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Effects of different levels of crude protein and protease on nitrogen utilization, nutrient digestibility, and growth performance in growing pigs

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Abstract

This study was conducted to evaluate the effects of different levels of crude protein (CP) and protease on nitrogen (N) utilization, nutrient digestibility, and growth performance in growing pigs. A total of six crossbred ([Landrace × Yorkshire] × Duroc) barrows were individually accepted in 1.2 m × 0.7 m × 0.96 m stainless steel metabolism cages. The pigs (average initial body weight of 27.91 ± 1.84 kg) randomly assigned to six diets with six weeks (6 × 6 Latin square design). The experiment was carried out in an environment with a temperature of 23 ± 1.5℃, a relative humidity of 83 ± 2.3% and a wind speed of 0.25 ± 0.03 m/s. The dietary treatments were arranged in a 2 × 3 factorial design with two levels of CP (15.3% or 17.1%) and three levels of protease (0 ppm, 150 ppm, or 300 ppm). The average daily gain and gain to feed ratio (G:F) tended to increase ($p = 0.074$) with increasing amounts of protease. The low CP level diet reduced ($p < 0.050$) urinary and fecal N concentrations, the total N excretion in feces, and increased ($p < 0.050$) N retention. Different protease levels in the diet did not affect ($p > 0.05$) at N intake, but supplementation of the diets with 300 ppm protease decreased ($p < 0.050$) the N concentration in urine and feces and tended to increase ($p = 0.061$) the percentage of N retention retained of the total N intake. The dietary CP level did not affect ($p > 0.050$) the apparent total tract digestibility (ATTD) of dry matter, digestible energy (DE), and metabolic energy (ME), but diet supplementation with 300 ppm protease showed higher ($p < 0.050$) ATTD of DE and ME than in the protease-free diet. Therefore, a low protein diet with protease could improve the utilization of nitrogen, thereby reducing the negative effect of N excretion into the environment while maintaining or increasing growth performance compared to a high protein diet.

Keywords: Protein, Protease, Nitrogen, Digestibility, Growing pigs

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

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

Authors' contributions
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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 concentration of dietary crude protein (CP) has been closely related to growth performance. However, undigested CP in pig diets causes environmental pollution and odor. A prior study reported that when the dietary protein content increased above a certain level, undigested protein increased, resulting in increased intestinal pathogenic microorganisms (*Escherichia coli, Clostridium,* and *Enterobacteriaceae*) and decreased numbers of beneficial *Lactobacillus* bacteria [1]. According to the US Environmental Protection Agency (US EPA, 2004), ammonia released during animal production constituted about 50% of the total anthropogenic ammonia emissions, causing eutrophication, soil acidification, and impaired visibility [2]. Pigs fed a high CP diet had higher urinary energy excretion and reduced on energy retention and the efficiency [3].

Many researchers have reported that lower CP with added crystalline amino acids-maintained growth performance and reduced nitrogen excretion in growing to finishing pigs [4–6]. The addition of crystalline amino acids to 12% CP diets for growing pig showed equal performance, and reduced nitrogen (N) emissions and cost compared to pigs fed a 16% CP diet [7,8].

Protease has been used in swine diets as part of enzyme cocktails [9]. Various studies have shown that dietary supplementation with protease had positive effects on nutrient digestibility and growth performance in weaning and growing pigs [9–14]. Also, protease has been available commercially and shown beneficial effects on nutrient digestibility and the growth performance of pigs [9,15–18].

Lower CP diets with protease supplementation are expected to show positive effects on the growth performance, nitrogen emission, and energy metabolism of pigs. However, it is not known how much protease supplementation is appropriate in low CP diets. Therefore, the main purpose of this experiment was to evaluate the effects of different levels of CP and protease on nitrogen utilization, nutrient digestibility, and growth performance in growing pigs.

MATERIALS AND METHODS

Experiment design and housing

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

A total of six crossbred ([Landrace × Yorkshire] × Duroc) barrows were individually accepted in 1.2 m × 0.7 m × 0.96 m stainless steel metabolism cages. The pigs (average initial body weight of 27.91 ± 1.84 kg) randomly assigned to six diets with six weeks (6 × 6 Latin square design). The experiment was carried out in an environment with a temperature of 23 ± 1.5℃, a relative humidity of 83 ± 2.3% and a wind speed of 0.25 ± 0.03 m/s.

Diets and feeding

The diets were adapted to exceed or meet the NRC [19] nutritional requirements for pigs. Table 1 shows the nutritional content of the main ingredients used in this experiment. The dietary treatments were arranged in a 2 × 3 factorial design with two levels of CP (15.3% or 17.1%) and three levels of protease (0 ppm, 150 ppm, or 300 ppm). The PT125TM a protease enzyme was supported by a commercial company (Eugene-Bio, Suwon, Korea). According to the supplier, protease PT125TM, an alkaline serine endopeptidase produced by a fermentation process by a *Streptomyces* bacterial strain at optimal pH 8.5, was purified from a crude solution produced by a *Streptomyces* spp. optimized to produce only proteases. The experiment was conducted for six weeks. The daily feed allowance was arranged to 2.7 times the requirement to maintain digestible energy (DE, 2.7 × 110 kcal of DE/kg $BW^{0.75}$) [19]. The daily diet was distributed in half and fed at 8:00 and 17:00 h.

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Table 1. Chemical composition of the basal 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	100

[1)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 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_{12}, 33 μg.

[2)]Provided per kg of complete diet: copper (as $CuSO_4 \cdot 5H_2O$), 12 mg; zinc (as $ZnSO_4$), 85 mg; manganese (as MnO_2), 8 mg; iodine (as KI), 0.28 mg; and selenium (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

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

Feed was always mixed with water in a 1 to 1 ration. During the experiment, pigs were freely supplied with water.

Sampling and analysis

Beginning of each week pigs were weighed individually, and daily feed supply and residual feed quantity were recorded. Each week, the experiment consisted of 4 days of adaptation period and 3 days of collection period to collect urine and feces. The total feces were immediately packaged in plastic bags when they were produced in the metabolism cages, and stored at −20℃ during experiment period. Urine was collected once a day on to buckets that filled with 50 mL of 6 mol/L HCl that under the metabolic cages. The total collected urine was weighed and stored at −20℃. Feces and urine collection were performed according to the method described in Song et al. [20]. The fecal sample was dried in a forced-air oven and then crushed on a 1-mm screen and thoroughly melded before sub-sample collection for chemical analysis. The gross energy of urine, feces and diet was analyzed by an adiabatic oxygen bomb calorimeter (Par Instruments, Moline, Il, USA). The nitrogen content in feces and urine was also analyzed [21]. The calculations of DE and metabolizable energy were conducted in the manner described by Lammers et al. [22].

Calculations

DE was calculated by subtracting the GE in the feces from the dietary GE. The DE calculated from dietary chemical composition (Eq. 1). The metabolic energy (ME) was calculated directly from the nutritional composition and the DE (Eq. 2).

$$DE = 1{,}161 + (0.749 \times GE) - (4.3 \times Ash) - (4.1 \times NDF) \quad \text{(Eq. 1) [23]}$$

$$ME = (1.00 \times DE) - (0.68 \times CP) \quad \text{(Eq. 2) [23]}$$

Statistical analysis

The data for the effects of different level of dietary CP with different level of protease supplementation on the apparent total tract digestibility (ATTD) of nitrogen, energy and growth performance in growing pigs were subjected to two-way ANOVA, with addition levels, types and their interactions as main effects and litter as covariate. The data were statistically analyzed by the generalized linear moder (GLM) procedure in IBM SPSS statistics v.25 (SPSS, Chicago, IL, USA). Each cage was used for each experimental unit. Differences between treatment groups was determined using Tukey's honest significant difference (HSD) test with a p-value of < 0.05 indicating significance.

RESULT

Growth performance

The growth performance data are shown in Table 2. Average daily gain (ADG) and gain to feed ratio (G:F) tended to increase ($p = 0.074$) with increasing dietary protease supplementation, but the dietary CP levels did not affect ($p > 0.050$) pig performance.

Nitrogen utilization

The N utilization data are shown in Table 3. The low CP level diet reduced ($p < 0.050$) the urine and fecal N concentration, fecal N excretion, and total N excretion, and increased ($p < 0.050$) N retention. Diet supplementation with protease did not affect N intake but decreased ($p < 0.050$) urine and fecal N concentration and tended to increase ($p = 0.061$) the proportion of N retained from the total N intake. However, there was no significant difference ($p > 0.050$) in nitrogen utilization in the interaction of CP and protease levels in the diets. Also, the biological values were not affected by the effects of CP, protease levels, and their interaction.

Nutrient digestibility

The apparent ATTD of the nutrients is shown in Table 4. The CP level in the diet did not affect ($p > 0.050$) the ATTD of DM, DE, and ME. Supplementation with 300 ppm protease showed higher ($p < 0.050$) DE and ME than the protease-free diet.

DISCUSSION

The need to reduce N excretion has become a very important topic in the pig industry. Urinary and fecal N excretion occupy the largest proportion of N excretion in the animal industry. Pfeiffer et al. [24] reported strong correlation was observed between an increase in CP intake and an increase in N content in the urine also excessive protein supply as well as excess amino acid is a source of large amounts of excreted urea and is responsible for low nitrogen absorption coefficient. N content in feces was also reported to be lower in low protein diets than in high protein diets. Consuming a low

Table 2. Effects of dietary crude protein level with protease supplementation on growth performance in growing pigs

Item	HP			LP			SE	p-value		
	PT 0	PT 150	PT 300	PT 0	PT 150	PT 300		CP	Protease	CP × protease
ADG (g/d)	511	523	555	495	526	576	12	0.958	0.068	0.645
ADFI (g/d)	1,350	1,415	1,394	1,374	1,380	1,386	44	0.949	0.950	0.969
G:F	0.361	0.370	0.386	0.352	0.375	0.405	0.010	0.863	0.074	0.468

Each value is the mean value of 6 replicates (1 pig/cage; 6 × 6 latin square).

HP, high crude protein (17.3%); LP, low crude protein (15.1%); CP, crude protein; PT, protease (ppm); ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency.

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Table 3. Effect of dietary crude protein level and supplementation protease on nitrogen utilization in growing pigs

Items	HP			LP			SE	*p*-value		
	PT 0	PT 150	PT 300	PT 0	PT 150	PT 300		CP	Protease	CP × protease
N intake (g/d)	39.77	39.65	39.53	35.16	35.03	34.69	0.54	< .001	0.934	0.992
Urine excretion (kg/d)	2.01	1.99	1.98	2.07	2.03	1.94	0.11	0.928	0.965	0.986
N concentration in urine (%)	0.157[a]	0.142[ab]	0.130[b]	0.135[a]	0.120[ab]	0.115[b]	0.004	0.010	0.034	0.905
N excretion in urine (g/d)	3.18	2.79	2.54	2.72	2.46	2.22	0.17	0.308	0.434	0.983
Feces excretion (g/d)	253	251	258	258	262	255	2	0.570	0.983	0.689
N concentration in feces (%)	3.73[a]	3.66[a]	3.46[b]	3.14[a]	3.08[a]	2.94[b]	0.05	< .001	< .001	0.649
N excretion in feces (g/d)	9.46	9.21	8.93	8.07	8.05	7.51	0.15	< .001	0.106	0.858
Total N excretion (g/d)	12.63	12.00	11.46	10.79	10.52	9.73	0.24	< .001	0.082	0.929
N retention (g/d)	27.14	27.65	28.07	24.38	24.52	24.96	0.42	< .001	0.689	0.971
N retention (% of N intake)	68.15	69.83	70.98	69.24	69.89	71.98	0.47	0.441	0.061	0.881
Biological value (%)[1)]	89.44	91.01	91.67	90.12	90.88	91.86	0.53	0.827	0.355	0.956

Each value is the mean value of 6 replicates (1 pig/cage; 6 × 6 latin square).

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

[1)](N intake – urinary N excretion – fecal N excretion) / (N intake – fecal N excretion) × 100.

HP, high crude protein (17.3%); LP, low crude protein (15.1%); CP, crude protein; PT, protease (ppm); N, nitrogen.

Table 4. Effects of dietary crude protein level and protease supplementation on the apparent total tract digestibility of nutrients in growing pigs

Items	HP			LP			SE	*p*-value		
	PT 0	PT 150	PT 300	PT 0	PT 150	PT 300		CP	Protease	CP* × protease
Dry matter	81.81	82.10	81.29	81.44	81.03	81.51	0.22	0.386	0.919	0.536
Digestible energy	72.90[a]	74.47[ab]	75.97[b]	74.08[a]	74.40[ab]	76.21[b]	0.35	0.485	0.008	0.708
Metabolic energy	71.52[a]	73.46[ab]	74.93[b]	72.60[a]	73.23[ab]	75.09[b]	0.34	0.549	0.001	0.625

Each value is the mean value of 6 replicates (1 pig/cage; 6 × 6 latin square).

[a,b]Means in the same row with different superscripts differ ($p < 0.05$).

HP, high crude protein (17.3%); LP, low crude protein (15.1%); PT, protease (ppm); CP, crude protein.

CP diet could reduce N excretion [25]. However, an adequate amount of CP for pig growth is essential. Therefore, it is important to have the optimal effect with a small amount of CP. It is a common strategy to use protein enzymes in pig diets to increase the efficiency of N utilization [26]. The method of stimulating the digestion of nutrients including nitrogen through dietary supplementation of exogenous enzymes has attracted the attention of the pig industry [14]. In several studies, protease has been shown to have positive effects on the nutrient digestibility or growth performance in pigs from weaning through finishing [9,11,15,18,27]. However, there are still something to be defined about protease in diets [13,16]. The effectiveness of supplying protease enzyme in pig diets can differ due to disparity between the ingredients, the age of the pigs, or enzyme products [13]. Therefore, this experiment was performed to investigate the effects of different levels of CP and protease on nitrogen utilization, nutrient digestibility, and growth performance in growing pigs.

In this study, G:F and ADG tended to increase ($p = 0.074$) with increasing dietary protease supplementation, but the level of dietary protein was not significant difference ($p < 0.050$) on pig performance. This observation corresponds with results reported by Omogbenigun et al. [12], who observed the effect of exogenous enzymes on pig ADG. This result might be due to the better digestibility of nutrients in protease-supplemented diets compared to basal diets. Table 4 shows that DE and ME were significantly higher ($p < 0.050$) at low CP levels with 300 ppm protease supplementation compared to the other treatments.

Many studies have demonstrated a correlation between protein and nitrogen emission. The use of relatively insufficient CP in growing pigs can cause an accumulation of organic compounds and manure that emits ammonia and odors [28]. The protease was expected to increase the digestibility of the protein by cleaving the peptide bond in the protein by hydrolysis to break down the protein into small polypeptides or single amino acids. Table 3 shows that the low CP diet decreased ($p < 0.050$) urinary and fecal N concentrations, fecal N excretion, and total N excretion. These results agreed with those of Dourmad and Henry [29] and Canh et al. [30], who reported a 10% reduction in nitrogen excretion per point CP reduction in the diet, seen as a change from 12.63 g/d to 10.52 g/d from a 2% reduction in CP content. The low CP diet showed a decrease ($p < 0.050$) in nitrogen retention (g/d) compared to the high CP diet. However, there was no significant difference ($p > 0.050$) in the percentage of nitrogen retained of the total N intake according to the dietary CP level. Nitrogen retention is the most important factor in reducing nitrogen excretion as much as possible while maintaining pig growth performance [31]. N retention and the percentage of N intake was not significantly different but tended to increase ($p = 0.061$) with increasing dietary protease supplementation. Also, protease supplementation reduced ($p < 0.050$) urinary and fecal N concentrations compared to those in the protease-free diet. N concentration in urine tended to decrease as the amount of protease addition increased. The fecal N concentration was not significantly different between the basal diet and the 150 ppm protease-supplemented diet, but there was a significant difference in the 300 ppm protease-supplemented diet.

Table 4 shows that the CP level did not affect ($p > 0.050$) nutrient digestibility. However, protease supplementation showed higher ($p < 0.050$) DE and ME compared to the basal diet. In this experiment, both DE and ME increased as the amount of added protease increased. Protease is now known as dietary enzyme that target tight protein binding to increase protein availability. In our experiment, protease supplementation on diets show positive effect on DE and ME during the experimental period but made no difference on ADG and G:F. It has been suggested that endogenous protease enzymes can improve the digestibility of starch and protein, but have no effect on growth performance in pigs [32]. Dietary protease significantly improved the apparent ileal digestibility (AID) of CP not only corn but sorghum-based diets [33]. Furthermore, corn-based diets with protease increased the AID of CP and amino acids in growing pigs [34]. The protease enzyme appears to be due to improved protein digestibility on in the corn-soybean meal diets by progressing deoxidation two sulfurs through hydrolysis to break the cystine disulfide bond in soy proteins such as glycinin and β-conglycinin [35].

CONCLUSION

The results of this experiment showed that a low CP (15.1%) diet with added protease (300 ppm) significantly lowered nitrogen emissions and increased energy utilization.

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