A Thesis
For the Degree of Master of Science

Effect of Protease in Low Protein Diet on Growth Performance, Nutrient Digestibility, Gas Emission, and Intestinal Morphology of Weaned Pigs

이유자돈 사료 내 프로테이즈의 첨가가
이유자돈의 성장성적, 영양소 소화율, 악취 발생,
소장의 형태학적 변화에 미치는 영향

August, 2022

By
Gim, Min Jin

College of Agriculture and Life Sciences
Seoul National University

Effect of Protease in Low Protein Diet on Growth Performance, Nutrient Digestibility, Gas Emission, and Intestinal Morphology of Weaned Pigs

지도교수 **김 유 용**

이 논문을 농학석사 학위논문으로 제출함

2022 년 8 월

서울대학교 대학원 농생명공학부

김 민 진

김민진의 농학석사 학위논문을 인준함

2022 년 6 월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Summary

This study was conducted to evaluate the effects of protease in improving the growth performance, nutrient digestibility, gas emission, and intestinal morphology of weaned piglets fed low protein diets. A total of 120 weaned pigs [Duroc × (Yorkshire × Landrace); 12.86 ± 0.13 kg of initial body weight (BW); 21 day old] were randomly assigned to 4 dietary treatments (6 pigs with 3 barrows and 3 gilts per pen and 5 replicated pens per treatment) in a randomized completely block design. The experimental dietary treatments were 1) HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (HCP; CP=17.5% at phase 1, 17% at phase 2), 2) LCP: a low protein diet as a negative control (LCP; CP=16.5% at phase 1, 16% at phase 2), AA 7% down, 3) ASIA: LCP+0.03% KEMZYME® PLUS(ASIA) and 4) EU: LCP+0.03% KEMZYME® PLUS(EU). Pigs were fed each experimental diet for 5 weeks (Phase 1: day 0 to 14, phase 2: day 14 to 35). There was no significant effect on BW, ADG, ADFI and G:F ratio at day 0 to 14. But in overall period, the G:F ratio was increased (P=0.03) by protease supplementation. Digestibility of CP was increased by supplementation of protease with a low level of dietary protein and EU showed much higher digestibility of CP compared to ASIA (P=0.02). Weaned pigs fed EU had the lowest, HCP had the highest ammonia gas emission during the growth of piglets (P=0.01). There was no significant difference in villus height, crypt depth and VH:CD ratio. Consequently, the present study

demonstrated that supplementation of 0.03% KEMZYME® PLUS(EU) could improve G:F ratio of piglets and decrease the ammonia gas emission of slurry.

Keywords: Protease, Weaned piglet, Growth performance, Nutrient digestibility, Gas emission, Intestinal morphology

Contents

List of Tables

III. Experiment

List of Abbreviations

ADG	:	Average daily gain
ADFI	:	Average daily feed intake
ANF	:	Anti-nutritional factors
AOAC	:	Association of official analytical chemists
ATTD	:	Apparent total tract digestibility
BW	:	Body weight
CP	:	Crude protein
EU	:	European union
FAO	:	Food and Agriculture Organization
FCR	:	Feed conversion ratio
G:F	:	Gain : Feed
GLM	:	General linear model
GIT	:	Gastro intestinal tract
NRC	:	National research council
SAS	:	Statistical analysis system
SEM	:	Standard error of the mean
VH:CD	:	Villus height to crypt depth ratio

I. Introduction

Protein is a nitrogen source for the supply and synthesis of essential amino acids for maintaining the life of livestock, and an appropriate level of supply through feed is essential for the growth and maintenance of pigs (NRC, 2012). In general, high-protein feed is fed to weaned pigs, but undigested intestinal protein causes the growth of pathogens in the intestinal tract, and the generated intestinal toxins lead to reduced immunity, diarrhea, and, in severe cases, death. In addition, there is a decrease in productivity, the occurrence of diseases, and environmental problems due to the emitted nitrogen and gases (Wang et al., 2018). In order to solve this problem, amendments and research are being conducted. Among the amendments to the Feed Management Act (2016), the registration of crude protein content in feed has been changed from the minimum required amount to the maximum limit, and the NRC (2012) has reduced the crude protein content and increased the amount of synthetic amino acids. It has been reported that the addition of synthetic amino acids improves gut health, reduces feed costs and reduces nitrogen emissions without inhibiting piglet growth (Ha et al., 2021). In addition, low-protein feed is known as a good way to improve gut health when feeding piglets with antibiotic-free feed by suppressing the production of toxic substances in the intestine (Zhou et al., 2020).

Soybean meal used as a protein source in the pig industry contains anti-nutritive factors that lower the availability of raw materials, such as phytin,

lectin, and trypsin inhibitors, which have a negative effect on feed efficiency and are known to be an important factor in increasing feed cost as a result. In addition, the ability to secrete digestive enzymes is not sufficient in weaning piglets, and especially in the case of protein digestibility, pepsin activity is low because the ability to secrete hydrochloric acid in the stomach is low (Lee, 2014). Therefore, in order to solve this problem, the use of an enzyme agent has been proposed as a method. Studies related to proteolytic enzymes, such as protease, have reported that feed costs are reduced along with improvement in the growth rate of livestock (Duarte et al., 2019).

Therefore, this study was conducted to evaluate the effects of protease in improving the growth performance, nutrient digestibility, gas emission, and intestinal morphology of weaned piglets fed low protein diets.

II. Review of Literature

1. Introduction

1.1 Weaning stress

Due to the nature of economic animals, weaning of piglets inevitably leads to a more rapid transition. It is perhaps natural for piglets to go through various difficulties while weaning.

A typical phenomenon after weaning is 'changes in the villi in the small intestine'. While breastfeeding, long, well-developed villi are bluntly and short-cut immediately after weaning. In the end, it developed into a situation where it was difficult to eat even if you wanted to eat feed. If the feed intake is low, nutrients to be used in the intestine are also insufficient, and a vicious cycle occurs in which the function of the mucous membrane is deteriorated. Intestinal epithelial cells are damaged, which facilitates the penetration of pathogens and toxins. In addition, the ability to secrete digestive enzymes through intestinal juice is lowered, resulting in poor digestion and absorption. In the end, the increase in the expression of inflammation in the intestinal cells and the increase in abnormal fermentation in the intestine led to soft stool and diarrhea.

As unfavorable conditions are created immediately after weaning, the number of individuals who eventually develop diarrhea increases. Since

various factors overlap, it can lead to a difficult situation in which diarrhea is hardly caught even when trying all kinds of methods. Diarrhea once developed does not improve well after one or two days of treatment, so it often persists for several days.

Diarrhea reduces feed intake and retards growth. If diarrhea persists for about 4 days, the shipping time will increase by 2 weeks or more. In order to minimize weaning stress and reduce diarrhea, it is important to understand the digestive physiology of piglets and apply nutritional solutions to them. Diarrhea occurring in weaning pigs can be divided into nutrient/physiological diarrhea, disease diarrhea and environmental diarrhea. In the end, it is necessary to minimize the frequency of diarrhea by applying nutritional solutions along with methods such as proper facility environment, breeding management, and hygiene management.

A low-protein nutritional design is an excellent solution to not only reduce odors, but also reduce the piglets' susceptibility to diarrhea. In the past, when precise nutrition and establishment of individual amino acid requirements were insufficient, there was a time when the nutrient content of feed was designed with crude protein. However, from 2005 to 2011, the use of antibiotics in feed was gradually banned, causing many difficulties in the field. At this time, a very positive effect was directly experienced by introducing a low-protein, precision amino acid design as a nutritional solution. In particular, weaned pigs lack the ability to secrete hydrochloric acid in the stomach, so the acidity is relatively weak. When hydrochloric acid is secreted from the

stomach, it triggers the activity of a digestive enzyme called pepsin that breaks down proteins. In other words, protein digestion is poor. So, the amino acid type design that relieves the burden of protein digestion helps to improve digestion and reduce diarrhea. The most notable change is that the distribution and expression of pathogenic microorganisms is increased when fed with high protein.

The ability of piglets to secrete digestive enzymes is still insufficient. In particular, immediately after weaning, the secretion of all digestive enzymes temporarily and sharply decreases. This is because the secretion of digestive enzymes through the intestinal juice is restricted as the epithelial cells of the small intestine are damaged. Overall, the activity of protease is the lowest. During breastfeeding, the activity of lactase is maintained at the highest level, and the secretion of enzymes that break down carbohydrates and fats is gradually increased. (Yoon, 2020)

1.2 Feed protein level and weaned pigs

Nursery piglets are sensitive to the ANF in soybean, which may cause diarrhea. Low protein diet is able to reduce feed cost and nitrogen emission. Low-protein diet is able to reduce the diarrhea incidence of weaned piglets without affecting growth and gut health because of the decreased amount of soybean meal(SBM) and ANF in the diet (Opapeju et al., 2008). Numerous studies have found that low protein diet with balanced AA increases the feed conversion ratio (FCR), and has no detrimental effects on growth performance (Opapeju et al., 2008; Peng et al., 2016). But pigs fed reduced crude protein diet without supplemented AA got poor growth performance and lower feed conversion because the animals could not get their essential AA requirement fulfilled (Kerr et al., 1995). Exogenous protease has been used as mono-component or as a part of multi-enzyme in feed industry to increase nutrient digestibility, especially protein and AAs (Adebiyi and Olukosi, 2015). Protease can also increase the activities of digestive enzymes and body weight gain in nursery pigs (Murugesan et al., 2014). Additionally, protease plays an important role in gut health by decreasing diarrhea (Zhang et al., 2014).

The cost of pork production mainly comes from the feed, and the significant increases of feed cost during the last decade have reduced profit margins of pork production (Schmit et al., 2009). The use of exogenous feed enzymes has been one of the most widely used strategies to improve nutrient

utilization efficacy and reduce the feed cost in the animal industry (Adeola and Cowieson, 2011). Proteases have been routinely included to swine diets for many years as part of enzyme cocktails containing xylanases, celluase, amlyase and glucanases (Yin et al., 2001, 2004; Omogbenigun et al., 2004). An enzyme cocktail (b-glucanase, xylanase and protease) improved the digestibillity of crude protein and energy at ileal and the total tract levels of the hulless barley-based diets for young piglets (Yin et al., 2001). Similarly, an enzyme cocktail (arabinoxylanase and protease) improved the nutritional value of diets containing wheat bran or rice bran for growing pig (Yin et al., 2004). Dietary supplementation with enzyme cocktails including proteases improved nutrient utilization and growth performance in weaned pigs (Omogbenigun et al., 2004). A beta-glucanase-protease enzyme blend product improved the ileal digestibility of crude protein and other nutrients (Opapeju et al., 2008). Supplementation of 0.05% of enzyme cocktails (a-amylase, b-mannanase, and protease) to a corn and soybean meal (SBM) diet or a complex diet improved the performance of growing pigs (Zhou et al., 2020).

Recently, proteases have been used alone in the pig diets with the availability of several commercial stand-alone proteases, and new mechanisms of action have been proposed (O'Doherty and Forde, 1999; McAlpine et al., 2012). However, efficacy of protease in weaned piglets and its mechanisms behind are still not clear especially when low digestible protein sources are used.

2. Protein reduction trend in feed industry

2.1 Livestock odor control

Odor complaints and increasing regulations are very difficult challenges to solve in running the pig industry. According to the Ministry of Environment's announcement, complaints about the smell of livestock are increasing every year from 2,838 cases in 2011 to 6,398 cases in 2016. This trend is ultimately serving as a background for tightening environmental regulations. For example, regulations such as limiting distances for raising livestock are being applied. Therefore, for a sustainable pig industry, a lot of effort is needed to reduce the odor of livestock.

Livestock odor is a gaseous substance generated in livestock houses, livestock manure, and composting facilities. It is defined as an odor that irritates the nose and gives discomfort and disgust at concentrations higher than the standard. In order to reduce the occurrence of odors in pig farms, it is necessary to identify the causes of odors and apply solutions that can be taken for each cause. The causes of odor in pig farms can be divided into three main causes. The smell generated inside farm, the smell emitted from the inside of farm to the outside, and the smell generated from the composting facility.

In fact, in order to solve these complex causes of odors, a lot of effort is required throughout the farm, such as breeding management, inside and outside the pig house, and livestock manure management, along with

improving the facility environment. There are a lot of substances that cause bad odors in pig houses. Among them, the substances with the highest odor contribution rate are ammonia, hydrogen sulfide, methyl mercaptan, butyraldehyde, n-valeraldehyde, and i-valeraldehyde. The main source of these substances is related to 'undigested proteins'. In the case of ammonia, as shown in the figure below, protein that is not used in the pig body is converted to urea through a metabolic process (liver). It is then released into the air.

On the other hand, other odor-causing substances such as hydrogen sulfide and methyl mercaptan are produced when microbes in pig manure ferment micronized proteins. (Yoon, 2020)

2.2 Rising feed cost

Korea is a country with a very high dependence on foreign feed for feed, so it is necessary to constantly monitor import prices. Currently, representative international agricultural product price indexes include FAO's Food Price Index and IGC's Grain and Oil Price Index. Since the FAO price index is for finished products, there is a limit to representing raw material prices. Since the IGC price index is calculated by weighting the FOB price for grain raw materials, it can be said to represent the raw material price, but it has the disadvantage that freight rates and time lag are not reflected.

As of 2022, the war in Ukraine has twisted the global agricultural supply chain and caused a food crisis. The war between Russia and Ukraine, which accounts for 29% of world wheat exports, further accelerated rising world grain prices. Corn prices, where Russia and Ukraine account for about 14% of the world market, have risen 27% since early 2022, and soybeans also rose by 28%. The World Food Price Index compiled by the UN Food and Agriculture Organization already reached a record high of 140.7 in February, and the figure has risen further since March when the effects of the war began in earnest. International oil prices rose due to concerns about supply disruptions due to the US and UK embargoes on Russian oil, the review of import restrictions by major countries such as the EU, and geopolitical issues in the Middle East due to the prolonged Russia-Ukraine war.

Fertilizer supply is also aggravating the food shortage. Russia is a major exporter of major fertilizer components, and especially in the case of potassium carbonate, Russia and Belarus account for more than 40% of global exports. According to the Food and Agriculture Organization of the United Nations, it is predicted that if the fertilizer problem is not resolved, there will be a serious supply shortage next year. Natural gas prices, which play a pivotal role in nitrogen fertilizer production and prices, surged in 2021. Coal and renewable energy production decreased due to extreme weather around the world, resulting in increased demand for natural gas and increased prices. Export restrictions by major exporting countries due to increased global fertilizer demand and rising domestic prices accelerated the rise in international fertilizer prices at the end of 2021.

For both corn and soybeans, the war is driving up prices, but the drought in South America is also at the bottom. In the case of Korea, which is highly dependent on imported grains, food security may be shaken. The Ministry of Agriculture, Food and Rural Affairs held three meetings this year to discuss countermeasures. However, short-term prescriptions are all it takes, such as raising funds for raw material purchases in the feed and food industries, and increasing the quota of raw materials that can replace feed grains.

Ⅲ. Effect of Protease in Low Protein Diet on Growth Performance, Nutrient Digestibility, Gas Emission, and Intestinal Morphology of Weaned Pigs

ABSTRACT: This study was conducted to evaluate the effects of protease in improving the growth performance, nutrient digestibility, gas emission, and intestinal morphology of weaned piglets fed low protein diets. A total of 120 weaned pigs [Duroc × (Yorkshire × Landrace); 12.86 ± 0.13 kg of initial body weight (BW); 21 day old] were randomly assigned to 4 dietary treatments (6 pigs with 3 barrows and 3 gilts per pen and 5 replicated pens per treatment) in a randomized completely block design. The experimental dietary treatments were 1) HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (HCP; CP=17.5% at phase 1, 17% at phase 2), 2) LCP: a low protein diet as a negative control (LCP; CP=16.5% at phase 1, 16% at phase 2), AA 7% down, 3) ASIA: LCP+0.03% KEMZYME® PLUS(ASIA) and 4) EU: LCP+0.03% KEMZYME® PLUS(EU). Pigs were fed each experimental diet for 5 weeks (Phase 1: day 0 to 14, phase 2: day 14 to 35). There was no significant effect on BW, ADG, ADFI and G:F ratio at day 0 to 14. But in overall period, the G:F ratio was increased (P=0.03) by protease supplementation. Digestibility of CP was increased by supplementation of protease with a low level of dietary protein and EU showed much higher digestibility of CP compared to ASIA (P=0.02).

Weaned pigs fed EU had the lowest, HCP had the highest ammonia gas emission during the growth of piglets (P=0.01). There was no significant difference in villus height, crypt depth and VH:CD ratio. Consequently, the present study demonstrated that supplementation of 0.03% KEMZYME® PLUS(EU) could improve G:F ratio of piglets and decrease the ammonia gas emission of slurry.

Keywords: Protease, Weaned piglet, Growth performance, Nutrient digestibility, Gas emission, Intestinal morphology

Introduction

Exogenous feed enzymes have been in commercial use in swine diets for almost 30 years. Although proteases represented a small proportion of total sales compared to carbohydrases and phytases, their application in swine diets has gained interested, particularly on utilizing amino acids (AA) in plant-based sources and reducing the detrimental effects of nitrogen (N) excretion on the environment.

Feed cost constitutes approximately 55 to 75% of total cost of pig production (Nuguyen et al., 2017). As feed prices continued to rise, the search for efficient feed additives to reduce feed costs has been done constantly. Soybean meal (SBM) is a major protein source in swine diets due to its excellent balance of amino acids. The use of exogenous feed enzymes has been one of the most widely used strategies to improve nutrient utilization efficacy and reduce the feed cost in the animal industry (Zuo et al., 2015). Exogenous protease can improve digestibility of protein in monogastric animals and shift the site of protein digestion to more proximal intestinal segments (Cowieson and Roos, 2016). Exogenous proteases have been reported to reduce anti-nutritional effects of various proteinaceous anti-nutrients and to increase intestinal resilience by reducing inflammatory responses and improving tight junction and mucin integrity (Cowieson, 2016). Therefore, exogenous proteases may be included in diets to improve nutrient utilization and leading to reduce feed cost. Indeed, the addition of protease

to a diet resulted in a significant improvement in ADG and feed conversion ratio (FCR) of pigs and broilers (O'Doherty and Forde 1999; Cowieson, 2016).

In addition, the growing public concerns in the environment impact of livestock has driven the need to reduce nutrients in waste generated by food animal production. The poor bioavailability of protein in diets leads to reduce the absorption of protein in the GIT of animals. This results in greater excretion of protein-derived fermentation by-products, which have negative effects on environmental integrity (Sutton et al., 1999; O'Shea et al., 2014). Protease supplementation to corn-soybean meal-based diet resulted in degradation of proteinous anti-nutritive factors and improved live weight gain in piglets (Rooke et al., 1998) and enhanced the total tract digestibility of DM and N in sows (De Souza et al., 2007). Furthermore, protease supplementation can raise the energy value of the feed because proteases can help to degrade starch bound proteins by increasing starch digestibility (Wang et al., 2008).

The objective of this study to evaluate the effects of protease in improving the growth performance, nutrient digestibility, gas emission and intestinal morphology of weaned piglets fed low protein diets.

Materials and Methods

Experimental animals and management

This experiment was conducted in Daewoo farm, Muan, Jeollanam-do, Korea. A total of 120 weaned pigs [Duroc × (Yorkshire × Landrace); 12.86 ± 0.13 kg of initial body weight (BW); 21 day old] were randomly assigned to 4 dietary treatments (6 pigs with 3 barrows and 3 gilts per pen and 5 replicated pens per treatment) in a randomized completely block design (block=BW and sex). Temperature and ventilation were managed automatically. All pigs were housed in an environmentally controlled room with a slatted plastic floor and allowed ad libitum access to diets and water throughout the entire experiment period. Phase 1 was from day 0 to 14, and phase 2 was from day 15 to 35.

Experimental diets

The experimental dietary treatments were 1) HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (CP=17.5% at phase 1, 17% at phase 2), 2) LCP: a low protein diet as a negative control (CP=16.5% at phase 1, 16% at phase 2), AA 7% down, 3) ASIA: LCP+0.03% KEMZYME® PLUS(ASIA) and 4) EU: LCP+0.03% KEMZYME® PLUS(EU). The dietary treatments did not include antibiotics and antimicrobials. Pigs were fed the diets for 35 days (Phase 1: 0-2 weeks,

phase 2: 3-5 weeks). Formulas and chemical composition of experimental diets were presented in table 1 and 2.

Digestibility trial

Total collection method was used for the apparent nutrient digestibility. After 5 days adaptation period, 5 days of collection period was followed. To determine the first and last day of collection days, 5% of ferric oxide and chromium oxide were added in the first and last experimental diet as selection marker, respectively. During the experimental period, water was provided ad libitum and all pigs were fed a daily level of 1.6 times the estimated maintenance requirement for energy (i.e. 106 kcal of ME per kg of $BW^{0.75}$; NRC, 1998). Total urine was collected daily in a plastic container containing 50 mL of 4N H_2SO_4 to avoid nitrogen evaporation and frozen during the 5 days of collection period for nitrogen retention analysis

Sample collection and measurements

Body weight was recorded initially, and at the 2nd, 5th week of the experimental period. Feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days prior to fecal collection to calculate nutrient digestibility. Each sample was stored in a freezer at -20℃until analyzed. The fecal samples were thawed and dried at

60℃ for 72h, after which they were finely grounded to pass through a 1 mm screen. The procedures to determine nutrient digestibility were in accordance with the methods established by the AOAC (2002).

Feces and urine were kept in sealed containers and were immediately stored at -4℃ for the duration of the period. After the collection period, feces and urine samples were pooled and each mixed well for each replication. Then the slurry was prepared by mixing urine and feces in 1:1 ratio. The samples were permitted to ferment for at 25℃ for 7 days. After the fermentation period a gas sampling pump (Model GV-100; Gastec corp., Ayase, Japan) was utilized for gas detection (Gastec detector tube No.3La for ammonia). Before the measurements, slurry samples were shaken manually for approximately 30s to disrupt any crust formation on the surface of the slurry sample and to homogenize them.

Intestinal morphology

A total of 12 pigs were selected and slaughtered for the collection of the aseptic ileum samples (about 5 cm fragment in ileum as 1/3 distal part) were conserved in 4% paraformaldehyde for 24 h and embedded in paraffin blocks. Paraffin blocks were sectioned into 5 μm slices, installed on glass slides, and dyed with hematoxylin-eosin (HE) for microscopic examination. Select approximate 20 well-oriented villi and their adjoining crypts on each

slice to measure and calculate the average villus height, crypt depth and of villus height to crypt depth ratio via a light microscope using a calibrated 10-fold eyepiece reticle.

Chemical analysis

Diets were grounded by a Cyclotec 1093 Sample Mill (Foss Tecator, Hillerod, Denmark) and grounded diets were analyzed. All analyses were performed in duplicate samples and analyses were repeated if results from duplicate samples varied more than 5% from the mean. Experimental diet was analyzed for contents of dry matter (procedure 930.15; AOAC, 1995), crude ash (procedure 942.05; AOAC, 1995), ether extract (procedure 920.39; AOAC, 1995), N by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden).

Statistical analysis

All experimental data were analyzed using the GLM (General Linear Model) procedure as a completely randomized design (SAS Inst. Inc., Cary, NC, USA). The pen was used as the experimental unit. Significance test was conducted and LSD (Least Significant Difference) multi range test was used. Differences were declared significant at $P<0.05$, while $0.05 \leq P<0.10$ was considered to indicate a trend in the data.

Results and Discussion

Growth Performance

Effect of different protease on growth performance was presented in table 3. There were no significant differences in BW, ADG, ADFI in phase 1 and 2, and the overall period of the experiment. Similarly, there was no significant differences between the treatment groups in the G:F ratio of phase 1. However, in phase 2, the HCP and EU treated group showed a tendency to had significantly higher G:F ratio (P=0.09), and the G:F ratio of the HCP and EU group was significantly higher during the overall experimental period(P=0.03).

In previous studies, there were no significant differences in BW, ADG, ADFI when 0.05% of protein was added to corn-based feed (Chen, 2017). According to Yi et al (2013), feed intake was not affected by the addition of protease but G:F ratio was linearly increased by 0.015%. Dierick et al. (2004) showed a non-significant trend for piglets to perform better especially at BW added 0.0125% protease. According to Graham et al. (2016), there were no differences for ADG and ADFI when supplemented with 0.05% protease from day 0 to 7. In study of Duarte et al. (2019), the using of either xylanase (45,000 XU/kg) or protease (300,000 U/kg) did not affect the BW at d 10 after weaning. Ma et al. (2020) showed pigs fed the diet with alkaline protease had no significant differences between in ADFI among the treatments. Perez-

Palencia et al. (2021) showed that no significant difference was observed between supplementation of amino acid supply and 0.0125% protease on pig growth performance from weaning to finishing period. During the first 2 weeks postweaning, there were no differences among dietary treatments for overall growth performance. The study of Tactacan et al. (2016) when piglets fed 0.02% protease diet, no differences in BW of d 7 and 21 were observed. Zuo et al. (2015) reported that there was no significant difference between the low-protein diet and 0.01% protease with low-protein diet.

Consequently, when CP level was reduced in weaning diet, protease supplementation in diet showed improved the G:F ratio of weaning pigs. But numerically, the G:F ratio of EU was higher than ASIA.

Nutrient Digestibility

Effect of different protease on nutrient digestibility and amino acid digestibility were shown in table 4. There was no significant difference in nutrient digestibility and amino acid digestibility.

In previous studies, dietary supplementation with 0.0125% protease showed no significant difference between treatments (Nuguyen et al., 2019). According to Duarte et al. (2019) the digestibility of DM, gross energy (GE), and CP were not affected by using xylanase (45,000 XU/kg) and protease (300,000 U/kg). Also, Ma et al. (2020) showed the apparent digestibility of DM, GE, calcium and phosphorus were not influenced by 10 IU/kg of alkaline, acidic and neutral protease supplementation. Park et al. (2019) showed there was no difference on AID of DM, CP, and energy of weaned pigs of 0.2% addition of protease. Zuo et al. (2016) reported that there was no significant difference in the digestibility of CP between the control group and the groups with inclusion of protease. The CP level in the diet did not affect the ATTD of DM and energy in growing pigs (Kim et al., 2020). Pigs offered diets supplemented with protease had increased coefficients of ileal digestibility of N compared to pigs offered diet without protease supplementation. There was xylanase and protease interaction on the AID of GE (O'Shea and O'Doherty, 2013). Yin et al. (2004) reported that there was no significant difference in the apparent ileal digestibility of amino acid when 0.02% protease was added at maze-wheat bran-based diet. According to Ha et al. (2021), there was no significant difference in nutrient digestibility according

to protein level until 2 weeks after weaning. When the digestibility test was conducted when the protein content in the feed was over 20%, the digestibility was different when the protein content was lowered and protease was added due to the low digestible protein ratio. However, in the current experiment, it is considered that the difference in digestibility did not appear as the experiment was conducted in a state in which the protein content was sufficiently lowered.

In conclusion, when CP level was reduced in weaning diet, protease supplementation in diet had no effect on nutrient digestibility.

Gas Emission

Effect of different protease on ammonia gas emission was shown in table 5. The ammonia gas emission was the lowest at the EU group, and significantly higher in the HCP group with high protein level.

In previous studies, dietary supplementation with 0.0125 % protease showed no significant difference between treatments on ammonia gas emission (Nuguyen et al., 2019). Total mercaptans and hydrogen sulfide emission did not differ between dietary treatments. Lei et al. (2017) showed the effect of supplementation with protease alone or in combination with fructo-oligosaccharide did not affect total mercaptans and hydrogen sulfide emission. According to the study of Upadhaya et al. (2011), the supplementation of 0.0125% protease in the diet did not influence ammonia gas emission, total mecaptans and H_2S emission during experiment period. Dietary protease supplementation can improve N utilization and reduce N excretion in manure or ammonia emissions (O'Shea et al., 2014; Upadhaya et al., 2016). Tactacan et al. (2016) showed the ammonia emission from the feces was reduced in piglets fed the diet with 0.02% protease relative to those fed the control diet. On the other hand, McAlpine et al (2012) reported that finishing pigs offered protease supplemented diets had significantly higher ammonia emissions compared to basal fed pigs. Pigs fed low-protein diet and the treatments with 0.05% protease had lower fecal ammonia emissions compared with pigs fed high-protein diet. In the study by Ha et al. (2021), when a low-protein feed was fed, the amount of hydrogen sulfide in

manure was significantly reduced, and the amount of ammonia produced also tended to decrease. In addition, in the treatment group to which protease was added, the amount of ammonia was significantly reduced, and the amount of hydrogen sulfide also tended to decrease. Pigs fed 0.05% proteases had lower fecal ammonia emissions compared with pigs fed higher protein diet at finishing pigs (Lei et al., 2017). The supplementation of protease enzyme reduced the ammonia emission from the feces in piglets fed the experimental diet relative to those fed the control diet. However, R.SH emission was the same in both groups. The effectiveness protease in swine diets has been associated with the type of protease used, the dose, feed ingredients used in formulation and interactions with other enzymes (Cowieson and Roos, 2016; Lee et al., 2018; Torres-Pitarch et al., 2019).

It was reported that undigested proteins cause hydrogen sulfide, ammonia, and malodorous substances by harmful bacteria in the intestine (Neis et al., 2015; Fortune et al., 2016). Wang et al. (2018) reported that for every 10 g/kg decrease in protein in feed, the amount of ammonia generated in the minute decreased by 8%.

In conclusion, when feed CP was reduced, supplementation of protease decreases the ammonia gas emission on weaned piglets. Also, the EU was more effective at ammonia gas emission than ASIA.

Intestinal Morphology

Effect of different protease on intestinal morphology was shown in table 6. There was no significant difference in villus height, crypt depth and VH:CD ratio.

The VH:CD ratio can be an indicator to evaluate nutrient digestion and absorption capacity of the small intestine. The reduction of villus height could induce decreased absorption of nutrients, which may be responsible for the reduced growth performance. Park et al. (2019) showed weaned pigs fed protease had tendency to have higher VH:CD ratio among treatments. This result was similar to the result reported by Wang et al. (2011) and Rooke et al. (2003). However, Kim et al. (2017) the addition of 0.05% mixture of xylanase, protease, pectinase α-amylase and β-glucanase had no influence on nutrient VH:CD ratio. In the study of Ha et al. (2021), there was no significant difference in the morphological change of the villi in the small intestine between the treatment groups according to the protein level of the feed and the addition of protease. Zuo et al. (2015) reported there were no significant differences in the crypt width of duodenum and jejunum related to the protein content. According to Chen (2017), there were no significant differences in crypt depth and villus height, and VH:CD ratio of jejunum. In the study of Ndazigaruye et al. (2019), VH:CD ratio was not affected by the exogenous protease added to the low protein diets at broiler. On the other hand, inclusions of 0.02 and 0.03% protease with low protein diet significantly

reduced the crypt width of duodenum and ileum when compared with the low protein diet without protease.

In conclusion, when CP level was reduced in weaning diet, protease supplementation in diet had no effect on intestinal morphology.

Conclusion

During the feeding trial, there were no significant effect on BW, ADG, ADFI and G:F ratio at phase 1. But in phase 2 and overall period, the G:F ratio was increased by protease supplementation (P=0.03). Numerically, the G:F ratio of EU was higher than ASIA. The supplementation of protease had no effect on nutrient digestibility and intestinal morphology. The ammonia emission was decreased by the protease supplementation, and the EU had significantly lower ammonia gas emission of slurry(P=0.01).

Consequently, the present study demonstrated that supplementation of 0.03% KEMZYME® PLUS(EU) could improve G:F ratio of piglets and decrease the ammonia gas emission of slurry.

Table 1. Formula and chemical composition of experimental diet in phase 1.

Ingredient, %	Treatment[1)]			
	HCP	LCP	ASIA	EU
Corn	58.55	60.98	60.92	60.92
Soybean meal	8.14	5.71	5.71	5.71
Soy oil	1.11	1.08	1.10	1.10
Whey base	5.00	5.00	5.00	5.00
Lactose base	10.00	10.00	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00
Blood plasma	2.00	2.00	2.00	2.00
Fermented Soybean meal	8.00	8.00	8.00	8.00
L-Lysine Sulfate, 55%	0.34	0.33	0.33	0.33
DL-met, 98%	0.10	0.09	0.09	0.09
L-threonine, 98.5%	0.02	0.02	0.02	0.02
L-Tryptophan, 99%	0.04	0.05	0.05	0.05
DCP	1.21	1.27	1.27	1.27
Limestone	0.69	0.68	0.68	0.68
KEMZYME® PLUS(ASIA)	0.00	0.00	0.03	0.00
KEMZYME® PLUS(EU)	0.00	0.00	0.00	0.03
Vit. Mix[2)]	0.10	0.10	0.10	0.10
Min. Mix[3)]	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Zinc oxide	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00
Chemical composition				
ME, kcal/kg[4)]	3400.00	3400.00	3400.00	3400.00
CP, %[5)]	17.50	16.50	16.50	16.50
Lys, %[5)]	1.15	1.08	1.08	1.08
Met, %[5)]	0.37	0.35	0.35	0.35
Ca. %[5)]	0.80	0.80	0.80	0.80
Total P, %[5)]	0.65	0.65	0.65	0.65

[1)] HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (HCP; CP=17.5% at phase 1, 17% at phase 2), LCP: a low protein diet as a negative control (LCP; CP=16.5% at phase 1, 16% at phase 2), ASIA: LCP+0.03% KEMZYME® PLUS(ASIA), EU: LCP+0.03% KEMZYME® PLUS(EU).
[2)]Provided per kg of complete diet: vitamin A, 11,000 IU; vitamin D, 920 IU; vitamin E, 65 mg; vitamin K3, 7.5 mg; vitamin B1, 2.8 mg; vitamin B2, 8.5 mg; vitamin B6, 4 mg; vitamin B12, 45μg; Biotin, 190 mg; Ca-Pan, 37 mg; Niacin, 40 mg; Folic acid, 0.55 mg
[3)] Provided per kg of complete diet: Mn, 30 mg; Cu, 32 mg; Zn, 23 mg; Fe, 75 mg; I, 0.25mg; Co, 0.25 mg; Se, 0.1 mg
[4)]Calculated values
[5)]Analyzed values.

Table 2. Formula and chemical composition of experimental diet in phase 2.

Ingredient, %	Treatment[1)]			
	HCP	LCP	ASIA	EU
Corn	65.64	68.07	68.01	68.01
Soybean meal	12.74	10.31	10.32	10.32
Soy oil	0.78	0.76	0.77	0.77
Whey base	2.50	2.50	2.50	2.50
Lactose base	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00
Fermented Soybean meal	8.00	8.00	8.00	8.00
L-Lysine Sulfate, 55%	0.44	0.44	0.44	0.44
DL-met, 98%	0.09	0.09	0.09	0.09
L-threonine, 98.5%	0.05	0.05	0.05	0.05
L-Tryptophan, 99%	0.04	0.05	0.05	0.05
DCP	1.33	1.39	1.39	1.39
Limestone	0.58	0.56	0.56	0.56
KEMZYME® PLUS(ASIA)	0.00	0.00	0.03	0.00
KEMZYME® PLUS(EU)	0.00	0.00	0.00	0.03
Vit. Mix[2)]	0.10	0.10	0.10	0.10
Min. Mix[3)]	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Zinc oxide	0.30	0.30	0.30	0.30
Sum	100.00	100.00	100.00	100.00
Chemical composition				
ME, kcal/kg[4)]	3350.00	3350.00	3350.00	3350.00
CP, %[5)]	17.00	16.00	16.00	16.00
Lys, %[5)]	1.10	1.03	1.03	1.03
Met, %[5)]	0.35	0.33	0.33	0.33
Ca. %[5)]	0.70	0.70	0.70	0.70
Total P, %[5)]	0.60	0.60	0.60	0.60

[1)] HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (HCP; CP=17.5% at phase 1, 17% at phase 2), LCP: a low protein diet as a negative control (LCP; CP=16.5% at phase 1, 16% at phase 2), ASIA: LCP+0.03% KEMZYME® PLUS(ASIA), PLUS: LCP+0.03% KEMZYME® PLUS(EU).
[2)]Provided per kg of complete diet: vitamin A, 11,000 IU; vitamin D, 920 IU; vitamin E, 65 mg; vitamin K3, 7.5 mg; vitamin B1, 2.8 mg; vitamin B2, 8.5 mg; vitamin B6, 4 mg; vitamin B12, 45μg; Biotin, 190 mg; Ca-Pan, 37 mg; Niacin, 40 mg; Folic acid, 0.55 mg
[3)] Provided per kg of complete diet: Mn, 30 mg; Cu, 32 mg; Zn, 23 mg; Fe, 75 mg; I, 0.25mg; Co, 0.25 mg; Se, 0.1 mg
[4)]Calculated values
[5)]Analyzed values.

Table 3. Effect of protease on growth performance in piglets[1)]

Criteria	Treatment[2)]				SEM[3)]	P-value
	HCP	LCP	ASIA	EU		
BW, kg						
Initial	12.88	12.96	12.84	12.92		
Day 14	18.26	18.01	18.51	17.87	0.352	0.94
Day 35	32.17	30.20	32.11	31.63	0.684	0.75
ADG, g						
Day 0-14	384.29	360.57	403.94	353.84	8.235	0.11
Day 15-35	665.05	577.16	647.50	655.48	20.864	0.46
Day 0-35	551.26	493.06	550.08	534.83	12.311	0.32
ADFI, g						
Day 0-14	741.76	723.81	760.86	709.95	18.983	0.82
Day 15-35	1262.40	1245.20	1275.20	1208.80	28.478	0.78
Day 0-35	1054.20	1065.40	1069.40	1009.20	21.785	0.78
G:F ratio						
Day 0-14	0.53	0.50	0.53	0.50	0.011	0.75
Day 15-35	0.53^{B}	0.45^{A}	0.51^{AB}	0.54^{B}	0.015	0.09
Day 0-35	0.53^{b}	0.46^{a}	0.51^{b}	0.53^{b}	0.010	0.03

[1)]A total of 120 weaned pigs [Duroc × (Yorkshire × Landrace); 12.86 ± 0.13 kg of initial body weight (BW); 21 day old] were randomly assigned to 4 dietary treatments

[2)] HCP: a diet based on corn and SBM to meet or exceed the requirement of CP as a positive control (HCP; CP=17.5% at phase 1, 17% at phase 2), LCP: a low protein diet as a negative control (LCP; CP=16.5% at phase 1, 16% at phase 2), ASIA: LCP+0.03% KEMZYME® PLUS(ASIA), EU: LCP+0.03% KEMZYME® PLUS(EU).

[3)]Standard error of means

[ab]Means in a same row with different superscript letters were significantly different (P<0.05).

Table 4. Effect of protease on nutrient digestibility in piglets[1)]

Digestibility, %	Treatment				SEM[2)]	P-value
	HCP	LCP	ASIA	EU		
Nutrient digestibility, %						
DM	72.36	72.11	78.13	72.60	1.274	0.30
Protein	57.31	52.97	63.75	52.19	2.318	0.29
Ash	26.18	13.75	27.42	16.17	3.129	0.30
Fat	76.45	72.75	73.92	70.63	1.250	0.47
Amino acid digestibility, %						
His	91.34	89.67	89.98	88.70	0.726	0.70
Thr	88.90	86.76	87.81	85.66	0.978	0.74
Arg	91.76	89.93	90.57	89.24	0.675	0.66
Val	89.52	86.74	87.46	85.61	0.950	0.59
Met	88.34	86.91	86.54	84.97	0.925	0.70
Phe	91.01	88.76	89.76	87.93	0.779	0.60
Ile	89.32	86.15	87.09	85.30	0.960	0.55
Leu	91.69	90.27	90.98	89.21	0.705	0.70
Lys	90.38	87.82	89.42	87.40	0.961	0.73

[1)]Standard error of means
[2)]A total of 12 barrow and initial body weight 12.48±1.53

Table 5. Effect of protease on ammonia gas emission in piglets

Item	Treatment				SEM[1)]	P-value
	HCP	LCP	ASIA	EU		
NH_3, ppm	12.89[d]	10.39[c]	9.28[b]	6.78[a]	0.672	0.01

[1)]Standard error of means
[abc]Means in a same row with different superscript letters were significantly different (P<0.05).

Table 6. Effect of protease on intestinal morphology in piglets

Item	Treatment				SEM[1)]	P-value
	HCP	LCP	ASIA	EU		
VH,μm	434.60	412.67	406.01	376.56	15.165	0.66
CD,μm	309.74	302.59	295.82	289.26	11.522	0.95
VH:CD ratio	1.52	1.39	1.42	1.36	0.034	0.45

[1)]Standard error of means

Literature Cited

Adebiyi, A. O and O. A. Olukosi. 2015. Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without carbohydrases and protease for broilers and turkeys. Poultry Science. 94:1270-1276.

Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzyme to improve non-ruminant animal production. J. Anim. Sci. 89:3189-3218.

AOAC. Official methods of analysis. 18th ed. Association of Official Analytical Chemists International, Gaithersburg, MD, U. S. A. 2007.

Chen, H. 2017. Use of feed enzyme on Growth and Health of Pigs.

Cheng, L. and IH. Kim. 2019. Effects of dietary supplementation with Bacillus lichenformis derived-protease on growth performance, nutrient digestibility, and fecal microbial chedding in post-weaned growing pigs. Journal of Applied Animal Research. 47(1): 322-325.

Cowieson, A. J. 2016. 1st international round-table on protein nutrition of nun-ruminants. Anim. Feed. Sci. Technol. 221: 243-244.

Cowieson, A. J. and F. F. Roos. 2016. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. Anim. Feed. Sci. Technol. 221: 331-340.

De Souza, A. L. P., M. D. Lindemann, and G. L. Cromwell. 2007. Supplementation of dietary enzymes has varying effects on apparent protein and amino acid digestibility in reproducing sows. Livest Sci. 109: 122-124

Dierick, N., J. Decuypere, K. Molly and E. Vanderbeke. 2004. Microbial protease addition to a soybean meal diet for weaned piglets: effects on performance, digestion, gut flora and gut function. EAAP publication. 10.

Duarte, E. M., X. Z. Fang, W. M. Dutra and S. W. Kim. 2019. Dietary supplementation of xylanase and protease on growth performance, digesta viscosity, nutrient digestibility, immune and oxidative stress status, and gut health of newly weaned pigs. Animal Nutrition. 5: 351-358.

Graham, A., B. Knopf, L. Ochoa, R. J. Harrell and Y. Ma. 2016. Effect of feeding protease at two soybean meal inclusion rates for nursery pigs. J. Anim. Sci. 97: 117-117.

Ha, S. H., Y. H. Choi, A. Hosseindoust, D. J. Ha, J. Moturi, J. Y. Mun, T. Habeeb, C. B. Lee, J. W. Park, J. S. Kim and S. J. Sa. 2021. Effects of dietary protein level and supplementation of protease on growth performance, nutrient digestibility, gut microflora, intestinal morphology and fecal noxious gas emission in weanling pigs. Ann. Anim. Resour. Sci. 32(3): 48-56.

Hong, SM., HD. Jang, HJ. Kim, JS. Yoo, JH. Lee and IH Kim. 2011. Effects of Enyzyme Complex Supplementation on Growth Performance, Nutrient Digestibility, Blood Profiles and Economic Feed Cost in Growing Pigs. J. Anim. Sci. Technol. 53(2): 113-118.

Karr-Lilienthal, L. K., C. T. Kadzere, C. M. Grieshop, and G. C. Fahey Jr. 2005. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: a review. Livest. Prod. Sci. 97:1-12.

Kim, Y. J., T. H. Kim, M. H. Song, J. S. An, W. Yun, J. H. Lee, H. J. Oh, J. S. Lee, G. M. Kim, H.B. Kim and J. H. Cho. 2020. Effects of different levels of

crude protein and protease on nitrogen utilization, nutrient digestibility, and growth performance in growing pigs. J. Anim. Sci. Technol. 62(5): 659-667.

Lee, S. A., M. R. Bedford, and C. L. Walk. 2018. Meta-analysis: explicit value of mono-component proteases in monogastric diets. Poult. Sci. 97: 2078-2085.

Lee. 2019. Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers. Animals. 9(5): 226.

Lei, X. J.., J. Y. Cheong, J. H. Park and I. H. Kim. 2017. Supplementation of protease, alone and in combination with fructooligosaccharide to low protein diet for finishing pigs. Japanese Society of Animal Science.

Long, S., J. Hu, S. Hahfuz, H. Ma, and X. Piao. 2020. Effects of dietary supplementation of compound enzymes on performance, nutrient digestibility, serum antioxidant status, immunoglobulins, intestinal morphology and microbiota community in weaned pigs. Archives of Animal Nutrition. 75(1): 31-47.

Ma, W., L. Yi, L. Guo, Z. Wang and F. Zhao. 2020. Effects of three kinds of protease on growth performance, apparent digestibility and faecal microbial counts in weanling pigs. Czech J. Anim. Sci. 10: 373-379.

Mc Alpine, P. O., C. J. O'Shea, P. F. Varley, and J. V. O'Doherty. 2012. The effect of protease and xylanase enzymes on growth performance and nutrient digestibility in finisher pigs. J. Anim. Sci. 90: 375-377.

Min. Y., Y. Choi, Y. Kim, Y. Jeong, D. Kim, H. Jung, M. Song. 2019. Effects of protease supplementation on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. J Anim Sci Technol. 2019;61(4):234-238.

Murugesan, G. R., L. F. Romero and M. E. Persia. 2014. Effects of Protease, Phytase and a Bacillus sp. Direct-Fed Microbial on Nutrient and Energy Digestibility, Ileal Brush Border Digestive Enzyme Activity and Cecal Short-Chain Fatty Acid Concentration in Broiler Chickens

Neis, E. P., Dejong, C. H. and Rensen, S. S. 2015. The role of microbial amino acid metabolism in host metabolism. Nutr. 7(4): 7930-2946.

Nuguyen, D. H., J. W. Park and I. H. Kim. 2017. Effect of crumbled diet on growth performance, market day age and meat quality of growing-finishing pigs. J. Appl. Anim. Res. 45: 396-399.

O'doherty, J. V., and S. Forde. 1999. The effect of the protease and alpha-galactosidase supplementation on the nutritive value of peas for growing and finishing pigs. Ir. J. Agric. Food Res. 1: 217-226.

O'Shea, C. J., P. O. Mc Alpine, P. Solan, P. F. Varley, A. M. Walsh and J. V. O. Doherty. 2014. The effect of protease and xylanase enzymes on growth performance, nutrient digestibility, and manure odour in grower-finisher pigs. Anim. Feed Sci. Technol. 189: 88-97.

Park, S. W., J. J. Lee, B. M. Yang, J. H. Cho, S. Kim, J. Kang, S. Oh, D. J. Park, R. Perez-Maldonaldo, J. Y. Cho, I. H. Park, H. B. Kim and M. Song. 2020. Dietary protease improves growth performance, nutrient digestibility, and intestinal morphology of weaned pigs. J. Anim. Sci. Technol, 62(1): 21-30.

Perez-Palencia, J. Y., R. S. Samuel, and C. L. Levesque. 2021. Supplementation of protease to low amino acid diets containing superdose

level of phytase for wean-to-finish pigs: effects on performance, postweaning intestinal health and carcass characteristics. Transl. Anim. Sci. 2021.5:1-14.

Rooke, J. A., M. Slessor, H. Fraser and J. R. Thomson. 1998. Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soya-bean meal. Anim. Feed Sci. Technol. 70: 175-190.

Sutton, A. L., K. B. Kephart, M. W. Verstegen, T. T. Canh, and P. J. Hobbs. 1999. Potential for reduction of odorous compounds in swine manure through diet modification. J. Anim. Sci. 77: 430-439.

Tactacan, G. B., S. Y. Cho, J. H. Cho, and I. H. Kim. 2016. Performance responses, nutrient digestibility, blood characteristics and measures of gastrointestinal health in weanling pigs fed protease enzyme. Asian-Australas. J. Anim. Sci. 29: 998-1003.

Torress-Pitarch, A., E. G. Manzanilla, G. E. Gardiner, J. V. O. Doherty, and P. G. Lawlor. 2019. Systematic review and meta-analysis on the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: effect of enzyme type and cereal source. Anim. Feed. Sci. Techno.. 251: 153-165.

Upadhaya, S. D., H. M. Yun and I. H. Kim. 2016. 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. 216: 281-287

Wang, L. L., J. J. Chung, E. C. Li, S. Uman, P. Atluri, J. A. Burdick. 2018. Injectable and protease-degradable hydrogel for siRNA sequestration and triggered delivery to the heart. 285:152-161.

Wang, S. L., C. H. Yang, T. W. Liang and Y. H. Yen. 2008. Optimization of conditions for protease production by Chryseobacterium taeanense TKU001. Bioresource Technology. 99(9):3700-3707

Yi, J. Q, X. S. Piao, Z. C. Li, H. Y. Zhang, Y. Chen, Q. Y. Li, J. D. Liu, Q. Zhang, Y. J. Ru, and B. Dong. 2013. The Effects of Enzyme complex on performance, intestinal health and nutrient digestibility of weaned pigs. Asian Australas. J. Anim. Sci. 26(8): 1181-1188

Yin. Y. L., Z. Y. Deng, H. L. Huang, T. J. Li and H. Y. Zhong. 2003. The effect of arabinoxylanase and protease supplementation on nutritional value of diets containing wheat bran or rice bran in growing pig. Journal of Animal and Feed Science. 13: 445-461.

Zhang, D., D. Liu, X. Lv, Y. Wang, Z. Xun, Z. Liu, F. Li, H. Lu. 2014. The Cysteine Protease CEP1, a Key Executor Involved in Tapetal Programmed Cell Death, Regulates Pollen Development in Arabidopsis. The Plant Cell. 26(7):2939-2961.

Zuo, J., B. Ling, L. Long, T. Li, L. Lahaye, C. Yang, and D. Feng. 2015. Effect of dietary supplementation with protease on the growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Anim. Nutri.

Ⅴ. Summary in Korean

본 연구는 단백질 저감 사료를 급여한 이유자돈의 성장성적, 영양소 소화율, 가스 배출량 및 소장의 형태학적 변화에 프로테이즈의 첨가가 미치는 영향을 평가하기 위해 수행하였다. 사양실험을 위해 평균체중 12.86 ± 0.13kg 의 삼원교잡종 [Duroc × (Yorkshire × Landrace) 이유자돈 120 두를 공시하여 총 5 주간 (자돈 전기 2 주와 자돈 후기 3 주) 실험을 수행하였다. 실험돈들은 체중과 성별에 따라 4 처리 5 반복, 펜당 6 마리씩 난괴법 (RCBD; randomized complete block design)으로 배치하여 수행되었다. 실험 처리구는 다음과 같다. 1] HCP: 옥수수-대두박 위주의 기초 사료 (CP 함량 자돈 전기 17.5%, 자돈 후기 17%, 2) LCP: 옥수수-대두박 위주의 기초 사료중 단백질 함량 1% 저감 (CP 함량 자돈 전기 16.5%, 자돈 후기 16%), 3) ASIA: LCP+0.03% KEMZYME® PLUS(ASIA) and 4) EU: LCP+0.03% KEMZYME® PLUS(EU).

사양실험 결과 자돈 전기와 후기에서 체중, 일당증체량, 일당사료섭취량 및 사료효율에서는 유의적인 영향이 나타나지 않았다. 하지만 실험 전체 기간에서 프로테이즈의 첨가는 사료효율(ASIA, EU, P=0.03)을 향상시켰다. 건물, 단백질, 회분, 지방, 아미노산 소화율 및 소장의 형태학적 변화에서는 처리구간 유의적인 차이가 나타나지 않았다.

암모니아 가스 배출량은 단백질 수준이 가장 높은 HCP 에서 가장 높은 배출량을 보였고, EU 에서 가장 낮은 배출량을 보였다.

결론적으로, 프로테이즈의 첨가는 사료효율을 향상시키고 암모니아 배출량을 감소시켜 이유자돈의 성장성적과 암모니아 배출량에 긍정적인 영향을 미쳤다. 특히 KEMZYME® PLUS(EU)는 암모니아의 배출량이 유의적으로 가장 낮았기 때문에 KEMZYME® PLUS(EU) 가 KEMZYME® PLUS(ASIA)에 비해 성장성적과 악취저감에 더 효율적인 것으로 사료된다.