 0.770 |
| Final BW (kg) | 111.16 | 114.83 | 1.59 | 0.108 |
| ADG (g/d) | 866.30 | 910.96 | 14.36 | 0.033 |
| ADFI (g/d) | 2,745.30 | 2,802.56 | 41.66 | 0.395 |
| G:F (g/g) | 0.316 | 0.325 | 0.01 | 0.273 |

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

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

**Table 3. Effects of dietary protease on complete blood count of growing-finishing pigs[1)]**

| Items[2)] | CON | PRO | SEM | *p*-value |
|---|---|---|---|---|
| Phase Ⅰ (60 d) | | | | |
| RBC (× $10^6$/μL) | 7.54 | 7.43 | 0.16 | 0.661 |
| WBC (× $10^3$/μL) | 18.77 | 20.14 | 1.47 | 0.582 |
| NE (%) | 33.69 | 30.67 | 1.81 | 0.303 |
| LY (%) | 58.04 | 60.90 | 1.86 | 0.329 |
| MO (%) | 5.85 | 5.57 | 0.38 | 0.620 |
| EO (%) | 2.33 | 2.79 | 0.27 | 0.280 |
| BA (%) | 0.10 | 0.07 | 0.05 | 0.728 |
| Phase Ⅱ (88 d) | | | | |
| RBC (× $10^6$/μL) | 7.25 | 7.36 | 0.19 | 0.701 |
| WBC (× $10^3$/μL) | 21.50 | 21.93 | 1.43 | 0.845 |
| NE (%) | 30.49 | 27.36 | 2.33 | 0.390 |
| LY (%) | 58.39 | 61.93 | 2.16 | 0.307 |
| MO (%) | 7.33 | 6.70 | 0.59 | 0.495 |
| EO (%) | 3.71 | 3.60 | 0.32 | 0.807 |
| BA (%) | 0.09 | 0.04 | 0.03 | 0.227 |

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

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



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Table 4. Effects of dietary protease on carcass characteristics of finishing pigs[1)]

| Items[2)] | CON | PRO | SEM | *p*-value |
|---|---|---|---|---|
| Live weight (kg) | 117.05 | 117.42 | 1.84 | 0.890 |
| Hot carcass weight (kg) | 84.85 | 84.82 | 1.50 | 0.990 |
| Dressing percentage (%) | 72.49 | 72.21 | 0.33 | 0.556 |
| Backfat thickness (mm) | 27.00 | 25.55 | 1.47 | 0.491 |
| Carcass grade | 1.54 | 1.73 | 0.24 | 0.587 |

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

[2)]CON, basal diet; PRO, CON + 0.01% protease; Based on a scale with 1, grade 2; 2, grade 1; 3, grade 1$^{+}$; SEM, standard error of means.

## Conclusion

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

### Competing interests

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

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Not applicable.

### 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.
Software: Kim DW, Kim JW.
Validation: Jung HJ.
Investigation: Jeong YD, Min YJ.
Writing - original draft: Min YJ.
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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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