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Supplementation of protease and different nutrient density diets in growing-finishing pigs

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Abstract

This study was conducted to investigate the effects of protease supplementation and different nutrient density of diets in growing-finishing pigs. A total of one hundred-eight crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial body weight (BW; 18.74 ± 3.46 kg) were used for 15 weeks. Pigs were randomly assigned to six dietary treatments with 6 replicates of 3 pigs per pen in a 3 × 2 factorial through the following arrangement: Three groups of protease (1, Basal diets; 2, Protease A: 125 mg/kg protease derived from *Streptomyces* sps; 3, Protease B: 100 mg/kg protease derived from *Bacillus licheniformis*) at two different nutrient density diets (1, Basal requirement; 2, 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy). High nutrient (HN) diets showed higher average daily gain (ADG) ($p < 0.05$) and apparent total tract digestibility (ATTD) of crude protein (CP) ($p < .0001$) compared to basal nutrient (BN) diets during growing periods. Supplementation of protease showed higher BW ($p < 0.05$) and ADG ($p < 0.05$) compared to non-supplementation of protease during growing periods. Also, supplementation of protease showed higher ATTD of CP ($p < 0.01$), ATTD of gross energy ($p < 0.05$) and decreased blood urea nitrogen (BUN) level ($p = 0.001$) compared to non-supplementation of protease during finishing periods. Pigs which fed the protease showed decreased ammonia (NH_3) emissions ($p < 0.05$) during experiment periods and decreased hydrogen sulfide (H_2S) emissions ($p < 0.01$) during finishing periods. Interactions between nutrient density and protease were observed, which decreased the feed conversion ratio ($p < 0.05$) in HN diets without protease compared to BN diets without protease during weeks 4 to 6. Also, interaction between nutrient density and protease was observed, which resulted in improved ATTD of CP ($p < 0.01$) in response to PTA supplementation with HN diets during the finishing period. In conclusion, supplementation of protease reduces NH_3 in feces and BUN in whole blood by increasing the digestibility of CP and improves growth performance. Also, diets with high nutrient density improved growth performance and nutrient digestibility in growing periods.

Keywords: Nutrient density, Protease, Growing-finishing pig

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Ethics approval and consent to participate
The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1740-22-02).

INTRODUCTION

Growing-finishing pigs are considered as a main cause of environmental problems such as eutrophication and acidification due to nitrogen emissions [1,2]. Over-supplementation of diets with nutrients is a major factor contributing to the excretion of nutrients into feces and urine and emissions of nitrogen (N) [3]. Therefore, researchers are constantly attempting to reduce N emission from feces and urine by reducing dietary protein and energy levels in pig diets [4,5]. Researchers have reported that reducing dietary protein concentration to be under 4% [6–9] and reducing dietary energy (150 kcal) can decrease N emission [10,11].

However, the most concerning challenge of reducing dietary protein and energy is a decrease in growth performance [4,12–14]. When dietary protein and energy levels are reduced in feeds at growing-finishing periods, pigs attempt to maintain energy consumption by eating more feed [11,15]. Reduction of dietary protein and energy levels can also lead to extension of feeding term for growing-finishing pigs to reach market weights due to poor growth performance [16–18]. Consequently, reduction of dietary protein and energy could increase feed consumption, which might cause poor profitability to swine producers.

Dietary nutrient density could affect feed consumption of pigs. Previous studies have reported feed intake is decreased when dietary nutrient density is increased [19,20]. It means that uptake of exogenous protease might be influenced by nutrient density. However, few studies have evaluated the relationship between protease and nutrient density.

Exogenous protease known to hydrolyze proteins to peptides, is being widely used to enhance growth performance and improve protein digestion [21–24]. Several studies have reported that improved digestion and absorption of crude protein (CP) might reduce losses of nutrients associated with strategies to reduce N emissions [25,26]. Furthermore, supplementation of exogenous protease to high protein diets can reduce N excretion into urine in growing pigs [27]. Therefore, supplementation of exogenous protease could be an ideal strategy to reduce N emissions when pigs are fed with high protein diets during growing-finishing periods. Many studies have reported effects of protease based on *Bacillus licheniformis*, *Aspergillus* and *Peniophora* [28,29], while there are few studies on effects of exogenous protease based on *Streptomyces* sps. We hypothesized that increased nutrient density diets and supplementation of exogenous protease would positively affect growth performance and nutrient digestibility without increasing excretion of residual nutrients, which could negatively cause environmental impacts. Thus, the objective of this study was to investigate the effects of nutrient density and protease on growth performance, nutrient digestibility, blood profiles, gas emissions, and fecal microflora.

MATERIALS AND METHODS

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1740-22-02).

Source of protease

The protease A (PTA) enzyme was supported by a commercial company. According to the supplier, PTA, 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* sps. PTA contains 60,000 protease units/g produced by a *Streptomyces* sps. PTB commercial

product, containing 75,000 protease units/g derived from *Nocardiopsis prasina* produced in *Bacillus licheniformis*.

Experimental design, animals, and housing

A total of one hundred-eight crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial body weight (BW; 18.74 ± 3.46 kg) were used for 15 weeks. Pigs were randomly assigned to six dietary treatments with 6 replicates of 3 pigs per pen in a 3 × 2 factorial through arrangement: three groups of protease (1, Basal diets; 2, PTA: 125 mg/kg protease derived from *Streptomyces* sps; 3, PTB: 100 mg/kg protease derived from *Bacillus licheniformis*) at two different nutrient density diets (1; Basal requirement 2; 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy). Feeds were provided by Woosung (Daejeon, Korea). The feed was formulated to meet or exceed the nutritional requirements of pigs according to NRC (Table 1) [3]. Each pigs had *ad libitum* access to water. A single-sided stainless-steel feeder and nipple drinker were placed with each pen.

Measurements and sampling

Growth performance

BW, and feed consumption were measured at initial, 2, 4, 6, 9, 13 and 15 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Nutrient digestibility

In experiment periods, fresh fecal samples from each treatment are collected by rectal massage at weeks 6 and 13 from 3 pigs in a pen. Fresh fecal samples were transferred to the laboratory in an icebox for analysis. Diets and feces were analyzed for dry matter (DM), CP, and gross energy (GE) using AOAC methods [30]. We analyzed the GE of the diets and feces using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL, USA). To evaluate the apparent total tract digestibility (ATTD) of CP, DM, and GE, 0.2% chromium oxide (Cr_2O_3) was analyzed immediately as an indigestible marker that added in pig's diet. Using a spectrophotometer, the acid digestion method was used to determine Cr_2O_3.

Calculating the ATTD used the following formula:

"Digestibility (%) = [1 – {(Nf × Cd) / (Nd × Cf)}] × 100"

Nf =nutrient concentration in feces (DM%), Nd = nutrient concentration in diet (DM%), Cd =chromium concentration in diet (DM%), and Cf = chromium concentration in feces (DM%).

Blood profiles

At 6 and 13 weeks, blood samples from the anterior vena cava of 6 pigs per treatment were collected to evaluate the levels of total protein and blood urea nitrogen (BUN). Blood samples were collected into both nonheparinized tubes and vacuum tubes containing K_3EDTA (Becton, Dickinson and CO., Franklin Lakes, NJ, USA) to obtain serum and whole blood. After collection, serum samples were centrifuged (3,000 rpm) for 20 min at 4℃. BUN (Urease GLDH, Roche, Mannheim, Germany) and total protein concentration (Colorimetric assay, Roche) were analyzed using a blood analyzer (Cobas C702, Roche).

Gas emissions

To analysis fecal ammonia (NH_3), and hydrogen sulfide (H_2S) concentration, feces were collected at weeks 6, 13. The feces (300 g) that collected for each treatment were stored in a plastic box with small holes, and the holes were sealed with plaster. Feces were fermented for 24 hours and 48 hours

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in room temperature (25℃). Before the measurement, feces with plastic boxes are shaken 20s to break down any crust formation. NH_3, and H_2S concentrations were determined in the ranges of 50.0 to 100.0 ppm (Gastec, Gastec detector tube No. 3La for NH_3; No. 4LK for H_2S and No. 81L for acetic acid, detector tube, Gastec, Kanagawa, Japan).

Fecal microflora

Fresh fecal samples from each treatment are collected by rectal massage at weeks 6 and 13 from 3 pigs in a pen. Fresh fecal samples were transferred to the laboratory in an icebox for analysis. For counting bacterial colonies, the pour plate method was used. To count the number of *Lactobacillus* and *Escherichia coli* (*E. coli*), de Man, Rogosa and Sharpe (MRS) agar for *Lactobacillus*, and MacConkey agar for *E. coli* were used, and *E. coli* was cultured at 37℃ for 24 h, and *Lactobacillus* was cultured for 48 h.

Statistical analysis

Data for effects of two types of feedstuffs (High nutrient diet [HN], basal nutrient diet [BN]) added with three different types (CON, PTA and PTB) of protease on growth performance, nutrient digestibility, blood profiles and fecal microflora of pigs were conducted with two-way ANOVA, with different nutrient density, different types of proteases, and their interactions as main effects and litter as a covariate. All data was analyzed with the general linear model procedure of SAS (Version 9.4, 2013, SAS, Cary, NC, USA). Differences between treatment groups was considered by using Duncan's multiple range test. Statistical significance and tendency were determined at $p < 0.05$ and $0.05 \leq p < 0.10$.

RESULTS

Growth performance

As shown in Table 2, BW was significantly increased ($p < 0.05$) in PTA added treatment compared with CON at weeks 4. Also, at weeks 6, BW was significantly increased ($p < 0.01$) in PTA and PTB added treatment compared with CON. From weeks 2 to 4, ADG was significantly increased ($p < 0.05$) in PTA added treatment compared with CON. From weeks 0 to 6, ADG was significantly increased ($p < 0.01$) in PTA and PTB added treatment compared with CON. From weeks 0 to 2, 4 to 6 and 0 to 6, ADG was significantly increased ($p < 0.05$) in HN treatment than for BN treatment. There was an interaction ($p < 0.05$) between nutrient density of diets and protease in FCR at weeks 4 to 6. Pigs supplemented with BN, and no additive had higher FCR levels compared to pigs supplemented with HN and no additive. From weeks 13 to 15, ADG was higher ($p < 0.05$) HN treatment than for BN treatment. From weeks 9 to 13 and 6 to 15, ADG was significantly increased ($p < 0.05$) in PTA added treatment compared with PTB. From weeks 13 to 15, ADG was significantly increased ($p < 0.05$) in PTA and PTB added treatment compared with CON. From weeks 9 to 13, FCR was less ($p < 0.05$) for PTB added treatment than PTA added treatment. From weeks 6 to 9, ADFI showed a tendency to increase ($p < 0.10$) when the BN diets were supplied. Also, from weeks 0 to 15, FCR showed a tendency to increase ($p < 0.10$) when the BN diets were supplied.

Nutrient digestibility

As shown in Table 3, pigs supplemented with HN showed higher ($p < .0001$) levels of CP digestion compared to BN at weeks 6. Also, CP digestion was significantly increased ($p < 0.05$) in supplementation of PTA protease compared to CON at weeks 6. Supplementation of PTA

Table 1. Composition of basal diets in growing period and finishing periods (as fed basis)

Items	Growing period		Finishing period	
	Basal	High	Basal	High
Ingredients (%)	100	100	100	100
Corn	53.479	51.115	53.959	55.776
Soybean meal	15.660	18.466	15.466	13.100
Wheat (11%)	3.750	3.750	3.750	3.750
Rice bran	6.500	6.500	6.500	6.500
DDGS	11.500	10.500	10.500	11.500
Limestone	1.270	1.116	0.883	0.910
Vegetable oil	1.320	1.966	2.000	1.720
Sugar	4.590	4.550	4.950	4.870
Poultry oil	0.200	0.200	0.200	0.200
Salt	0.358	0.361	0.378	0.376
Choline chloride	0.040	0.066	0.066	0.040
Lysine sulfate	0.724	0.725	0.731	0.711
L-Methionine (99%)	0.083	0.101	0.128	0.077
Tryptophan (98%)	0.049	0.042	0.043	0.038
Emulsifier	0.050	0.050	0.050	0.050
MDCP	0.061	0.120	0	0
Threonine	0.146	0.152	0.176	0.162
Vitamin and mineral premix[1)]	0.220	0.220	0.220	0.220
Calculated composition				
Dry matter (%)	86.69	86.72	86.45	86.47
Protein (%)	15.90	16.78	14.89	15.58
Fat (%)	5.51	5.98	5.75	5.94
Fiber (%)	3.83	3.83	3.79	3.78
Ash (%)	5.19	5.07	4.41	4.49
Calcium (%)	0.72	0.64	0.46	0.46
Phosphorus (%)	0.49	0.50	0.44	0.44
Na (%)	0.20	0.20	0.20	0.20
Cl (%)	0.35	0.35	0.35	0.35
NE (kcal/kg)	2,475.5	2,525.5	2,475.5	2,525.5
Total AA (g/kg)				
Arg	8.68	9.40	7.92	8.51
Ile	5.80	6.21	5.38	5.71
Leu	14.32	14.77	13.59	13.90
Lys	10.35	10.99	9.55	10.16
Met + Cys	6.42	6.79	6.12	6.75
Met	3.60	3.87	3.41	3.97
Thr	7.03	7.42	6.77	7.17
Trp	1.99	2.06	1.76	1.92
Val	7.34	7.75	6.81	7.11
AID AA (g/kg)				
Arg	7.40	8.11	6.70	7.28
Ile	4.63	5.01	4.26	4.58
Leu	12.07	12.48	11.48	11.77
Lys	8.90	9.50	8.20	8.80
Met + Cys	5.25	5.61	5.00	5.63
Met	3.17	3.44	3.01	3.57
Thr	5.43	5.80	5.25	5.63
Trp	1.60	1.66	1.39	1.54
Val	5.71	6.08	5.28	5.56

[1)]Provided per kilogram of complete diet: vitamin A, 11,025 U; vitamin D_3,1103 U; vitamin E, 44 U; 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; Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and selenium (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

High, high nutrient density diet; Basal, basal nutrient density diet; DDGS, distiller's dried grains with solubles; MDCP, monodicalcium phosphate; NE, net energy; AA, amino acids; Arg, arginine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Cys, cysteine; Thr, tryptophan; Val, valine; AID, apparent ileal digestibility.

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Table 2. Effects of different protease and nutrient density feed on growth performance in growing-finishing pigs

Items	High[1)]			Basal			SE	Den		Pro			*p*-value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den× Pro
BW (kg)															
Initial BW	18.79	18.80	18.56	18.70	18.81	18.73	0.126	18.72	18.75	18.74	18.81	18.64	0.778	0.449	0.624
2 wk	27.91	28.02	27.91	27.46	27.99	27.50	0.187	27.95	27.65	27.68	28.01	27.71	0.071	0.188	0.496
4 wk	38.46	39.66	38.84	37.89	39.29	38.89	0.399	38.99	38.69	38.18^{b}	39.48^{a}	38.86^{ab}	0.378	0.015	0.737
6 wk	51.56	52.63	51.99	50.40	52.06	52.14	0.387	52.06	51.54	50.98^{b}	52.35^{a}	52.06^{a}	0.114	0.006	0.266
9 wk	71.96	71.92	70.56	70.82	71.35	71.41	0.931	71.48	71.19	71.39	71.64	70.99	0.710	0.784	0.558
13 wk	98.02	98.46	95.22	96.71	98.76	96.60	1.022	97.23	97.36	97.36	98.61	95.91	0.881	0.052	0.434
15 wk	111.71	113.50	110.43	110.42	111.93	111.20	0.884	111.88	111.19	111.07	112.72	110.82	0.347	0.092	0.372
0 to 2 wk															
ADG (kg)	0.65	0.66	0.67	0.63	0.66	0.63	0.011	0.66	0.64	0.64	0.66	0.65	0.025	0.300	0.294
ADFI (kg)	1.25	1.23	1.21	1.21	1.24	1.21	0.004	1.23	1.22	1.23	1.23	1.21	0.813	0.873	0.843
FCR (kg/kg)	1.92	1.86	1.82	1.94	1.89	1.93	0.087	1.86	1.92	1.93	1.87	1.87	0.536	0.865	0.575
2 to 4 wk															
ADG (kg)	0.75	0.83	0.78	0.75	0.81	0.81	0.021	0.79	0.79	0.75^{b}	0.82^{a}	0.80^{ab}	0.999	0.013	0.412
ADFI (kg)	1.70	1.73	1.70	1.63	1.76	1.77	0.061	1.71	1.72	1.67	1.75	1.73	0.890	0.373	0.478
FCR (kg/kg)	2.26	2.08	2.18	2.18	2.18	2.17	0.051	2.17	2.18	2.22	2.13	2.18	0.936	0.088	0.165
4 to 6 wk															
ADG (kg)	0.94	0.93	0.94	0.89	0.91	0.95	0.009	0.93	0.92	0.91^{b}	0.92^{ab}	0.94^{a}	0.046	0.015	0.053
ADFI (kg)	2.02	2.19	2.19	2.20	2.13	2.19	0.050	2.13	2.17	2.11	2.16	2.19	0.385	0.315	0.073
FCR (kg/kg)	2.15^{b}	2.36^{ab}	2.33^{ab}	2.47^{a}	2.34^{ab}	2.30^{ab}	0.060	2.29	2.37	2.31	2.35	2.32	0.109	0.768	0.011
0 to 6 wk															
ADG (kg)	0.78	0.81	0.80	0.75	0.79	0.80	0.007	0.79	0.78	0.77^{b}	0.80^{a}	0.80^{a}	0.048	0.001	0.279
ADFI (kg)	1.66	1.72	1.70	1.68	1.71	1.72	0.033	1.69	1.70	1.67	1.71	1.71	0.671	0.339	0.902
FCR (kg/kg)	2.13	2.12	2.13	2.24	2.17	2.15	0.045	2.13	2.18	2.17	2.15	2.15	0.255	0.672	0.754
6 to 9 wk															
ADG (kg)	0.97	0.92	0.88	0.97	0.92	0.92	0.042	0.92	0.94	0.97	0.92	0.90	0.744	0.239	0.904
ADFI (kg)	2.40	2.39	2.39	2.48	2.46	2.49	0.032	2.39	2.48	2.44	2.42	2.44	0.005	0.772	0.881
FCR (kg/kg)	2.48	2.60	2.70	2.55	2.67	2.71	0.118	2.59	2.65	2.51	2.64	2.71	0.476	0.416	0.972
9 to 13 wk															
ADG (kg)	0.93	0.95	0.88	0.94	0.98	0.90	0.025	0.92	0.93	0.93^{ab}	0.97^{a}	0.89^{b}	0.321	0.025	0.921
ADFI (kg)	2.59	2.60	2.59	2.64	2.62	2.64	0.041	2.60	2.64	2.62	2.61	2.61	0.244	0.981	0.955
FCR (kg/kg)	2.79	2.74	2.94	2.81	2.68	2.93	0.107	2.82	2.82	2.82^{ab}	2.71^{b}	2.94^{a}	0.439	0.034	0.764
13 to 15 wk															
ADG (kg)	0.98	1.07	1.09	0.93	0.94	1.02	0.037	1.05	0.99	0.98^{b}	1.01^{ab}	1.06^{a}	0.016	0.046	0.508
ADFI (kg)	3.15	3.09	3.13	2.99	3.06	3.11	0.045	3.12	3.05	3.07	3.08	3.12	0.066	0.456	0.258
FCR (kg/kg)	3.22	2.88	2.88	3.21	3.26	3.04	0.111	2.98	3.09	3.13	3.05	2.93	0.067	0.064	0.231
6 to 15 wk															
ADG (kg)	0.95	0.96	0.93	0.95	0.95	0.93	0.010	0.95	0.95	0.95^{ab}	0.96^{a}	0.93^{b}	0.695	0.031	0.695
ADFI (kg)	2.65	2.64	2.65	2.67	2.66	2.69	0.027	2.64	2.67	2.66	2.65	2.67	0.201	0.788	0.834
FCR (kg/kg)	2.78	2.74	2.85	2.80	2.80	2.89	0.041	2.79	2.82	2.79	2.77	2.86	0.243	0.056	0.849
0 to 15 wk															
ADG (kg)	0.89	0.90	0.87	0.87	0.89	0.88	0.008	0.89	0.88	0.88	0.89	0.88	0.286	0.098	0.535
ADFI (kg)	2.26	2.27	2.27	2.27	2.28	2.31	0.016	2.27	2.29	2.26	2.28	2.29	0.109	0.360	0.611
FCR (kg/kg)	2.55	2.53	2.59	2.60	2.57	2.63	0.027	2.55	2.6	2.57	2.55	2.61	0.057	0.104	0.890

[1)]High nutrient density is 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density.

[a,b]Means in the same row with difference superscripts differ at $p < 0.05$.

High, high nutrient density diet; Basal, basal nutrient density diet; Den, different nutrient density diet; Pro, supplementation of protease; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; Den × Pro, different nutrient density diet × supplementation of protease; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table 3. Effects of different protease and nutrient density feed on nutrient digestibility in growing-finishing pigs

Items (%)	High[1)]			Basal			SE	Den		Pro			*p*-value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den× Pro
DM															
6 wk	88.29	88.88	89.01	88.54	89.18	88.78	0.325	89.06	88.83	88.91	89.03	88.89	0.402	0.909	0.290
13 wk	88.60	89.03	88.63	88.08	88.80	88.45	0.346	88.75	88.44	88.34	88.91	88.54	0.289	0.267	0.862
CP															
6 wk	70.78	72.06	71.48	67.75	69.69	68.59	0.539	71.44	68.68	69.26^{b}	70.87^{a}	70.04^{ab}	< .0001	0.027	0.816
13 wk	67.34^{c}	71.39^{a}	69.48^{b}	70.96^{ab}	70.88^{ab}	72.10^{a}	0.501	69.40	71.32	69.15^{b}	71.13^{a}	70.79^{a}	0.001	0.002	0.002
GE															
6 wk	74.91	74.35	74.51	74.19	74.73	74.16	0.356	74.59	74.36	74.55	74.54	74.33	0.435	0.786	0.311
13 wk	70.68	72.63	71.93	70.55	72.13	71.33	0.545	71.74	71.33	70.61^{b}	72.38^{a}	71.63^{ab}	0.371	0.016	0.900

[1)]High nutrient density is 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density.

[a–c]Means in the same row with difference superscripts differ at $p < 0.05$.

High, high nutrient density diet; Basal, basal nutrient density diet; Den, different nutrient density diet; Pro, supplementation of protease; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; Den × Pro, different nutrient density diet × supplementation of protease; DM, dry matter; CP, crude protein; GE, gross energy.

Table 4. Effects of different protease and nutrient density feed on blood profiles in growing-finishing pigs

Items	High[1)]			Basal			SE	Den		Pro			*p*-value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den× Pro
Total protein (g/dL)															
6 wk	6.02	6.02	6.28	5.78	6.00	6.06	0.137	6.11	5.95	5.90	6.01	6.17	0.165	0.161	0.678
13 wk	6.54	6.70	6.40	6.26	6.70	6.46	0.193	6.53	6.47	6.40	6.70	6.41	0.707	0.231	0.601
BUN (mg/dL)															
6 wk	7.80	6.60	7.20	8.00	6.74	7.00	0.716	7.20	7.25	7.90	6.67	7.10	0.937	0.239	0.956
13 wk	14.60	11.80	13.20	14.20	11.40	12.60	0.645	13.20	12.73	14.40^{a}	11.60^{b}	12.90^{b}	0.385	0.001	0.984

[1)]High nutrient density is 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density.

[a,b]Means in the same row with difference superscripts differ at $p < 0.05$.

High, high nutrient density diet; Basal, basal nutrient density diet; Den, different nutrient density diet; Pro, supplementation of protease; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; Den × Pro, different nutrient density diet × supplementation of protease; BUN, blood urea nitrogen.

diets showed significant increase of GE ($p < 0.05$) than CON diets at weeks 13. However, there was no significant difference in DM at weeks 13. Pigs supplemented with BN showed higher ($p < 0.001$) CP digestion levels compared to HN at weeks 13. Also, supplementation of PTA and PTB showed higher ($p < 0.05$) levels of CP digestion compared to CON at weeks 13. There was an interaction ($p < 0.05$) between nutrient density of diets and protease in CP digestibility at weeks 13. Supplementation of PTA showed higher ($p < 0.05$) concentrations of CP digestibility in HN diets compared to CON and PTA at weeks 13.

Blood profiles

As shown in Table 4, there was no significant difference between HN and BN diets treatment and supplementation of protease at growing periods. In finishing period, there was significant increased ($p < 0.05$) in BUN between supplementation of protease. PTA and PTB showed lesser than CON. Total protein was not significantly ($p > 0.05$) affected by nutrient density of diets and supplementation of protease.

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Table 5. Effects of different protease and nutrient density feed on gas emission in growing-finishing pigs

Items (ppm)	High[1)]			Basal			SE	Den		Pro			*p*-value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den× Pro
H_2S															
6 wk	4.73	4.38	4.53	4.55	4.53	4.48	0.131	4.54	4.52	4.64	4.45	4.50	0.819	0.358	0.475
13 wk	5.30	4.80	5.00	5.40	4.60	5.10	0.146	5.04	5.03	5.35[a]	4.73[b]	5.03[b]	0.890	0.002	0.554
NH_3															
6 wk	3.45	3.18	3.35	3.53	3.13	3.30	0.126	3.33	3.32	3.49[a]	3.15[b]	3.33[ab]	0.936	0.049	0.850
13 wk	3.40	3.10	3.30	3.60	3.20	3.20	0.129	3.28	3.34	3.52[a]	3.16[b]	3.26[ab]	0.587	0.039	0.476

[1)]High nutrient density is 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density.

[a,b]Means in the same row with difference superscripts differ at $p < 0.05$.

High, high nutrient density diet; Basal, basal nutrient density diet; Den, different nutrient density diet; Pro, supplementation of protease; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; Den × Pro, different nutrient density diet × supplementation of protease; H_2S, hydrogen sulfide; NH_3, ammonia.

Table 6. Effects of different protease and nutrient density feed on fecal microflora in growing-finishing pigs

Items (Log_{10} CFU/g)	High[1)]			Basal			SE	Den		Pro			*p*-value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den× Pro
E. coli															
6 wk	6.48	6.52	6.60	6.51	6.46	6.50	0.131	6.54	6.49	6.50	6.49	6.55	0.644	0.882	0.856
13 wk	6.52	6.58	6.61	6.52	6.51	6.51	0.149	6.57	6.51	6.52	6.54	6.56	0.633	0.969	0.929
Lactobacillus															
6 wk	7.81	7.90	7.09	7.39	7.93	7.84	0.275	7.57	7.78	7.65	7.91	7.47	0.588	0.238	0.116
13 wk	9.33	9.57	9.35	9.19	9.56	9.13	0.260	9.41	9.29	9.26	9.57	9.24	0.573	0.341	0.923

[1)]High nutrient density is 0.94%–0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density.

High, high nutrient density diet; Basal, basal nutrient density diet; Den, different nutrient density diet; Pro, supplementation of protease; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; Den × Pro, different nutrient density diet × supplementation of protease; *E. coli*, *Escherichia coli*.

Gas emission

As shown in Table 5, addition of PTA diets showed lesser than CON diets ($p < 0.05$) NH_3 emissions at weeks 6. There was no significant difference ($p < 0.05$) in H_2S emission. At weeks 13, there was significant difference ($p < 0.05$) in H_2S between supplementation of protease. Supplementation of PTA and PTB decreased significantly in H_2S emission compared with CON. In NH_3, PTA showed lowest ($p < 0.05$) emissions and CON showed highest emissions.

Fecal microflora

As shown in Table 6, there was no significant difference ($p > 0.05$) in *E. coli* and *Lactobacillus* concentration in feces between density of diets and supplementation of protease at weeks 6. Likewise, there was no significant difference ($p > 0.05$) in *E. coli* and *Lactobacillus* in feces between density of diets and supplementation of protease at weeks 13.

DISCUSSION

The current study focused on the following two mechanisms to assess positive effects of high nutrient density and protease; 1) providing HN diets could improve nutrient digestibility with

ADG due to increased dietary protein with energy, and 2) supplementation of protease for hydrolyzing dietary proteins into peptides could increase digestion and absorption.

Pigs in groups with HN diets showed improvement in BW and ADG compared to those in groups with BN diets. These observations are consistent with previous studies showing that providing high dietary protein (0.97% and 1.29%, respectively) and energy levels (100 kcal/kg and143 kcal/kg, respectively) could improve ADG during growing periods [31,32]. Supporting the current study's results, numerous studies have reported a positive correlation between increased dietary protein with energy, which could improve growth performance [31,33,34]. Thus, the increase of ADG in the present study might be due to the increased nutrient density diets.

During the growing period, we observed increased ATTD of CP in HN diets. Previous studies have reported a positive correlation between improved ATTD of CP and improved ADG [13,32]. Our results were consistent with previous reports, suggesting that providing HN diets could lead to positive correlation between increase of ATTD of CP and increase of ADG.

Dietary energy level is highly related with feed intake and considered as first determinant of ADFI [19,20]. Yan et al. has reported a decrease of ADFI when growing-finishing pig are fed 100 kcal/kg higher energy density diets than basal energy density diets [31]. However, in the present study, we observed decreased ADFI only at weeks 6 to 9. According to Meng et al. [13], HN diets can improve growth performance without affecting ADFI of growing-finishing pigs which might be reasonable of different results in this study.

Supplementation of protease improved BW and ADG compared to non-supplementation protease groups in this study. These results are consistent with a previous study, reporting that 125 mg/kg of protease (*Streptomyces* sps and *Bacillus licheniformis*) supplementation could improve ADG [35]. Increase of ADG with ATTD of CP was observed in the present study, which is consistent with results of previous study [13,32]. The improvement in ATTD of CP and ADG by protease supplementation could be due to the effective breakdown of protein molecules into more useable peptides that can be degraded into amino acids by carboxypeptidases [36]. Thus, increased ATTD of CP might be reasonable by using protease which can improve the utilization of dietary proteins, leading to increase of ADG in this study.

BUN is a waste of protein, which produced by mechanism of N digestion in liver and kidney [37]. Several studies have indicated that BUN concentration and total protein in blood can be used as indicators of protein utilizations and N excretion [35,38]. In the present study, we observed the supplementation of protease decreased BUN concentration and improved ATTD of CP during finishing periods. Following the mechanism of BUN, we considered that the current study's decreased BUN concentration could be explained by effects of protease, which could improve digestion and utilization of dietary protein.

In aspects of nutrient density, HN diets did not show significant effects on BUN in blood, consistent with results of Yan and Kim results [32]. According to previous studies, differences in nutrient density were 1.2% and 2.1% dietary protein, respectively, with 100 kcal/kg energy level [32,39]. However, studies of Yan and Kim [32] and Wang et al. [39] did not show significant difference of BUN level in the blood. Thus, we hypothesized that more difference between dietary protein and energy levels should be considered to demonstrate the effects of nutrient density on blood profiles in this study.

Significant differences of total protein in blood were not observed in the current study. Consistently, Yan et al. [31] have reported that 1% difference in dietary protein does not reduce total protein in blood. Liu et al. [26] have also reported that supplementation of protease, which derived from *Pseudoalteromonas arctica* can reduce total protein in blood. The potential mechanism of reduced total protein in blood might be due to increased ATTD of CP which can improve

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utilization of proteins. Thus, concentration of nutrient density and types of proteases should be considered to identify differences of total protein in blood in this study.

According to Ferket et al. [40] and Yan et al. [41], improved nutrient digestibility could result in decreased gas emissions by reducing substrates for microbial fermentation in the large intestine. Previous studies have reported that protease can reduces gas emissions due to improved utilization and absorption of dietary proteins [25,26]. Gas emissions, which occurred by undigested protein, many studies have reported that the increased ATTD of CP by providing HN diets effects in reducing gas emissions [13,32]. Thus, we considered that the reduction of gas emission might be due to improved ATTD of CP by providing HN diets and protease in this study.

Reduction of pathogenic bacterial population in the gastrointestinal tract and improvement of beneficial microbial activity are considered as main factors that decrease gas emissions by improving microbial fermentation [42]. Although decreased gas emissions were observed, there were no significant differences in fecal microflora in this study. In the aspects of nutrient density diets, Zhao et al. [20] have reported that under 80 kcal/kg energy level difference does not show improvement of fecal microflora, consistent with the current study. Many studies have also revealed that supplementation of protease can provide positive effects in weanling period than in growing-finishing periods due to enhanced resistance to intestinal pathogens [43,44]. Thus, higher more concentration of protease and more nutrient density be needed to identify differences in fecal microflora.

In the current study, we observed an interaction between protease and nutrient density diet in FCR and nutrient digestibility. The possible reason for decreased FCR in HN diets without protease could be explained by higher energy concentration. Pigs consume more feeds to meet their energy requirement when a low energy concentration feed is supplied [20]. Due to this explanation, we considered that the decrease of FCR in HN might be due to energy concentration differences between HN and BN diets. Higher improvement in ATTD of CP was observed in the finishing period in response to PTA supplementation with HN diets. When pigs consume HN diets with PTA (*Streptomyces* sps), they showed greater digestion of CP compared to CON and PTB (*Bacillus licheniformis)*. This result was consistent with a study of Cheng et al. [45] reporting that supplementation of *Bacillus licheniformis* in diets with different nutrient densities (0.74% differences in CP) did not show significant difference in ATTD of CP. According to results of the present study, providing HN diets with supplementation of PTA produced by *Streptomyces* sps could be ideal ways to maximize production while reducing environmental pollution.

CONCLUSION

The result of this study coincides with the hypothesis regarding the effect of high-nutrient density diets and exogenous protease supplementation in diets. In this study, our results showed that the supplementation of exogenous protease could improve growth performance, nutrient digestibility, blood profiles, and gas emission. Also, high nutrient density diets showed beneficial effects on growth performance and nutrient digestibility compared to basal nutrient density diets. In addition, we observed a higher ATTD of CP in HN diets with *Streptomyces* sps compared to *Bacillus licheniformis*. In conclusion, providing HN diets with PTA could be ideal strategy to reduce environmental pollution with maintaining productivity.

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