RESEARCH ARTICLE

J Anim Sci Technol 2022;64(5):854-862

https://doi.org/10.5187/jast.2022.e51

JAST

Journal of Animal Science and Technology

pISSN 2672-0191 eISSN 2055-0391

Effects of protease enzyme supplementation in weanling pigs' diet with different crude protein levels on growth performance and nutrient digestibility

Olivier Munezero and In Ho Kim*

Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea

Received: Mar 24, 2022
Revised: Jun 14, 2022
Accepted: Jun 14, 2022

***Corresponding author**
In Ho Kim
Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea.
Tel: +82-41-550-3652
E-mail: inhokim@dankook.ac.kr

ORCID
Olivier Munezero
https://orcid.org/0000-0003-4574-0494
In Ho Kim
https://orcid.org/0000-0001-6652-2504

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

Funding sources
Not applicable.

Acknowledgements
Not applicable.

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

Abstract

The purpose of this study is to determine the impact of different levels of crude protein (CP) diets supplemented with dietary protease on the growth performance and nutrient digestibility of the weanling pigs. In a 5-week study, 100 crossbred ([Landrace × Yorkshire] × Duroc) of weaner pigs that have an average initial body weight (BW) of 7.17±1.06 kg were assigned to one of four dietary treatments with 5 replications and 5 pigs (3 gilts and 2 castrated male pigs) per pen in a randomized complete block design. The dietary treatments were as follows: Phase 1: CON: basal diets (20.60% CP); low protein (LP): CON − 0.30% CP; PLP1: (CON − 0.30% CP) + 0.05% protease; PLP2: (CON − 0.50% CP) + 0.05% protease. Phase 2: CON: basal diets (18.88% CP); LP: CON − 0.30% CP; PLP1: (CON − 0.30% CP) + 0.05% protease; PLP2: (CON − 0.50% CP) + 0.05% protease. The addition of protease to low CP diets significantly increased the feed conversion ratio (FCR) (p = 0.039), BW (p = 0.046), average daily gain (ADG) (p = 0.049), and average daily feed intake (ADFI) tended to increase (p = 0.053) in the young pigs during phase 1. However, FCR tended to increase throughout the experiment but did not change during phase 2, whereas BW, ADG, and ADFI stayed unchanged throughout phase 2 and overall. There was no significant difference in dry matter, nitrogen (N), and gross energy of nutrient digestibility in all phases and overall in weaned pigs with low CP when protease was fed. In contrast, adding protease to the low CP diets increased the tendency of N digestibility (p = 0.059) during phase 1. It is concluded that dietary protease supplementation tended to increase N retention during the first phase of the weaning period, hence increasing piglet performance.

Keywords: Crude protein, Growth performance, Nutrient digestibility, Protease, Weanling pigs

INTRODUCTION

The pig's weaning period is possibly the most stressful time of its productive life and is the foundation of a thriving lifetime of growth performance [1]. During this critical period, weaned piglets face a variety of problems, including post-weaning diarrhea and reduced feed intake due to an immature

corresponding author.

Authors' contributions
Conceptualization: Munezero O, Kim IH.
Data curation: Munezero O.
Formal analysis: Munezero O.
Methodology: Munezero O.
Software: Munezero O.
Validation: Kim IH.
Investigation:Munezero O, Kim IH.
Writing - original draft: Munezero O.
Writing - review & editing: Munezero O, Kim IH.

Ethics approval and consent to participate
The Institutional Animal Care and Use Committee at Dankook University in Cheonan, Korea, examined and approved the outlined protocols (Protocol number DK-1-2106).

immune system and limited enzyme secretory capacity [2], both of which are economically significant in today's pig industry. Therefore, it is critical to improve the weanling pigs' ability to digest nutrients and establish a healthy and strong foundation for long-term growth and profitability. The addition of exogenous enzymes to the pig diets with reduced crude protein (CP) can improve the growth performance. This is due to improved protein digestibility and amino acid levels, allowing weanling pigs to thrive and continue to benefit later in life. As a result, excessive nutrient excretion is prevented, allowing for lower production costs. Exogenous enzymes supplemented as pig diet additives hydrolyze non-starch polysaccharides (NSP), break the cell wall that encapsulates them, degrade anti-nutritional factors (protease inhibitors, antigenic proteins, non-protein amino acids), and break glycolytic bonds that are not hydrolyzed by endogenous enzymatic activity [3–5]. Exogenous enzymes that can be introduced into pig diets include phytases, carbohydrases, proteases, and lipases [6]. Pigs can produce digestive proteases such as pepsin, trypsin, and chymotrypsin to break down proteins [7]. However, the secretion of endogenous proteases (trypsin, chymotrypsin) decreases dramatically during the weaning stage [8]. In a study by Lindemann et al. [2], weanling piglets experienced a severe and rapid drop in endogenous protease (trypsin and chymotrypsin) secretion after weaning. This drop was due to post-weaning stress or a lack of solid feed intake after weaning and it required more than two weeks to return to normal secretion levels. Because of the decrease in endogenous protease production, there is a possibility of an increase in the flow of undigested protein to the hindgut, leading to the production of ammonia, which is harmful to gut health and digestive capacity. It has been suggested that adding exogenous enzymes (such as protease) to post-weaning diets can aid in controlling post-weaning challenges by compensating for underdeveloped endogenous enzyme secretory capacity and enhancing nutrient digestibility in weaned pigs [1]. Proteases are enzymes that can be produced in the digestive tract [9]. Based on their catalytic mechanisms, proteases can be divided into six groups: Metalloproteases, cysteine, serine, and threonine proteases, aspartic, and glutamic [10]. Several studies have shown that protease improves the apparent ileal digestibility of gross energy (GE), dry matter (DM), CP, starch, NSP, phytate, isoleucine, valine, and aspartic acid during the weaning stage. Apparent total tract digestibility of DM, GE, CP, starch, NSP, phytate, and utilization of phosphorus; nutrient digestibility and modification of microbial communities in the posterior intestine; concentrations of acetic, propionic, and butyric acids in the cecum and colon; fermentation capacity in the intestine and a longer transit time are improved by protease [5,11–13]. Many studies have shown that exogenous protease supplementation in diets improves growth performance and nutrient digestibility, particularly for CP and amino acids [14,15]. Even though the objective of exogenous enzyme supplementation in the diet is to promote growth performance and nutrient digestibility, a review of the literature revealed that the protease results published to date are inconsistent [7]. The numerous functions of proteases may be affected by dietary formulation and ingredients [16]. Nevertheless, dietary protease supplementation can compensate for decreased endogenous protease secretion and boost digestive capacity in weanling pigs. Therefore, the focus of this research was to assess the effect of protease supplementation on growth performance and nutrient digestibility of the weanling pigs.

MATERIALS AND METHODS

This experiment was conducted at Dankook University's Swine Research Unit in Jeonui (Sejong, Korea). Dankook University's Institutional Animal Care and Use Committee in Cheonan, Korea, examined and approved the outlined protocols (Protocol number DK-1-2106) and weanling pigs used in this study.

Protease enzyme

The protease enzyme used in this study was a commercial product purchased from (Ronozyme ProAct®, Novozymes A/S, Bagavaerd, Denmark) synthesized from *Bacillus licheniformis* submerged fermentation.

Experimental design, animal housing, and diets

In the 5-weeks of the experiment, a total of a hundred crossbred [(Landrace × Yorkshire) × Duroc] weanling pigs were assigned to one of four dietary treatments based on the average initial body weight (BW) of (7.17 ± 1.06 kg) and sex. They were then divided into 5 replications, with each pen containing 5 pigs (3 gilts and 2 castrated barrows) in a randomized complete block design. The nutritional diets were provided in two distinct phases, phase 1 (0 to 14 days) and phase 2 (15 to 35 days). The dietary treatments were as follows:

Phase 1: CON: basal diets (20.60% CP); low protein (LP): CON - 0.30% CP; PLP1: (CON – 0.30% CP) + 0.05% protease; PLP2: (CON – 0.50% CP) + 0.05% protease.
Phase 2: CON: basal diets (18.88% CP); LP: CON – 0.30% CP; PLP1: (CON – 0.30% CP) + 0.05% protease; PLP2: (CON – 0.50% CP) + 0.05% protease.

The basal diets were prepared following National Research Council (NRC) standards and were designed to meet or exceed the nutrient requirements of pigs [17]. The dietary composition of weanling pigs utilized in this experiment is illustrated in Table 1. The room temperature was kept at 28℃, and each pen was outfitted with a self-feeder and nipple drinker, allowing the weanling pigs' free access to feed and water throughout the research.

Sampling, measurements, and laboratory procedures

To compute the growth performance, the individual piglet's BW was measured using a GL-6000S balance machine (G-Tech International, Uijiengbu, Korea) on days 14 and 35 to determine the average daily gain (ADG). Meanwhile, the amount of feed ingested and feed residues (on a per-pen basis) were recorded to calculate the average daily feed intake (ADFI) and feed conversion ratio (FCR).

Seven days before fecal sample collection, 2 g/kg of indigestible marker chromium oxide (Cr_2O_3) was added to weanling pig diets to determine nutrient digestibility of nitrogen (N), DM, and GE. Fresh fecal samples were collected by qualified personnel at the end of week 5 from two pigs per pen (1 gilt and 1 barrow) using a rectal massage technique, immediately placed in an ice box, delivered to the laboratory, and maintained at −20℃ until examined. Then the samples were dried for 72 hours in a forced-air oven at 70℃ before being finely powdered to pass through a 1-mm screen sieve. The AOAC recommended procedures were used to analyze the DM. UV absorption spectrophotometry was used to determine the amount of chromium in the sample (UV-1201, Shimadzu, Kyoto, Japan) and the findings were recorded for statistical analysis. 2 grams of feed and fecal sample were measured and assigned in an oxygen bomb calorimeter Parr 6400 to calculate GE (Parr Instrument Company, Moline, Il, USA). A Kjeltec 8600 analyzer was used to determine the N content (Foss Tecator AB, Hoeganaes, Sweden). The following formula was used to calculate the nutrient digestibility: N digestibility = 1 – [(Nf × Cd) / (Nd × Cf)]; N – nitrogen, Nf – nutrient concentration in feces, Nd – nutrient concentration in diet, Cd – chromium concentration in diet, and Cf – chromium concentration in feces.

https://doi.org/10.5187/jast.2022.e51

Table 1. Diets composition (as-fed basis)

	Phase 1 (0–14 days)				Phase 2 (15–35 days)			
	CON[1)]	LP	PLP1	PLP2	CON	LP	PLP1	PLP2
Corn	36.17	36.87	36.78	37.28	62.56	63..30	63.20	63.68
Expeller-pressed corn	15.00	15.00	15.00	15.00	5.00	5.00	5.00	5.00
Lactose	10.00	10.00	10.00	10.00	3.00	3.00	3.00	3.00
Dehulled soybean meal	12.14	11.49	11.50	11.03	10.36	9.66	9.68	924
Hamlet protein	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Plasma protein	6.00	6.00	6.00	6.00	4.00	4.00	4.00	4.00
Whey protein	5.00	5.00	5.00	5.00	-	-	-	-
Soybean oil	1.26	1.26	1.29	1.28	0.70	0.69	0.72	0.72
Limestone powder	1.20	120	120	120	0..99	0..99	0..99	0..99
Mono-calcium phosphate	1.23	123	123	123	133	133	133	133
Methionine	0.15	0.14	0.14	0.14	0.12	0.11	0.11	0.10
Lysine	0.32	028	028	026	0.41	0.39	0.39	036
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc oxide	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Vitamin-mineral mix[2)]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Saline	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Chromium oxide	0.30	030	030	030	030	030	030	030
Protease	-	-	0.05	0.05	-	-	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated value								
Moisture	9.85	9.87	9.86	9.87	11.43	11.45	11.44	11.45
Crude protein (%)	20.60	20.30	20.30	20.10	18.88	18.58	18.58	18.38
Crude fat (%)	3.30	3.32	3.35	3.35	324	325	328	329
Crude fiber (%)	1.66	1.66	1.65	1.65	1.82	1.81	1.81	1.80
Crude ash (%)	6.48	6.45	6.45	6.43	5.83	5.80	5.80	5.78
Calcium (%)	0.79	0.79	0.79	0.79	0.69	0.69	0.69	0.69
Phosphorus (%)	0.62	0.62	0.62	0.62	0.61	0.60	0.60	0.60
Lysine (%)	1.53	1.48	1.48	1.45	1.40	136	136	133
Methionine (%)	0.44	0. 43	0.43	0.42	0.40	0.39	0.39	0.38
Metabolizable energy (kcal)	3.400	3.400	3.400	3.400	3.350	3.350	3.350	3.350

[1)]CON, basal diet; LP, CON - 0.3% CP; PLP1, LP + 0.05% protease; PLP2, (CON - 0.50% CP) + 0.05% protease.

[2)]Provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E,48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; d-pantothenic, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 mg, Fe (as $FeSO_4 \cdot 7H_2O$), 90 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 15 mg; Zn (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se(as $Na_2SeO_3 \cdot 5H_2O$), 0.25 mg.

LP, low protein; CP, crude protein.

Statistical analysis

The data from the experiments were cleaned and exported to SAS software (SAS Institute, Cary, NC, USA) for analysis. They were analyzed using a completely randomized block design by using SAS's Generalized Linear Model procedure. The pen was regarded as the experimental unit. The GLM approach was used to assess significant differences in mean values among dietary treatments using repeated measurements and Duncan's tests. The standard error means were used to express data variability. Probabilities less than 0.05 were deemed significant.

RESULTS

Growth performance

Table 2 displays the growth performance data. The findings indicated that the inclusion of protease in low CP diets enhanced the weanling pigs' BWG (p = 0.046), ADG (p = 0.049), FCR (p = 0.039), and ADFI tended to increase (p = 0.053) during phase 1. However, FCR tended to increase (p = 0.076) throughout the experiment but was not affected during phase 2, and also BW, ADG, and ADFI remained unaltered throughout phase 2 and overall.

Nutrient digestibility

The nutrient digestibility of N, DM, and GE is illustrated in Table 3. Dietary protease supplementation in weaned pigs with low CP had no effect on N, DM, and GE on nutrient digestibility in all phases and overall. However, the addition of protease to the low CP diets increased tendency of N digestibility (p=0.059) during phase 1.

DISCUSSION

Growth performance

Exogenous protease enzyme supplementation to the diets to promote protein usage has attracted the pig industry's interest. Generally, dietary protease has been utilized as a mono-component or as part of an enzyme combination in poultry and swine diets [13,18]. Protein is required for the metabolism and physiology of animals, and changing CP levels in isoenergetic diets, on the other hand, is linked to changes in energy-to-protein ratios, which can have a big impact on body composition such as body fat content [19]. Exogenous enzymes should be studied because they have been found to assist lower the amount of nutrients in animal diets, especially CP. Various

Table 2. Effect of supplementing dietary protease to low CP diet on growth performance in weanling pigs

Items	CON[1)]	LP	PLP1	PLP2	SEM	*p*-value
Body weight (kg)						
Initial	7.17	7.17	7.17	7.17	0.003	0.887
Phase 1	11.74[a]	11.38[b]	11.61[ab]	11.34[b]	0.100	0.046
Phase 2	21.66	21.17	21.54	21.39	0.220	0.463
Phase 1 (0–14 days)						
ADG (g)	326[a]	300[b]	317[ab]	297[b]	7.0	0.049
ADFI (g)	413	385	405	385	8.0	0.053
FCR	1.266[b]	1.284[ab]	1.279[ab]	1.296[a]	0.006	0.039
Phase 2 (15–35 days)						
ADG (g)	472	466	473	479	11.0	0.867
ADFI (g)	718	724	724	742	15.0	0.717
FCR	1.521	1.553	1.530	1.550	0.014	0.312
Overall						
ADG (g)	414	400	410	406	6.0	0.476
ADFI (g)	596	588	596	599	6.0	0.858
FCR	1.441	1.472	1.453	1.475	0.010	0.076

[1)]CON, basal diet; LP, CON − 0.3% CP; PLP1, LP + 0.05% protease; PLP2, (CON − 0.50% CP) + 0.05% protease.

[a,b]Means groups that have different superscripts in the same row are distinct (p < 0.05).

CP, crude protein; LP, low protein; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

https://doi.org/10.5187/jast.2022.e51

Table 3. Effect of dietary protease supplementation on nutrient digestibility in weanling pigs fed a low-CP diet

Items	CON[1)]	LP	PLP1	PLP2	SEM	*p*-value
Phase 1 (0–14 days)						
Dry matter	77.28	76.71	78.11	78.17	0.725	0.373
Nitrogen	69.26	70.98	73.26	72.17	1.008	0.059
Gross energy	74.89	75.02	75.79	75.42	0.670	0.814
Phase 2 (15–35 days)						
Dry matter	74.87	74.89	75.26	76.81	1.187	0.625
Nitrogen	67.85	68.66	68.56	69.88	1.838	0.888
Gross energy	72.97	72.40	72.62	74.38	1.340	0.727

[1)]CON, basal diet; LP, CON − 0.3% CP; PLP1, LP + 0.05% protease; PLP2, (CON − 0.50% CP) + 0.05% protease.

[a,b]Means groups that have different superscripts in the same row are distinct ($p < 0.05$).

CP, crude protein; LP, low protein.

researchers have reported that the inclusion of protease in the diet of swine can enhance their growth performance. Protease supplementation increased ADG and G: F ratio ($p < 0.05$) in finishing pigs fed a protease-supplemented diet during weeks 6–12 [20]. Moreover, Lei et al. [21] reported that supplementing protease to a low CP diet increased growth performance. In the current study, weanling pigs fed low CP diets and treated with protease had better BW, ADG, and FCR during phase 1, and also the ADFI tended to increase when protease was added to the weaned pigs' diets. Contrary to expectations, this study found no significant differences in growth performance between the four groups during phase 2 or throughout the trial. The release of endogenous enzymes is lower in younger animals due to post-weaning stress or a lack of solid feed intake after weaning [22]. As a result, young animals are more responsive to exogenous enzyme supplementation, which is likely why phase 1exhibited such a great positive result. Although these findings differ from those of some previously published studies [9,23], they are consistent with a study conducted on broiler chickens by Yan et al. [24] who found that supplementing protease to broiler chickens' diet during the starter phase had a stronger effect than during other growth phases. Moreover, the current study's findings are consistent with those of Lei et al. [21], who discovered improved growth performance in finishing pigs fed protease-supplemented diets. Further research on the effect of protease on pig growth performance is needed to resolve the inconsistencies in available data.

Nutrient digestibility

Protease is recognized as a dietary enzyme that aims to increase protein availability by targeting tight protein binding. Protease improves protein digestibility by hydrolyzing proteins into free amino acids and peptides that can be absorbed in the small intestine [25]. Protease supplementation has been shown to improve performance in both normal and reduced protein diets [26]. In the current experiment, we discovered that protease had no effect on N, DM, and GE on the nutrient digestibility in either each phase or overall. The lack of effect of the supplemented protease in the second phase could be attributed to the post-weaning phase, where pancreatic protease secretions were in an exponential phase [18] and the endogenous enzyme metabolism could have reduced the activity of the supplemented protease. However, adding protease to the low CP diets, increased the tendency of N digestibility during phase 1. This negative nutrient digestibility response in the current study could be attributed to the substrate released by the enzyme, which may have been used by commensal bacteria rather than the pig's metabolism, as improvements in nutrient digestibility do not always translate to significant increases in metabolic energy. Although many prior studies

claimed considerable improvements in nutrient digestibility [11,27–29]. For example, Tactacan et al. [11] discovered that pigs fed the protease diet had higher apparent total tract digestibility of DM and N ($p < 0.05$) than pigs fed the CON diet. In addition, Guggenbuhl et al. [18] found that dietary serine protease generated by *B. licheniformis* fermentation increased apparent ileal amino acid digestibility in weaned piglets. However, other research studies produces the results that are consistent with our current findings. For instance, Mohammadigheisar and Kim [9] discovered that the addition of dietary protease did not affect N digestibility. It is possible that young animals are more sensitive to supplemental protease, in agreement with the findings of this study.

CONCLUSIONS

By incorporating protease into the feed of weaned pigs, we were able to reduce dietary CP levels while having no negative effect on piglets' performance. The introduction of exogenous protease to the diets of weanling pigs improved their BW, ADG, and FCR during phase 1, and also the ADFI tended to increase when protease was added to the weaned pigs' diets. Furthermore, the findings revealed that adding exogenous protease to weaned pig feed tended to improve N digestibility during phase 1. Therefore, supplementing dietary protease into piglets' diets should be considered, as the results of the current study show that it can improve piglet performance.

REFERENCES

1. Torres-Pitarch A, Hermans D, Manzanilla EG, Bindelle J, Everaert N, Beckers Y, et al. Effect of feed enzymes on digestibility and growth in weaned pigs: a systematic review and meta-analysis. Anim Feed Sci Technol. 2017;233:145-59. https://doi.org/10.1016/j.anifeedsci.2017.04.024
2. Lindemann MD, Cornelius SG, El Kandelgy SM, Moser RL, Pettigrew JE. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J Anim Sci. 1986;62:1298-307. https://doi.org/10.2527/jas1986.6251298x
3. Kim JC, Sands JS, Mullan BP, Pluske JR. Performance and total-tract digestibility responses to exogenous xylanase and phytase in diets for growing pigs. Anim Feed Sci Technol. 2008;142:163-72. https://doi.org/10.1016/j.anifeedsci.2007.07.004
4. Masey O'Neill HV, Smith JA, Bedford MR. Multicarbohydrase enzymes for non-ruminants. Asian-Australas J Anim Sci. 2014;27:290-301. https://doi.org/10.5713/ajas.2013.13261
5. Recharla N, Kim D, Ramani S, Song M, Park J, Balasubramanian B, et al. Dietary multi-enzyme complex improves in vitro nutrient digestibility and hind gut microbial fermentation of pigs. PLOS ONE. 2019;14:e0217459. https://doi.org/10.1371/journal.pone.0217459
6. Ravindran V. Feed enzymes: the science, practice, and metabolic realities. J Appl Poult Res. 2013;22:628-36. https://doi.org/10.3382/japr.2013-00739
7. Aranda-Aguirre E, Robles-Jimenez LE, Osorio-Avalos J, Vargas-Bello-Pérez E, Gonzalez-Ronquillo M. A systematic-review on the role of exogenous enzymes on the productive performance at weaning, growing and finishing in pigs. Vet Anim Sci. 2021;14:100195. https://doi.org/10.1016/j.vas.2021.100195
8. 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. https://doi.org/10.1016/j.aninu.2015.10.003
9. Mohammadigheisar M, Kim IH. Addition of a protease to low crude protein density diets of

https://doi.org/10.5187/jast.2022.e51

broiler chickens. J Appl Anim Res. 2018;46:1377-81. https://doi.org/10.1080/09712119.2018.1512862
10. Rawlings ND, Tolle DP, Barrett AJ. MEROPS: the peptidase database. Nucleic Acids Res. 2004;32:D160-4. https://doi.org/10.1093/nar/gkh071
11. Tactacan GB, Cho SY, Cho JH, Kim IH. Performance responses, nutrient digestibility, blood characteristics, and measures of gastrointestinal health in weanling pigs fed protease enzyme. Asian-Australas J Anim Sci. 2016;29:998-1003. https://doi.org/10.5713/ajas.15.0886
12. Yi JQ, Piao XS, Li ZC, Zhang HY, Chen Y, Li QY, et al. The effects of enzyme complex on performance, intestinal health and nutrient digestibility of weaned pigs. Asian-Australas J Anim Sci. 2013;26:1181-8. https://doi.org/10.5713/ajas.2013.13129
13. Zhang GG, Yang ZB, Wang Y, Yang WR, Zhou HJ. Effects of dietary supplementation of multi-enzyme on growth performance, nutrient digestibility, small intestinal digestive enzyme activities, and large intestinal selected microbiota in weanling pigs. J Anim Sci. 2014;92:2063-9. https://doi.org/10.2527/jas.2013-6672
14. Cowieson AJ, Roos FF. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. Anim Feed Sci Technol. 2016;221:331-40. https://doi.org/10.1016/j.anifeedsci.2016.04.015
15. O'Shea CJ, Mc Alpine PO, Solan P, Curran T, Varley PF, Walsh AM, et al. The effect of protease and xylanase enzymes on growth performance, nutrient digestibility, and manure odour in grower–finisher pigs. Anim Feed Sci Technol. 2014;189:88-97. https://doi.org/10.1016/j.anifeedsci.2013.11.012
16. Kocher A, Choct M, Ross G, Broz J, Chung TK. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. J Appl Poult Res. 2003;12:275-83. https://doi.org/10.1093/japr/12.3.275
17. NRC [National Research Council]. Nutrient requirements of domestic animals: No. 2. nutrient requirements of swine. Washington, DC: The National Academies Press; 1953.
18. Guggenbuhl P, Waché Y, Wilson JW. Effects of dietary supplementation with a protease on the apparent ileal digestibility of the weaned piglet. J Anim Sci. 2012;90:152-4. https://doi.org/10.2527/jas.53835
19. Swennen Q, Janssens GPJ, Decuypere E, Buyse J. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: energy and protein metabolism and diet-induced thermogenesis. Poult Sci. 2004;83:1997-2004. https://doi.org/10.1093/ps/83.12.1997
20. 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. https://doi.org/10.1016/j.anifeedsci.2016.04.003
21. 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. https://doi.org/10.1111/asj.12849
22. Rui Y, Su, Yan-min ZH, Wen-jun A, Rajput N, et al. Effect of dietary supplementation of compound enzymes on endogenous digestive enzymes in cherry valley ducks fed miscellaneous meal diet. Pak J Zool. 2012;44:1561-6.
23. Mohammadi GM, Foroudi F, Ghazikhani SA. Effect of using L–threonine and reducing dietary levels of crude protein on egg production in layers. Iran J Appl Anim Sci. 2011;1:65-8.
24. Yan F, Garribay L, Arce J, Lopez-Coello C, Camacho D, Disbennet P, et al. Effects of protease supplementation on broiler performance and in vitro protein digestibility. In: Proceedings of

the 23rd Annual Australian Poultry Science Symposium; 2012; Sydney, Australia. p. 134-7.
25. Shahir MH, Rahimi R, Taheri HR, Heidariniya A, Baradaran N, Asadi Kermani Z. Effect of protein source and protease addition on performance, blood metabolites and nutrient digestibility of turkeys fed on low-protein diets from 28 to 55 d post hatch. Br Poult Sci. 2016;57:390-6. https://doi.org/10.1080/00071668.2016.1172202
26. 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. https://doi.org/10.2527/jas.53979
27. Kim YJ, Kim TH, Song MH, An JS, Yun W, Lee JH, et al. 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;62:659-67. https://doi.org/10.5187/jast.2020.62.5.659
28. Liu SY, Selle PH, Court SG, Cowieson AJ. Protease supplementation of sorghum-based broiler diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. Anim Feed Sci Technol. 2013;183:175-83. https://doi.org/10.1016/j.anifeedsci.2013.05.006
29. Wang D, Zeng Z, Piao X, Li P, Xue L, Zhang Q, et al. Effects of keratinase supplementation of corn-soybean meal based diets on apparent ileal amino acid digestibility in growing pigs and serum amino acids, cytokines, immunoglobulin levels and loin muscle area in nursery pigs. Arch Anim Nutr. 2011;65:290-302. https://doi.org/10.1080/1745039X.2011.586140

https://doi.org/10.5187/jast.2022.e51