

# Effect of low protein diets added with protease on growth performance, nutrient digestibility of weaned piglets and growing-finishing pigs

Yong Ju Kim<sup>1#</sup>, Ji Hwan Lee<sup>1#</sup>, Tae Heon Kim<sup>1#</sup>, Min Ho Song<sup>2#</sup>, Won Yun<sup>1</sup>, Han Jin Oh<sup>1</sup>, Jun Soeng Lee<sup>1</sup>, Hyeun Bum Kim<sup>3\*</sup> and Jin Ho Cho<sup>1\*</sup>

<sup>1</sup>Division of Food and Animal Science, Chungbuk National University, Cheongju 28644, Korea

<sup>2</sup>Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea

<sup>3</sup>Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea



Received: Nov 12, 2020  
 Revised: Feb 2, 2021  
 Accepted: Feb 21, 2021

#These authors contributed equally to this work.

#### \*Corresponding author

Hyeun Bum Kim  
 Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea.  
 Tel: +82-41-550-3653  
 E-mail: hbkim@dankook.ac.kr

Jin Ho Cho  
 Division of Food and Animal Science, Chungbuk National University, Cheongju 28644, Korea.  
 Tel: +82-43-261-2544  
 E-mail: jinhcho@chungbuk.ac.kr

Copyright © 2021 Korean Society of Animal Sciences and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

#### ORCID

Yong Ju Kim  
<https://orcid.org/0000-0002-0960-0884>

#### Abstract

The objective of this study was to evaluate the effects of low protein diets added with protease on growth performance, nutrient digestibility, and blood profiles of weaned piglets and growing-finishing pigs. A total of 96 weaned pigs ([Yorkshire × Landrace] × Duroc) with average body weight (BW) of 6.99 ± 0.21 kg were used in a 20-week experiment. The dietary treatments were arranged in a 2 × 3 factorial design. Treatments were as follows: In phase 1 (1–2 weeks), two protein levels as high protein (HP; 19.0%), low protein (LP; 17.0%), and three protease (PT) levels (PT0, 0%; PT1, 0.3%; and PT2, 0.5%); in phase 2 (3–4 weeks), protein levels (HP, 18.05%; LP, 16.15%) and protease levels (0%, 0.3%, and 0.5%); in phase 3 (5–12 weeks), protein levels (HP, 17.1%; LP, 15.3%) and protease level (0%, 0.15%, and 0.3%); in phase 4 (13–20 weeks), protein levels (HP, 16.15%; LP, 14.45%) and protease level (0%, 0.15%, and 0.3%). At 4 weeks and 20 weeks after treatment, BW was higher ( $p < 0.050$ ) in the PT2 group than PT0 group. From weeks 0 to 4, average daily gain (ADG) and feed efficiency (G/F) were higher ( $p = 0.006$  and  $p = 0.014$ ;  $p = 0.014$  and  $p = 0.044$ , respectively) in the PT2 group than PT0 and PT1 groups. From weeks 16 to 20, ADG and G/F were higher ( $p < 0.001$  and  $p = 0.009$ ;  $p = 0.004$  and  $p = 0.033$ , respectively) in the PT2 group than PT0 and PT1 groups. Crude protein (CP) digestibility was higher ( $p = 0.013$ ,  $p = 0.014$ , and  $p = 0.035$ , respectively) in the low protein (LP) group than high protein (HP) group at weeks 4, 12, and 20. At weeks 4 and 20, the LP diet group had lower ( $p < 0.001$  and  $p = 0.001$ , respectively) blood urea nitrogen (BUN) levels than the HP diet group. Therefore, a low CP diet added with protease could increase growth performance and CP digestibility of weaned piglets and growing-finishing pigs.

**Keywords:** Protein, Protease, Growth performance, Nutrient digestibility, Pigs

Ji Hwan Lee  
<https://orcid.org/0000-0001-8161-4853>  
 Tae Heon Kim  
<https://orcid.org/0000-0001-9054-5781>  
 Min Ho Song  
<https://orcid.org/0000-0002-4515-5212>  
 Won Yun  
<https://orcid.org/0000-0002-1835-2640>  
 Han Jin Oh  
<https://orcid.org/0000-0002-3396-483X>  
 Jun Soeng Lee  
<https://orcid.org/0000-0002-2497-6855>  
 Hyeun Bum Kim  
<https://orcid.org/0000-0003-1366-6090>  
 Jin Ho Cho  
<https://orcid.org/0000-0001-7151-0778>

#### Competing interests

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

#### Funding sources

The present research was supported by Eugene-Bio in 2020.

#### 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: Kim YJ, Lee JH, Kim TH, Song MH.  
 Data curation: Kim YJ, Lee JH.  
 Formal analysis: Kim YJ, Lee JH.  
 Investigation: Lee JH, Kim TH, Yun W, Oh HJ, Lee JS.  
 Writing - original draft: Kim YJ, Kim TH.  
 Writing - review & editing: Kim HB, Cho JH.

#### Ethics approval and consent to participate

The experimental protocol was approved and conducted under the guidelines of the Animal Care and Use Committee of Chungbuk National University (CBNUA-1428-20-02).

## INTRODUCTION

The world is affected by environmental pollution by rapid development of industries including the swine industry. Among contaminants identified in manure, minerals such as potassium, calcium, zinc, copper, cadmium, and lead are harmful to the environment [1]. In addition, the two most harmful contaminants are nitrogen and phosphorus [1]. Torrallardona [2] studied about the improvement of precision in nutrient requirements and reported that if nutrients in the feed supplied to animals exceed animal's nutrient requirements, they are not available and excreted. Kerr [3] reported that total nitrogen excretion decreases by 8% for every 1% decrease in nitrogen intake. From an economic and environmental point of view, decreasing crude protein (CP) and supplementing an enzyme cocktail in a diet could be an effective strategy for the pig industry to reduce production cost and pollution [4,5]. However, some studies have reported that low protein diets decrease growth performance on weaned piglets and growing-finishing pigs [6,7]. Exogenous enzymes are expected to solve these problems. Protease is a generic term for an enzyme that breaks down proteins. Supplementation of protease in diets can improve protein utilization in livestock animals [8,9]. Many studies have also shown that protease supplementation in pig diets can improve nutrient digestibility and growth performance [8,10–12].

Therefore, the objective of this study was to evaluate growth performance, nutrient digestibility, and blood profiles of weaned piglets and growing-finishing pigs according to the level of protease supplementation to high or low protein diets.

## MATERIALS AND METHODS

The experimental protocol was approved (CBNUA-1428-20-02) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

### Animals and facilities

A total of 96 weaned pigs ([Yorkshire × Landrace] × Duroc) with an average body weight (BW) of  $6.99 \pm 0.21$  kg were used in a 20-week experiment. The dietary treatments were arranged in a  $2 \times 3$  factorial design with two levels of crude protein (CP) and three levels of protease (PT). Pigs were allotted to one of six dietary treatments in a completely randomized block design based on initial BW. There were four pigs in a pen with four replicate pens for each treatment. Each pen has a single-sided feeder and a nipple drinker. Pigs easily got water and feed *ad libitum*.

### Dietary treatments

Experimental diets (treatments) were corn-soybean meal with different CP and exogenous PT levels. Table 1,2 and 3 showed the nutritional content of the main ingredients used in this experiment. Treatments were as follows: In phase 1 (1–2 weeks), two protein levels as high protein (HP; 19.0%), low protein (LP; 17.0%), and three PT levels (PT0, 0%; PT1, 0.3%; and PT2, 0.5%); in phase 2 (3–4 weeks), two protein levels (HP, 18.05%; LP, 16.15%) and three PT level (0%, 0.3%, and 0.5%); in phase 3 (5–12 weeks), two protein levels (HP, 17.1%; LP, 15.3%) and three PT level (0%, 0.15%, and 0.3%); in phase 4 (13–20 weeks), two protein levels (HP, 16.15%; LP, 14.45%) and three PT level (0%, 0.15%, and 0.3%). PT125TM, an alkaline serine endopeptidase generated by a fermentation progress of a *Streptomyces* bacterial strain at optimal pH of 8.5, was obtained from a commercial company (Eugene-Bio, Suwon, Korea). According to the supplier, PT125TM was depurated from a crude solution created by a *Streptomyces* spp. optimized to manufacture only proteases. All diets in pelleted form were formulated to meet or exceed nutrient requirements for

**Table 1. Chemical composition of the basal weanling diets (as-fed basis)**

Items	Content			
	Phase 1		Phase 2	
	HP	LP	HP	LP
Ingredient (%)				
Corn	366.00	410.82	524.1	564.02
Barely	50.00	50.00	-	-
Soybean meal	263.81	212.43	258.00	209.60
Fish meal	40.00	40.00	20.00	20.00
Soybean oil	38.02	38.33	32.80	33.13
Monocalcium phosphate	7.21	8.16	7.95	8.87
Limestone	11.14	11.13	11.70	13.38
Wheat bran	-	-	50.00	50.00
Sugar	30.00	30.00	20.00	20.00
Vitamin premix <sup>1</sup>	2.50	2.50	2.50	2.50
Mineral premix <sup>2</sup>	2.00	2.00	2.00	2.00
L-Lysine-HCl (78%)	4.80	6.39	6.14	7.64
DL-Methionine (50%)	2.71	2.95	2.74	2.98
L-Threonine (89%)	2.03	2.83	2.35	3.12
L-Tryptophan (10%)	6.83	9.49	7.24	9.76
ZnO	1.20	1.20	1.20	1.20
Salt	1.75	1.77	1.28	1.80
Sweet whey powder	120.00	120.00	50.00	50.00
Lactose	50.00	50.00	-	-
Total	1,000.00	1,000.00	1,000.00	1,000.00

<sup>1</sup>Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D<sub>3</sub>, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg.

<sup>2</sup>Provided per kg of complete diet: copper (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 12 mg; zinc (as ZnSO<sub>4</sub>), 85 mg; manganese (as MnO<sub>2</sub>), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O), 0.15 mg.

HP, high crude protein (19.00% [days 1–14]; 17.00% [days 15–28]); LP, low crude protein (18.05% [days 1–14]; 16.15% [days 15–28]).

pigs [13].

### Data and sample collection

BWs of pigs, amount of feed offered, and amount of remnant feed in each pen were weighed at the initial and end day of each experiment period (weaned, growing, and finishing periods). Growth performance (average daily gain [ADG], average daily feed intake [ADFI], and [G/F]) was measured throughout the experiment. At weeks 4, 12, and 20, each experimental diet was provided, and each contained 0.2% of chromic oxide as an indigestible marker. Fecal samples from randomly selected two pigs per pen were collected by rectal palpation. Diet samples were taken from each of the prepared diets and stored at -20 °C before analysis. Before chemical analysis, fecal samples were unfrozen and desiccated at 70 °C for 75 hours, after that was crushed fine enough to pass through a 1 mm screen. All analysis items (feed and fecal samples) were analyzed for dry matter (DM) and CP according to the AOAC [14] procedure. Chromium was analyzed with an ultraviolet absorption spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. [15]. For the analysis of the serum profile, 5 pigs were randomly selected from each treatment and blood samples were collected by thorough venipuncture at the end of 4 and 20 weeks. At the time of collection, to collect whole blood and serum, blood samples were gathered

**Table 2. Chemical composition of the basal growing diets (as-fed basis)**

Items	Phase 3	
	HP	LP
Ingredient (%)		
Corn	64.95	72.43
Wheat	7.00	5.00
Soybean meal	22.00	17.50
Wheat bran	3.00	2.00
Monocalcium phosphate	1.00	1.00
Limestone	1.00	1.00
Vitamin premix <sup>1</sup>	0.10	0.10
Mineral premix <sup>2</sup>	0.20	0.20
L-Lysine-HCl (78%)	0.30	0.32
DL-Methionine (50%)	0.10	0.10
L-Threonine (89%)	0.20	0.20
Salt	0.15	0.15
Total	100.00	100.00

<sup>1</sup>Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D<sub>3</sub>, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg.

<sup>2</sup>Provided per kg of complete diet: copper (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 12 mg; zinc (as ZnSO<sub>4</sub>), 85 mg; manganese (as MnO<sub>2</sub>), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O), 0.15 mg.

HP, high crude protein (17.3%); LP, low crude protein (15.1%).

**Table 3. Chemical composition of the basal finishing diets (as-fed basis)**

Items	Phase 4	
	HP	LP
Ingredient (%)		
Corn	68.95	76.42
Wheat	5.00	3.00
Soybean meal	20.00	15.60
Wheat bran	3.00	2.00
Monocalcium phosphate	1.00	1.00
Limestone	1.00	1.00
Vitamin premix <sup>1</sup>	0.10	0.10
Mineral premix <sup>2</sup>	0.20	0.20
L-Lysine-HCl (78%)	0.31	0.33
DL-Methionine (50%)	0.10	0.10
L-Threonine (89%)	0.20	0.20
Salt	0.15	0.12
Total	100.00	100.00

<sup>1</sup>Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D<sub>3</sub>, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; and vitamin B<sub>12</sub>, 33 µg.

<sup>2</sup>Provided per kg of complete diet: copper (as CuSO<sub>4</sub>·5H<sub>2</sub>O), 12 mg; zinc (as ZnSO<sub>4</sub>), 85 mg; manganese (as MnO<sub>2</sub>), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O), 0.15 mg.

HP, high crude protein (16.15%); LP, low crude protein (14.45%).

in non-heparinized tubes and vacuum tubes with K3EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), respectively. After collection, serum samples were centrifuged at 3,000×g for 15 min at 4°C. White blood cells (WBC), red blood cells (RBC), blood urea nitrogen (BUN),

and immunoglobulin G (IgG) concentrations in whole blood were measured using an automatic blood analyzer (ADVIA 120, Bayer, Leverkusen, Germany).

### Statistical analysis

Data for effects of different levels of dietary CP added with different levels of protease on digestibility of DM, CP, growth performance, and blood profiles of weaned piglets and growing-finishing pigs were subjected to two-way ANOVA, with the protein level, the protease addition level, and their interactions as main effects and litter as a covariate. All data were statistically analyzed with PROC General Linear Models (GLM) of SAS (SAS Institute, Cary, NC, USA). Differences between treatment groups were measured using Tukey's honest significant difference (HSD) test with a  $p$ -value of less than 0.05 designating statistical significance.

## RESULTS

### Growth performance

Growth performance data are shown in Table 4. BWs were higher ( $p < 0.05$ ) for the PT2 group of pigs at 4 weeks and 20 weeks than those for the PT0 group of pigs. From weeks 0 to 4, ADG and G/F ratio were higher ( $p < 0.001$  and  $p = 0.009$ ;  $p = 0.004$  and  $p = 0.033$ , respectively) for PT2 group of pigs than for PT0 and PT1 groups of pigs. From weeks 16 to 20, ADG and G/F ratio were higher ( $p < 0.001$  and  $p = 0.009$ ;  $p = 0.004$  and  $p = 0.033$ , respectively) for the PT2 group of pigs than for the PT0 and PT1 groups of pigs. Throughout the experiment, from weeks 0 to 20, ADG and G/F ratio were higher ( $p = 0.044$  and  $p = 0.049$ , respectively) for the PT2 group of pigs than for the PT0 group. There was no significant ( $p > 0.05$ ) difference in growth performance between groups with different CP levels in diets. For growth performance data, there was no interaction between CP level and protease supplement level.

### Nutrient digestibility

Nutrient digestibility data are shown in Table 5. DM digestibility was not significantly ( $p > 0.050$ ) affected by CP level or protease supplemented level at week 4, 12, or 20. CP digestibility was higher ( $p = 0.013$ ,  $p = 0.014$ , and  $p = 0.035$ , respectively) in LP group than for HP group at weeks 4, 12, and 20. Protease supplementation did not significantly affect CP digestibility. There was no interaction between CP level and protease supplement level.

### Blood profiles

Results of blood profiles are shown in Table 6. At weeks 4 and 20, LP diet groups had lower BUN levels than HP diet groups ( $p < 0.001$  and  $p = 0.001$ , respectively). WBC, RBC, and IgG were not significantly ( $p > 0.050$ ) affected by CP level or protease supplement level at weeks 4 and 20. There was no interaction between CP level and protease supplement level.

## DISCUSSION

Due to the importance of environmental issues, the pig industry is also subjected to be inspected based on reducing environmental pollution. One of such efforts is to reduce the amount of nitrogen in feed. Although limiting nitrogen in feed is important, maintaining productivity is also essential. Diets designed to reduce pigs' nitrogen excretion will only be acceptable to the pig industry if they can maintain pig performance [16]. In this study, the effects of LP and HP diets on growth performance showed no significant difference throughout the experiment, similar to findings of

**Table 4.** Effects of crude protein level with protease supplementation level on growth performance in weaned piglets and growing-finishing pigs

Item	Main effects					SE	p-value		
	Protein level		Protease level				CP	Protease	CP × protease
	HP	LP	PT0	PT1	PT2				
Body weight (kg)									
Initial	7.0	7.0	7.0	7.0	7.0	0.1	0.688	0.977	0.997
4 wk	15.7	15.7	15.3 <sup>a</sup>	15.4 <sup>a</sup>	16.5 <sup>b</sup>	0.2	0.944	0.010	0.941
8 wk	32.2	32.3	31.9	32.0	32.8	0.4	0.875	0.695	0.803
12 wk	57.8	57.1	57.6	56.8	58.0	0.7	0.662	0.786	0.706
16 wk	88.4	88.4	88.4	88.1	88.6	0.9	0.980	0.974	0.958
20 wk	114.2	113.6	111.9 <sup>a</sup>	113.2 <sup>ab</sup>	116.6 <sup>b</sup>	0.8	0.730	0.049	0.987
Weeks 0–4									
ADG (g)	310	313	296 <sup>a</sup>	300 <sup>a</sup>	340 <sup>b</sup>	6	0.779	0.003	0.917
ADFI (g)	435	428	420	421	453	7	0.592	0.082	0.895
G/F	0.71	0.73	0.70 <sup>a</sup>	0.71 <sup>a</sup>	0.75 <sup>b</sup>	0.01	0.168	0.011	0.981
Weeks 4–8									
ADG (g)	587	592	594	594	581	12	0.860	0.885	0.785
ADFI (g)	1,130	1,147	1,161	1,159	1,096	25	0.753	0.517	0.793
G/F	0.52	0.52	0.51	0.52	0.53	0.01	0.890	0.615	0.958
Weeks 8–12									
ADG (g)	914	887	917	885	900	19	0.483	0.802	0.293
ADFI (g)	2,104	2,124	2,164	2,113	2,066	34	0.779	0.519	0.411
G/F	0.44	0.42	0.42	0.42	0.44	0.01	0.248	0.515	0.677
Weeks 12–16									
ADG (g)	1,094	1,115	1,101	1,118	1,095	24	0.679	0.926	0.790
ADFI (g)	2,867	2,927	2,914	2,891	2,886	35	0.406	0.941	0.607
G/F	0.38	0.38	0.38	0.39	0.38	0.01	0.855	0.861	0.889
Weeks 16–20									
ADG (g)	921	903	840 <sup>a</sup>	799 <sup>a</sup>	1,000 <sup>b</sup>	15	0.472	< 0.001	0.667
ADFI (g)	3,140	3,016	2,997	3,081	3,157	33	0.062	0.141	0.974
G/F	0.29	0.30	0.28 <sup>a</sup>	0.29 <sup>a</sup>	0.32 <sup>b</sup>	0.01	0.534	0.011	0.846
Weeks 0–8									
ADG (g)	449	453	445	447	460	7	0.813	0.673	0.773
ADFI (g)	783	787	790	790	775	13	0.867	0.868	0.748
G/F	0.58	0.58	0.57	0.57	0.60	0.01	0.886	0.134	0.989
Weeks 8–16									
ADG (g)	1,004	1,001	1,009	1,002	997	13	0.898	0.940	0.746
ADFI (g)	2,486	2,525	2,539	2,502	2,476	22	0.377	0.512	0.314
G/F	0.40	0.58	0.40	0.40	0.40	0.01	0.426	0.888	0.868
Weeks 0–20									
ADG (g)	806	802	789 <sup>a</sup>	799 <sup>ab</sup>	824 <sup>b</sup>	6	0.750	0.044	0.987
ADFI (g)	1,935	1,928	1,931	1,933	1,932	10	0.744	0.997	0.697
G/F	0.42	0.42	0.41 <sup>a</sup>	0.41 <sup>ab</sup>	0.43 <sup>b</sup>	0.01	0.889	0.049	0.843

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

<sup>a,b</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).

HP, high crude protein (19.00% [days 1–14 phase], 18.05% [days 15–28 phase], 17.10% [growing phase] and 16.15% [finishing phase]); LP, low crude protein (17.00% [days 1–14], 16.15% [days 15–28], 15.30% [growing phase] and 14.45% [finishing phase]); PT0, protease 0 ppm (weanling-finishing phase); PT1, protease 300 ppm (weanling phase) and 150 ppm (growing-finishing phase); PT2, protease 500 ppm (weanling phase) and 300 ppm (growing-finishing phase); CP, crude protein; ADG, average daily gain; ADFI, average daily feed intake; G/F, feed efficiency.

**Table 5.** Effects of crude protein level and supplementation protease level on digestibility of nutrients in weaned piglets and growing-finishing pigs

Item	Main effects					SE	p-value		
	Protein level		Protease level				CP	Protease	CP × protease
	HP	LP	PT0	PT1	PT2				
Week 4									
DM	78.58	78.58	78.98	78.64	78.12	0.26	0.992	0.453	0.734
CP	72.42	74.21	73.53	73.76	72.66	0.36	0.013	0.362	0.794
Week 12									
DM	80.66	81.24	81.17	81.01	80.66	0.17	0.095	0.440	0.661
CP	75.95	77.45	76.51	76.88	76.71	0.29	0.014	0.858	0.938
Week 20									
DM	82.63	82.77	82.63	83.00	82.48	0.16	0.664	0.432	0.736
CP	79.15	80.69	79.79	80.41	79.56	0.37	0.035	0.575	0.295

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

HP, high crude protein (19.00% [days 1–14 phase], 18.05% [days 15–28 phase], 17.10% [growing phase] and 16.15% [finishing phase]); LP, low crude protein (17.00% [days 1–14], 16.15% [days 15–28], 15.30% [growing phase] and 14.45% [finishing phase]); PT0, protease 0 ppm (weanling-finishing phase); PT1, protease 300 ppm (weanling phase) and 150 ppm (growing-finishing phase); PT2, protease 500 ppm (weanling phase) and 300 ppm (growing-finishing phase); CP, crude protein; DM, dry matter.

**Table 6.** Effects of crude protein level and supplementation protease level on blood profiles in weaned piglets and finishing pigs

Item	Main effects					SE	p-value		
	Protein level		Protease level				CP	Protease	CP × protease
	HP	LP	PT0	PT1	PT2				
Weeks 4									
WBC ( $10^3$ per $\mu$ L)	17.19	17.36	17.58	17.07	17.19	0.14	0.588	0.362	0.591
RBC ( $10^6$ per $\mu$ L)	7.17	7.08	7.19	7.12	7.06	0.12	0.758	0.929	0.975
BUN (mg/dL)	14.5	8.6	11.6	11.3	10.9	0.7	< 0.001	0.268	0.746
IgG (mg/dL)	319	327	313	325	329	4	0.318	0.235	0.681
Weeks 20									
WBC ( $10^3$ per $\mu$ L)	21.13	21.25	21.31	20.77	21.49	0.53	0.915	0.866	0.500
RBC ( $10^6$ per $\mu$ L)	7.45	7.63	7.13	7.79	7.70	0.12	0.758	0.063	0.930
BUN (mg/dL)	12.5	9.8	11.9	11.3	10.4	0.4	0.001	0.204	0.895
IgG (mg/dL)	307	318	308	308	321	6	0.394	0.605	0.688

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

HP, high crude protein (19.00% [days 1–14 phase], 18.05% [days 15–28 phase], 17.10% [growing phase] and 16.15% [finishing phase]); LP, low crude protein (17.00% [days 1–14], 16.15% [days 15–28], 15.30% [growing phase] and 14.45% [finishing phase]); PT0, protease 0 ppm (weanling-finishing phase); PT1, protease 300 ppm (weanling phase) and 150 ppm (growing-finishing phase); PT2, protease 500 ppm (weanling phase) and 300 ppm (growing-finishing phase); CP, crude protein; WBC, white blood cells; RBC, red blood cells; BUN, blood urea nitrogen; IgG, immunoglobulin G.

previous studies [17,18]. Over the past 10 years, the genetic potential of pigs has been improved dramatically, very different from rates of growth and protein deposition in pigs in experiments used to create NRC [13] requirements. These results suggested that nitrogen content in feed is measured more than necessary and that sufficient growth performance can be guaranteed even with low protein content. Pigs diets supplemented with protease showed higher growth performance than those fed with protease-free diets. In the current study, phase of weaned piglet, the greater growth performance in protease supplemented diet was in agreement with previous studies [12,19]. It was reported that the activity of digestive enzymes in gastrointestinal and pancreatic tissues decreases rapidly after weaning [20]. Therefore, adding proteases to weaned piglets diet can help them digest certain types of proteins that are resistant to pig digestive enzymes and neutralize protease inhibitors, thus improving nutrient digestibility and growth performance [21]. At weeks 16 to 20

in present study, better growth performance was shown in protease supplemented diets, the same context has been recently reported [8,22,23]. During the entire period of the experiment from week 0 to 20, the addition of protease resulted in significant increases of growth performance especially ADG and G/F. Thus, the addition of protease had a positive effect on growth performance.

Protease addition is expected to increased digestibility of DM and CP by breaking down protein molecules that are not well decomposed. However, in the present study, protein level and protease supplementation had no significant effect on DM digestibility or CP digestibility. Contrary to this experiment, previous studies have shown that the addition of protease can increase nutrient digestibility [12,24,25].

Pigs fed with LP diets showed significantly higher CP digestibility than pigs fed with HP diets during the whole growth section. Le Bellego [26] also reported that a 6.5-point reduction for protein content in the diet resulted in a 60% reduction in nitrogen emission with the same nitrogen retention level. Therefore, high protein diets might lead to discharge of a high amount of protein without being sufficiently utilized during digestion process. It has been shown that low protein diets are superior in usability and environmental aspects than general high protein diets currently in use.

Results of blood profiles revealed that WBC, RBC, and IgG were not affected by CP or protease level. However, BUN levels were significantly lower in groups fed with LP than in groups fed with HP at weeks 4 and 20, consistent with the results of Chen et al. [27] and Gó mez et al. [6]. They reported that there was a positive relationship between CP concentration and serum urea concentration, indicating that excess dietary nitrogen intake decreased. Blood or plasma urea nitrogen concentration can be useful indicators for formulating diet or for identifying feeding programs and nitrogen utilization problems. It can be used as an indicator of protein status in animal treatment [28,29]. There was a significant negative correlation between BUN content and protein or amino acid utilization [30]. This seems to be due to the fact that when CP content in feed is low, the digestibility of nitrogen is high, so the amount discharged is reduced.

## CONCLUSION

Results of this experiment showed that a low CP diet with added protease could increase growth performance and CP digestibility of weaned piglets to finishing pigs.

## REFERENCES

1. Jongbloed AW, Lenis NP. Excretion of nitrogen and some minerals by livestock. Wageningen, Netherlands: EAAP Publication; 1993. p. 22-36.
2. Torrallardona D. Reduction of nitrogen excretion in pigs. Improvement of precision in nutrient requirements. *Cah Opt Mediterr*. 1999;37:265-74.
3. Kerr BJ. Dietary manipulation to reduce environmental impact. Paper presented at: 9th International Symposium on Digestive Physiology in Pigs; 2003; Banff, AI, Canada. p. 139-58.
4. Kendall DC, Gaines AM, Kerr BJ, Allee GL. True ileal digestible tryptophan to lysine ratios in ninety-to one hundred twenty-five-kilogram barrows. *J Anim Sci*. 2007;85:3004-12. <https://doi.org/10.2527/jas.2007-0013>
5. Chen HY, Yi XW, Zhang G, Lu N, Chu LC, Thacker PA, et al. Studies on reducing nitrogen excretion: i. net energy requirement of finishing pigs maximizing performance and carcass quality fed low crude protein diets supplemented with crystalline amino acids. *J Anim Sci Biotechnol*. 2011;2:84-93.



6. Gómez RS, Lewis AJ, Miller PS, Chen HY. Growth performance, diet apparent digestibility, and plasma metabolite concentrations of barrows fed corn-soybean meal diets or low-protein, amino acid-supplemented diets at different feeding level. *J Anim Sci.* 2002;80:644-53. <https://doi.org/10.2527/2002.803644x>
7. Zhang S, Qiao S, Ren M, Zeng X, Ma X, Wu Z, et al. Supplementation with branched-chain amino acids to a low-protein diet regulates intestinal expression of amino acid and peptide transporters in weanling pigs. *Amino Acids.* 2013;45:1191-205. <https://doi.org/10.1007/s00726-013-1577-y>
8. Guggenbuhl P, Waché Y, Wilson JW. Effects of dietary supplementation with a protease on the apparent ileal digestibility of the weaned piglet. *J Anim Sci.* 2012;90 suppl 4:152-4. <https://doi.org/10.2527/jas.53835>
9. Adebisi AO, Olukosi OA. Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without a mixture of carbohydrases and protease for broilers and turkeys. *Poult Sci.* 2015;94:1270-6. <https://doi.org/10.3382/ps/pev089>
10. Omogbenigun FO, Nyachoti CM, Slominski BA. Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. *J Anim Sci.* 2004;82:1053-61. <https://doi.org/10.1093/ansci/82.4.1053>
11. 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. <https://doi.org/10.2527/jas.2007-0262>
12. 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. <https://doi.org/10.1016/j.aninu.2015.10.003>
13. NRC [National Research Council]. Nutrient requirement of pigs. 11th ed. Washington, DC: The National Academy Press; 2012.
14. AOAC [Association of Official Analytical Chemists] International. Official method of analysis of AOAC International. 16th ed. Arlington, VA: AOAC International; 1995.
15. Williams CH, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J Agric Sci.* 1962;59:381-5. <https://doi.org/10.1017/S002185960001546X>
16. Kay RM, Lee PA. The performance of growing and finishing pigs offered diets formulated to reduce nitrogen excretion. In: Proceedings of the British Society of Animal Science; 1996; Cambridge, UK.
17. Reyna L, Figueroa JL, Zamora V, Cordero JL, Sánchez-Torres MT, Cuca M. Addition of protease to standard diet or low protein, amino acid-supplemented, sorghum-soybean meal diets for growing-finishing pigs. *J Anim Vet Adv.* 2006;5:1202-8.
18. Zhao Y, Tian G, Chen D, Zheng P, Yu J, He J, et al. Effect of different dietary protein levels and amino acids supplementation patterns on growth performance, carcass characteristics and nitrogen excretion in growing-finishing pigs. *J Anim Sci Biotechnol.* 2019;10:75. <https://doi.org/10.1186/s40104-019-0381-2>
19. 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. <https://doi.org/10.5187/jast.2020.62.1.21>
20. 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. <https://doi.org/10.1080/0039420310001656677>

21. 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. <https://doi.org/10.4314/sajas.v47i5.13>
22. Jo JK, Ingale SL, Kim JS, Kim YW, Kim KH, Lohakare JD, et al. Effects of exogenous enzyme supplementation to corn-and soybean meal-based or complex diets on growth performance, nutrient digestibility, and blood metabolites in growing pigs. *J Anim Sci.* 2012;90:3041-8. <https://doi.org/10.2527/jas.2010-3430>
23. Murugesan GR, Romero LF, Persia ME. Effects of protease, phytase and a *Bacillus* sp. Direct-fed microbial on nutrient and energy digestibility, ileal brush border digestive enzyme activity and cecal short-chain fatty acid concentration in broiler chickens. *PLOS ONE.* 2014;9:e101888. <https://doi.org/10.1371/journal.pone.0101888>
24. 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 Technol.* 2014;189:88-97. <https://doi.org/10.1016/j.anifeedsci.2013.11.012>
25. Pan L, Shang QH, Ma XK, Wu Y, Long SF, Wang QQ, et al. Coated compound proteases improve nitrogen utilization by decreasing manure nitrogen output for growing pigs fed sorghum soybean meal-based diets. *Anim Feed Sci Technol.* 2017;230:136-42. <https://doi.org/10.1016/j.anifeedsci.2017.05.014>
26. Le Bellego L, Noblet J, van Milgen J. Effects of dietary crude protein level and meal frequency on energy utilization in growing pigs. *Cah Opt Mediterr.* 2002;16:78-80.
27. Chen HY, Lewis AJ, Miller PS, Yen JT. The effect of excess protein on growth performance and protein metabolism of finishing barrows and gilts. *J Anim Sci.* 1999;77:3238-47. <https://doi.org/10.2527/1999.77123238x>
28. Whang KY, Kim SW, Donovan SM, McKeith FK, Easter RA. Effects of protein deprivation on subsequent growth performance, gain of body components, and protein requirements in growing pigs. *J Anim Sci.* 2003;81:705-16. <https://doi.org/10.2527/2003.813705x>
29. Kohn RA, Dinneen MM, Russek-Cohen E. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *J Anim Sci.* 2005;83:879-89. <https://doi.org/10.2527/2005.834879x>
30. Coma J, Zimmerman DR, Carrion D. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. *J Anim Sci.* 1995;73:3649-56. <https://doi.org/10.2527/1995.73123649x>