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Permalink
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Journal
Journal of animal science and technology, 62(3)

ISSN
2055-0391

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Publication Date
2020-05-01

DOI
10.5187/jast.2020.62.3.313

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RESEARCH ARTICLE

J Anim Sci Technol 2020;62(3):313-320

https://doi.org/10.5187/jast.2020.62.3.313

JAST

Journal of Animal Science and Technology

pISSN 2672-0191 eISSN 2055-0391

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

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Received: Dec 16, 2019
Revised: Mar 12, 2020
Accepted: Mar 15, 2020

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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 (NCPRO). 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 NCPRO 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 NCPRO 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-

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

Funding sources
The present study was supported by the Individual Basic Science & Engineering Research Program (No. NRF-2019R1F1A1063306) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education and supported throughout by DSM Nutrition Korea Ltd., Seoul, Korea and DSM Singapore Nutritional Products.

Acknowledgements
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: Choe J, Perez-Maldonado R, Park IH, Song M.
Data curation: Lee JJ, Kang J, Park S.
Formal analysis: Lee JJ, Choe J, Cho JY, Park IH.
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Writing - review & editing: Cho JH, Kim HB, Song M.

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).

mance that has been used for decades [1,2]. Therefore, the swine industry has been used antibiotic growth promoters (AGP) which has two major impacts; growth promoting and disease prevention [1,3]. However, there is a potential risk and a concern by the society about the emergence of antimicrobial resistance in human pathogenic bacteria and their transmission into the human population by the abuse of AGP [1–4]. Thus the use of AGP in animal diets has been banned or reduced internationally and at the same time alternatives for AGP have been increasingly demanded. Moreover, the cost of main ingredients in swine diets, such as soybean meal and corn, have been remarkably increased due to the competition among animal feed, human food, and biofuel industries [5–8]. Especially, soybean meal which is one of main protein sources for swine, but unfortunately it contains various anti-nutritional factors (ANFs) that can inhibit protein utilization by pigs [9–11].

Potential solutions for the issues mentioned above are strongly required to replace the AGP without the risk regarding antimicrobial resistant microbes and to improve the utilization of protein and other nutrients in pig diets efficiently. Inclusion of exogenous enzyme in pig diets is one of plausible strategies for improvement of nutrient utilization efficiency [12,13]. Among the exogenous enzymes available, dietary protease (PRO) is an enzyme to catalyze the hydrolysis of proteins with ANFs to small peptides or amino acids and is generally used as a part of enzyme cocktails [14–19] or used as a mono-component protease [20–22] in non-ruminant diets. Previous studies reported the supplementation of PRO which improved growth performance and nutrient digestibility at various stages of different age of pigs [14,17,22–24], but the beneficial effects of PRO on pig performance and nutrient digestibility have not been further confirmed particularly at the latest pig growth period. Therefore, the objective of this study was to verify whether addition of PRO could improve growth performance and nutrient digestibility of growing-finishing pigs.

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

 https://doi.org/10.5187/jast.2020.62.3.313

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 D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 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.

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℃ until analysis. Diet and fecal samples were dried in a forced-air drying oven at 60℃ and ground using a cyclone mill (Foss Tecator Sycltec 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.

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 vertebras and between the last thoracic vertebras 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[1)]

Item	Treatments			SEM	*p*-value
	PC	PCPRO	NCPRO		
Growing period (6 weeks after weaning period)					
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[b]	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[ab]	0.462[b]	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[b]	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[b]	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[b]	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[b]	0.01	0.015

[1)]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.

 https://doi.org/10.5187/jast.2020.62.3.313

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[1)]

Item	Treatments			SEM	*p*-value
	PC	PCPRO	NCPRO		
Growing period (6 weeks after weaning period)					
DM (%)	73.77	80.57	80.22	2.57	0.487
CP (%)	69.61[a]	76.33[b]	77.53[b]	2.21	0.046
GE (%)	72.68	79.63	79.26	2.46	0.327
Finishing period (5 weeks after growing period)					
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

[1)]Each value is the mean value of 7 replicates (2 pigs/replicate).

[a,b]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.

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

Item	Treatments			SEM	*p*-value
	PC	PCPRO	NCPRO		
Final live body weight (kg)	97.50	100.41	100.89	1.68	0.324
Hot carcass weight (kg)	87.71	87.56	89.10	1.18	0.603
Dressing percentage (%)	78.76	78.65	78.69	0.08	0.571
Backfat depth (mm)	21.94	21.01	21.78	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.

CONCLUSION

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

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https://doi.org/10.5187/jast.2020.62.3.313