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Article Title (within 20 words without abbreviations)	Effect of low protein diets added with protease on growth performance, nutrient digestibility of weaned piglets and growing-finishing pigs
Running Title	Effects of low protein diets added with protease on pigs
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Competing interests	The authors declare no conflict of interest.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	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 Please specify the authors' role using this form.	# These authors contributed equally to this work. Conceptualization: Yong Ju Kim, Ji Hwan Lee, Min Ho Song ,Tae Heon Kim Data curation: Yong Ju Kim, Ji Hwan Lee Formal analysis: Yong Ju Kim, Ji Hwan Lee Investigation: Won Yun, Ji Hwan Lee, Han Jin Oh, Jun Soeng Lee, Tae Heon Kim, Writing - original draft: Yong Ju Kim, Tae Heon Kim Writing - review & editing: Jin Ho Cho, Hyeun Bum Kim
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.

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

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), two protein levels (HP, 18.05%; LP, 16.15%) and three protease level (0, 0.3, and 0.5%); in phase 3 (4-12 weeks), two protein levels (HP, 17.1%; LP, 15.3%) and three protease level (0, 0.15, and 0.3%); in phase 4 (13-20 weeks), two protein levels (HP, 16.15%; LP, 14.45%) and three protease level (0, 0.15, and 0.3%). Pigs were allotted to one of six dietary treatment groups in a completely randomized block design based on initial BW. There were four pigs in a pen with four replicate pens for each treatment. At 4 weeks and 20 weeks after treatment, BW was higher ($p < 0.05$) in the PT2 group than PT0 group. From weeks 0 to 4, average daily gain (ADG) and feed efficiency (G/F) ratio 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 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) in the PT2 group than 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) in the PT2 group than PT0 group of pigs. CP digestibility was higher in the low protein (LP) group of pigs than high protein (HP) group at weeks 4, 12, and 20 ($p = 0.013$, $p = 0.014$, and $p = 0.035$, respectively). Supplementation with protease did not significantly affect CP digestibility. At weeks 4 and 20, the LP diet group of pigs had lower blood urea nitrogen (BUN) levels than the HP diet group of pigs ($p < 0.001$ and $p = 0.001$, respectively). 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

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 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 of 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 (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 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. 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), two protein levels (HP, 18.05%; LP, 16.15%) and three protease level (0, 0.3, and 0.5%); in phase 3 (4-12 weeks), two protein levels (HP, 17.1%; LP, 15.3%) and three protease level (0, 0.15, and 0.3%); in phase 4 (13-20 weeks), two protein levels (HP, 16.15%; LP, 14.45%) and three protease 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, Youngtong-Ku, Suwon city, Gyeonggi-do, Korea). According to the supplier, protease 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 pigs [13].

Data and sample collection

BWs of pigs, amount of feed offered, and amount of remanent feed in each pen were weighed at the initial day and end of each experiment period (weaned, growing, and finishing periods). Growth performance [average daily gain (ADG), average daily feed intake (ADFI), and feed

efficiency (G:F, gain to feed ratio)] 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 (Shimadzu, 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 in non-heparinized tubes and vacuum tubes with K3EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), respectively. After collection, serum samples were centrifuged at 3000g 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, NY, USA).

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 Inst, 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 affected by CP level or protease supplemented level at week 4, 12, or 20. CP digestibility was higher in LP group than for HP group at weeks 4, 12, and 20 ($p = 0.013$, $p = 0.014$, and $p = 0.035$, respectively). Protease supplementation did not significantly affect CP digestibility. There was no interaction effect between CP level and protease supplement level on nutrient digestibility.

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 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 accepted by 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 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 fed with 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 week 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 increase 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. 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 Gomez 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.

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

Items	Content			
	Days 1-14		Days 15-28	
	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 ¹⁾	2.50	2.50	2.50	2.50
Mineral premix ²⁾	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	1000.00	1000.00	1000.00	1000.00

¹⁾ Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D₃, 1103 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 B₁₂, 33 µg.

²⁾ Provided per kg of complete diet: copper (as CuSO₄·5H₂O), 12 mg; zinc (as ZnSO₄), 85 mg; manganese (as MnO₂), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na₂SeO₃·5H₂O), 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)).

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

Items	Content	
	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 ¹⁾	0.10	0.10
Mineral premix ²⁾	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

¹⁾Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D₃, 1103 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 B₁₂, 33 µg.

²⁾Provided per kg of complete diet: copper (as CuSO₄·5H₂O), 12 mg; zinc (as ZnSO₄), 85 mg; manganese (as MnO₂), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na₂SeO₃·5H₂O), 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	Content	
	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 ¹⁾	0.10	0.10
Mineral premix ²⁾	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

¹⁾Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D₃, 1103 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 B₁₂, 33 µg.

²⁾Provided per kg of complete diet: copper (as CuSO₄·5H₂O), 12 mg; zinc (as ZnSO₄), 85 mg; manganese (as MnO₂), 8 mg; iodine (as KI), 0.28 mg; and selenium (as Na₂SeO₃·5H₂O), 0.15 mg.

HP, High Crude Protein (16.15%); LP, Low Crude Protein (14.45%).

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 ^a	7.0 ^a	7.0 ^b	0.1	0.688	0.977	0.997
4 wk	15.7	15.7	15.3	15.4	16.5	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 ^a	113.2 ^{ab}	116.6 ^b	0.8	0.730	0.049	0.987
Weeks 0-4									
ADG (g)	310	313	296 ^a	300 ^a	340 ^b	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 ^a	0.71 ^a	0.75 ^b	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)	1130	1147	1161	1159	1096	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)	2104	2124	2164	2113	2066	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)	1094	1115	1101	1118	1095	24	0.679	0.926	0.790
ADFI (g)	2867	2927	2914	2891	2886	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 ^a	799 ^a	1000 ^b	15	0.472	< 0.001	0.667
ADFI (g)	3140	3016	2997	3081	3157	33	0.062	0.141	0.974
G/F	0.29	0.30	0.28 ^a	0.29 ^a	0.32 ^b	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)	1004	1001	1009	1002	997	13	0.898	0.940	0.746
ADFI (g)	2486	2525	2539	2502	2476	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 ^a	799 ^{ab}	824 ^b	6	0.750	0.044	0.987
ADFI (g)	1935	1928	1931	1933	1932	10	0.744	0.997	0.697
G/F	0.42	0.42	0.41 ^a	0.41 ^{ab}	0.43 ^b	0.01	0.889	0.049	0.843

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

^{a,b}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 0ppm (weanling-finishing phase); PT1, protease 300ppm (weanling phase) and 150ppm (growing-finishing phase); PT2, protease 500ppm (weanling phase) and 300ppm (growing-finishing phase); ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency

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

Running page

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 0ppm (weanling-finishing phase); PT1, protease 300ppm (weanling phase) and 150ppm (growing-finishing phase); PT2, protease 500ppm (weanling phase) and 300ppm (growing-finishing phase); DM, dry matter; CP, crude protein

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 μL)	17.19	17.36	17.58	17.07	17.19	0.14	0.588	0.362	0.591
RBC (10 ⁶ per μL)	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 μL)	21.13	21.25	21.31	20.77	21.49	0.53	0.915	0.866	0.500
RBC (10 ⁶ per μL)	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 0ppm (weanling-finishing phase); PT1, protease 300ppm (weanling phase) and 150ppm (growing-finishing phase); PT2, protease 500ppm (weanling phase) and 300ppm (growing-finishing phase)