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

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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
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 ± 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. 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 process 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...
Data and sample collection

BW 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 ℃ before analysis. Before chemical analysis, fecal samples were unfrozen and desiccated at 70 ℃ 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 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 D₃, 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 B₁₂, 33 μg.

<sup>2</sup>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]).
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),

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

| Ingredients       | Phase 3 |   | 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 premix1   | 0.10   |   | 0.10    |   |
| Mineral premix2   | 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  |   |

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

2Provided 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)

| Ingredients       | Phase 4 |   | 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 premix1   | 0.10    |   | 0.10    |   |
| Mineral premix2   | 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  |   |

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

2Provided 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%).
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
Effects of low protein diets added with protease on pigs

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

| Item                      | Protein level       | Main effects | Protease level | SE | p-value |
|---------------------------|---------------------|--------------|----------------|----|---------|
|                           | HP (g) LP (g)       | PT0 (g)      | PT1 (g)        | PT2 (g) | CP | Protease | CP × protease |
| Body weight (kg)          |                     |              |                |     |         |          |               |
| Initial                   | 7.0                 | 7.0          | 7.0            | 7.0 | 0.1     | 0.888    | 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>ab</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>a</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>a</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>a</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>a</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>a</sup> | 0.01    | 0.889    | 0.049         | 0.843         |

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

<sup>a</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.
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

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

| Item | Protein level | Main effects | SE | p-value | p-value | p-value |
|------|---------------|--------------|----|---------|---------|---------|
|      | HP | LP | PT0 | PT1 | PT2 | CP | Protease | CP × protease |
| 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 | Protein level | Main effects | SE | p-value | p-value | p-value |
|------|---------------|--------------|----|---------|---------|---------|
|      | HP | LP | PT0 | PT1 | PT2 | CP | Protease | CP × protease |
| 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 | 0.663 | 0.930 |
|      | BUN (mg/dL) | 12.5 | 9.9 | 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.
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 Gómez 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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