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Article Title (within 20 words without abbreviations)	Supplementation of protease with low protein diets improves incidence of frequency and nitrogen metabolism in weaned pigs
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Ethics approval and consent to participate	The experimental protocol was approved (CBNUA-2008-22-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

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Abstract

The purpose of this study was to determine the effect of different crude protein (CP) levels and protease (PT) supplementation on effect in weaned pigs. A total of 24 barrow weaned pig, 7-week-old and with an initial weight of 10.94 ± 1.26 kg, were performed in the 2-week experiment. The experiment followed a 2 × 2 factorial design with two levels of CP (18.78% and 16.92%) and two levels of PT supplementation (0, 0.1%). No difference was observed in growth performance by the variations in CP and PT levels. In fecal score, the low CP (LP) diet showed an increased frequency of 0 scores compared to the high CP (HP) diet. The apparent total tract digestibility (ATTD) of dry matter (DM) increased during the first week in the LP diet compared to the HP diet. At second week, the ATTD of DM was increased in the LP diet compared to the HP diet and the ATTD of CP increased in the PT10 compared to the PT0. Also, DM had an interaction between CP and PT. In the ATTD of amino acids in the first week, the LP diet had a higher methionine digestibility than the HP diet, and the tryptophan digestibility was higher in the HP diet than the LP diet. In second week, the PT10 diet had a higher digestibility than the PT0 diet for indispensable amino acids excluding lysine and tryptophan, and for methionine, the LP diet had a higher digestibility than the HP diet. In the ATTD of indispensable amino acids, the digestibility of threonine, isoleucine, leucine, arginine, and methionine was higher in the PT10 diet compared to the PT0 diet. Blood urea nitrogen (BUN) and hydrogen sulfide (H_2S) gas emissions were decreased in the LP diet and PT10 compared to HP and PT0. In the nitrogen utilization, total excretion decreased in the LP and PT10 compared to HP and PT0, and the nitrogen retention ratio increased at second week. In this study, supplementation of PT in the LP diet can improve weaned pig's fecal score, nutrients digestibility, BUN, H_2S emission, and nitrogen utilization in weaned pigs.

Keywords: Protease, protein levels, nutrients digestibility, nitrogen utilization, weaned pigs

Introduction

Post-weaning diarrhea (PWD) is a serious disease that causes low growth performance and mortality in weaned pig [1,2]. High concentrations of crude protein (CP) can cause undigested proteins to pass the gastrointestinal tract, increasing intestinal permeability and encouraging the growth of harmful bacteria due to an elevation in intestinal pH [3-5] (Fig. 1). Anti-nutritional factors (ANF) (e.g., trypsin inhibitor, antigens, lectins) present in soybean are sensitive factors causing diarrhea in weaned pigs [6].

The low CP (LP) diet reduces the incidence of diarrhea in weaned pigs due to its LP content and ANF contents [6,7]. According to the results of previous study, the LP diet reduced blood urea nitrogen (BUN) in pigs [8]. However, feeding a LP diet without any supplementation reduced the growth performance of weaned pigs [7,9]. Protease (PT) is an exogenous enzyme that helps in the digestion of proteins [10]. Exogenous PT improves intestinal villus height, crypt depth and increases digestive enzyme activity in weaned pigs, with these effects increasing digestibility, decreasing the incidence of diarrhea and finally improving growth performance [11]. Moreover, the supplementation of PT in the diet of weaned pigs increases protein utilization and reduces nitrogen excretion [12]. This study hypothesizes that the decrease in growth performance caused by the feeding of an LP diet will be improved by supplementing with PT, causing a greater expected effect on weaned pigs. Thus, the aim of this study was to determine whether PT supplementation in weaned pig diets at different protein levels could improve growth performance, fecal score, nutrients digestibility, blood characteristics, odor emission, bacteria count, and nitrogen utilization.

Materials and Methods

Ethics

The experimental protocol was approved (CBNUA-2008-22-01) by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea.

Animal and facilities

A total of 24 weaned pigs ([Yorkshire × Landrace] × Duroc), 7-week-old, with an initial weight of 10.94 ± 1.26 kg, were selected for the study. The metabolic experiment was conducted over a period of 14 days. Pigs were individually and completely randomized in metabolic cages of 45 × 55 × 45 cm, with 6 replicates per treatment and

1 pig per replicate based on the initial body weight (BW). The experiment was carried out in an environment with a temperature of 28 ± 1.5℃, a relative humidity of 72 ± 2.3% and a wind speed of 0.26 ± 0.04 m/s.

Dietary treatments

The nutrient composition of all diets is formulated to meet or exceed the requirements set by the National Research Council [13]. The nutrient composition of all diets (Table 1) is formulated. The analyzed nutrient levels in CP were determined using the AOAC method 988.05, as specified in the AOAC [14] method. Amino acids were assayed using ion-exchange chromatography with an automatic amino acid analyzer (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan) following hydrolysis with 6 M HCl at 110℃ for 24 hours. Cystine was quantified as cysteic acid, and methionine was measured as methionine sulphone, both after peroxidation with performic acid and pre-column derivatization using phenylisothiocyanate (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). Tryptophan analysis was performed by hydrolyzing the samples with 4 M NaOH at 110℃ for 22 hours, followed by derivatization using phenylisothiocyanate (Model 76337, Agilent Technologics, Waldbronn, Germany). The experimental design employed a 2 × 2 factorial design based on the following factors: (1) CP content in the basal diet (18.78%) or a LP diet (16.92%) with a 10% reduction compared to the CP content in the basal diet, and (2) Non-supplementation of PT or supplementation of 0.1% PT. The PT was provided by a commercial company (Pearlzyme Inc., Incheon, Korea). According to the supplier, the *Bacillus* sp. that produces alkaline PT was dried after fermentation, mixed with excipients, and quantified to an enzyme activity of 10,000 U/g.

Sampling and measurements

Growth performance and fecal score

BW was measured at the beginning of the experiment, as well as at 7 and 14 days. Feed intake (FI) was calculated by measuring the daily diet supply amount and residual amount. Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) were calculated for each period: 0-7 days, 7-14 days, and 0-14 days. The fecal score was observed and recorded twice a day (at 8:00 and 17:00) by the same individual. The fecal score was assigned as follows: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.

Nutrients digestibility and nitrogen utilization

After the start of the experiment, chromium oxide (Cr_2O_3) was added to the diet at a rate of 0.2% on 5 and 12 days as a marker for a two-day period, and all feces and urine with the markers were collected. Urine was collected in a plate filled with 50 mL of 6 mol/L HCl under the metabolic cage. The total weight of the collected fresh diet, fecal, and urine samples were measured and stored at -20°C, and the collected samples were dried in a dryer at 60°C for 72 hours, then pulverized with a Wiley mill and used for analysis. Dry matter (DM) was determined using AOAC method 930.15 and CP and amino acids were determined using "**Dietary treatments"** were analyzed as described. Gross energy was measured with an adiabatic oxygen bomb calorimeter (Parr Isoperibol Bomb Calorimeter 6400, Parr Instrument Co., Moline, IL, USA).

For calculating the apparent total tract digestibility (ATTD) of the nutrients, we used the following equation: Digestibility = 1 - [(Nf × Cd)/(Nd × Cf)] × 100, where Nf = concentration of nutrient in fecal, Nd = concentration of nutrient in the diet, Cd = concentration of chromium in the diet, and Cf = concentration of chromium in the fecal.

Blood profiles

Blood was collected from the jugular veins of all pigs using serum separator tube, respectively, to collect of blood. Serum samples were centrifuged at 4° C at 3,000 rpm for 15 minutes after collection. The total protein level was determined using a colorimetric assay, while the BUN level was analyzed using the glutamate dehydrogenase (GLDH) method for urea. Both total protein and BUN levels in the blood samples were determined using a fully automated chemistry analyzer (Cobas C702, Hofmann-La Roche, Switzerland) for clinical chemistry analysis. Serum biochemical test was analyzed for Immunoglobulin G using an automatic biochemical blood analyzer (Hitachi 747, Hitachi, Japan).

Odor emission

For the analysis of odor emission, 150 g of fresh feces collected through rectal massage from all pigs on the 7 days and 14 days of the experiment and 100 g of urine + sawdust was mixed, stored in a 4.2 L plastic box, and fermented at 34°C for 72 hours. The amount of ammonia (NH_3) and hydrogen sulfide (H_2S), which are odor-causing substances, was analyzed through a gas detector (GV-110S, Gastec Corp., Ayase, Japan) using an NH_3 detector tube and a H_2S detector tube, respectively.

Bacteria count

After collecting feces from all pigs by rectal massage on the 7 days and 14 days of the experiment, the feces were suspended in sterile physiological saline and homogenized, and then diluted from 10^{-3} to 10^{-7} as samples for measuring the number of microorganisms. was used. To measure the number of *Lactobacillus* and *Escherichia coli* (*E. coli*) bacteria in feces by experimental treatment, de man, rogosa and sharpe (MRS) agar was used for *Lactobacillus* and

MacConkey agar (Difco, USA) was used for *E. coli*. At 37°C, *Lactobacillus* for 48 hours, *E. coli* for After culturing for 24 hours, the number of bacteria was measured.

Statistical analysis

Data on growth performance, nutrient digestibility, blood characteristics, bacteria count, and nitrogen utilization were tested for significance between the means using the two-way analysis of variance of SAS (SAS Institute, Cary, NC, USA). Means were separated using the Tukey test. Fecal score was Chi-squared test using SAS's FREQ method. The other analysis content a statistically significant difference was recognized at a significance level of 0.05 or less, and a $0.05 \leq p < 0.10$ was considered tendency.

Results

Growth performance and fecal score

There was no significant difference in growth performance due to different CP levels and PT supplementation (Table 2). The fecal score significantly decreased ($p < 0.05$) with varying CP levels and showed a significant interaction ($p < 0.05$) between CP levels and PT supplementation (Fig. 2).

Nutrients digestibility

Table 3. shows the effect of supplemental PT on nutrient digestibility with different CP levels in weaned pig. The different CP levels and supplementation of PT showed no significant differences in ATTD in the first week. The high CP (HP) was significantly increased ($p < 0.05$) the ATTD of DM compared to the LP in the second week, and DM was a significant interaction ($p < 0.01$) between CP and PT. The PT10 increased ($p < 0.05$) ATTD of CP compared to the PT0 in the second week.

Table 4. show the effect of supplemental PT on amino acids digestibility with different CP levels at 7 days in weaned pig. In indispensable amino acids, the LP showed a significant increase ($p < 0.05$) in ATTD of methionine compared to the HP. In the ATTD of tryptophan, the HP was significantly increased ($p < 0.05$) compared to the LP. The different CP levels and supplementation of PT showed no significant differences in the ATTD of dispensable amino acids.

The PT10 increased ($p < 0.05$) ATTD of threonine, valine, isoleucine, leucine, phenylalanine, and total indispensable amino acids compared to the PT0 at days 14 (Table 5). The ATTD of histidine and arginine was increased ($p < 0.05$) in the PT10 compared to the PT0, and there was a significant interaction ($p < 0.05$) between CP and PT. The LP increased ($p < 0.01$) ATTD of methionine compared to the HP. Also, the ATTD of methionine showed a significant increase ($p < 0.05$) in the PT10 compared to the PT0. The ATTD of tryptophan had a significant interaction ($p < 0.01$) between CP and PT.

In the dispensable amino acids, the aspartic acid, glutamic acid, alanine, and total dispensable amino acids showed significant increases ($p < 0.05$) in the PT10 compared to the PT0. The ATTD of serine and tyrosine were increased ($p < 0.05$) in the PT10 compared to the PT0, and there was a significant interaction ($p < 0.01$) between CP and PT.

Blood profiles

The LP decreased ($p < 0.01$) BUN compared to HP (Table 6). Also, the PT10 decreased ($p < 0.05$) BUN compared to the PT0.

Odor emission

The PT10 decreased ($p < 0.05$) H_2S compared to the PT0 (Table 7). Also, the LP significantly decreased ($p < 0.01$) H_2S compared to the HP, and H_2S was a significant interaction ($p < 0.05$) between CP and PT.

Bacteria count

The PT0 decreased ($p < 0.05$) *E. coli* population compared to the PT10 at 14 days (Table 8). Different CP levels and supplementation of PT showed no significant difference ($p > 0.05$) in the population of *E. coli* and *Lactobacillus* at 7 days.

Nitrogen utilization

The HP had higher ($p < 0.05$) nitrogen intake than the LP at 7 days (Table 9). In the nitrogen concentration in feces ratio, the HP was significantly higher ($p < 0.01$) than the LP. Also, the PT10 significantly lowered ($p < 0.01$) nitrogen concentration in feces ratio compared to the PT. The HP showed significantly higher ($p < 0.01$) nitrogen retention than LP.

The HP showed significantly higher ($p < 0.01$) nitrogen intake than the LP (Table 10). In feces excretion, the HP was significantly higher ($p < 0.01$) than the LP, and the feces excretion showed a significant interaction ($p < 0.01$) between CP and PT. The HP showed significantly higher ($p < 0.01$) nitrogen concentration ratio than the LP. Also, PT10 significantly lowered ($p < 0.01$) nitrogen concentration ratio compared to PT0, and nitrogen concentration ratio showed a significant interaction ($p < 0.01$) between CP and PT. Nitrogen excretion in feces, total nitrogen excretion and nitrogen retention were significantly higher ($p < .001$) in the HP compared to the LP. In addition, there were significantly lower ($p < 0.01$) in the PT10 compared to the PT0. In nitrogen retention ratio, the LP was significant increased (p < 0.05) compared to the HP. The PT10 was significant increased (p < 0.01) nitrogen retention ratio compared to the PT0.

Discussion

Exogenous enzymes are commonly used to improve growth performance, nutrient utilization, and digestibility of animals [15,16]. In pigs, protein is essential for metabolism and physiology. Changes in protein levels can affect their growth [6]. In this study, we hypothesized that supplementing an LP diet with PT could improve the growth performances of weaned pigs. However, our findings showed that neither protein levels nor PT supplementation had a significant effect on growth performance.

Kim et al. [17] have reported that adding PT at different levels of CP in diets of growing pigs does not affect ADG, ADFI, or G:F. However, supplementing PT to an LP diet improved ADFI and ADG in another study [6]. This improvement might be because PTs could hydrolyze proteins, increasing their digestibility by breaking them down into free amino acids and peptides that can be absorbed in the small intestine [18]. Such differences in the results of various studies might be due to factors such as the type of experiment conducted (metabolic vs feeding), the composition of the diet, and the type of PT used.

Excessive proteins from feeding are not digested in the ileum, but digested in the hindgut [19]. Fermentation of proteins and amino acids by intestinal microflora is a major cause of diarrhea [20-22]. Nyachoti and Jayaramand [23] have reported the need to eradicate PWD by reducing CP content in the diet of weaned pigs. The mechanism by which PTs metabolize proteins is by enhancing hydrolytic capacity in the small intestine [24]. In the present study, the PT diet reduced the incidence of diarrhea during the entire experimental period and an interaction tendency between the LP diet and PT was found, similar to results reported by Yu et al. [6]. The LP diet reduced influx of proteins into the intestine by reducing nitrogen intake. PT also increased CP digestibility. The reduction in the incidence of diarrhea might be due to two factors, LP and PT. In previous studies, an LP diet resulted in a lower incidence of diarrhea than an HP diet, leading to increased DM digestibility [19,25]. Additionally, Zuo et al. [11] have reported that supplementation with PTs can decrease diarrhea score due to improved intestinal development, digestive enzyme activity, and nutrient digestibility.

HP diets contain a lot of ANF. Trypsin inhibitors can reduce the digestibility of pigs [26]. LP diet should be fed as an alternative of the HP diet to increase piglets' nutrient availability from ANF [6]. In the present study, the LP diet improved DM digestibility. However, many studies have suggested that feeding an LP diet can decrease nutrient digestibility in pigs [27,28]. Zhou et al. [29] have reported that an LP diet can reduce nutrient fermentation in the hindgut, thereby reducing nutrient digestibility. This seems to show various results according to CP levels due to the complex intestinal association of pigs with CP % reduction and amino acids supplementation. PTs are usually enzymes that aid in the digestion of proteins. They can also neutralize ANF and improve nutrient digestibility [11,30,31]. As shown in a previous study, the ATTD of CP can be improved in pigs supplemented with PT [11]. PT in basal diets and LP diets has been reported to have an advantage [32,33]. PTs can increase the ileal digestibility of amino acids in weaned pigs and hydrolyze proteins and convert proteins to free amino acids and peptides for good absorption in the small intestine, thereby increasing protein digestibility [18]. Through this action, it seemed that PT could improve the digestibility of many amino acids and total essential and non-essential amino acids in this study.

In blood profiles, BUN is known to be an index that can predict nitrogen excretion and nitrogen utilization efficiency in pigs [34]. It is also used as a method to fine-tune the diet or to identify problems with the nutrient compositions of the diet [35]. Figueroa et al. [36] have reported that when the CP level in a diet is decreased, the BUN is also decreased. In the present study, BUN was decreased after feeding the LP diet. PT improved the ATTD of CP, consistent with results of increased protein utilization. Nitrogen excretion is reduced due to low nitrogen intake from an LP diet. As a result, BUN is reduced by improving protein utilization. Reduction of CP in the diet and supplementation of PT show that it is an ideal diet for weaned pig.

As the livestock industry has become increasingly interested in the effects of environmental pollution around the world, the need to reduce pollutants generated from livestock production has increased. When nutrients in the diet exceed the required amount, they are not fully utilized as they are excreted [37]. H_2S in particularly high concentrations in animal facilities is a lethal gas that can cause death to animals and humans [38,39]. In our experiments, we saw a decrease in H_2S with LP diets and PT supplementation. Nguyen et al. [40] have reported that LP diet and LP supplementation with PT treatments can release less H_2S than basal diet treatment. This is seen as a decrease in H_2S due to the improvement of methionine and sulfur-containing amino acid digestibility.

Nitrogen utilization is used as an indicator of how efficiently pigs use proteins. In addition, there is a comparable model closely related to nitrogen utilization in this experiment, allowing accurate comparative analysis. Nitrogen excretion has a positive correlation with BUN. When BUN decreases, nitrogen excretion also decreases [34]. Whittemore et al. [41] have reported that nitrogen retention can be predicted by calculating it as (ADG × 0.16)/6.25. The growth performance results showed an ADG value of 0.58 kg in CP and PT treatment groups during the entire experimental period. Comparing nitrogen retention and predicted values shown in Table 10, it could be seen that the

HP group in the CP factor and the PT10 group in the PT factor came out similarly. Although HP contains high levels of CP in the diet itself, nitrogen retention seems to be high. The PT10 group showed a high value due to the improvement of nitrogen utilization caused by PT. Supplementation of PT in feed can improve nitrogen utilization by improving the ATTD of CP in weaned pigs. A decrease in BUN can prove the result of nitrogen metabolism.

Conclusion

There were no differences in growth performance with different CP levels and supplementation of PT in weaned pig. The LP diet showed a low fecal score and appeared to be effective in preventing diarrhea through interaction with PT. The DM also showed interaction with the LP diet and PT supplementation. Supplementation of PT improved protein and amino acids digestibility, and it can be seen that BUN was decreased. The digestibility of methionine, a sulfur-containing amino acid, was increased by supplementation with LP diet and PT, which resulted in a decrease in H_2S. As a conclusion of this study, supplementation of PT to the LP diet successfully prevented PWD and improved nitrogen utilization with improved digestibility.

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Tables and Figures

Table 1. Compositions of the basal and low crude protein diets (as-fed basis).

Ingredient, %	Basal diet	Low crude protein diet
Corn	60.81	66.12
Extruded corn	5.00	5.00
Lactose	3.00	3.00
Dehulled soybean meal, 51% CP[1)]	13.00	8.07
Soy protein concentrate, 65% CP	10.00	10.00
Plasma powder	3.00	2.50
Soy oil	2.00	2.00
Monocalcium phosphate	1.15	1.15
Limestone	0.99	0.99
L-Lysine-HCl (78%)	0.04	0.07
DL-Methionine (50%)	0.11	0.30
Choline chloride (25%)	0.10	0.10
Vitamin premix[1)]	0.20	0.20
Trace mineral premix[2)]	0.20	0.20
Salt	0.40	0.30
Total	100	100
Calculated nutrient levels		
ME, kcal/kg	3,386	3,385
CP, %	18.78	16.92
Lysine	1.23	1.23
Methionine	0.36	0.36
Ca, %	0.70	0.70
P, %	0.60	0.60
Analyzed nutrient levels		
CP, %	20.53	18.51
Lysine	1.23	1.23
Methionine	0.36	0.36
Tryptophan	0.19	0.15
Arginine	0.70	0.65
Isoleucine	0.76	0.74
Leucine	1.47	1.42
Phenylalanine	0.86	0.81
Threonine	0.78	0.74
Valine	0.88	0.87
Histidine	0.53	0.50

Abbreviation: ME, metabolizable energy; CP, crude protein

[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 without Zinc: Cu (as $CuSO_4 \bullet 5H_2O$), 12 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \bullet 5H_2O$), 0.15 mg.

Table 2. Effect of supplemental protease on growth performance with different CP levels in weaned pig.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
BW, kg												
Initial	10.962	10.925	10.940	10.943	0.563	10.944	10.942	10.951	10.934	0.998	0.977	0.972
1W	13.073	12.940	12.990	13.255	0.432	13.01	13.12	13.03	13.10	0.791	0.880	0.650
2W	15.680	15.591	15.514	15.680	0.432	15.64	15.60	15.60	15.64	0.930	0.930	0.771
0-1wk, kg												
ADG	0.302	0.288	0.293	0.331	0.040	0.295	0.312	0.298	0.310	0.675	0.765	0.524
ADFI	0.551	0.551	0.550	0.554	0.002	0.551	0.552	0.551	0.553	0.549	0.294	0.255
G:F	0.548	0.523	0.533	0.597	0.072	0.536	0.565	0.541	0.560	0.695	0.798	0.547
1-2wk, kg												
ADG	0.373	0.379	0.361	0.346	0.014	0.376	0.354	0.367	0.363	0.141	0.775	0.480
ADFI	0.595	0.599	0.599	0.600	0.003	0.597	0.600	0.597	0.600	0.372	0.341	0.598
G:F	0.627	0.633	0.603	0.577	0.023	0.630	0.590	0.615	0.605	0.107	0.695	0.495
0-2wk, kg												
ADG	0.337	0.333	0.327	0.338	0.019	0.335	0.333	0.332	0.336	0.894	0.840	0.697
ADFI	0.573	0.575	0.574	0.577	0.002	0.574	0.576	0.574	0.576	0.314	0.237	0.853
G:F	0.588	0.580	0.569	0.586	0.033	0.584	0.578	0.579	0.583	0.850	0.896	0.704

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 3. Effect of supplemental protease on the ATTD of nutrients with different CP levels in weaned pig.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
1wk, %												
DM	78.98	80.22	80.80	82.09	0.82	79.60	81.45	79.89	81.16	0.036	0.138	0.976
CP	77.96	78.43	77.90	77.15	1.00	78.20	77.53	77.93	77.79	0.510	0.886	0.549
GE	81.18	81.34	82.30	81.20	0.82	81.26	81.75	81.74	81.27	0.561	0.571	0.455
2wk, %												
DM	81.07	80.32	81.13	82.27	0.26	80.70	81.70	81.10	81.30	0.001	0.473	0.002
CP	76.57	78.41	77.06	78.79	0.37	77.49	77.93	76.82	78.60	0.246	<.001	0.884
GE	82.17	82.19	81.98	82.95	0.42	82.18	82.47	82.08	82.57	0.501	0.251	0.267

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; DM, dry matter; GE, gross energy; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 4. Effect of supplemental protease on the ATTD of amino acids with different CP levels in weaned pig at 7 days.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
Indispensable amino acids, %												
Threonine	74.94	74.89	76.04	73.92	1.58	74.92	74.98	75.49	74.41	0.967	0.511	0.530
Valine	73.93	74.35	75.61	73.28	1.90	74.14	74.45	74.77	73.82	0.877	0.629	0.488
Isoleucine	73.88	74.69	76.19	74.32	1.87	74.29	75.26	75.04	74.51	0.620	0.784	0.493
Leucine	79.20	80.28	79.45	79.14	1.19	79.74	79.30	79.33	79.71	0.720	0.757	0.577
Phenylalanine	79.83	80.75	79.72	79.79	1.25	80.29	79.76	79.78	80.27	0.683	0.701	0.743
Histidine	88.26	88.88	85.37	88.16	1.52	88.57	86.77	86.82	88.52	0.269	0.293	0.495
Lysine	78.37	78.40	77.82	76.60	1.74	78.39	77.21	78.10	77.50	0.520	0.741	0.728
Arginine	90.00	90.60	86.38	89.63	1.71	90.30	88.01	88.19	90.12	0.217	0.294	0.460
Methionine	75.24	74.18	82.33	83.09	1.73	74.71	82.71	78.79	78.64	0.002	0.934	0.616
Tryptophan	81.15	82.77	77.38	77.18	1.63	81.96	77.28	79.27	79.98	0.021	0.067	0.594
Total	79.64	80.20	79.67	79.48	1.24	79.92	79.58	79.66	79.84	0.787	0.886	0.771
Dispensable amino acids, %												
Aspartic acid	79.60	79.64	79.13	78.34	1.25	79.62	78.74	79.37	78.99	0.498	0.772	0.750
Serine	83.95	84.43	81.97	82.98	1.07	84.19	82.48	82.96	83.71	0.147	0.508	0.809
Glutamic acid	84.50	85.14	82.75	84.02	1.16	84.82	83.39	83.63	84.58	0.248	0.432	0.792
Proline	84.61	85.47	83.20	84.28	1.22	85.04	83.74	83.91	84.88	0.317	0.451	0.930
Glycine	73.30	73.10	74.34	71.61	2.28	73.20	72.98	73.82	72.36	0.923	0.947	0.596
Alanine	69.09	69.45	71.92	67.96	2.73	69.27	69.94	70.51	68.71	0.512	0.528	0.451
Tyrosine	75.84	76.80	76.07	74.85	1.95	76.32	75.46	75.96	75.83	0.671	0.948	0.590
Cysteine	83.87	84.20	82.18	81.82	1.16	84.04	82.00	83.03	83.01	0.117	0.992	0.775
Total	80.71	81.15	80.02	79.84	1.16	80.93	79.93	80.37	80.50	0.410	0.912	0.797

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 5. Effect of supplemental protease on the ATTD of amino acids with different CP levels in weaned pig at 14 days.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
Indispensable amino acids, %												
Threonine	74.16	76.72	75.75	76.63	0.44	75.44	76.19	74.96	76.68	0.125	0.004	0.093
Valine	72.49	74.91	73.81	75.27	0.68	73.70	74.54	73.15	75.09	0.253	0.021	0.497
Isoleucine	73.36	75.77	74.20	76.35	0.80	74.57	75.28	73.78	76.06	0.398	0.021	0.877
Leucine	78.91	81.21	79.92	80.96	0.50	80.06	80.44	79.42	81.09	0.468	0.010	0.242
Phenylalanine	79.50	81.87	80.91	81.50	0.53	80.69	81.21	80.21	81.69	0.356	0.023	0.131
Histidine	88.77	90.88	89.97	90.27	0.29	89.83	90.12	89.37	90.58	0.327	0.003	0.014
Lysine	76.24	78.39	78.34	79.14	0.87	77.32	78.74	77.29	78.77	0.139	0.127	0.458
Arginine	89.90	92.45	91.53	91.30	0.26	91.18	91.42	90.72	91.88	0.376	0.002	<.001
Methionine	76.77	79.85	81.66	85.18	1.11	78.31	83.42	79.22	82.52	0.002	0.017	0.846
Tryptophan	79.46	84.19	86.23	79.20	0.75	81.83	82.72	82.85	81.70	0.268	0.162	<.001
Total	78.96	81.44	80.67	81.52	0.53	80.20	81.10	79.82	81.48	0.133	0.014	0.165
Dispensable amino acids, %												
Aspartic acid	78.54	80.12	79.61	80.77	0.50	79.33	80.19	79.08	80.45	0.125	0.026	0.692
Serine	83.58	86.09	85.11	85.02	0.33	84.84	85.07	84.35	85.56	0.503	0.006	0.004
Glutamic acid	83.71	85.68	84.91	85.73	0.40	84.70	85.32	84.31	85.71	0.158	0.008	0.191
Proline	84.42	85.70	85.02	85.56	0.45	85.06	85.29	84.72	85.63	0.624	0.077	0.431
Glycine	70.22	72.97	72.76	73.31	0.85	71.60	73.04	71.49	73.14	0.129	0.089	0.233
Alanine	66.35	69.98	68.81	70.28	0.79	68.17	69.55	67.58	70.13	0.120	0.012	0.210
Tyrosine	72.86	78.54	77.62	75.44	0.58	75.70	76.53	75.24	76.99	0.193	0.017	<.001
Cysteine	83.01	85.35	84.72	84.79	0.65	84.18	84.76	83.87	85.07	0.403	0.102	0.120
Total	79.55	81.79	81.06	81.73	0.47	80.67	81.40	80.31	81.76	0.157	0.014	0.131

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 6. Effect of supplemental protease on blood profiles with different CP levels in weaned pig.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
Total protein, g/dL	5.47	5.40	4.97	5.37	0.24	5.44	5.17	5.22	5.39	0.285	0.500	0.348
BUN, mg/dL	8.17	7.33	7.00	5.83	0.42	7.75	6.42	7.59	6.58	0.005	0.027	0.695
IgG, mg/dL	370.0	389.3	368.7	307.3	50.61	379.7	338.0	369.3	348.3	0.420	0.683	0.435

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; BUN, blood urea nitrogen; IgG, immunoglobulin G; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

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Table 7. Effect of supplemental protease on odor emission with different CP levels in weaned pig.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
NH_3, ppm	11.67	10.50	11.83	11.50	0.81	11.09	11.67	11.75	11.00	0.367	0.481	0.614
H_2S, ppm	7.33	3.00	1.67	1.83	0.83	5.17	1.75	4.50	2.42	0.001	0.021	0.014

Abbreviation: CP, crude protein; PT, protease; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 8. Effect of supplemental protease on bacteria count with different CP levels in weaned pig.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
1wk, g/CFU												
E. coli	5.72	5.78	5.52	5.43	0.35	5.75	5.48	5.62	5.60	0.442	0.956	0.838
Lactobacillus	8.87	8.64	8.77	8.79	0.21	8.76	8.78	8.82	8.72	0.927	0.630	0.559
2wk, g/CFU												
E. coli	6.11	6.61	6.18	6.50	0.16	6.36	6.34	6.15	6.56	0.906	0.023	0.594
Lactobacillus	9.45	9.87	9.63	9.61	0.29	9.66	9.62	9.54	9.74	0.885	0.489	0.456

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; CFU, colony-forming unit; *E. coli*, *Escherichia coli*; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 9. Effect of supplemental protease on nitrogen utilization with different CP levels in weaned pig at 7 days.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
N intake, g/day	18.29	18.32	16.38	16.71	0.14	18.31	16.55	17.34	17.52	<.001	0.209	0.291
Urine excretion, g/day	368.7	372.4	366.8	367.7	4.25	370.5	367.3	367.7	370.1	0.451	0.589	0.745
N concentration in urine, %	0.15	0.14	0.14	0.13	0.01	0.15	0.14	0.15	0.14	0.062	0.154	0.627
N excretion in urine, mL/day	0.53	0.53	0.52	0.49	0.01	0.53	0.51	0.53	0.51	0.057	0.367	0.673
Feces excretion, g/day	99.16	99.18	93.82	101.34	3.72	99.17	97.58	96.49	100.26	0.673	0.323	0.325
N concentration in feces, %	4.06	3.98	3.84	3.77	0.01	4.02	3.81	3.95	3.88	<.001	<.001	0.904
N excretion in feces, g/day	4.03	3.95	3.61	3.82	0.14	3.99	3.72	3.82	3.89	0.067	0.654	0.322
Total N excretion, g/day	4.56	4.48	4.12	4.31	0.15	4.52	4.22	4.34	4.40	0.051	0.739	0.367
N retention, g/day	13.73	13.84	12.26	12.41	0.26	13.79	12.34	13.00	13.13	<.001	0.624	0.944
N retention, % of N intake	75.04	75.55	74.76	74.22	1.05	75.30	74.49	74.90	74.89	0.452	0.986	0.620

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; N, nitrogen; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

Table 10. Effect of supplemental protease on nitrogen utilization with different CP levels in weaned pig at 14 days.

CP level[1]	Basal		Low		SE	CP		PT		*P*-value		
PT level[2]	0%	0.1%	0%	0.1%		HP	LP	0	10	CP	PT	CP x PT
N intake, g/day	18.79	18.99	17.36	17.69	0.13	18.89	17.53	18.08	18.34	<.001	0.051	0.609
Urine excretion, g/day	379.9	378.9	372.4	375.2	4.08	379.4	373.8	376.1	377.1	0.185	0.821	0.652
N concentration in urine, %	0.18	0.16	0.16	0.16	0.01	0.17	0.16	0.17	0.16	0.195	0.075	0.296
N excretion in urine, g/day	0.67	0.60	0.60	0.58	0.02	0.64	0.59	0.64	0.59	0.089	0.089	0.291
Feces excretion, g/day	99.38	104.63	101.78	96.05	0.99	102.01	98.92	100.58	100.34	0.005	0.811	<.001
N concentration in feces, %	4.43	3.92	3.91	3.91	0.04	4.18	3.91	4.17	3.92	<.001	<.001	<.001
N excretion in feces, g/day	4.40	4.10	3.98	3.75	0.05	4.25	3.87	4.19	3.93	<.001	<.001	0.479
Total N excretion, g/day	5.07	4.70	4.57	4.33	0.05	4.89	4.33	5.07	4.52	<.001	<.001	0.204
N retention, g/day	13.73	14.29	12.79	13.36	0.14	14.01	13.08	13.26	13.83	<.001	<.001	0.089
N retention, % of N intake	73.03	75.28	73.63	75.51	0.33	74.16	74.57	73.33	75.40	0.029	<.001	0.111

Abbreviation: CP, crude protein; PT, protease; HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease; N, nitrogen; SE, standard of error

[1]CP level: Basal, diet containing 18.78% CP; Low, diet containing 16.92% CP

[2]PT level: supplementation of protease

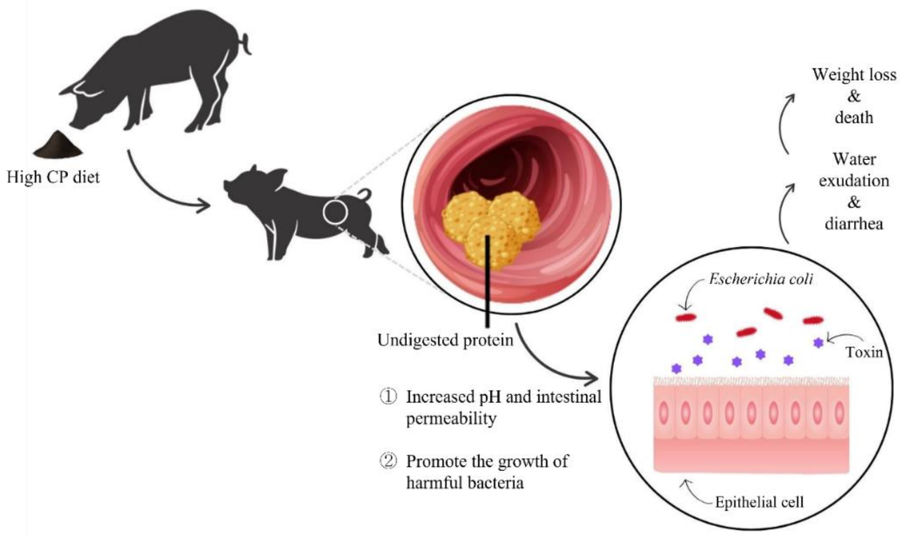

Fig. 1. Effects of high crude protein diet on weaned pig.

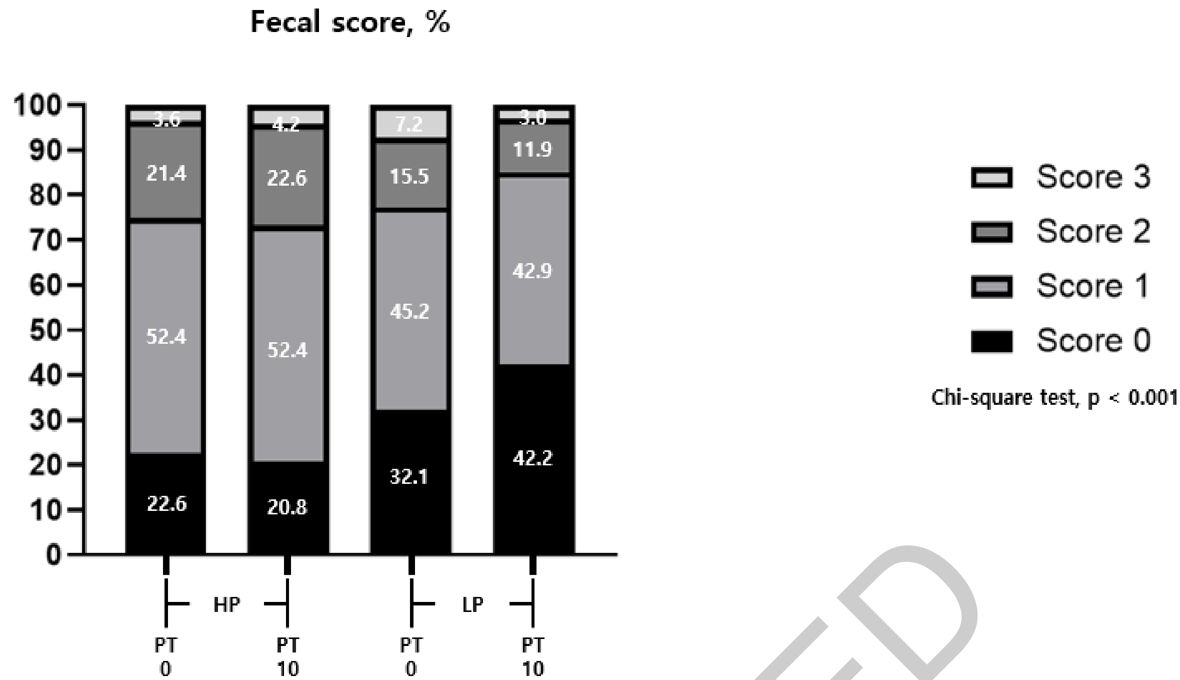

Fig. 2. Effect of supplemental protease on fecal score with different CP levels in weaned pig. Score 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. HP, high protein; LP, low crude protein; PT0, non-supplementation of protease; PT10, supplementation of 0.1% protease.