

# JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
<b>Article Type</b>	Research Article
<b>Article Title (within 20 words without abbreviations)</b>	Supplementation of protease and different nutrient density diets in growing-finishing pigs
<b>Running Title (within 10 words)</b>	Effects of protease with different nutrient density
<b>Author</b>	Sehyun Park <sup>1, #</sup> , Jihwan Lee <sup>2, #</sup> , Won Yun <sup>3</sup> , Seokman Hong <sup>3</sup> , Hanjin Oh <sup>1</sup> , Dongcheol Song <sup>1</sup> , Seyeon Chang <sup>1</sup> , Jaewoo An <sup>1</sup> , Hyunah Cho <sup>1</sup> , Kyeongho Jeon <sup>1</sup> , Jinho Cho <sup>1, *</sup> <i># These authors contributed equally to this work</i>
<b>Affiliation</b>	<sup>1</sup> Department of Animal Science, Chungbuk National University, Cheongju, Republic of Korea, 28644  <sup>2</sup> Department of Poultry Science, University of Georgia (UGA), Athens, GA, United States, 30602  <sup>3</sup> Central Research Institute, WOOSUNG FEED CO., LTD, Daejeon, Republic of Korea, 34379
<b>ORCID (for more information, please visit <a href="https://orcid.org">https://orcid.org</a>)</b>	Sehyun Park / parksae0808@naver.com ( <a href="https://orcid.org/0000-0002-6253-9496">https://orcid.org/0000-0002-6253-9496</a> ) Jihwan Lee / junenet123@naver.com ( <a href="https://orcid.org/0000-0001-8161-4853">https://orcid.org/0000-0001-8161-4853</a> ) Seokman Hong / smhong@woosung.kr ( <a href="https://orcid.org/0000-0003-4235-4061">https://orcid.org/0000-0003-4235-4061</a> ) Won Yun / wyoun@woosung.kr ( <a href="https://orcid.org/0000-0002-1835-2640">https://orcid.org/0000-0002-1835-2640</a> ) Hanjin Oh / dhgkswls17@naver.com ( <a href="https://orcid.org/0000-0002-3396-483X">https://orcid.org/0000-0002-3396-483X</a> ) Dongcheol Song / paul741@daum.net ( <a href="https://orcid.org/0000-0002-5704-603X">https://orcid.org/0000-0002-5704-603X</a> ) Seyeon Chang / angella2425@naver.com ( <a href="https://orcid.org/0000-0002-5238-2982">https://orcid.org/0000-0002-5238-2982</a> ) Jaewoo An / blueswing547@naver.com ( <a href="https://orcid.org/0000-0002-5602-5499">https://orcid.org/0000-0002-5602-5499</a> ) Hyunah Cho / hannah0928@naver.com ( <a href="https://orcid.org/0000-0003-3469-6715">https://orcid.org/0000-0003-3469-6715</a> ) Kyeongho Jeon / jeonkh1222@gmail.com ( <a href="https://orcid.org/0000-0003-2321-3319">https://orcid.org/0000-0003-2321-3319</a> ) Jinho Cho/ jinhcho@cbnu.ac.kr ( <a href="http://orcid.org/0000-0001-7151-0778">http://orcid.org/0000-0001-7151-0778</a> )
<b>Competing interests</b>	No potential conflict of interest relevant to this article was reported.
<b>Funding sources</b> State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
<b>Acknowledgements</b>	This work was carried out with the support of the WOOSUNG FEED CO., LTD, Republic of Korea.
<b>Availability of data and material</b>	All data generated or analysed during this study are included in this published article.
<b>Authors' contributions</b> Please specify the authors' role using this form.	Conceptualization: Cho JH. Data curation: Oh HJ, Song DC Formal analysis: Chang SY, Jeon KH. Methodology: An JW, Yun W, Hong SM.

	Software: Cho HA, Chang SY. Validation: Oh HJ. Investigation: Cho JH. Writing - original draft: Cho JH, Park SH, Lee JH. Writing - review & editing: Cho JH, Park SH, Lee JH.
<b>Ethics approval and consent to participate</b>	Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-1740-22-02).

#### CORRESPONDING AUTHOR CONTACT INFORMATION

<b>For the corresponding author (responsible for correspondence, proofreading, and reprints)</b>	<b>Fill in information in each box below</b>
First name, middle initial, last name	Jinho Cho
Email address – this is where your proofs will be sent	jinhcho@chungbuk.ac.kr
Secondary Email address	
Address	Chungbuk National University, Cheongju 28644, Korea
Cell phone number	+82-10-8014-8580
Office phone number	+82-43-261-2544
Fax number	+82-43-273-2240

ACCEPTED

## 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;  $18.74 \pm 3.46$  kg) were used for 15 weeks. Pigs were randomly assigned to six dietary treatments with 6 replicates of 3 pigs per pen in a  $3 \times 2$  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 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 crude protein (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<sub>3</sub>) emissions ( $p < 0.05$ ) during experiment periods and decreased hydrogen sulfide (H<sub>2</sub>S) 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<sub>3</sub> 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 (3 to 6):** Nutrient density, Protease, Growing-Finishing pig.

## 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 (150kcal) 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 [15, 16]. 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 [17-19]. 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 [20, 21]. 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 [22-25]. 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 [26, 27]. Furthermore, supplementation of exogenous protease to high protein diets can reduce N excretion into urine in growing pigs [28]. 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* [29, 30], 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. Protease B (PTB), commercial product, containing 75,000 protease units/g derived from *Nocardioopsis 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;  $18.74 \pm 3.46$  kg) were used for 15 weeks. Pigs were randomly assigned to six dietary treatments with 6 replicates of 3 pigs per pen in a  $3 \times 2$  factorial through 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). Feeds were provided by Woosung (Daejeon, Korea). The feed was formulated to meet or exceed the nutritional requirements of pigs according to NRC [3]. Each pig 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), crude protein (CP), and gross energy (GE) using AOAC methods [31]. 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, dry matter (DM), and gross

energy (GE), 0.2% chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) 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<sub>2</sub>O<sub>3</sub>.

Calculating the ATTD used the following formula:

$$\text{“Digestibility (\%)} = [1 - \{(Nf \times Cd)/(Nd \times Cf)\}] \times 100\text{”}$$

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<sub>3</sub>EDTA (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°C. BUN (Urease GLDH, Roche, Germany) and total protein concentration (Colorimetric assay, Roche, Germany) were analyzed using a blood analyzer (Cobas C702, Roche, Germany).

## **Gas emissions**

To analysis fecal ammonia (NH<sub>3</sub>), and hydrogen sulfide (H<sub>2</sub>S) 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 in room temperature (25°C). Before the measurement, feces with plastic boxes are shaken 20s to break down any crust formation. NH<sub>3</sub>, and H<sub>2</sub>S concentrations were determined in the ranges of 50.0 to 100.0 ppm (Gastec Corp., Gastec detector tube No. 3La for NH<sub>3</sub>; No. 4LK for H<sub>2</sub>S and No. 81L for acetic acid, Gastec Corp, detector tube, 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°C for 24 h, and *Lactobacillus* was cultured for 48 h.

## **Statistical analysis**

Data for effects of two types of feedstuffs (HN, 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 3, 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 4, 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 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 5, 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.

### **Gas emission**

As shown in Table 6, addition of PTA diets showed lesser than CON diets ( $p < 0.05$ )  $\text{NH}_3$  emissions at weeks 6. There was no significant difference ( $p < 0.05$ ) in  $\text{H}_2\text{S}$  emission. At weeks 13, there was significant difference ( $p < 0.05$ ) in  $\text{H}_2\text{S}$  between supplementation of protease. Supplementation of PTA and PTB decreased significantly in  $\text{H}_2\text{S}$  emission compared with CON. In  $\text{NH}_3$ , PTA showed lowest ( $p < 0.05$ ) emissions and CON showed highest emissions.

### **Fecal microflora**

As shown in Table 7, 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 and 143 kcal/kg, respectively) could improve ADG during growing periods [32,33]. Supporting the current study's results, numerous studies have reported a positive correlation between increased dietary protein with energy, which could improve growth performance [32,34,35]. 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 [13,33]. 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 [20, 21]. 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 [32]. 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 [36]. Increase of ADG with ATTD of CP was observed in the present study, which is consistent with results of previous study [13,33]. 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 [37]. 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 [38]. Several studies have indicated that BUN concentration and total protein in blood can be used as indicators of protein utilizations and N excretion [36,39]. 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 [33]. According to previous studies, differences in nutrient density were 1.2% and 2.1% dietary protein, respectively, with 100 kcal/kg energy level [33,40]. However, studies of Yan and Kim. [33] and Wang et al. [40] 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. [32] have reported that 1% difference in dietary protein does not reduce total protein in blood. Liu et al. [27] 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 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. [41] and Yan et al. [42], 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 reduce gas emissions due to improved utilization and absorption of dietary proteins [26,27]. 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 [13,33]. 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 [43]. 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. [21] 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 weaning period than in growing-finishing periods due to enhanced resistance to intestinal pathogens [44,45]. 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 [21]. 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* spp), they showed greater digestion of CP compared to CON and PTB (*Bacillus licheniformis*). This result was consistent with a study of Cheng et al. [46] 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* spp 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.

## **Acknowledgments**

This work was carried out with the support of the WOOSUNG FEED CO., LTD, Republic of Korea.

ACCEPTED

## References

1. Van Meensel J, Lauwers L, Van Huylenbroeck G. Communicative diagnosis of cost-saving options for reducing nitrogen emission from pig finishing. *Journal of Environmental Management*. 2010;91: 2370-7. doi: 10.1016/j.jenvman.2010.06.026
2. Dourmad JY, Jondreville C. Impact of nutrition on nitrogen, phosphorus, Cu and Zn in pig manure, and on emissions of ammonia and odours. *Livest Sci*. 2007;112: 192-8. doi: 10.1016/j.livsci.2007.09.002
3. NRC [National Research Council] Nutrient requirement of swine. 11th ed. Washington, DC: The National Academies Press; 2012.
4. Zamora V, Figueroa JL, Reyna L., Cordero JL, Sánchez-Torres MT, Martínez M. Growth performance, carcass characteristics and plasma urea nitrogen concentration of nursery pigs fed low-protein diets supplemented with glucomannans or protease. *J Appl Ani Res*. 2011;39: 53-6. doi: 10.1080/09712119.2011.565217
5. Kim YJ, Lee JH, Kim TH, Song MH, Yun W, Oh HJ, Lee JS, Kim HB, Cho JH. Effect of low protein diets added with protease on growth performance, nutrient digestibility of weaned piglets and growing-finishing pigs. *J Anim Sci Technol*. 2021;63: 491. doi: 10.5187/jast. 2021.e49
6. Kerr BJ, Easter RA. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. *J Anim Sci*. 1995;73: 3000-8. doi:10.2527/1995.73103000x
7. Figueroa JL, Lewis AJ, Miller PS, Fischer RL., Gómez RS, Diedrichsen RM. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J Anim Sci*. 2002;80: 2911-9. doi:10.2527/2002.80112911x
8. Otto E R, Yokoyama M, Ku PK, Ames NK, Trottier N L. Nitrogen balance and ileal amino acid digestibility in growing pigs fed diets reduced in protein concentration. *J Anim Sci*. 2003; 81: 1743-53. doi: 10.2527/2003.8171743x
9. He L, Wu L, Xu Z, Li T, Yao K, Cui Z, Yin Y, Wu G. 2016. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino Acids*. 2016; 48: 21-30. doi: 10.1007/s00726-015-2059-1
10. Carter SD, Cromwell G L, Lindemann MD, Turner LW, Bridges TC. Reducing N and P excretion by dietary manipulation in growing and finishing pigs. *J Anim Sci*. 1996; 74: 59.
11. Lan R, Tran H, Kim IH. Effects of probiotic supplementation in different nutrient density diets on growth performance, nutrient digestibility, blood profiles, fecal microflora and noxious gas emission in weaning pig. *J Sci Food Agric* 2017; 97: 1335-41. doi 10.1002/jsfa.7871
12. Hosseini SM, Akbary SM, Maheri-Sis N, Aghsaghali AM. Effect of different energy levels of diet on feed efficiency, growth rate and carcass characteristics of fattening Bahmaei lambs. *J Anim Vet Adv*. 2008; 7: 1551-4

13. Meng QW, Yan L., Ao X, Zhou TX, Wang JP, Lee JH, Kim IH. Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. *J Anim Sci Technol*. 2010; 88: 3320-6. doi:10.2527/jas.2009-2308
14. Upadhaya SD, Lee KY, Kim IH. Influence of protected organic acid blends and diets with different nutrient densities on growth performance, nutrient digestibility and faecal noxious gas emission in growing pigs. *Vet Med*. 2014; 59. doi:10.17221/7779-vetmed
15. Nyachoti CM, Zijlstra RT, De Lange CFM, Patience JF. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. *Can J Anim Sci*. 2004; 84: 549-66. doi:10.4141/a04-001
16. Lan R, Tran H, Kim IH. Effects of probiotic supplementation in different nutrient density diets on growth performance, nutrient digestibility, blood profiles, fecal microflora and noxious gas emission in weaning pig. *J Sci Food Agric*. 2014; 97: 1335-41. doi: 10.1002/jsfa.7871
17. Douglas SL, Edwards SA, Kyriazakis I. Management strategies to improve the performance of low-birth-weight pigs to weaning and their long-term consequences. *J Anim Sci*. 2014; 92: 2280-8. doi: 10.2527/jas.2013-7388
18. Douglas SL., Wellock I, Edwards SA, Kyriazakis I. High specification starter diets improve the performance of low-birth-weight pigs to 10 weeks of age. *J Anim Sci*. 2014; 92: 4741-50. doi: 10.2527/jas.2014-7625
19. Camp Montoro J, Solà-Oriol D, Muns R, Gasa J, Llanes N, Manzanilla EG. High levels of standardized ileal digestible amino acids improve feed efficiency in slow-growing pigs at late grower-finisher stage. *J Anim Physiol Anim Nutr*. 2022; 106: 276-83. doi:10.1111/jpn.13610
20. Park JW, Kim IH. Effect of dietary fermented corn in different energy dense diets on growth performance, nutrient digestibility, ileal microorganisms, and fecal noxious gas emission of growing pigs. *Can J Anim Sci*. 2018; 98: 325-32. doi: 10.1139/cjas-2016-0075
21. Zhao Y, Tian G, Chen D, Zheng P, Yu J, He J, Yu B. Effects of varying levels of dietary protein and net energy on growth performance, nitrogen balance and faecal characteristics of growing-finishing pigs. *Revista Brasileira de Zootecnia*. 2019; 48. doi: 10.1590/rbz4820180021
22. Carpenter DA, O'Mara FP, O'Doherty JV. The effect of dietary crude protein concentration on growth performance, carcass composition and nitrogen excretion in entire grower-finisher pigs. *Ir J Agric Food*. 2004; 227-36.
23. Mc Alpine PO, O'shea CJ, Varley PF, O'Doherty JV. The effect of protease and xylanase enzymes on growth performance and nutrient digestibility in finisher pigs. *J Anim Sci*. 2012; 90: 375-7. doi: 10.2527/jas.53979
24. Zuo J, Ling B, Long L, Li T, Lahaye L, Yang C, et al. Effect of dietary supplementation with protease on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes, and gene expression of weaned piglets. *Anim Nutr*. 2015; 1: 276-82. doi: 10.1016/j.aninu.2015.10.003

25. Choe J, Kim KS, Kim HB, Park S, Kim J, Kim S, ... & Song, M. Effect of protease on growth performance and carcass characteristics of growing-finishing pigs. *S Afr J Anim Sci.* 2017; 47: 697-703. doi:10.4314/sajas.v47i5.13
26. Lei XJ, Cheong JY, Park JH, Kim IH. Supplementation of protease, alone and in combination with fructooligosaccharide to low protein diet for finishing pigs. *Anim Sci J.* 2017; 88: 1987-93. doi: 10.1111/asj.12849
27. Liu, X., Yin, J, Kim IH. Effect of protease derived from *Pseudoalteromonas arctica* supplementation on growth performance, nutrient digestibility, meat quality, noxious gas emission and blood profiles in finishing pigs. *J Anim Physiol Anim Nutr.* 2019; 103: 1926-33. doi: 10.1111/jpn.13202
28. Kim YJ., Kim TH., Song MH, An JS, Yun W, Lee JH, Lee JS, Kim GM, Kim TH, Cho, J. H. Effects of different levels of crude protein and protease on nitrogen utilization, nutrient digestibility, and growth performance in growing pigs. *J Anim Sci Technol.* 2020; 62: 659. doi: 10.5187/jast.2020.62.5.659
29. Kim JM, Choi JH, Han DJ, Choi YS, Jeong JY, Choi GH, Paik HD Kim CJ. Effect of protease produced from *Bacillus polyfermenticus* SCD on quality of jerky. *Food Sci Biotechnol.* 2008; 17: 389-95.
30. Kim JM, Choi YS, Choi JH, Choi GH, Lee JH, Paik HD, Kim CJ. Effects of crude proteases extracted from *Bacillus polyfermenticus* on tenderizing pork meat. *Food Sci of Anim Resour.* 2011; 31: 491-6.
31. AOAC, M. [Association of official analytical chemists] International. *Official methods of analysis. AOAC International.* 16th ed. Washington, DC: AOAC International; 1995.
32. Yan L, Wang JP, Kim HJ, Meng QW, Ao X, Hong SM, Kim IH. Influence of essential oil supplementation and diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, meat quality and fecal noxious gas content in grower–finisher pigs. *Livest Sci.* 2010; 128: 115-22. doi: 10.1016/j.livsci.2009.11.008
33. Yan L, Kim IH. Effect of probiotics supplementation in diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, faecal microbial population and faecal noxious gas content in growing pigs. *J Appl Anim Res.* 2013; 41: 23-8. doi: 10.1080/09712119.2012.739092
34. Eittle T, Roth-Maier DA, Roth FX. Effect of apparent ileal digestible lysine to energy ratio on performance of finishing pigs at different dietary metabolizable energy levels. *J Anim Physiol Anim Nutr.* 2003; 87: 269-79. doi: 10.1046/j.1439-0396.2003.00436.x
35. Cho SB, Han IK, Kim YY, Park SK, Hwang OH, Choi CW, Yang SH, Park KH, Choi DY, Yoo YH. Effect of lysine to digestible energy ratio on growth performance and carcass characteristics in finishing pigs. *Asian-Australas J Anim Sci.* 2012; 25: 1582-7. doi: 10.5713/ajas.2012.12311
36. Upadhaya SD, Yun HM, Kim IH. Influence of low- or high-density corn and soybean meal-based diets and protease supplementation on growth performance, apparent digestibility, blood characteristics and noxious gas emission of finishing pigs. *Anim Feed Sci Technol.* 2016; 216: 281-7. doi: 10.1016/j.anifeedsci.2016.04.003
37. Nguyen DH, Upadhaya SD, Lei XJ, Yin J, Kim IH. Influence of dietary protease supplementation to corn–soybean meal-based high-and low-energy diets on growth performance, nutrient digestibility, blood profiles, and gas emission in growing pigs. *Can J Anim Sci.* 2019; 99: 482-8. doi: 10.1139/cjas-2017-0104

38. Fang LH, Jin YH, Do SH, Hong JS, Kim BO, Han TH, Kim YY. Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs. *Asian-Australas J Anim Sci.* 2019; 32: 556. doi: 10.5713/ajas.18.0294
39. Defu Yu, Weiyun Zhu, Suqin Hang. Effects of low-protein diet on the intestinal morphology, digestive enzyme activity, blood urea nitrogen, and gut microbiota and metabolites in weaned pigs. *Arch Anim Nutr.* 2019; 73: 4, 287-305, doi: 10.1080/1745039X.2019.1614849
40. Wang YM, Yu HT, Zhou JY, Zeng XF, Wang G, Cai S, Huang S, Zhu ZP, Tan JJ, Levesque CL, Qiao S.Y. Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. *Anim Feed Sci Technol.* 2019; 256: 114256. doi: 10.1016/j.anifeedsci.2019.114256
41. Ferket PR, van Heugten E, van Kempen TATG, Angel R. Nutritional strategies to reduce environmental emissions from non-ruminants. *J. Anim. Sci.* 2002; 80: E168–E182
42. Yan L, Meng QW, Kim IH. The effect of an herb extract mixture on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas content in growing pigs. *Livest Sci.* 2011; 141: 143-7. doi: 10.1016/j.livsci.2011.05.011
43. Nguyen DH, Lee SI, Cheong JY, Kim IH. Influence of low-protein diets and protease and bromelain supplementation on growth performance, nutrient digestibility, blood urine nitrogen, creatinine, and faecal noxious gas in growing–finishing pigs. *Can J Anim Sci.* 2018; 98: 488-97. doi: 10.1139/cjas-2016-0116
44. Giang HH, Viet TQ, Ogle B, Lindberg JE. Effects of supplementation of probiotics on the performance, nutrient digestibility and faecal microflora in growing-finishing pigs. 2011; *Asian-Australas J Anim Sci.* 24: 655-61. doi: 10.5713/ajas.2011.10238
45. Kim YJ, Cho SB, Song MH, Lee SI, Hong SM, Yun W, Lee JH, Oh HJ, Chang SY, An JW, Go YB, Song DC, Cho HA, Cho JH. Effects of different *Bacillus licheniformis* and *Bacillus subtilis* ratios on nutrient digestibility, fecal microflora, and gas emissions of growing pigs. *J Anim Sci Technol.* 2022; 64: 291. doi: 10.5187/jast.2022.e12
46. Cheng L, Jin YH, Kim IH. Effects of *Bacillus licheniformis* derived-protease supplementation, alone or in combination, with valine to low protein diet on growth performance and carcass quality grade in growing-finishing pigs. *J Appl Anim Res.* 2021; 49: 180-4. doi: 10.1080/09712119.2021.1933494

## Tables and Figures

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

Items	Growing period		Finishing period	
	Basal	High	Basal	High
<b>Ingredients (%)</b>				
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 <sup>x</sup>	0.220	0.220	0.220	0.220
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Calculated composition</b>				
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)	2475.5	2525.5	2475.5	2525.5
<b>Total AA (g/kg)</b>				
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
<b>AID AA (g/kg)</b>				
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

Abbreviation: High, high nutrient density diet; Basal, basal nutrient density diet; DDGS, Distiller's dried grains with solubles; MDCP, monocalcium 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.

<sup>x</sup> Provided per kilogram of complete diet: vitamin A, 11,025 U; vitamin D<sub>3</sub>, 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<sub>12</sub>, 33 µg; Cu (as CuSO<sub>4</sub> · 5H<sub>2</sub>O), 12 mg; Zn (as ZnSO<sub>4</sub>), 85 mg; Mn (as MnO<sub>2</sub>), 8 mg; I (as KI), 0.28 mg; and selenium (as Na<sub>2</sub>SeO<sub>3</sub> · 5H<sub>2</sub>O), 0.15 mg.

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

Items	High			Basal			SE	Den			Pro			<i>p</i> -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 w	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 w	38.46	39.66	38.84	37.89	39.29	38.89	0.399	38.99	38.69	38.18b	39.48a	38.86ab	0.378	0.015	0.737	
6 w	51.56	52.63	51.99	50.40	52.06	52.14	0.387	52.06	51.54	50.98b	52.35a	52.06a	0.114	0.006	0.266	
9 w	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 w	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 w	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 2w																
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 4w																
ADG, kg	0.75	0.83	0.78	0.75	0.81	0.81	0.021	0.79	0.79	0.75b	0.82a	0.80ab	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 6w																
ADG, kg	0.94	0.93	0.94	0.89	0.91	0.95	0.009	0.93	0.92	0.91b	0.92ab	0.94a	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	2.15b	2.36ab	2.33ab	2.47a	2.34ab	2.30ab	0.060	2.29	2.37	2.31	2.35	2.32	0.109	0.768	0.011	
0 to 6w																
ADG, kg	0.78	0.81	0.80	0.75	0.79	0.80	0.007	0.79	0.78	0.77b	0.80a	0.80a	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 9w															
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 13w															
ADG, kg	0.93	0.95	0.88	0.94	0.98	0.90	0.025	0.92	0.93	0.93ab	0.97a	0.89b	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	2.82ab	2.71b	2.94a	0.439	0.034	0.764
13 to 15w															
ADG, kg	0.98	1.07	1.09	0.93	0.94	1.02	0.037	1.05	0.99	0.98b	1.01ab	1.06a	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 15w															
ADG, kg	0.95	0.96	0.93	0.95	0.95	0.93	0.010	0.95	0.95	0.95ab	0.96a	0.93b	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 15w															
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

Abbreviation: High, high nutrient density diet; Basal, basal nutrient density diet; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SE, standard error; Den, different nutrient density diet; Pro, supplementation of protease; Den × Pro, different nutrient density diet × supplementation of protease

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  $p < 0.05$

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

Items, %	High			Basal			SE	Den		Pro			<i>p</i> -value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den×Pro
DM															
6w	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
13w	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															
6w	70.78	72.06	71.48	67.75	69.69	68.59	0.539	71.44	68.68	69.26b	70.87a	70.04ab	<.0001	0.027	0.816
13w	67.34c	71.39a	69.48b	70.96ab	70.88ab	72.10a	0.501	69.40	71.32	69.15b	71.13a	70.79a	0.001	0.002	0.002
GE															
6w	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
13w	70.68	72.63	71.93	70.55	72.13	71.33	0.545	71.74	71.33	70.61b	72.38a	71.63ab	0.371	0.016	0.900

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

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  $p < 0.05$

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

Items	High			Basal			SE	Den		Pro			<i>p</i> -value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den×Pro
Total protein, g/dL															
6w	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
13w	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															
6w	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
13w	14.60	11.80	13.20	14.20	11.40	12.60	0.645	13.20	12.73	14.40a	11.60b	12.90b	0.385	0.001	0.984

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

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  $p < 0.05$

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

Items, ppm	High			Basal			SE	Den			Pro			<i>p</i> -value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den×Pro	
<b>H<sub>2</sub>S</b>																
6w	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	
13w	5.30	4.80	5.00	5.40	4.60	5.10	0.146	5.04	5.03	5.35a	4.73b	5.03b	0.890	0.002	0.554	
<b>NH<sub>3</sub></b>																
6w	3.45	3.18	3.35	3.53	3.13	3.30	0.126	3.33	3.32	3.49a	3.15b	3.33ab	0.936	0.049	0.850	
13w	3.40	3.10	3.30	3.60	3.20	3.20	0.129	3.28	3.34	3.52a	3.16b	3.26ab	0.587	0.039	0.476	

Abbreviation: High, high nutrient density diet; Basal, basal nutrient density diet; CON, basal diet; PTA, CON + 0.0125% protease A; PTB, CON + 0.010% protease B; H<sub>2</sub>S, hydrogen sulfide; NH<sub>3</sub>: ammonia; SE, standard error; Den, different nutrient density diet; Pro, supplementation of protease; Den × Pro, different nutrient density diet × supplementation of protease

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  $p < 0.05$

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

Items, (log <sub>10</sub> cfu/g)	High			Basal			SE	Den			Pro			<i>p</i> -value		
	CON	PTA	PTB	CON	PTA	PTB		High	Basal	CON	PTA	PTB	Den	Pro	Den×Pro	
<i>E. coli</i>																
6w	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	
13w	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	
<i>Lactobacillus</i>																
6w	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	
13w	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	

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

High nutrient density is 0.94-0.98% higher than requirement in dietary protein and 50 kcal/kg in energy than basal nutrient density