Dietary protease improves growth rate and protein digestibility of growing-finishing pigs

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Abstract

This research was performed to investigate the hypothesis that dietary mono-component protease (PRO) might improve growth performance, nutrient digestibility, and carcass characteristics of growing-finishing pigs. A total of eighty-four pigs [Duroc × (Landrace × Yorkshire), 25.3 ± 2.16 kg initial body weight] were randomly assigned to three dietary treatments (7 replicates/treatment; 2 barrows and 2 gilts/replicate) in a randomized complete block design (block = sex). The dietary treatments were prepared as follows; (1) a positive control (PC) as a typical growing-finishing diet based on corn and soybean meal, (2) PC added with 0.015% of PRO (PCPRO), and (3) a negative control (NC) added with 0.015% of PRO (NC PRO). The NC had a lower concentration of crude protein (CP) compared with PC. The PRO was a commercial product that contained 75,000 protease units/g and derived from Nocardiopsis prasina produced in Bacillus licheniformis. Dietary treatments were offered to pigs during growing and finishing periods. Measurements were growth performance, apparent total tract digestibility (ATTD) of nutrients, and carcass characteristics. The PCPRO and/or NC PRO increased average daily gain (ADG) and gain to feed ratio (G:F) during growing (p < 0.10), finishing (p < 0.05), and growing-finishing periods (p < 0.10) compared with PC. Furthermore, pigs fed PCPRO and NC PRO had higher (p < 0.05) ATTD of CP and energy during growing and/or finishing periods than those fed PC. In conclusion, the supplementation of PRO in diets improved growth performance and protein digestibility of growing-finishing pigs.

Keywords: Carcass characteristics, Dietary protease, Growing-finishing pigs, Growth performance, Nutrient digestibility

INTRODUCTION

One of the main goals in swine production is to maximize pork productivity. Consequently, the swine industry constantly requires efficient livestock and nutritional management to increase growth perfor-
Dietary protease for growing-finishing pigs

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

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The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea. This study was performed at the research facility of Chungnam National University.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors’ contributions
Conceptualization: Choe J, Perez-Maldonado R, Park IH, Song M.
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Ethics approval and consent to participate
The experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval #CNU-00910).

MATERIALS AND METHODS

The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea. This study was performed at the research facility of Chungnam National University.

Experimental design, animals, and diets
A total of eighty-four pigs [Duroc × (Landrace × Yorkshire); average initial body weight (BW) of 25.3 ± 2.16 kg] were used and randomly assigned to three diets (7 replicates/treatment, 2 barrows and 2 gilts/replicate) in a randomized complete block design (block = sex). The diets were as follows; (1) positive control (PC) as a typical growing-finishing diet based on corn and soybean meal, (2) PC added with 0.015% of PRO (PCPRO), and (3) negative control (NC) added with 0.015% of PRO (NCPRO) (Table 1). The NC had a lower level of crude protein compared with PC. The PRO was a commercial product (Ronozyme® ProAct, DSM Nutritional Products, Kaiseraugst, Switzerland) that contained 75,000 protease units/g derived from Nocardiopsis prasina produced in Bacillus licheniformis. There were 2 experimental periods which consisted of 6 weeks of growing period followed by a 5 weeks finishing period. All pigs were housed in an environmentally controlled room with free access to feed and water during the overall experimental period.

Data and sample collection
The BW of pigs and the amount of feed offered and feed residual in each pen were weighed at the initial and end of each experiment period (growing and finishing periods). Growth performance [average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F ratio, gain-to-feed ratio)] was measured throughout. In the last week of each experimental period, a respective
experimental diet containing 0.2% of chromic oxide as an indigestible marker was provided during that week. Fecal samples from randomly selected two pigs per pen were collected daily by rectal palpation for the last 3 d after 4 d adaptation period. Diet samples were collected from each batch of manufactured feed and subsequently pooled and stored at –20°C until analysis. Diet and fecal samples were dried in a forced-air drying oven at 60°C and ground using a cyclone mill (Foss Tecator Syctec 1093, Hillerød, Denmark) before analysis.

In the last day of the experiment, all feed was withdraw 4 hours previous to all pigs being transported from the farm to the nearest local commercial slaughterhouse. The slaughter procedure and carcass characteristics measurements were performed under the supervision of the Korea Institute for Animal Products Quality Evaluation. The pigs were washed with water for reducing stress. To all pigs, water was freely accessible during lairage, and were allowed to rest for about 4 hours. The final BW of each pig was noted and processed with conventional slaughter process with the electrical stunning and scalding-singeing.

Table 1. Composition of experimental diets for growing-finishing pigs (as-fed basis)

| Item                        | Experimental diets | Growing   | Finishing  |
|-----------------------------|--------------------|-----------|------------|
|                             |                    | PC        | NC         | PC         | NC         |
| Ingredients (%)             |                    |           |            |            |            |
| Corn (8%)                   | 46.34              | 46.58     | 49.62      | 54.80      |
| Soybean meal (44%)          | 18.00              | 17.70     | 10.30      | 9.30       |
| Wheat (10%)                 | 14.60              | 15.00     | 15.60      | 11.00      |
| DDGS                        | 9.70               | 11.10     | 14.30      | 15.60      |
| Rapeseed meal               | 4.00               | 4.10      | 5.50       | 5.60       |
| Full fat soya               | 2.70               | 0.50      | 1.00       | 0.60       |
| Tallow                      | 1.20               | 1.50      | 0.80       | 0.70       |
| Mono-dicalcium phosphate    | 0.90               | 0.90      | 0.47       | 0.49       |
| Limestone                   | 1.20               | 1.30      | 1.30       | 1.30       |
| Salt                        | 0.36               | 0.35      | 0.28       | 0.27       |
| Vitamin-mineral premix 1)   | 0.30               | 0.30      | 0.30       | 0.30       |
| L-Lysine-HCl                | 0.40               | 0.40      | 0.36       | 0.35       |
| DL-Methionine               | 0.13               | 0.12      | 0.05       | 0.03       |
| L-Threonine                 | 0.14               | 0.12      | 0.11       | 0.09       |
| L-Tryptophan                | 0.03               | 0.03      | 0.01       | 0.01       |
| Total                       | 100                | 100       | 100        | 100        |

Calculated composition

| Metabolizable energy (Mcal/kg) | 3.21 | 3.21 | 3.16 | 3.16 |
| Crude protein (%)              | 18.43| 17.93| 16.53| 16.18|
| Lysine (%)                     | 1.15 | 1.10 | 0.94 | 0.90 |
| Methionine (%)                 | 0.43 | 0.41 | 0.33 | 0.31 |
| Cysteine (%)                   | 0.29 | 0.27 | 0.28 | 0.26 |
| Ca (%)                         | 0.76 | 0.76 | 0.72 | 0.72 |
| P (%)                          | 0.58 | 0.58 | 0.53 | 0.53 |

1)Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 2,500 IU; vitamin E, 30 IU; vitamin K₃, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B₁₂, 12 μg; Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

PC, positive control; NC, negative control.
Sample analyses and measurements
Diet and fecal samples were analyzed for dry matter (DM) [25], crude protein (CP) [25], gross energy (GE) using a bomb calorimeter (Parr 1281 Bomb Calorimeter, Parr Instrument Co., Moline, IL, USA), and chromium contents using an absorption spectrophotometer (Hitachi Z-5000 Absorption Spectrophotometer, Hitachi High-Technologies Co., Tokyo, Japan). Apparent total tract digestibility (ATTD) of DM, CP, and GE of growing and finishing pigs was calculated based on Stein et al. [26]. The dressing percentage was calculated by comparing final live BW and hot carcass weight. After splitting, the backfat thickness was directly measured between 11th and 12th thoracic vertebra and between the last thoracic vertebra as well as the 1st lumbar vertebra. The average of two measurements was expressed as a backfat thickness for each pig.

Statistical analysis
All data was analyzed with the PROC General Linear Models (GLM) of SAS (SAS Inst., Cary, NC, USA). The pen was used as an experimental unit. The statistical model for growth performance, nutrient digestibility, and carcass characteristics included dietary treatments as a fixed effect and sex as a covariate. The results are presented as a mean ± standard error of the mean. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p \leq 0.10$, respectively.

RESULTS AND DISCUSSION
Pigs fed PCPRO and NCPRO had higher ADG and G:F ratio during growing ($p < 0.10$) and finishing ($p < 0.05$) periods than those fed PC (Table 2). Similarly, the PCPRO and NCPRO

| Table 2. Effects of dietary protease on growth performance of growing-finishing pigs$^\dagger$ |
|--------------------------|--------------------------|--------------------------|
| Item                     | PC                       | PCPRO                    | NCPRO                    |
| **Growing period (6 weeks after weaning period)** | **PC** | **PCPRO** | **NCPRO** | **SEM** | **p-value** |
| Initial body weight (kg) | 25.34                    | 25.20                    | 25.45                    | 0.77    | 0.974       |
| Final body weight (kg)   | 63.96                    | 65.23                    | 65.07                    | 1.29    | 0.756       |
| ADG (kg/d)               | 0.920$^a$                | 0.953$^b$                | 0.943$^a$                | 0.01    | 0.099       |
| ADFI (kg/d)              | 2.128                    | 2.113                    | 2.043                    | 0.05    | 0.460       |
| G:F ratio (kg/kg)        | 0.432$^a$                | 0.451$^b$                | 0.462$^a$                | 0.01    | 0.099       |
| **Finishing period (5 weeks after growing period)** | | | | | |
| Initial body weight (kg) | 63.96                    | 65.23                    | 65.07                    | 1.29    | 0.756       |
| Final body weight (kg)   | 97.50                    | 100.41                   | 100.89                   | 1.68    | 0.324       |
| ADG (kg/d)               | 0.958$^a$                | 1.005$^b$                | 1.023$^a$                | 0.02    | 0.045       |
| ADFI (kg/d)              | 3.721                    | 3.528                    | 3.508                    | 0.11    | 0.312       |
| G:F ratio (kg/kg)        | 0.257$^a$                | 0.285$^b$                | 0.292$^a$                | 0.01    | 0.014       |
| **Growing-finishing period (11 weeks after weaning period)** | | | | | |
| Initial body weight (kg) | 25.34                    | 25.20                    | 25.45                    | 0.77    | 0.974       |
| Final body weight (kg)   | 97.50                    | 100.41                   | 100.89                   | 1.68    | 0.324       |
| ADG (kg/d)               | 0.937$^a$                | 0.977$^{ab}$             | 0.980$^a$                | 0.01    | 0.098       |
| ADFI (kg/d)              | 2.852                    | 2.756                    | 2.707                    | 0.07    | 0.356       |
| G:F ratio (kg/kg)        | 0.329$^a$                | 0.354$^b$                | 0.362$^a$                | 0.01    | 0.015       |

$^\dagger$Each value is the mean value of 7 replicates (4 pigs/replicate).
$^a,b$Means with different superscripts within the same row differ ($p < 0.10$).
PC, positive control; PCPRO, positive control + 0.015% of dietary protease; NCPRO, negative control + 0.015% of dietary protease; SEM, standard error of mean; ADG, average daily gain; ADFI, average daily feed intake; G:F ratio, gain-to-feed ratio.
increased ADG ($p < 0.10$) and G:F ratio ($p < 0.05$) during overall experimental period compared with PC. These results are consistent with the results from previous studies reported that PRO improved growth rate of growing-finishing pigs [20,21,27]. These beneficial effects of PRO may be contributed by improved availability of more degraded protein with improved apparent ileal N digestibility reduced ANFs by PRO in pig diets [22,28–30]. The beneficial effect of mono-component protease on pig performance which was observed in this study was also reported by the work done by O’Doherty and Forde [27]. Those workers found improvements in the weight gain (0.862 vs 0.887 kg/day) of growing and finishing pigs. Generally, weaned pigs have a low activity of digestive enzymes in the stomach and pancreatic epithelium tissue, but growing or finishing pigs have a more developed digestive system [31–33]. Supplementation of dietary enzymes including PRO was complementary to the endogenous digestive systems. PRO was able to degrade certain nutrients that were not well digested by endogenous enzymes secreted from the animal digestive systems. Similarly, the application of PRO in the diets of animals may be able to neutralize certain ANFs, resulting in an increase of nutrient digestibility and utilization efficiency [14,28,31].

Pigs fed PCPRO and NCPRO had higher ATTD of CP during growing period ($p < 0.05$) and ATTD of CP and GE during finishing period ($p < 0.05$) than those fed PC (Table 3). These results are consistent with previous work that showed the improvement of nutrient digestibility of growing-finishing pigs [29,30,34], but did not show an improvement of growth performance of pigs fed PRO. However, some studies did not observe the positive effects of PRO on nutrient digestibility of growing pigs, but found only improvement of growth performance of pigs fed PRO [20–22]. The beneficial impact of PRO in present study may be related to more opportunities for pigs to better utilize more hydrolyzed protein by PRO in the experimental diets, resulting in the improvement of both growth performance and nutrient digestibility.

There were no significant differences between treatments on carcass characteristics of pigs by addition of PRO in the pig diets (Table 4). Similar results were observed in previous studies [20,21,35], but other previous work did show changed of carcass characteristics of pigs when PRO was added in the diets [35,36]. The inconsistent results may be due to different type of PRO used and the conditions and stages of pig age when PRO were added into the diets. Future research is needed to investigate in detail amino acid digestibility profile and to verify whether PRO can modify carcass characteristics of pigs.

### Table 3. Effects of dietary protease on apparent total tract digestibility of growing-finishing pigs

| Item               | Treatments | SEM | $p$-value |
|--------------------|------------|-----|-----------|
|                    | PC    | PCPRO | NCPRO  |
| DM (%)             | 73.77 | 80.57 | 80.22   | 2.57 | 0.487  |
| CP (%)             | 69.61$^a$ | 76.33$^a$ | 77.53$^b$ | 2.21 | 0.046  |
| GE (%)             | 72.68 | 79.63 | 79.26   | 2.46 | 0.327  |
| DM (%)             | 78.33 | 85.86 | 83.36   | 1.93 | 0.108  |
| CP (%)             | 74.26$^a$ | 83.61$^b$ | 83.36$^b$ | 1.99 | 0.041  |
| GE (%)             | 74.86$^a$ | 83.84$^b$ | 83.99$^b$ | 2.06 | 0.043  |

$^a$Each value is the mean value of 7 replicates (2 pigs/replicate).

$^*$Means with different superscripts within the same row differ significantly ($p < 0.05$).

PC, positive control; PCPRO, positive control + 0.015% of dietary protease; NCPRO, negative control + 0.015% of dietary protease; SEM, standard error of mean; DM, dry matter; CP, crude protein; GE, gross energy.
CONCLUSION

The present study indicates supplementation of PRO in diets improved growth performance and protein digestibility of growing-finishing pigs.

REFERENCES

1. Dibner JJ, Richards JD. Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci. 2005;84:634-43.
2. Fouhse JM, Zijlstra RT, Willing BP. The role of gut microbiota in the health and disease of pigs. Anim Front. 2016;6:30-6.
3. Brown K, Uwiera RRE, Kalmokoff ML, Brooks SPJ, Inglis GD. Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. Int J Antimicrob Agents. 2017;49:12-24.
4. Modi CM, Mody SK, Patel HB, Dudharta GB, Kumar A, Sheikh TJ. Growth promoting use of antimicrobial agents in animals. J Appl Pharm Sci. 2011;01:33-6.
5. Kim J, Seo J, Kim W, Yun HM, Kim SC, Jang Y, et al. Effects of palm kernel expellers on productive performance, nutrient digestibility, and white blood cells of lactating sows. Asian-Australas J Anim Sci. 2015;28:1150-4.
6. Kim S, Kim B, Kim Y, Jung S, Kim Y, Park J, et al. Value of palm kernel co-products in swine diets. Korean J Agric Sci. 2016;43:761-8.
7. Hoffman LA, Baker AJ. Estimating the substitution of distillers’ grains for corn and soybean meal in the U.S. feed complex. Washington, DC: United States Department of Agriculture; 2011. Report No.: FDS-11-I-01.
8. Lawrence JD, Mintert J, Anderson JD, Anderson DP. Feed grains and livestock: impacts on meat supplies and prices. Choices. 2008;23:11-5.
9. Florou-Paneri P, Christaki E, Giannenas I, Bonos E, Skoufos I, Tsinias A, et al. Alternative protein sources to soybean meal in pig diets. J Food Agric Environ. 2014;12:655-60.
10. Min BJ, Cho JH, Chen YJ, Kim HJ, Yoo JS, Wang Q, et al. Effects of replacing soy protein concentrate with fermented soy protein in starter diet on growth performance and ileal amino acid digestibility in weaned pigs. Asian-Australas J Anim Sci. 2009;22:99-106.
11. Anderson RL, Rackis JJ, Tallent WH. Biologically active substances in soy products. In: Wilcke HL, Hopkins DT, Waggle DH, editors. Soy protein and human nutrition. New York, NY: Academic press; 1979. p. 209-33.
12. Park S, Kim B, Kim Y, Kim S, Jang K, Kim Y, et al. Nutrition and feed approach according to pig physiology. Korean J Agric Sci. 2016;43:750-60.
13. Adeola O, Cowieson AJ. Board-invited review: opportunities and challenges in using exoge-

Table 4. Effects of dietary protease on carcass characteristics of growing-finishing pigs

| Item                          | Treatments | SEM | p-value |
|-------------------------------|------------|-----|---------|
| Final live body weight (kg)   | 97.50      | 1.68| 0.324   |
| Hot carcass weight (kg)       | 87.71      | 1.18| 0.603   |
| Dressing percentage (%)       | 78.76      | 0.08| 0.571   |
| Backfat depth (mm)            | 21.94      | 0.69| 0.605   |

1) Each value is the mean value of 7 replicates; The growing-finishing period was 11 weeks after weaning.

PC, positive control; PCPRO, positive control + 0.015% of dietary protease; NCPRO, negative control + 0.015% of dietary protease; SEM, standard error of means.
nous enzymes to improve nonruminant animal production. J Anim Sci. 2011;89:3189-218.
14. Zuo J, Ling B, Long L, Li T, Lahaye L, Yang C, et al. Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim Nutr. 2015;1:276-82.
15. Kim Y, Baek J, Jang K, Kim J, Kim S, Mun D, et al. Effects of dietary enzyme cocktail on growth performance, intestinal morphology, and nutrient digestibility of weaned pigs. Korean J Agric Sci. 2017;44:513-8.
16. Kang J, Cho J, Jang K, Kim J, Kim S, Mun D, et al. Effects of dietary enzyme cocktail on diarrhea and immune responses of weaned pigs. Korean J Agric Sci. 2017;44:525-30.
17. Kim SK, Cho MW, Kim JS, Jang KB, Kim SA, Mun DY, et al. Effects of eco-friendly multi-enzyme on growth performance, intestinal morphology, and nutrient digestibility of weaned pigs. Korean J Org Agric. 2018;26:141-9.
18. Min YJ, Kim JS, Kim SA, Jang KB, Mun DY, Kim BH, et al. Effects of eco-friendly multi-enzyme on diarrhea and immune response of weaned pigs. Korean J Org Agric. 2018;26:151-61.
19. Min Y, Choi Y, Choe J, Kim Y, Jeong Y, Kim D, et al. Effects of dietary mixture of protease and probiotics on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. J Anim Sci Technol. 2019;61:272-7.
20. Choe J, Kim KS, Kim HB, Park S, Kim J, Kim S, et al. Effect of protease on growth performance and carcass characteristics of growing-finishing pigs. S Afr J Anim Sci. 2017;47:697-703.
21. Min Y, Choi Y, Kim Y, Jeong Y, Kim D, Kim J, et al. Effects of protease supplementation on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. J Anim Sci Technol. 2019;61:234-8.
22. Park S, Lee JJ, Yang BM, Cho JH, Kim S, Kang J, et al. Dietary protease improves growth performance, nutrient digestibility, and intestinal morphology of weaned pigs. J Anim Sci Technol. 2020;62:21-30.
23. Yin YL, Baidoo SK, Jin LZ, Liu YG, Schulze H, Simmins PH. The effect of different carbohydrase and protease supplementation on apparent (ileal and overall) digestibility of nutrients of five hullless barley varieties in young pigs. Livest Prod Sci. 2001;71:109-20.
24. Yin YL, Deng ZY, Huang HL, Li TJ, Zhong HY. The effect of arabinoxylanase and protease supplementation on nutritional value of diets containing wheat bran or rice bran in growing pig. J Anim Feed Sci. 2004;13:445-61.
25. AOAC [Association of Official Analytical Chemists] International. Official methods of analysis of AOAC International. Gaithersburg, MD: AOAC International; 2005.
26. Stein HH, Seve B, Fuller MF, Moughan PJ, de Lange CFM. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci. 2007;85:172-80.
27. O’doherty JV, Forde S. The effect of protease and alpha-galactosidase supplementation on the nutritive value of peas for growing and finishing pigs. Ir Agric Food Res. 1999;217-26.
28. Cowieson AJ, Roos FF. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. Anim Feed Sci Tech. 2016;221:331-40.
29. O’Shea CJ, Mc Alpine PO, Solan P, Curran T, Varley PF, Walsh AM, et al. The effect of protease and xylanase enzymes on growth performance, nutrient digestibility, and manure odour in grower-finisher pigs. Anim Feed Sci Tech. 2014;189:88-97.
30. Pan L, Zhao PF, Yang ZY, Long SF, Wang HL, Tian QY, et al. Effects of coated compound proteases on apparent total tract digestibility of nutrients and apparent ileal digestibility of ami-
no acids for pigs. Asian Australas J Anim Sci. 2016;29:1761–7.

31. Hedemann MS, Jensen BB. Variations in enzyme activity in stomach and pancreatic tissue and digesta in piglets around weaning. Arch Anim Nutr. 2004;58:47–59.

32. Ngoc TTB, Len NT, Ogle B, Lindberg JE. Influence of particle size and multi-enzyme supplementation of fibrous diets on total tract digestibility and performance of weaning (8–20 kg) and growing (20–40 kg) pigs. Anim Feed Sci Technol. 2011;169:86–95.

33. Zhang GG, Yang ZB, Wang Y, Yang WR, Zhou HJ. Effect of dietary supplementation of multi-enzyme on growth performance, nutrient digestibility, small intestinal digestive enzyme activities, and large intestinal selected microbiota in weanling pigs. J Anim Sci. 2014;92:2063–9.

34. Ji F, Casper DP, Brown PK, Spangler DA, Haydon KD, Pettigrew JE. Effects of dietary supplementation of an enzyme blend on the ileal and fecal digestibility of nutrients in growing pigs. J Anim Sci. 2008;86:1533–43.

35. Stephenson EW, DeRouchey JM, Escobar J, Woodworth JC, Tokach MD, Goodband RD, et al. Effects of a novel protease enzyme (CIBENZA DP100) on finishing pig growth performance and carcass characteristics. Kans Agric Exp Stn Res Rep. 2014;69:69–76.

36. Wang D, Zeng Z, Piao X, Li P, Xue L, Zhang Q, et al. Effects of keratinase supplementation of corn-soybean meal based diets on apparent ileal amino acid digestibility in growing pigs and serum amino acids, cytokines, immunoglobulin levels and loin muscle area in nursery pigs. Arch Anim Nutr. 2011;65:290–302.