

Effect of feed technologies on the digestibility of energy and nutrients in diets fed to growing
pigs

by

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B.S., Universidad Nacional de Colombia, 2018
M.S., University of Illinois at Urbana-Champaign, 2020

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Manhattan, Kansas

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Abstract

A total of 6 experiments were conducted and structured in 4 chapters to evaluate the effects of feed technologies on the digestibility of energy and nutrients in diets fed to growing pigs. For Chapter 1, four dietary treatments were used in 2 experiments, consisting of a mash diet and 3 pelleted diets that were conditioned at 30, 60, and 180 seconds to determine the effects of conditioning time on AID of CP and AA and concentration of DE and ME. The results indicated that increasing conditioning time up to 180 seconds improved the SID of CP and most AA, and pelleting increased the ATTD of GE, but there was no evidence of difference in DE and ME concentrations. For Chapter 2, a total of 12 cannulated pigs were used, and 4 dietary treatments were manufactured, consisting of a mash diet and 3 pelleted diets using 3 different pellet dies of length-to-diameter ratio (L:D) of 6, 8, and 10. Results from this experiment indicated that pelleting diets leads to improvements in SID of CP and AA; however, this improvement is dependent on pellet die thickness, with thicker dies, such as L:D 8 or 10, improving SID of AA. For Chapter 3, a group of 10 pigs was used to test the effect of myristic acid, protected benzoic acid, and *aspergillus niger* cultures on the ATTD of GE and nitrogen retention in high fiber diets fed to growing pigs. Results indicate a tendency ($P < 0.15$) of myristic acid and protected benzoic acid diets to have increased concentration of ME compared with the negative control diet, with the aspergillus culture diet being intermediate. However, no effect was observed due to the inclusion of feed additives on ATTD of GE or nitrogen retention. For Chapter 4, 2 groups of growing pigs were used, and 2 experiments were designed to determine the effect of protease inclusion on the ATTD of GE and nutrients and SID of CP and AA. These results indicate that the inclusion of protease at 4,000 U/kg increases the digestibility of nutrients when included in

diets fed to growing pigs. These data can be used during feed formulation to ensure that adequate concentrations of digestible nutrients are being provided to the animals to support their growth.

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Approved by:

Major Professor
Dr. Chad B. Paulk

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Chapter 1 - Effect of feed conditioning time prior to pelleting on standardized ileal digestibility of amino acids and total tract digestibility of energy in diets fed to growing pigs

ABSTRACT

Two experiments were conducted to determine the effect of increasing retention time in the conditioner prior to pelleting on digestibility of energy, protein, and amino acids (AA) in diets for growing pigs. Four dietary treatments were used in both experiments and consisted of a mash diet, and 3 pelleted diets that were conditioned for 30, 60, or 180 s. In Exp. 1, 12 ileal cannulated barrows with an initial average body weight of 44.9 ± 2.70 kg were allotted to a triplicated 4×4 Latin square with 4 dietary treatments and 4 experimental periods in each square for a total of 12 replicate pigs per treatment. Each period consisted of 5 days of adaptation and 2 days of collection of ileal digesta. In Exp. 2, 12 barrows (initial average body weight: 34.1 ± 1.03 kg) were allotted to a triplicated 4×3 incomplete Latin square design with 4 dietary treatments and 3 experimental periods. Each period consisted of 5 days of adaptation and 5 days of collection of feces and urine. Pigs were individually housed in metabolism crates and feces and urine were collected. Results of Exp. 1 indicated an overall treatment effect ($P < 0.05$) on standardized ileal digestibility (SID) of crude protein and all AA except Lys, Met, and Trp. The SID of crude protein, Arg, Leu, Thr, Ala, Ser, and Tyr was greater ($P < 0.05$) in all pelleted diets compared with the mash diet. The SID of His, Ile, Phe, Val, Asp, and Glu was less ($P < 0.05$) in the mash diet compared with the pelleted diets conditioned for 60 s or 180 s. The SID of Cys was less ($P < 0.05$) in the mash diet compared with the pelleted diet conditioned for 180 s. Increasing the retention time in the conditioner from 30 to 180 s increased (linear; $P < 0.05$) the SID of CP and

most AA. Results of Exp. 2 demonstrated that pelleted diets had greater ($P < 0.05$) apparent total tract digestibility (**ATTD**) of gross energy compared with the mash diet, but no treatment effects were observed for ATTD of dry matter or on digestible energy, metabolizable energy or the metabolizability of digestible energy. These results indicate that increasing conditioning time up to 180 s improved AA digestibility for most AA and pelleting also increased ATTD of gross energy.

INTRODUCTION

Conditioning feed with steam prior to pelleting is a key process to prepare the feed for pellet formation in the pellet die (Wang et al., 2019). Proper mash feed conditioning is the first major step of the pelleting process and is necessary to achieve optimum production rates, pellet quality, energy usage, and die and roll life. Increasing the amount of steam used in the conditioning process will in turn result in increased conditioning temperature and moisture added to the feed. Addition of heat and moisture has been attributed to changing the physicochemical properties of the feed, typically resulting in improved binding properties between particles (Thomas and van der Poel, 1996), which may increase nutrient digestibility (Svihus and Zimonja, 2011).

Parameters that can be adjusted during feed conditioning prior to pelleting include steam addition (temperature and moisture), retention time, and steam pressure. Conditioner retention time is influenced by the length of the conditioner, the angle and placement of the mixing paddles, and the operating speed. When steam is added to the conditioner, moisture absorption occurs at a slower rate than heat transfer (Thomas and van der Poel, 2020), which supports the

hypothesis that longer retention times may influence pellet durability and nutrient digestibility by promoting physicochemical changes through increased moisture absorption.

Increasing the retention time during conditioning has also been considered a strategy to reduce microbial contamination of feed (Boroojeni et al., 2016; Cochrane et al., 2017; Boltz et al., 2019). Although research has shown the capacity of feed to transmit pathogens such as viruses or bacteria, temperatures and retention times sufficient to neutralize those pathogens are or can be achieved during conditioning (Cochrane et al., 2017). Increasing the temperature during conditioning, can be an effective method of control, however, it might lead to heat damage of nutrients (González-Vega et al., 2011) therefore, the alternative is to use lower temperatures for a longer retention time. To increase feed retention time in the conditioner, operators can adjust the paddle configuration to reduce forward movement, reduce conditioner shaft speed, or reduce feed production rate to decrease the flow rate of feed through the conditioner. Hygienizers or double- and triple-pass conditioners can also be used to increase retention time during conditioning. These systems provide greater flexibility to control the duration of feed exposure to high temperature and moisture.

Feed mills may explore opportunities to maximize feed retention time in the conditioner to improve pellet durability and microbial control. However, to our knowledge, no research has been conducted to determine the effect of extended retention time on nutrient and energy digestibility. Therefore, the objective of this work was to test the hypothesis that extended retention time of feed in the conditioner prior to pelleting will increase the standardized ileal digestibility (**SID**) of amino acids (**AA**) and the apparent total tract digestibility (**ATTD**) of energy of diets fed to growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Kansas State University approved the protocol for two experiments. Castrated male pigs (241 × 600, DNA Genetics, Columbus, NE, USA) were used in both experiments. Pigs were housed individually in metabolism crates (1.50 m × 0.56 m) that were equipped with solid panels on the sides, a feeder, a water cup drinker, and metal diamond-slatted flooring.

Diet and diet preparation

A diet based on corn, soybean meal, and distillers dried grains with solubles was formulated (Tables 1 and 2) to meet or exceed all nutrient requirements for pigs between 40 and 65 kg (NRC, 2012). The diet was manufactured at the O.H. Kruse Feed Technology and Innovation Center in Manhattan, KS, USA, following their standard procedures. A total of 2,449 kg of a basal diet was mixed and used for both experiments. Four separate batches were mixed using a twin-shaft counterpoise mixer (Hayes and Stolz, model TRDB63-0152, Fort Worth, TX, USA). The mixing time was 60 s for dry ingredients and an extra 180 s after adding liquid ingredients. After mixing, one batch of 455 kg of feed was kept as mash and the remaining 3 batches of 664 kg of mixed feed were used in the manufacturing of pelleted treatments.

The mash diet was mixed and sacked off after mixing without further processing. To prepare the pelleted diets, the mash feed was conditioned for approximately 30, 60, or 180 s at a target temperature of 85 °C in a single-pass conditioner. To accomplish the desired retention time, the paddles of the conditioner were set at an angle of 10°, the feeder flow to the conditioner was adjusted to 10%, and the speed of the conditioner was adjusted to 85%, 60%, or 10%. Following condition, each of the 3 diets was pelleted on a 100 HP pellet mill (CPM, model 3016-4 Master, Crawfordsville, IN, USA) equipped with a 4.8 × 45.0 mm die. Each diet was pelleted

in a single run that took approximately 80 min. During pelleting, the temperature in the conditioner was monitored and recorded every 20 minutes (Table 3).

Once pelleted, diets were analyzed for pellet quality using the Holmen forced-air method (TekPro Ltd, model NHP 100, Norfolk, UK; Evans, 2023). Prior to analysis, samples were stored in commercial paper feed sacks. Each sample was analyzed in duplicate, and results were averaged. Collected pellet samples were sifted with a U.S. No. 5 (3.9 mm) sieve to allow for the separation of fines and pellets. The pellet durability was then assessed using the NHP 100 (TekPro Ltd, Norfolk, UK) pellet tester. The following equation was used to calculate the pellet durability index (**PDI**; S269.5; ASAE, 2012):

$$PDI (\%) = \frac{\text{Weight of recovered pellets}}{\text{Initial weight of pellets}} \times 100$$

Experiment 1: Amino Acid Digestibility

A total of 12 barrows (average initial body weight: 44.95 ± 2.70 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998). The animals were allotted to a triplicated 4×4 Latin square design (Kim and Stein, 2009) with four periods of 7 d and 4 dietary treatments. Therefore, each diet was fed to 3 pigs in each period for a total of 12 replicate pigs per dietary treatment for the 4 periods. Pigs were fed 3.0 times the maintenance requirement for metabolizable energy (**ME**; i.e. 197 kcal/kg body weight^{0.60}; NRC, 2012) and had free access to water throughout the experiment. The daily feed allowance was divided and fed in two equal meals at 0800 and 1400 h.

The first five days of each period were considered the adaptation period to the diet (Adeola, 2001), with the ileal sample collection taking place on days 6 and 7. On each collection day, samples were collected for 8 h by removing the lid of the cannula, attaching a plastic bag to

the barrel of the cannula, and securing it with a plastic cable tie. The digesta flowing into the bag were collected every 30 minutes or every time the bag was full. Digesta samples were immediately stored at -20 °C to prevent bacterial degradation of AA.

Diets were bagged, and a sample was collected by probing each bag and combining into one composite sample. Digesta samples were thawed, subsampled, lyophilized, and ground in preparation for laboratory analysis. Samples of diets and ileal digesta were analyzed for dry matter (**DM**; method 930.15; AOAC, 2019) and nitrogen using the combustion method in a LECO analyzer (method 990.03; AOAC, 2019). Crude protein (**CP**) was calculated as nitrogen $\times$ 6.25. Diet and ileal digesta samples were also analyzed for AA (method 982.30 E [a, b, c]; AOAC, 2019) and titanium was quantified following the method described by Myers et al. (2004). Diet samples were also analyzed for ash (method 942.05; AOAC, 2019), crude fiber (method 978.10; AOAC, 2019), and crude fat (method 920.39; AOAC, 2019).

Apparent ileal digestibility (**AID**) and SID of CP and AA were calculated for the four dietary treatments as described by Stein et al. (2007). The values for the basal endogenous losses of CP and AA used to calculate SID were obtained from Adeola (2016).

Experiment 2: Energy

Twelve barrows (average initial body weight: 34.11 ± 1.03 kg) were allotted to a 4×3 incomplete Latin square design with 4 treatments and three periods of 13 days, for a total of nine replicate pigs per dietary treatment. During each experimental period, the initial first five days were considered an adaptation period to the diets and the adaptation period was followed by 5 days of collection of feces and urine. Feeding was managed as described in Exp. 1.

During collection, a urine pan and a screen were installed underneath the floor of the crates to allow for the total, but separate, collection of fecal and urine samples. Fecal samples

were collected following the marker-to-marker approach (Adeola, 2001). In short, an indigestible marker was included in the meal of the morning of the first day of collection, and the collection of feces started the moment the marker was spotted in the feces. A second indigestible marker was included in the meal on the morning of the last day of collection, and then the fecal collection stopped the moment the second marker was spotted in the feces. Unconsumed feed during the collection period was weighed, collected, and recorded. Orts were collected as well. Urine was collected every morning during the collection period. A bucket with 50 mL of 6 *N* HCl to prevent volatilization was placed underneath the urine pan under each crate and covered with cheesecloth. The buckets with the urine content were weighed daily, and 10% of the content was subsampled. Both feces and urine samples were immediately stored at -20°C. After the subsample of urine was taken, the remaining urine was discarded and HCl was added to the buckets again.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet. A subsample was then taken for chemical analysis. Fecal samples were dried at 55°C in a forced-air oven (Jacobs et al., 2011), finely ground, mixed, and subsampled. Urine samples were filtered and lyophilized. Feed, urine, and fecal samples were analyzed for gross energy using bomb calorimetry (Parr 6200, Parr Instruments Company, Moline, IL, USA). Feed and fecal samples were analyzed for DM (Method 930,15; AOAC, 2019). Following analysis of diets, fecal samples, and urine samples, ATTD of gross energy and DM was calculated and digestible energy (**DE**) and ME were calculated for each diet (Adeola, 2001).

Statistical Analyses

Data were analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC, USA) using pig as the experimental unit. Diet was the fixed effect and pig and period were

random effects. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Studentized residuals were calculated for each observation. Observations with a studentized residual outside of ± 3 were considered outliers and removed. Contrast statements were used to test linear and quadratic effects of increasing retention time in the conditioner in pelleted treatments; therefore, the mash diet was not included in the contrasts. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

Because the four treatment diets were produced using the same ingredient composition, only small variations in chemical composition were observed among diets (Table 2). However, differences were observed in the concentration of GE in the mash diet compared with the pelleted diets.

The expected conditioning temperature was 85°C and actual conditioning temperatures averaged 83.6°C and pellet mill production rates averaged 488 kg/hr (Table 3). Increasing the retention time of feed in the conditioner from 30 to 180 s numerically improved PDI from 81.6 to 88.7%.

No overall treatment effects were observed for the AID of Lys, Met, or Trp (Table 4). The AID of DM, CP, Thr, Leu, and Ala in pelleted diets, regardless of retention time, was greater ($P < 0.05$) AID than in the mash diet. The AID of Arg, His, Ile, Phe, Val, Asp, Glu, Ser, Tyr, indispensable AA, dispensable AA, and total AA was greater ($P < 0.05$) in pelleted diets with retention times of 60 and 180s compared with the mash diet; however, no differences were observed between the 30 s and the mash diets for the AID of these AA. The AID of Cys and Gly

was greater ($P < 0.05$) only in the diet pelleted after condition for 180 s compared with the mash diet. A linear ($P < 0.05$) increase was observed in the AID of DM and most AA except for Lys and Trp as retention time in the conditioner increased from 30 to 180 s.

No overall treatment effects were observed for the SID of Lys or Trp (Table 5). All pelleted diets had a greater ($P < 0.05$) SID of CP, Thr, Arg, Leu, Ala, Ser, and Tyr compared with the mash diet. Diets pelleted with conditioner retention times of 60 or 180 s had greater ($P < 0.05$) SID of Arg, His, Ile, Leu, Phe, Val, Ala, Asp, Glu, Ser, Tyr, DAA, indispensable AA, and total AA compared with the mash diet. Increasing the retention time in the conditioner from 30 to 180 s increased (linear; $P < 0.05$) the SID of CP and most AA, except for Lys and Trp.

No differences were observed in the ATTD of DM, concentrations of DE and ME, or the metabolizability of DE (i.e., ME:DE; Table 6). However, the ATTD of GE was greater ($P < 0.05$) in the pelleted diets compared with the mash diet.

DISCUSSION

Although it has been suggested that the ideal retention time in the conditioner is in the range of 30 to 90 s, most conditioners have an estimated retention time shorter than 30 s (Schofield and American Feed Industry Association, 2005; Behnke, 2014; Dunmire et al., 2021). However, retention time in the conditioner can be increased. The angle of paddles is usually factory set at 30 to 45° forward angle, which can be modified to a lesser angle to reduce the speed at which feed will move through the conditioner. The closer the paddles are to be perpendicular to the shaft, the greater retention time can be accomplished (Schofield and American Feed Industry Association, 2005; Behnke, 2014). Modifying the shaft speed can also alter retention time. However, shaft speed works in combination with the paddle angle and there

are different combinations that might lead to different retention times, and achieving the desired retention time as a result of the combined effects of shaft speed and paddle angle is a trial-and-error process (Schofield and American Feed Industry Association, 2005; Behnke, 2014). With the equipment available to prepare diets for the present experiments, the desired retention times were accomplished by reducing the shaft speed of the conditioner, adjusting the angle of the paddles in the conditioner, and reducing the amount of feed flowing into the conditioner. The first retention time accomplished was 180 s, due to the degree of difficulty to accomplish this time. Once the paddles and the flow of feed into the conditioner were adjusted to reach 180 s of retention, they remained unchanged for the remaining pellet runs. Because the only parameter that was modified to achieve the different retention times was the conditioner shaft speed, it was theorized that the conditions for conditioning (i.e. temperature, steam quality, pressure, etc.) other than retention times were similar across treatments.

During pelleting, the exposure of feed to heat and moisture creates conditions for physicochemical changes that potentially affect its nutritional value. Improvements in ileal digestibility of CP and AA when pelleting swine diets has been reported (Rojas et al., 2016; Dunmire et al., 2022; Lee et al., 2025). In the case of protein, there is a degree of denaturation of protein and inactivation of antinutritional factors that can lead to these potential improvements in AA digestibility. However, if ingredients or complete feed are over processed, Maillard reactions may occur which could result in negative effects on protein digestibility (González-Vega et al., 2011).

The chemical reactions involved in Maillard reactions can render AA indigestible due to the link of its amino group with a reducing sugar (Martins et al., 2000). Because Maillard reactions require a free amino group to occur, amino acids differ in their susceptibility. For

example, lysine is particularly reactive due to the presence of both an α -amino group, like all amino acids, and an additional ϵ -amino group (Martins et al., 2000). Tryptophan is also susceptible due to the nitrogen within its indole ring. As a result, the Maillard reaction might reduce digestibility of Lys and Trp before affecting other amino acids, due to their greater susceptibility to heat-induced reactions compared with other AA (Ajandouz and Puigserver, 1999; Salazar-Villanea et al., 2018). The observation that there were differences between mash and pelleted diets for the digestibility of Lys and Trp contrast with previous experiments (Rojas et al., 2016; Dunmire et al., 2022; Lee et al., 2025) where AID or SID of Lys and Trp increased with pelleting. However, because no improvements or reduction in the digestibility of Lys or Trp were observed, it is hypothesized that the positive effects of pelleting on Lys or Trp digestibility were offset by the negative effects of the Maillard reactions. Under the conditions of the present experiment, the slower production rate during pelleting might have resulted in prolonged residence time of the feed in the pellet die, where higher temperatures are reached due to friction and pressure. The extended exposure to heat may also have promoted Maillard reactions to a greater extent compared with previous studies, and therefore, potentially explaining the differences observed in the AID and SID of Lys and Trp compared with the mash diet.

Protein denaturation, particularly changes in tertiary structure, can occur during processes involving high temperatures and the presence of moisture in the environment. The temperature at which denaturation occurs is directly influenced by environmental moisture; proteins exposed to high moisture can denature at temperatures as low as 60 to 70°C, whereas proteins exposed to lower moisture conditions require higher temperatures that can reach 100 to 200°C (Adams, 1991; Svihus and Zimonja, 2011; Zhang et al., 2022). During pelleting, the use of steam, increases the feed moisture concentration 3 to 6% (Schofield and American Feed Industry

Association, 2005), while also increasing the temperature during conditioning. This increase in the temperature and the presence of moisture indicates the capacity of pelleting of promoting protein denaturation, which can partially explain the positive effect of pelleting on SID of CP and AA.

Although increasing the conditioning temperature by adding steam increases the amount of moisture added to the feed, increasing the retention time allows more time for that moisture and heat to be absorbed and penetrate feed particles. Therefore, increasing the retention time during conditioning resulted in increased AID and SID of most AA. The observation of increased AID and SID of AA in the diets pelleted using 180 s of retention time, compared with the 30 s retention time, may be a result of the additional time that the moisture in the conditioner could penetrate the feed and enhance the reactions produced by the heat treatment. Water diffusion is 100 times slower than heat diffusion (Thomas and van der Poel, 2020), resulting in a longer time needed for a particle to be hydrated even in the presence of sufficient moisture, compared with the time needed to reach a higher temperature. Moisture is not only one of the most important contributors to the binding of particles in the pellet (Thomas and van der Poel, 1996) which results in a more durable pellet (Cutlip et al., 2008; Abdollahi et al., 2010; Abdollahi et al., 2013; Evans et al., 2021), but water is also an important factor in the reactions that might result in increased CP and AA digestibility.. It is hypothesized that the extended residence of the feed in the conditioner contributed to a greater absorption of moisture in the feed, which in turn facilitated the physicochemical reactions during the formation of the pellet that resulted in the observed increased digestibility of CP and AA.

The increase in the ATTD of GE in the pelleted diets compared with the meal diet is in agreement with published data (Wondra et al., 1995; Le Gall et al., 2009; Rojas et al., 2016; Lee

et al., 2024). Gelatinization of starch is considered the main reason for the increase in ATTD of GE in diets fed to pigs (Lancheros et al., 2020). The high heat and moisture conditions during pelleting can promote the process of gelatinization in the grains in the feed. Due to the swelling and leaking of starch out of the granules (Hoseney, 1986), starch becomes more available for the endogenous enzymes needed for digestion, which may result in increased absorption of glucose in the small intestine. Changes in conditioning temperature and time may contribute to increased starch gelatinization in pelleted diets (Lewis et al., 2015), but conditioning temperature may be less important than the effect of the feed being pushed through the pellet die where friction temperature is generated. Under the conditions of the present experiment, increasing the retention time in the conditioner did not translate into an increase in the ATTD of GE, which could be an indication of minimal change in the concentration of gelatinized starch with increasing conditioning retention time. Limited research has been conducted to determine the effect of pelleting on the ATTD of fat (Le Gall et al., 2009; Lee et al., 2024) although the contribution of fat to the total GE energy of diets is significant due to its caloric density. An increase in ATTD of fat in pelleted diets compared with mash diets has been observed (Lee et al., 2024). However, due to the low inclusion of fat in the diets used in this experiment it is unlikely this was a major contributor to the increased ATTD of GE.

The results observed for DE and ME contrast with reported values, indicating increased DE and ME in pelleted diets compared with mash diets (Rojas et al., 2016; Lee et al., 2024). However, results in the present experiment may have been influenced by the initial concentration of GE in the treatment diets. The mash diet had a greater concentration of GE compared with the pelleted diets, which in turn results in greater concentrations of DE and ME. This observation can be partially explained by mixing, sampling, and analysis variation. Additionally, a possible

explanation for the lower GE in pelleted diets could be explained by moisture gain during conditioning, however, the concentrations of DM did not differ between treatments. Although the differences in ATTD of GE between the mash treatment and the pelleted treatments were significant, the difference is not greater than 2.5 percent units of ATTD, whereas the difference in GE concentrations is greater than 100 kcal/kg. This results in similar concentrations of DE and ME regardless of the greater ATTD of GE observed in pelleted diets. Furthermore, the observation of no differences in the metabolizability of DE among treatments can be an explanation for the observation of no differences on the concentrations of DE and ME across pelleted treatments. This indicates that although the ATTD of GE was greater in pelleted diets compared with mash diets, it did not influence the capacity of the pigs to further utilize the energy once it was absorbed.

In conclusion, extended retention times in the conditioner prior to pelleting increased the AID and SID of CP and most AA. In contrast, the ATTD of GE was not affected by the extended conditioning time, but it was improved by pelleting. These results indicate that increasing retention time as a strategy to enhance pellet quality or reduce microbial load can be implemented without compromising the digestibility of energy or nutrients.

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Table 1.1. Ingredient composition of experimental diets (as-fed basis)¹

Item	Basal diet
Ingredient, %	
Corn	59.62
Soybean meal	16.14
Distillers dried grains with solubles	20.00
Soybean oil	1.00
Calcium carbonate	0.95
Monocalcium phosphate, 21% P	0.30
Sodium chloride	0.40
L-Lysine-HCl	0.45
DL-Methionine	0.05
L-Threonine	0.12
L-Tryptophan	0.05
L-Valine	0.02
Trace mineral premix ²	0.15
Vitamin premix ³	0.25
Phytase concentrate ⁴	0.01
Titanium dioxide	0.50
Total	100.00
Calculated analysis	
Standardized ileal digestible AA, %	
Lysine	1.00
Isoleucine:lysine	61
Leucine:lysine	156
Methionine:lysine	33
Methionine and cysteine:lysine	58
Threonine:lysine	70
Tryptophan:lysine	20.3
Valine:lysine	72
Histidine:lysine	42
Total lysine, %	1.16
Metabolizable energy, kcal/kg	3,803
Crude protein, %	18.70
Ca, %	0.57
STTD P, %	0.38

¹A basal diet was formulated and divided into 4 batches. One batch was used without further processing, but three batches were pelleted following conditioning at 30, 60, or 180 s).

²Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate.

³Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D₃; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B₁₂; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.

⁴Quantum Blue 10g (AB Vista, Marlborough, Wiltshire, UK) contained 10,000 FTU/g. At 0.01% inclusion, complete diets were expected to contain 500 FTU/kg with an estimated release of 0.10% STTD P.

Table 1.2. Analyzed composition of experimental diets (as-fed basis)

Item,	Mash diet	Pelleted diets – Retention time, s		
		30	60	180
Gross energy, kcal/kg	4,101	3,963	3,986	3,998
Dry matter, %	87.89	87.47	87.44	87.51
Crude protein, %	19.29	19.04	19.14	19.07
Crude fat, %	3.04	4.15	4.18	4.03
Crude fiber, %	3.06	4.06	4.33	5.09
Ash, %	5.81	4.98	5.06	5.04
Indispensable AA, %				
Arginine	1.07	1.02	0.99	1.02
Histidine	0.51	0.49	0.48	0.49
Isoleucine	0.82	0.77	0.76	0.80
Leucine	1.87	1.80	1.78	1.87
Lysine	1.32	1.25	1.18	1.22
Methionine	0.43	0.34	0.34	0.35
Phenylalanine	0.95	0.90	0.89	0.94
Threonine	0.80	0.80	0.76	0.80
Tryptophan	0.22	0.22	0.21	0.22
Valine	0.98	0.93	0.90	0.94
Dispensable AA, %				
Alanine	1.10	1.07	1.06	1.10
Aspartic acid	1.61	1.56	1.53	1.60
Cysteine	0.35	0.33	0.32	0.34
Glutamic acid	3.29	3.14	3.12	3.28
Glycine	0.77	0.74	0.72	0.74
Serine	0.79	0.75	0.74	0.78
Tyrosine	0.68	0.64	0.63	0.66

Table 1.3. Feed processing and pellet quality of pelleted diets^{1,2}

Item,	Pelleted diets – retention time, s		
	30	60	180
Production rate, kg/h	504	487	472
Conditioning temperature, °C	83.8	84.0	83.1
Pellet durability index (PDI), % ³	81.6	84.8	88.7

¹Treatments were pelleted using a 5-ton 100-horsepower pellet mill (Model PM3016-4, CPM, Crawfordsville, IN, USA) equipped with a 4.8 × 45-mm die.

²The values for production rate and conditioning temperatures are the averages of four measurements taken at evenly spaced intervals over the duration of the pellet run.

³Holmen NHP100 (Norfolk, UK) for 60 s; Samples were analyzed in triplicate.

Table 1.4. Apparent ileal digestibility (AID) of dry matter, crude protein and amino acid (AA) in experimental diets¹

Item, %	Mash	Retention time, s			SEM	<i>P</i> <	Contrast <i>P</i> -value ²	
		30	60	180			Linear	Quadratic
Dry matter	63.5 ^b	65.9 ^a	66.3 ^a	68.0 ^a	0.71	0.001	0.014	0.937
Crude protein	64.7 ^b	71.3 ^a	73.4 ^a	73.9 ^a	1.01	0.001	0.122	0.238
Indispensable AA								
Arginine	82.4 ^c	84.2 ^{bc}	85.3 ^{ab}	86.1 ^a	0.71	0.001	0.013	0.220
Histidine	75.1 ^c	76.3 ^{bc}	78.1 ^{ab}	79.1 ^a	0.89	0.001	0.005	0.128
Isoleucine	73.2 ^c	75.5 ^{bc}	77.5 ^{ab}	78.9 ^a	1.10	0.001	0.004	0.148
Leucine	76.2 ^c	79.6 ^b	81.5 ^{ab}	82.7 ^a	0.99	0.001	0.004	0.144
Lysine	78.6	79.5	78.7	79.8	0.99	0.488	0.451	0.336
Methionine	83.9	83.4	84.8	85.4	0.88	0.052	0.026	0.138
Phenylalanine	74.8 ^c	77.3 ^{bc}	79.3 ^{ab}	80.7 ^a	1.02	0.001	0.004	0.156
Threonine	67.0 ^b	70.2 ^a	71.0 ^a	72.9 ^a	1.05	0.001	0.017	0.838
Tryptophan	79.6	80.0	80.8	81.5	1.00	0.252	0.150	0.577
Valine	70.4 ^c	73.0 ^{bc}	74.4 ^{ab}	76.0 ^a	1.17	0.001	0.013	0.409
Mean	75.8 ^c	77.9 ^{bc}	79.1 ^{ab}	80.4 ^a	0.94	0.001	0.012	0.357
Dispensable AA								
Alanine	70.3 ^c	74.2 ^b	76.4 ^{ab}	77.6 ^a	1.20	0.001	0.009	0.136
Aspartic acid	68.6 ^c	70.8 ^{bc}	72.8 ^{ab}	74.2 ^a	1.04	0.001	0.008	0.191
Cysteine	58.8 ^b	57.7 ^b	61.0 ^{ab}	64.8 ^a	1.46	0.002	0.001	0.443
Glutamic acid	75.2 ^c	76.7 ^{bc}	78.7 ^{ab}	80.5 ^a	1.05	0.001	0.005	0.284
Glycine	52.3 ^b	53.6 ^{ab}	55.2 ^{ab}	58.4 ^a	1.67	0.022	0.035	0.730
Serine	72.2 ^c	74.4 ^{bc}	76.0 ^{ab}	77.5 ^a	0.92	0.001	0.002	0.233
Tyrosine	76.0 ^c	78.2 ^{bc}	79.7 ^{ab}	81.1 ^a	0.90	0.001	0.002	0.242
Mean	70.4 ^c	72.4 ^{bc}	74.3 ^{ab}	76.3 ^a	1.06	0.001	0.003	0.296
Total AA	73.0 ^c	75.0 ^{bc}	76.6 ^{ab}	78.2 ^a	0.98	0.001	0.005	0.318

^{a-c}Means within a row lacking a common superscript letter are different (*P* < 0.05).

¹ N = 12.

²Linear and quadratic effects of increasing conditioning time prior to pelleting (i.e. 30, 60, and 180 s).

Table 1.5. Standardized ileal digestibility of crude protein and amino acids (AA) in experimental diets^{1,2,3}

Item, %	Mash	Retention time, s			SEM	<i>P</i> <	Contrast <i>P</i> -value ³	
		30	60	180			Linear	Quadratic
Crude protein	73.5 ^b	79.2 ^a	81.2 ^a	81.9 ^a	1.01	0.001	0.044	0.132
Indispensable AA								
Arginine	87.3 ^c	89.2 ^b	90.5 ^{ab}	91.1 ^a	0.71	0.001	0.016	0.143
Histidine	78.0 ^c	79.3 ^{bc}	81.1 ^{ab}	82.1 ^a	0.89	0.001	0.006	0.110
Isoleucine	76.4 ^c	78.9 ^{bc}	80.9 ^{ab}	82.2 ^a	1.10	0.001	0.006	0.129
Leucine	78.5 ^c	82.0 ^b	83.9 ^{ab}	85.1 ^a	0.99	0.001	0.005	0.131
Lysine	81.2	82.3	81.7	82.7	0.99	0.436	0.441	0.431
Methionine	86.2 ^a	86.2 ^a	87.6 ^a	88.1 ^a	0.89	0.027	0.034	0.133
Phenylalanine	77.8 ^c	80.4 ^{bc}	82.4 ^{ab}	83.6 ^a	1.02	0.001	0.005	0.138
Threonine	72.7 ^b	75.9 ^a	76.9 ^a	78.6 ^a	1.05	0.001	0.022	0.619
Tryptophan	84.8	85.1	86.2	86.7	1.00	0.225	0.176	0.411
Valine	74.6 ^b	77.3 ^{ab}	78.9 ^a	80.3 ^a	1.17	0.001	0.017	0.330
Mean	79.2 ^c	81.5 ^{bc}	82.8 ^{ab}	83.9 ^a	0.94	0.001	0.016	0.293
Dispensable AA								
Alanine	74.8 ^c	78.9 ^b	81.1 ^{ab}	82.2 ^a	1.20	0.001	0.012	0.119
Aspartic acid	72.7 ^c	75.0 ^{bc}	77.1 ^{ab}	78.3 ^a	1.04	0.001	0.011	0.161
Cysteine	63.1 ^b	63.2 ^b	65.7 ^{ab}	69.2 ^a	1.46	0.002	0.001	0.381
Glutamic acid	77.7 ^c	79.4 ^{bc}	81.3 ^{ab}	83.0 ^a	1.05	0.001	0.007	0.269
Glycine	69.0 ^b	70.9 ^{ab}	72.9 ^{ab}	75.6 ^a	1.67	0.011	0.021	0.539
Serine	79.4 ^c	81.9 ^b	83.7 ^{ab}	84.8 ^a	0.92	0.001	0.006	0.169
Tyrosine	80.6 ^c	83.0 ^b	84.5 ^{ab}	85.7 ^a	0.90	0.001	0.004	0.193
Mean	79.2 ^c	81.5 ^{bc}	83.5 ^{ab}	85.1 ^a	1.06	0.001	0.006	0.219
Total AA	79.2 ^c	81.5 ^{bc}	83.2 ^{ab}	84.6 ^a	0.98	0.001	0.008	0.240

^{a-c}Means within a row lacking a common superscript letter are different (*P* < 0.05).

¹N = 12.

²Values for standardized ileal digestibility were obtained with the correction of apparent ileal digestibility values for basal endogenous losses of each individual AA and crude protein. These values were obtained from Adeola (2016) as (g/kg of dry matter intake): Crude protein, 17.28; Arg, 0.59; His, 0.17; Ile 0.30; Leu, 0.50; Lys, 0.40; Met, 0.11; Phe, 0.32; Thr, 0.52; Trp, 0.13; Val, 0.46; Ala, 0.57; Asp 0.75; Cys, 0.17; Glu, 0.94; Gly, 1.46; Ser, 0.65; Tyr, 0.35.

³Linear and quadratic effects of increasing conditioning time prior to pelleting (i.e. 30, 60, and 180 s).

Table 1.6. Digestible energy (DE), metabolizable energy (ME), and apparent total tract digestibility (ATTD) of dry matter and gross energy¹

Item,	Mash	Retention time, s			SEM	<i>P</i> <	Contrast <i>P</i> -value ²	
		30	60	180			Linear	Quadratic
ATTD of dry matter, %	86.76	87.79	87.42	87.50	0.32	0.135	0.676	0.433
ATTD of gross energy, %	85.14 ^b	87.48 ^a	87.06 ^a	87.66 ^a	0.63	0.001	0.545	0.413
DE, kcal/kg of dry matter	3,973	3,963	3,969	4,004	28.66	0.437	0.112	0.931
ME, kcal/kg of dry matter	3,781	3,787	3,767	3,812	43.26	0.671	0.337	0.464
ME:DE, %	95.07	95.66	94.92	95.19	0.60	0.655	0.683	0.260

^{ab} Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹N = 9.

²Linear and quadratic effects of increasing conditioning time prior to pelleting (i.e. 30, 60, and 180 s).

Chapter 2 - Effect of pellet die thickness on standardized ileal digestibility of amino acids when pelleting diets fed to growing pigs

ABSTRACT

Components of the pelleting process, such as steam conditioning and feed retention time in the conditioner and die, expose feed to various degrees of heat, moisture, pressure, and shear which changes its physical and chemical characteristics. These changes may influence nutrient digestibility. Therefore, the objective of this experiment was to determine the effect of different pellet die thicknesses on the apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of crude protein (**CP**) and amino acids (**AA**) in commercial diets fed to pigs. A total of 12 growing barrows with an initial average body weight of 77.1 ± 3.53 kg were allotted to a triplicated 4×4 Latin square design with 4 treatments and 4 periods for a total of 12 replicate pigs per treatment. The dietary treatments consisted of a mash diet and three separate diets that were pelleted using a 1-ton pellet mill equipped with different pellet die length:diameter ratios (**L:D**) of 6 (26.5- $\times$ 4.4-mm), 8 (35.2- $\times$ 4.4-mm), and 10 (44.0- $\times$ 4.4-mm). The pelleted diets were steam conditioned for 30 s (Wenger twin shaft pre-conditioner, Model 150), production rate was kept constant at 708 kg per hour, and conditioning temperature was adjusted to accomplish hot pellet temperature of 85°C. Ileal cannulated pigs were housed individually in pens equipped with a feeder, a drinker, smooth-side walls, and a fully slatted metal floor. For the AID and SID of CP and AA, the diets pelleted using the L:D 8 or L:D 10 pellet dies were greater ($P < 0.05$) than the mash diet and the diet pelleted using the L:D 6 pellet die, except for Lys, Met, Trp, and Gly. Diets pelleted using an L:D 10 pellet die had increased ($P < 0.05$) SID Lys compared with the mash control with other treatments being intermediate. Diets pelleted with the L:D 8 or L:D 10 die had increased ($P < 0.05$) SID of Met compared with the mash diet, whereas there was no

difference in SID of Met between the mash control and L:D 6 and L:D 8 pelleted diets. For Trp and Gly, the AID and SID of the diet pelleted using the L:D 8 pellet die was greater ($P < 0.05$) than if the L:D 6 die was used with the other treatments being intermediate. Results indicate that AA digestibility improvement resulting from pelleting diets depends on pellet die thickness with diets pelleted using an L:D of 8 or 10 having improved AA digestibility for specific AA.

INTRODUCTION

Key components of the pelleting process, such as steam conditioning and the time feed remains in the conditioner and die, subject feed to varying levels of heat, moisture, pressure, and shear. These conditions alter the physical and chemical properties of the feed. During steam conditioning and transit of feed through the die, heat and moisture plasticize the soluble fractions of the diet, promoting agglomeration of dietary components (Lundblad et al., 2009). Pelleting is widely used in swine nutrition and feeding to enhance nutrient utilization, feed efficiency, handling characteristics, and bulk density (Vukmirović et al., 2017). The primary improvement in feed efficiency for swine is often attributed to reduced feed wastage (Nemechek et al., 2015).

While reduced feed wastage is a major factor in improved feed efficiency, there is ongoing discussion about how much of the benefit from pelleting is due to enhanced nutrient digestibility. This has led to interest in optimizing specific pelleting parameters and settings, such as conditioning temperature, moisture addition, and die specification, to not only improve pellet quality but also enhance the nutritional value of the feed (Svihus and Zimonja, 2011; Abdollahi et al., 2013; Rojas et al., 2016; Rojas and Stein, 2017). Among these parameters, pellet die specifications play a critical role in determining the friction, heat, and pressure exerted on the feed during pellet formation (Behnke, 1994; Knarr et al., 2024a). A key aspect of die

specification is pellet die thickness, which is defined as the distance from the entry to the exit of each die hole and is commonly expressed as part of the length-to-diameter ratio (**L:D**), which also includes the hole diameter (Schofield and American Feed Industry Association, 2005).

Pelleting improves nutrient digestibility, particularly crude protein (**CP**) and amino acids (**AA**), largely due to protein denaturation and inactivation of antinutritional factors during processing (Rojas et al., 2016; Lee et al., 2023; Dunmire et al., 2024). However, these benefits can be counterbalanced by the risk of heat damage, which may reduce the availability and digestibility of key nutrients if processing parameters become excessive (González-Vega et al., 2011). One factor contributing to increased temperature, pressure, and shear is the use of thicker pellet dies, which extend feed retention time within the die. This increased residence time enhances pellet quality but also elevates the temperature of pellets as they exit the die (Saensukjaroenphon, 2019). Whereas thicker dies improve physical pellet quality (Saensukjaroenphon, 2019; Truelock et al., 2020), it remains unclear if these same conditions positively or negatively impact nutrient digestibility. To the authors' knowledge, no experiments have specifically determined effects of thicker pellet dies on nutrient digestibility in diets for pigs. Therefore, the objective of this experiment was to test the hypothesis that increased pellet die thickness will increase ileal digestibility of CP and AA by growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Kansas State University approved the use of animals for this experiment. Diets were mixed and processed at the Kansas State University O. H. Kruse Feed Technology and Innovation Center (Manhattan, KS).

Dietary treatments

Dietary treatments included a mash control, and three pelleted diets produced using pellet dies that varied in thickness, resulting in a total of four dietary treatments in the experiment. Pellet die diameters were held consistent; therefore, treatments will be expressed based on the pellet die L:D, as L:D 6, L:D 8, and L:D 10. A corn, soybean meal, and distillers dried grains with solubles-based diet was formulated (Table 1) to meet or exceed the nutrient requirements of pigs between 75 and 100 kg (NRC, 2012). Three batches of 726 kg and a fourth batch of 318 kg were mixing using a twin-shaft counterpoise mixer (Hayes and Stolz, model TRDB63-0152, Fort Worth, TX, USA) for a total of 2,495 kg. The mixing time was 60 s for dry ingredients and 120 s after liquid ingredients were added. The 318 kg-batch was kept as mash to be the control treatment. The remaining three batches were mixed, stored in a bin together, and used for further processing.

Feed processing

The experiment was designed to achieve pellet runs with a similar hot pellet temperature while using 3 different pellet dies. However, the increased thickness of the L:D 10 pellet die, resulted in increased hot pellet temperatures. Therefore, different conditioning temperatures were used to achieve similar hot pellet temperature across treatments. Pelleted diets were steam conditioned (25×140 cm Wenger twin shaft pre-conditioner, Model 150, Wenger, Sabetha, KS, USA) for 30 s at 79.6, 79.2, or 68.0°C, and subsequently pelleted using a 1-ton pellet mill (1112-2, California Pellet Mill, Crawfordsville, IN, USA) equipped with a 26.5- $\times$ 4.4-mm (L:D 6), 35.0- $\times$ 4.4-mm (L:D 8), or 44.0- $\times$ 4.4-mm (L:D 10; Table 2) pellet die, respectively. Reducing the temperature of conditioning in the pellet run using the L:D 10 die reduced the amount of steam and in turn the amount of moisture available to penetrate the feed during this run. Considering that a thicker pellet die might decrease production rate due to the increased pressure

needed to push the feed through a longer channel; by reducing the amount of moisture in the feed, it was possible to match the production rate of the L:D 6 and L:D 8 diets. Therefore, under the conditions of this experiment, it was possible to maintain the production rate (11.8 kg/min) and hot pellet temperature (83.9 to 85.5°C; Table 3) across treatments, at the expense of changes in conditioning temperature. After 15 min of warmup, each dietary treatment was pelleted in a single run that took approximately 40 min. After a completed pellet run, the pellet die was changed, and the mill would be allowed to warm up again.

During each pelleting run, the temperature in the conditioner, production rate, and hot pellet temperature were monitored and recorded every 8 minutes (Table 3). To record hot pellet temperature, a sample of pellets was placed into a pre-warmed double-wall thermos equipped with a thermometer. Retention time in the pellet die was calculated for each of the runs, according to Saensukjaroenphon (2019). Pellet samples were collected after cooling, sifted with a U.S. No. 5 (3.9 mm) sieve to separate pellets from fines, and stored in commercial paper feed sacks. Pellet durability index (**PDI**) was analyzed in duplicate, and the results were averaged, following the Holmen forced-air method (Evans, 2023) using a NHP 100 (TekPro Ltd, Norfolk, UK) pellet tester with each run lasting 30 s. For each dietary treatment, PDI was calculated using the following equation (S269.5; ASAE, 2012):

$$PDI (\%) = \frac{\text{Weight of recovered pellets}}{\text{Intitial weight of pellets}} \times 100$$

Animals and feeding

Twelve barrows (average initial body weight: 77.1 ± 3.53 kg; DNA 241 $\times$ 600, DNA, Columbus, NE) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a triplicated 4×4 Latin square design (Kim and Stein, 2009) with four periods of 7 d and 4 dietary treatments. Therefore, each diet was fed to 3 pigs in each period for a total of

12 replicate pigs per dietary treatment for the 4 periods. Feed allowance for the pigs was 3.0 times the maintenance requirement for metabolizable energy (i.e. 197 kcal/kg body weight^{0.60}; NRC, 2012) with free access to water throughout the experiment. The daily allowance of feed per day was divided into 2 equal meals that were provided to the animal at 0800 and 1400 h.

Experimental procedures

The first five days of each period were considered adaptation to the diet (Adeola, 2001), and ileal sample collection took place on days 6 and 7. During each collection day, the lid was removed from the cannulas, and a plastic bag was attached to the end of the cannula using a plastic cable tie. The digesta flowing into the bag was collected every 30 minutes or when the bag was full, for a total of 8 h each day. The samples collected were immediately stored at -20°C to prevent any bacterial degradation of AA or enzymatic activity.

Data collection and sample analysis

Ileal digesta samples were thawed, subsampled, lyophilized, and ground prior analysis. Diet and ileal samples were analyzed for nitrogen using the combustion method (LECO analyzer; method 990.03; AOAC, 2019) and CP was calculated as nitrogen $\times$ 6.25. Diet and ileal samples were also analyzed for dry matter (**DM**; method 930.15; AOAC, 2019), AA concentrations (method 982.30 E [a, b, c]; AOAC, 2019), and titanium concentrations (Myers et al., 2004). Diet samples were also analyzed for crude fat (method 920.39; AOAC, 2019), crude fiber (method 978.10; AOAC, 2019), and ash (method 942.05; AOAC, 2019).

Calculations and statistical analysis

Apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of CP and AA were calculated for the 4 dietary treatments using the equations described by Stein et al. (2007),

and the values for the basal endogenous losses of CP and AA were obtained from a previous experiment (Lopez et al., 2025) conducted with the same pigs used in the present experiment.

Data were analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC) using pig as the experimental unit and the random effects of pig and period and the fixed effect of dietary treatment. For each independent variable, least square means were calculated and then separated using the PDIF option. Studentized residuals were used to remove outliers, for any value outside of more than ± 3 . Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

The calculations of die retention time indicate that the feed spent 1.6, 2.2, or 3.3 s in the pellet die with L:D of 6, 8, and 10, respectively. Pellet durability index was analyzed for each dietary treatment, and the results indicate an increase in this measurement as the die thicknesses increased. The pellets manufactured using the die with L:D 6 had the lowest PDI (50.1%), followed by the L:D 8 (86.6%), and pellets produced using the L:D 10 die had the greatest PDI (93.1%).

There was an overall treatment effect ($P < 0.01$) on AID of DM, CP, and all AA. The AID of DM was greater ($P < 0.05$) in the diet pelleted using the L:D 8 or 10 die compared with the L:D 6 die, but AID of DM in the diet pelleted with the L:D 6 die was not different from the mash diet. No differences were observed between the mash diet and the diets pelleted using the L:D 6 or L:D 8 dies. Diets pelleted using the L:D 8 or the 10 dies had greater ($P < 0.05$) AID of CP and most AA, except for Lys, Met, Trp, and Gly, compared with the mash diet or the diet pelleted using the L:D 6 die. The diet pelleted using the L:D 10 die had greater ($P < 0.05$) AID of

Met than the mash diet and the L:D 6 diet, whereas the diet pelleted using the L:D 8 die had greater ($P < 0.05$) AID of Met than the mash diet, but not the other pelleted diets. The diets pelleted using the L:D 8 and the L:D 10 dies had greater ($P < 0.05$) AID of Lys compared with the mash diet, but no differences were observed between diets pelleted using the L:D 6 die and those pelleted using the L:D 8 or the L:D 10 or mash diets. No differences between the mash diet and the pelleted diets were observed for AID of Trp, however, the AID of Trp was greater ($P < 0.05$) in the diet pelleted using the L:D 8 die compared with the L:D 6 die. Additionally, no differences were observed for the AID of Trp between the L:D 10 diet and either the L:D 6 or the L:D 8 diet. The AID of Gly was greater ($P < 0.05$) in the L:D 8 diet compared with the L:D 6 diet, however, no differences were observed between the mash diet and the pelleted diets using either the L:D 6 or the L:D 10 die.

The SID of CP was greater ($P < 0.05$) in the diets pelleted using the L:D 8 or L:D 10 dies, compared with the L:D 6 die, but no differences were observed between the mash diet and the diet pelleted using the L:D 6 or the L:D 8 dies. Additionally, no differences were observed between the diet pelleted using the L:D 8 die and the L:D 10 die. The SID of most AA except Lys, Met, Trp, and Gly, were greater ($P < 0.05$) in the diets pelleted using the L:D 8 or the L:D 10 dies compared with the mash diet or the L:D 6 diet, but no differences were observed between the mash diet and the L:D 6 diet. The diet pelleted using the L:D 10 die had a greater ($P < 0.05$) SID of Lys than the mash diet, but there was no difference between the mash diet and the diets pelleted using the L:D 6 or the L:D 8 dies, or between the diet pelleted using the L:D 10 die and the diets pelleted using the L:D 6 or the L:D 8 dies. The SID of Met was less ($P < 0.05$) in the mash diet compared with the L:D 8 and the L:D 10 diets, but the SID of Met in the mash diet was no different from the L:D 6 diet. The SID of Met was also not different between the diet

pelleted using the L:D 6 die and the diet pelleted using the L:D 8 die, or between the diet pelleted using the L:D 8 die and the diet pelleted using the L:D 10 die. For the SID of Trp and Gly, no differences were observed between the mash diet and the pelleted diets, regardless of the pellet die. However, the SID of Trp and Gly in the diet pelleted using the L:D 8 pellet die was greater ($P < 0.05$) than in the L:D 6 diet, but not different from the L:D 10 diet. No differences were observed in the SID of Trp and Gly between the diet pelleted using the L:D 6 die or the L:D 10 die.

DISCUSSION

The pelleting parameters were selected for each pellet die treatment to balance production rate and hot pellet temperature across treatments. Production rate was standardized across the pellet runs to reduce possible variability that could confound the effect of the thickness of the pellet die. Changes in the residence time in the pellet die can be attributed to the effective length of the die, rather than changes in throughput or differences in conditioning time. Targeting similar hot pellet temperatures instead of similar conditioning temperatures decreased the possibility of heat damage or enzyme degradation in the pellets, which could confound the response in digestibility observed during the experiment. Hot pellet temperature is a better indicator for phytase degradation than conditioning temperature alone although reducing conditioning temperature reduced the amount of moisture in the feed during the pellet run using the L:D 10 die (Truelock et al., 2022). Thicker pellet dies require more pressure and friction for feed to pass through, but excess moisture can contribute to the formation of a thicker feed pad, which in turn can cause roll slips and die chokes (Evans, 2023).

Increasing die L:D from 5.6 to 8.0 improves pellet durability but increases pellet mill energy consumption (Truelock et al., 2020). Behnke (2014) described the same positive correlation between die L:D and pellet durability, which can be attributed to the increased pressure and resistance generated by a larger die L:D. When die hole diameter remains constant, a pellet die with a larger die L:D is thicker than a die with a smaller L:D. Thus, feed retention within the die is longer with a thicker die and is a primary factor in determining pellet durability. This is due to increased exposure to heat and pressure that leads to the physicochemical reactions experienced during pelleting (Bastiaansen et al., 2024), which enhance binding among particles and contribute to improved pellet durability (Saensukjaroenphon, 2019; Truelock et al., 2020; Knarr et al., 2024a). Therefore, the observation that increased pellet die thickness in the present experiment resulted in greater PDI is in agreement with previous data. The magnitude of increase in PDI as L:D increased from 6 to 8 is greater than the increase from L:D 8 to 10. This observation indicates that the pressure and friction as the feed went through the L:D 6 die was not enough to promote the physicochemical reactions that result in a more structurally sound pellet. Furthermore, the retention time in the pellet die was considerably shorter in the L:D 6 pellet die, compared with the L:D 8 and L:D 10. The time of residence along with the pressure, friction, and higher temperatures during the pellet runs using the L:D 8 and 10 pellet dies promoted the physicochemical changes to a greater degree than the L:D 6 die, which resulted in the increase in AA digestibility that was observed.

During pelleting, proteins are exposed to heat and mechanical forces that disrupt their tertiary structure, leading to irreversible denaturation and potential AA binding. In general, heat treatment improves protein digestibility by inactivating antinutritional factors such as enzyme inhibitors and unfolding proteins to enhance enzymatic access (Hancock and Behnke, 2001;

Svihus and Zimonja, 2011; Lancheros et al., 2020). However, it is not known if this effect is a result of denaturation of the proteins that become more available for digestion and absorption, or of elimination of antinutritional factors (Svihus and Zimonja, 2011). The combination of high temperature, shear force, and moisture during pelleting can potentially promote the Maillard reaction. This reaction involves the interaction of free amine groups in AA and proteins with the carbonyl groups of reducing sugars (e.g., glucose, galactose, and fructose), which can reduce the availability and utilization of AA (Thorpe and Baynes, 2003; Pahm et al., 2008; González-Vega et al., 2011; Peng et al., 2011). However, digestibility of CP and AA in swine diets is usually increased by pelleting (Rojas et al., 2016; Dunmire et al., 2024; Lee et al., 2025) indicating that formation of Maillard reaction products during pelleting likely is not a concern. Indeed, pelleting diets with different concentrations of reducing sugar and free AA did not induce Maillard reactions and improved SID of AA further indicating that pelleting diets at less than 90 °C has no negative impact on AA digestibility (Dunmire et al., 2024).

Because thicker pellet dies increase feed retention time, thicker dies expose feed to greater heat and pressure, which promotes physicochemical reactions (Bastiaansen et al., 2024). The observation that pelleting diets using the L:D 8 and 10 improved ileal AA digestibility is in agreement with reported data (Rojas et al., 2016; Dunmire et al., 2024; Lee et al., 2025). However, because no differences were observed between the diet pelleted using the L:D 6 die and the mash diet, it is hypothesized that diets pelleted using the L:D 6 die did not undergo the necessary physiochemical changes to result in improvements of nutrient digestibility. This is also demonstrated by the much poorer PDI achieved when pelleting diets using the L:D 6 die instead of the L:D 8 or the L:D 10 die.

To our knowledge, effects of pellet die thicknesses on ileal digestibility of nutrients in diets fed to growing pigs have not been determined. However, the AID of AA by broiler chickens is greater in diets pelleted using a pellet die with an L:D of 10 compared with a L:D 7.1 die (Knarr et al., 2024b). A longer retention time in the die allows friction and pressure to increase the temperature of the feed, and simultaneously allows more time for the moisture to diffuse into the feed which as mentioned before, is a slower process than heat diffusion (Thomas and van der Poel, 2020), potentially decreasing the threshold of temperature needed for protein denaturation and inactivation of anti-nutritional factors. Therefore, it is likely that it is the combination of both the elevated temperature in diets pelleted using the thicker dies and the increased moisture that resulted in the increased SID of AA that were observed.

In conclusion, pelleting diets using L:D 8 or L:D 10 pellet dies resulted in greater AID and SID of CP and most AA compared with a mash dietary treatment. However, no differences were observed between the diet pelleted using the L:D 6 die and the mash diet. These results indicate that the magnitude of the forces exerted on the feed during pelleting (i.e. temperature, pressure, friction) can influence the degree of the positive response observed from pelleting on CP and AA digestibility.

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Table 2.1. Ingredient and calculated nutrient composition of experimental diet (as-fed basis)¹

Item	Basal diet
Ingredient, %	
Corn	60.84
Soybean meal	15.00
Distillers dried grains with solubles	20.00
Soybean oil	1.00
Calcium carbonate	1.05
Monocalcium phosphate, 21% P	0.50
Sodium chloride	0.40
L-Lysine-HCl	0.30
Trace mineral premix ²	0.15
Vitamin premix ³	0.25
Phytase ⁴	0.01
Titanium dioxide	0.50
Total	100.00
Calculated composition	
Metabolizable energy, kcal/kg	3,337
Crude protein, %	17.9
Crude fat, %	5.1
Crude fiber, %	3.6
Ca, %	0.65
STTD P, %	0.41
Standardized ileal digestible AA, %	
Lysine	0.85
Isoleucine:lysine	69
Leucine:lysine	180
Methionine:lysine	32
Methionine and cysteine:lysine	61
Threonine:lysine	60
Tryptophan:lysine	17.4
Valine:lysine	81
Histidine:lysine	48
Total lysine, %	1.01

¹A basal diet was formulated and divided into 4 aliquots. One aliquot was used without further processing, and the remaining three aliquots were pelletized using different pellet dies (L:D 6, 8, and 10).

²Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate.

³Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D₃; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B₁₂; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.

⁴Quantum Blue 10g (AB Vista, Marlborough, Wiltshire, UK) contained 10,000 FTU/g. At 0.01% inclusion, complete diets were expected to contain 500 FTU/kg with an estimated release of 0.10% STTD P.

Table 2.2. Specifications of dies used during manufacturing of dietary treatments

Item,	Pellet die length:diameter		
	6	8	10
Die hole diameter, mm [D]	4.4	4.4	4.4
Die effective thickness, mm [L]	26.5	35.0	44.0
Internal die diameter, cm	30.5	30.5	30.5
Internal die width, cm	5.7	5.7	5.7
Internal die surface area, cm ²	547.2	547.2	547.2
Holes per cm ²	2.2	2.2	2.6
Total die holes ¹	1,188	1,188	1,442

¹Calculated according to Saensukjaroenphon (2019).

Table 2.3. Pellet mill parameters and subsequent pellet quality of pelleted diets^{1,2}

Item,	Pellet die length:diameter		
	6	8	10
Production rate, kg/min	11.8	11.8	11.8
Conditioning temperature, °C	79.6	79.2	68.0
Conditioning time, s	30.0	30.0	30.0
Hot pellet temperature, °C	83.9	85.5	84.3
Die retention, sec ³	1.6	2.2	3.3
Pellet durability index (PDI), % ⁴	50.1	86.8	93.1

¹Treatments were steam conditioned using a Wenger twin staff pre-conditioner (Model 150, Wenger, Sabetha, KS, USA) and subsequently pelleted using a California Pellet Mill (model 1112-2, Crawfordsville, IN, USA).

²The values for production rate and conditioning temperatures, and hot pellet temperature are the averages of six measurements taken at evenly spaced intervals over the duration of the pellet run.

³Calculated according to Saensukjaroenphon (2019) using the die specifications listed in Table 2.

⁴Holmen NHP100 (Norfolk, UK) method for 30 sec; Samples were analyzed in duplicated.

Table 2.4. Analyzed composition of experimental diets (as-fed basis)¹

Item, %	Mash diet	Pellet die length:diameter		
		6	8	10
Dry matter	89.92	88.57	87.77	87.82
Crude protein	18.33	18.45	17.67	18.16
Crude fat	4.20	4.92	4.72	4.79
Crude fiber	3.14	3.78	3.26	3.09
Ash	4.80	5.17	5.15	5.13
Indispensable AA				
Arginine	0.99	1.05	1.08	0.99
Histidine	0.49	0.50	0.51	0.48
Isoleucine	0.74	0.76	0.78	0.73
Leucine	1.85	1.77	1.86	1.79
Lysine	1.02	1.15	1.14	1.02
Methionine	0.33	0.33	0.33	0.31
Phenylalanine	0.90	0.91	0.94	0.88
Threonine	0.66	0.69	0.70	0.65
Tryptophan	0.16	0.16	0.17	0.15
Valine	0.88	0.92	0.93	0.87
Dispensable AA				
Alanine	1.12	1.09	1.13	1.09
Aspartic acid	1.50	1.61	1.64	1.53
Cysteine	0.36	0.36	0.37	0.35
Glutamic acid	3.24	3.22	3.33	3.18
Glycine	0.72	0.76	0.78	0.72
Serine	0.76	0.78	0.80	0.76
Tyrosine	0.65	0.65	0.68	0.65

¹Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.

Table 2.5. Apparent ileal digestibility (AID) of dry matter, crude protein and amino acid (AA) in dietary treatments¹

Item, %	Mash	Pellet die length:diameter			SEM	<i>P</i> <
		6	8	10		
Dry matter	69.2 ^{bc}	67.4 ^c	70.2 ^{ab}	72.2 ^a	0.69	0.001
Crude protein	75.4 ^b	75.1 ^b	77.4 ^a	77.5 ^a	0.73	0.001
Indispensable AA						
Arginine	86.1 ^b	87.0 ^b	88.3 ^a	88.2 ^a	0.54	0.001
Histidine	82.0 ^b	82.2 ^b	84.0 ^a	84.0 ^a	0.64	0.001
Isoleucine	78.7 ^b	79.8 ^b	82.0 ^a	82.0 ^a	0.75	0.001
Leucine	84.0 ^b	84.3 ^b	86.4 ^a	86.9 ^a	0.61	0.001
Lysine	81.4 ^b	82.3 ^{ab}	83.8 ^a	84.0 ^a	1.55	0.005
Methionine	84.3 ^c	85.4 ^{bc}	86.6 ^{ab}	86.9 ^a	0.61	0.001
Phenylalanine	81.6 ^b	82.4 ^b	84.4 ^a	84.6 ^a	0.66	0.001
Threonine	70.2 ^b	70.8 ^b	73.8 ^a	73.7 ^a	0.89	0.001
Tryptophan	84.4 ^{ab}	82.9 ^b	85.9 ^a	84.3 ^{ab}	0.97	0.012
Valine	76.0 ^b	77.7 ^b	79.9 ^a	80.0 ^a	0.79	0.001
Mean	81.1 ^b	81.8 ^b	83.8 ^a	83.9 ^a	0.68	0.001
Dispensable AA						
Alanine	79.3 ^b	79.8 ^b	82.3 ^a	83.0 ^a	0.73	0.001
Aspartic acid	75.8 ^b	76.8 ^b	79.3 ^a	79.0 ^a	0.82	0.001
Cysteine	70.7 ^b	70.9 ^b	73.8 ^a	73.2 ^a	1.00	0.001
Glutamic acid	84.7 ^b	84.4 ^b	86.7 ^a	86.7 ^a	0.66	0.001
Glycine	65.0 ^b	64.3 ^b	68.8 ^a	66.6 ^{ab}	1.39	0.003
Serine	78.8 ^b	79.3 ^b	81.5 ^a	82.0 ^a	0.66	0.001
Tyrosine	83.0 ^b	83.5 ^b	85.5 ^a	86.1 ^a	0.56	0.001
Mean	79.3 ^b	78.6 ^b	81.3 ^a	81.3 ^a	0.82	0.001
Total AA	80.1 ^b	80.1 ^b	82.4 ^a	82.4 ^a	0.75	0.001

^{a-c}Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹N = 12.

Table 2.6. Standardized ileal digestibility of crude protein (CP) and AA in dietary treatments^{1,2}

Item, %	Mash	Pellet die length:diameter			SEM	<i>P</i> <
		6	8	10		
Crude protein	83.0 ^{bc}	82.5 ^c	84.7 ^{ab}	85.1 ^a	0.73	0.001
Indispensable AA						
Arginine	90.8 ^b	91.3 ^b	92.6 ^a	92.8 ^a	0.54	0.001
Histidine	85.2 ^b	85.3 ^b	87.0 ^a	87.2 ^a	0.64	0.001
Isoleucine	82.5 ^b	83.4 ^b	85.5 ^a	85.7 ^a	0.75	0.001
Leucine	86.4 ^b	86.7 ^b	88.6 ^a	89.2 ^a	0.61	0.001
Lysine	84.8 ^b	85.3 ^{ab}	86.8 ^{ab}	87.4 ^a	1.55	0.006
Methionine	86.6 ^c	87.7 ^{bc}	88.8 ^{ab}	89.3 ^a	0.61	0.001
Phenylalanine	84.6 ^b	85.3 ^b	87.3 ^a	87.6 ^a	0.66	0.001
Threonine	77.2 ^b	77.4 ^b	80.2 ^a	80.7 ^a	0.89	0.001
Tryptophan	91.0 ^{ab}	89.4 ^b	92.1 ^a	91.3 ^{ab}	0.97	0.031
Valine	80.4 ^b	81.8 ^b	83.9 ^a	84.3 ^a	0.79	0.001
Mean	84.8 ^b	85.3 ^b	87.2 ^a	87.6 ^a	0.68	0.001
Dispensable AA						
Alanine	83.6 ^b	84.2 ^b	86.4 ^a	87.3 ^a	0.73	0.001
Aspartic acid	80.4 ^b	81.1 ^b	83.4 ^a	83.4 ^a	0.82	0.001
Cysteine	75.1 ^b	75.2 ^b	78.0 ^a	77.7 ^a	1.00	0.002
Glutamic acid	87.3 ^b	87.0 ^b	89.1 ^a	89.2 ^a	0.66	0.001
Glycine	83.9 ^{ab}	82.0 ^b	85.9 ^a	85.1 ^{ab}	1.39	0.014
Serine	84.5 ^b	84.7 ^b	86.8 ^a	87.6 ^a	0.66	0.001
Tyrosine	86.3 ^b	86.7 ^b	88.6 ^a	89.4 ^a	0.56	0.001
Mean	88.3 ^{bc}	87.4 ^c	89.8 ^{ab}	90.2 ^a	0.82	0.001
Total AA	86.7 ^b	86.5 ^b	88.6 ^a	89.0 ^a	0.75	0.001

^{a-c}Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹N = 12.

²Values for standardized ileal digestibility were obtained with the correction of apparent ileal digestibility values for basal endogenous losses of each individual AA and CP. These values were obtained from previously conducted research at the Swine Teaching and Research Center (STRC) at Kansas State University (g/kg of dry matter intake): Crude protein, 15.41; Arg, 0.52; His, 0.17; Ile 0.31; Leu, 0.48; Lys, 0.39; Met, 0.08; Phe, 0.30; Thr, 0.51; Trp, 0.12; Val, 0.42; Ala, 0.53; Asp 0.77; Cys, 0.18; Glu, 0.92; Gly, 1.52; Ser, 0.48; Tyr, 0.24.

Chapter 3 - Effect of the inclusion of myristic acid, protected benzoic acid, and *aspergillus niger* cultures on the apparent total tract digestibility of energy, concentration of digestible and metabolizable energy, and nitrogen retention

ABSTRACT

The objective of this experiment was to determine the effect of a source of myristic acid (**MA**), protected benzoic acid (**BA**), and cultures of *Aspergillus niger* (**AN**) on the apparent total tract digestibility (**ATTD**) of gross energy (**GE**), the concentration of digestible (**DE**) and metabolizable energy (**ME**), and N retention of commercial diets fed to growing pigs. Ten barrows (average initial body weight: 34.1 ± 1.03 kg) were allotted to a replicated 5×5 Latin square design with 5 treatments and 5 periods for a total of 10 replicate pigs per treatment. Treatments consist of a negative control basal corn, soybean meal, wheat middlings and DDGS-based diet, a positive control corn-soybean meal-based diet, and three additional diets adding either MA, BA, or AN to the negative control diet. Pigs were individually housed in metabolism crates. During each experimental period, pigs were fed one of the dietary treatments for a 5-d adaptation period and a 5-day collection period of fecal and urine samples. The ATTD of GE and dry matter was greatest ($P < 0.05$) in the positive control diet. However, no differences were observed between the negative control diet and the diet MA, BA, or AN. The concentration of DE was greatest ($P < 0.05$) in the positive control diet compared with the negative control diets with or without feed additives. The negative control diet containing MA had a greater ($P < 0.05$) concentration of DE compared with the negative control diet. There was a tendency ($P < 0.15$) for the BA, and AN diets to have a greater DE concentration than the negative control diet. The

concentration of ME was the greatest ($P < 0.05$) in the positive control diet when compared with the negative control diet with and without the feed additives. Diets containing MA and BA tended ($P < 0.15$) to have a greater concentration of ME compared with the negative control diet with diets containing AN being intermediate. There was a tendency of greater ($P < 0.15$) concentrations of ME in diets containing added MA and BA compared with the negative control diet. Pigs fed the positive control diet had the greatest ($P < 0.05$) N retention as percentage of intake and N digestibility. However, no evidence of differences between the negative control and the diets containing BA, MA, and AN were observed. This data suggests that the inclusion of MA and BA has the potential to increase the concentration of ME in commercial diets with high concentrations of fiber without impacting N retention.

INTRODUCTION

The inclusion of high-fiber ingredients in swine diets such as wheat middlings and distillers dried grains with solubles, has been shown to reduce apparent total tract digestibility (ATTD) of gross energy (GE) and dry matter (DM), as well as affect growth performance (Pedersen et al., 2007; Huang et al., 2013; Navarro et al., 2018). However, these feed ingredients are being included into swine diets due to their low cost (Woyengo et al., 2014). Their use is typically limited to grower-finishing pigs due to their resilience to the potential negative effects of lower-quality ingredients (Xu et al., 2010; Stewart et al., 2013). Different strategies have been implemented to capture additional value from diets that include high fiber ingredients, such as the use of feed additives. Among other modes of action, these feed additives aim to improve sanitary status or promote the digestion and absorption of nutrients directly or indirectly (Rao et al., 2023).

Acidifiers, organic acids, and direct fed microbials are traditionally used in weaning pig diets as alternatives of antibiotics (Torrallardona et al., 2007; Suiyanrayna and Ramana, 2015; Gaffield et al., 2024). However, less research has been conducted on the effect of these additives on diets fed to grower-finishing pigs, which includes the improvement of nutrient and energy digestibility. A recent literature review by Rao et al., (2023), suggests that some of the positive effects observed in weaning pigs, are observed also in grower-finishing pigs, although with less consistency in the response. Furthermore, it is still unclear if the possible response observed on growth performance is a result of changes in digestibility of nutrients and energy (Nguyen et al., 2020). Additionally, the capacity of these additives to modify the microbial populations in the gastrointestinal tract (**GIT**; Burdock and Garabin, 2006; Nguyen et al., 2020) along with the increased capacity for fermentation in grower-finishing pigs, might increase the utilization of the energy in high-fiber diets and therefore the concentrations of digestible and metabolizable energy. Therefore, the objective of this experiment is to determine the effect of three feed additives, a source of myristic acid (**MA**), protected benzoic acid (**BA**), and *aspergillus niger* (**AN**) on the digestibility of DM, GE, and N, and the concentrations of DE and ME in diets with a high concentration of fiber fed to growing-finishing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Kansas State University approved the use of animals for this experiment which was conducted at the Swine Teaching and Research Center at Kansas State University. A total of 10 barrows (average initial body weight: 34.1 ± 1.03 kg; DNA 200 $\times$ 400, DNA Genetics, Columbus, Nebraska, USA) were housed individually

in metabolic crates (1.50 m × 0.56 m) equipped with solid panels on the sides, a feeder, a water cup drinker, and metal diamond-slatted flooring.

A basal corn, soybean meal, wheat middlings, and corn DDGS-based diet was formulated and used in the experiment as a negative control treatment. A second corn-soybean meal-based diet was formulated and used as a positive control treatment. The diets were formulated to meet or exceed the recommended nutrient requirements of growing pigs (50-75 kg; NRC, 2012). Three additional diets were formulated by adding one source (Insighter Biotech, Guangzhou, China) of MA, BA, or AN to the negative control basal diet at inclusions of 0.07, 0.10, and 0.05% respectively. Therefore, a total of 5 dietary treatments were mixed at Kansas State University O.H. Kruse Feed Technology Innovation Center and used in this experiment. Samples of dietary treatments and ingredients were collected during mixing.

A total of 10 barrows (initial BW: 34.1 ± 1.03 kg) were used in this experiment. Each pig was allotted to a replicated 5×5 Latin squared design with 5 dietary treatments and 5 periods of 10-d in each square. Therefore, there were 10 replicated pigs per treatment. Each period consisted of a 5-d adaptation period, followed by a 5-d collection period. Pigs had ad libitum access to water throughout the experiment and were fed 3.0 times the estimated requirement for maintenance energy (i.e., 197 kcal of ME per kg of BW^{0.60}) divided into two equal meals fed at 0800 and 1700 h.

During the collection period, fecal samples were collected using the marker-to-marker approach described by Adeola (2001). In short, a screen and a urine pan were installed below the floor of the metabolic crates, and an indigestible marker (ferric oxide) was included in the meal fed in the morning of d 6 to signal the start the collection and in the morning of d 10 to signal the stop of the collection. Fecal samples and orts were collected twice daily during the collection

period and each morning a bucket that contained 50 mL of 6 N HCl to preserve samples, was placed under the urine pan of each crate. Urine buckets were daily weighed, and the value was recorded prior to taking a subsample of 20% of the total volume of urine in the bucket. The remaining content of the bucket was emptied and HCl was reapplied to the buckets. Fecal, orts, and urine samples were stored at -20°C after collection.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was taken for chemical analyses. Fecal samples were dried in a forced-air oven at 55°C (Jacobs et al., 2011), finely ground, mixed, and subsampled. Fecal, urine, ingredient, and feed samples were analyzed for DM (Method 930.15; AOAC, 2019) and GE using bomb calorimetry (Parr 6200, Parr Instruments Company, Moline, IL, USA). Feed and ingredient samples were analyzed for N using the combustion method in a LECO analyzer (method 990.03; AOAC, 2019), and crude protein (**CP**) was then calculated as $N \times 6.25$. Additionally, feed and ingredient samples were analyzed for Ash (Method 942.05; AOAC, 2019), crude fiber (Method 978.10; AOAC, 2019), neutral detergent fiber (Van Soest et al., 1991), and acid detergent fiber (Method 973.18 [A-D]; AOAC, 2019). Ingredients samples were analyzed for crude fat by ether extraction (EE; Method 920.39 [A]; AOAC, 2019) and feed samples by acid hydrolysis (AEE; Method 954.02; AOAC, 2019).

Concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) in each diet were calculated using the following equations:

$$DE_{Diet}, kcal/kg = GE_{Diet} - GE_{Feces} ; ME_{Diet} = DE_{Diet} - GE_{Urine}$$

where GE_{Diet} , GE_{Feces} , and GE_{Urine} represent the concentration of energy (kcal/kg) in the feed, fecal samples, and urine samples respectively.

The ATTD of GE, DM, and N for each diet was calculated using the following equation:

$$ATTD_{Nutrient}, \% = [(Nutrient_{intake} - Nutrient_{feces}) / Nutrient_{intake}] \times 100$$

where $Nutrient_{intake}$ and $Nutrient_{feces}$ represent the daily intake and fecal output (g/d) of the nutrient of interest, respectively.

Lastly, N retention (Nr) was calculated using the following equation:

$$Nr, \% = [(N_{intake} - \{N_{feces} + N_{urine}\}) / N_{intake}] \times 100$$

where N_{intake} , N_{feces} , and N_{urine} represent the daily intake, fecal output, and urine output (g/d) of N, respectively.

Experimental data was analyzed using the GLIMIX procedure of SAS (SAS Institute Inc., Cary, NC) using pig as the experimental unit and pig and period as the random effects. Least square means were calculated for each independent variable and means were separated using the PDIF option. Treatment differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.15$.

RESULTS

Analyzed compositions of ingredients are presented in Table 2. Nutrient concentrations in corn and corn DDGS are in agreement with published data (NRC, 2012). However, concentrations of GE in SBM and wheat middlings appear to be greater than previously reported values (NRC, 2012). Similarly, the concentration of ADF in wheat middlings was greater than reported values (NRC, 2012). In contrast, the concentration of CP in SBM was lower than reported values (NRC, 2012). There was less than 1% variation between the calculated nutrient concentrations, and the analyzed concentrations of nutrients in dietary treatments (Table 3). However, the analyzed concentration of fat was greater than the calculated values. This could be partially explained by the analysis of fat in diets which was performed by EE, instead of AEE.

Throughout the experiment, all pigs remained healthy and minimal feed refusal was observed. Pigs fed the positive control had the greatest ($P < 0.05$; Table 4) ATTD of DM and GE. However, no differences were observed between the negative control diet and the diet containing MA, BA, or AN. Pigs fed the positive control diet had the greatest ($P < 0.05$) daily GE intake, and the pigs fed the AN diet had the lowest ($P < 0.05$) daily GE intake. However, no differences were observed between the control diet and the MA or BA diet. Pigs fed the positive control diet had the lowest ($P < 0.05$) GE in feces, but no differences were observed between the negative control diet and the diets containing MA, BA, or AN. No differences were observed in the concentration of GE in urine.

The concentration of DE (as-is or DM basis) was the greatest ($P < 0.05$) in the positive control diet and the lowest ($P < 0.05$) in the negative control diet. The diet containing MA had a greater ($P < 0.05$) DE concentration than the negative control diet, but lower ($P < 0.05$) than the positive control diet. No differences were observed between the BA or AN diets and the positive or negative control diets. However, a tendency ($P < 0.15$) was observed for the BA and AN diets to have greater DE concentration than the negative control diet. Similarly, the concentration of ME (as-is or DM basis) was the greatest ($P < 0.05$) in the positive control diet, but no differences were observed between the negative control diet and the MA, BA, or AN diets. However, a tendency ($P < 0.15$) was observed for the MA or BA diets to have greater ME concentrations compared with the negative control diet but no tendency was observed for a greater concentration of ME in the AN diet compared with the negative control.

No differences were observed in the daily feed intake or daily g of N retained (Table 5). The daily g of N intake was the greatest ($P < 0.05$) for the pigs fed the AN diet and the lowest ($P < 0.05$) for the pigs fed the positive control diet. However, no differences were observed between

the negative control diet and the MA or BA diets. The concentration of N in feces was the lowest ($P < 0.05$) in pigs fed the positive control diet, but no differences were observed between the negative control diet and the MA, BA, or AN diets. The concentration of N in urine was greater ($P < 0.05$) in pigs fed the AN diet than the positive control diet. However, no differences were observed between the AN diet and the negative control, MA, or BA diets, or between the positive control and the negative control, MA, or BA diets. The N retention as percentage of intake was lower ($P < 0.05$) in pigs fed the AN diet than the positive control diet. However, no differences were observed between positive control and the negative control, MA, or BA diets, or between the AN diet and the negative control, MA, or BA diets. The ATTD of N was the greatest ($P < 0.05$) for the positive control diet, but no differences were observed between the negative control diet and the MA, BA, or AN diets.

DISCUSSION

This experiment was designed to test the effect of three feed additives when added to high fiber diets fed to growing-finishing pigs. The observation of greater ATTD of DM and GE in the positive control diet compared with the negative control diet agrees with previously published data due to the inclusion of high fiber ingredients (Pedersen et al., 2007; Huang et al., 2013; Navarro et al., 2018; Li et al., 2021). The decrease in digestibility of DM and GE as the concentration of fiber in the diet increases is consistent among studies. However, the composition of the fiber might play a role in the degree of the decrease in ATTD of DM and GE. Data published by Lyu et al., (2018) suggest that the digestibility of fiber in dietary treatments increased with greater concentrations of soluble dietary fiber (**SDF**), due to the greater availability for fermentation, compared with insoluble dietary fiber (**IDF**). Similarly, Renteria-

Flores et al., (2008) showed that high concentrations of SDF in diets fed to sows, resulted in greater ATTD of GE compared with diets with high concentrations of IDF and a corn-SBM control diet. This indicates that as the capacity for fermentation of the pig increases, the composition of the fiber plays a bigger role in the ATTD of GE. Although the ingredients and diets for the present experiment were not analyzed for IDF and SDF, the ingredients used in the formulation of the diet (i.e. corn, soybean meal, distillers dried grains with solubles, and wheat middlings) have consistent high concentrations of IDF and low SDF (Abelilla and Stein, 2019; Navarro et al., 2018). Therefore, it is theorized that the negative control diet with and without the addition of feed additives, have a high concentration of IDF and a considerably low concentration of SDF. Additionally, due to the greater inclusion of corn in the positive control diet, it is likely that the concentrations of both IDF and SDF were lower compared with the other diets. Because across all diets, the concentrations of IDF were consistently and significantly greater than SDF, the composition of the fiber could partially explain the lack of differences in ATTD of GE between the negative control diet and the diets with added additives. Moreover, due to the greater concentration of fiber in the negative control diet with or without additives compared with the positive control diet, the fiber content might also contribute to the differences in ATTD of GE between the treatments.

The observation of greater concentrations of DE and ME in the positive control diet compared with the negative control diet due to their differences in fiber concentration is in agreement with previously published data (Lyu et al., 2018; Navarro et al., 2018). Pigs have the capacity for fermentation of fiber in the hindgut, which allows them to better utilize the energy present in high-fiber ingredients, as shown in research conducted by Renteria-Flores et al. (2008), where diets with a high concentration of fiber had a greater concentration of DE and ME

than the control diet. However, the capacity for fermentation of a fully developed sow is greater than a grower-finishing pig (Grieshop et al., 2001). This suggests that the pigs used in the present experiment have not reached the optimum capacity for fermentation to be able to better utilize the energy present in high-fiber ingredients. However, the concentrations of ME observed in this experiment across all treatments are greater than the calculated values using previously reported data (NRC, 2012), indicating a better utilization of the energy and the nutrients available in the feed, compared with the reported values used to estimate the nutrient and energy composition of the diets during formulation.

Research has suggested the capacity of benzoic acid to modulate the microbiome in the hindgut (Bühler et al., 2014) and the immune status in portions of the GIT (Outlaw et al., 2023) and in turn, the potential changes in the microbiome present in the GIT can affect intestinal functions and nutrient digestibility (Zhou et al., 2021). In an experiment conducted to determine the effect of benzoic acid and concentrations of fiber on growth performance and nutrient digestibility, Cho et al. (2015) observed no effect of the inclusion of benzoic acid in the ATTD of GE or protein. Similarly, Bühler et al. (2014) observed similar results with no effect of the supplementation of benzoic acid on diets with different concentrations of fiber on ATTD of GE. These findings are generally consistent with Outlaw et al. (2023), who reported no effect of protected benzoic acid on ATTD of GE in corn, SBM-based diets, though direct comparisons are limited by differences in dietary composition. Additionally, the observation of a tendency of the BA diet to have a greater DE and ME concentration compared with the negative control diet, might be explained by similar modes of actions (Kiarie et al., 2024).

Fatty acids such as MA, are known for their antimicrobial activity (Burdock and Carabin, 2007; Qi et al., 2023) which have been shown to positively influence the intestinal microbiota

and growth performance of nursery pigs (Choi et al., 2025). Under the conditions of this experiment, the supplementation with MA showed the capacity to increase the concentration of DE and ME compared with the negative control diet. This observation contrasts with the lack of differences observed on the ATTD of GE between the negative control diet and the MA diet. However, the numerical difference in the ATTD of GE in the negative control diet being 0.52% lower than the MA diet, could explain the observed differences in DE concentrations. The observation of increased DE and ME in the MA diet might be explained by the influence of MA on the microbiome in the GIT, which might have led to improvements in intestinal health and proliferation of bacteria that can benefit from the fiber content of the diet, which in turn, can also positively influence the ATTD of GE.

Previously published data Sharma et al. (2022) suggest that the use of cultures of *AN* used as a probiotic, successfully reduced the pathogenic bacterial load in the GIT of laying hens (Sharma et al., 2022). Therefore, the tendency observed for increasing concentrations of DE or ME by AN can be due to antimicrobial activity, capacity to promote gut health, or the modulation of immune status in the GIT. However, extrapolation of results across species might not be accurate and to our knowledge, limited research has been conducted using AN as a feed additive in diets fed to pigs of different ages, therefore, more research is warranted.

Because the dietary treatments for the present experiment were not formulated to be isoproteic, the difference in N intake among treatments was expected. Similar to the response in ATTD of GE, the ATTD of N being greater in the positive control diet compared with the rest of the dietary treatments is in agreement with previously published data (Huang et al., 2013) with the values decreasing as the concentration of fiber increases. However, it has been reported that the inclusion of high concentrations of fiber may increase apparent ileal digestibility of CP and

amino acids (AA) due to an increase in viscosity, which in turn decreases passage rate (Li et al., 2021) giving more time for enzymes to break down nutrients. Because the apparent ileal digestibility of CP was not measured in the present experiment, it is not possible to compare or draw conclusions without the possible influence of the fermentation of undigested CP in the hindgut, therefore the ATTD of N should not be considered as a reliable estimation of the amount of protein that the animal is benefiting from the diet. In addition to the difference in fiber, the concentration of CP in the diet might have also influenced some of the results observed in the N balance of the pigs. Different results have been reported in the literature about the effect of increasing CP concentrations in diets fed to growing pigs (Noblet et al., 1987; Kerr et al., 2003; Zhao et al., 2018) likely due to the differences in the use of crystalline AA or energy levels. However, the observation of decrease N intake, fecal N, and urine N when lower CP diets are fed, is in agreement with the results observed in the present experiment. Because all diets were formulated to meet or exceed the requirements for SID AA and the pigs were fed a fixed amount of energy, the observation of no differences in N retention is in agreement with previously published data (Valaja and Silijander-Rasi, 1998). However, the supplementation of feed additives in the present experiment did not affect the N retention of the pigs fed the dietary treatments.

In conclusion, the supplementation with MA, BA, and cultures of AN has the potential to positively influence the concentrations of DE and ME in high fiber diets fed to growing pigs. However, to the knowledge of the authors no research has been conducted previously to determine optimal levels of inclusion or the possibility of using the additives together as a strategy to improve nutrient utilization, therefore, more research in this area is warranted.

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Table 3.1. Analyzed composition of feed ingredients (as-is basis)

Item	Corn	Soybean meal	Corn DDGS ¹	Wheat Middlings
Gross energy, kcal/kg	4,263	4,719	4,879	4,447
Dry matter, %	87.39	88.75	88.05	88.21
Crude protein, %	7.98	44.11	28.16	15.83
AEE, % ²	3.75	3.90	7.46	2.96
Ash, %	1.17	6.56	5.10	5.45
Crude fiber, %	1.62	4.58	7.92	7.98
NDF, % ³	8.62	9.94	25.36	36.96
ADF, % ⁴	2.67	5.81	12.27	11.00

¹DDGS = Distillers dried grains with solubles.

²AEE = Acid-hydrolysis ether extract.

³NDF = Neutral detergent fiber.

⁴ADF = Acid detergent fiber.

Table 3.2. Ingredient and calculated nutrient composition of experimental diets containing myristic acid, protected benzoic acid, and *Aspergillus niger* (as-fed basis)¹

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	<i>Aspergillus niger</i>
Ingredient, %					
Corn	36.74	78.24	36.67	36.64	36.69
Soybean meal	11.99	18.98	11.99	11.99	11.99
Corn distillers dried grains w/ s.	30.00	-	30.00	30.00	30.00
Wheat middlings	19.00	-	19.00	19.00	19.00
Calcium carbonate	1.00	0.77	1.00	1.00	1.00
Monocalcium phosphate, 21% P	-	0.55	-	-	-
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Trace mineral premix ²	0.15	0.15	0.15	0.15	0.15
Vitamin premix ³	0.25	0.25	0.25	0.25	0.25
L-Lysine-HCl	0.40	0.40	0.40	0.40	0.40
DL-Methionine	-	0.05	-	-	-
L-Threonine	0.02	0.10	0.02	0.02	0.02
L-Tryptophan	0.02	0.03	0.02	0.02	0.02
L-Valine	-	0.05	-	-	-
Phytase ⁴	0.03	0.03	0.03	0.03	0.03
Myristic acid	-	-	0.07	-	-
Protected benzoic acid	-	-	-	0.10	-
<i>Aspergillus niger</i>	-	-	-	-	0.05
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
Metabolizable energy, kcal/kg	3,245	3,310	3,245	3,245	3,245
Crude protein, %	20.4	16.1	20.4	20.4	20.4
Crude fiber, %	4.8	2.3	4.8	4.8	4.8
Fat, %	4.7	3.0	4.7	4.7	4.7
SID AA, %					
Lysine	0.96	0.96	0.96	0.96	0.96
Isoleucine:lysine	66	56	66	66	66
Leucine:lysine	173	131	173	173	173
Methionine:lysine	32	30	32	32	32
Methionine and cysteine:lysine	60	54	60	60	60
Threonine:lysine	60	60	60	60	60
Tryptophan:lysine	18.9	18.8	18.9	18.9	18.9
Valine:lysine	80	69	80	80	80
Histidine:lysine	47	39	47	47	47
Total lysine, %	1.16	1.07	1.16	1.16	1.16
Ca, %	0.55	0.55	0.55	0.55	0.55
STTD P, %	0.41	0.36	0.41	0.41	0.41

¹Myristic acid, protected benzoic acid, and *aspergillus niger* provided by Insighter Biotech, Guangzhou, China.

² Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate.

³ Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D3; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.

⁴ RONOZYME HiPhos 2700 (DSM, Parsippany, NJ, USA) contained 2,700 FYT/kg. At 0.03% inclusion, complete diets were expected to contain 1,000 FYT/kg with an estimated release of 0.13% STTD P.

Table 3.3. Analyzed composition of experimental diets (as-fed basis)

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	<i>Aspergillus niger</i>
Gross energy, kcal/kg	4,448	4,187	4,517	4,512	4,500
Dry matter, %	87.80	87.68	87.59	87.85	87.40
Crude protein, %	19.80	15.21	19.52	20.32	21.39
Crude fat, %	2.89	1.84	2.56	2.50	5.26
Ash, %	5.07	3.67	5.29	5.23	5.39
Crude fiber, %	5.11	2.19	5.04	5.26	5.31
NDF, % ¹	16.70	7.49	16.80	17.80	16.86
ADF, % ²	7.09	3.43	7.10	7.18	7.09

¹NDF = Neutral detergent fiber.

²ADF = Acid detergent fiber.

Table 3.4. Digestible energy (DE), metabolizable energy (ME), and apparent total tract digestibility (ATTD) of gross energy (GE) in experimental diets¹

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	<i>Aspergillus niger</i>	SEM	<i>P</i> <
ATTD of DM, %	80.8 ^{b,x}	91.6 ^{a,w}	80.6 ^{b,x}	80.8 ^{b,x}	80.7 ^{b,x}	0.41	0.001
ATTD of GE, %	80.0 ^{b,x}	90.3 ^{a,w}	80.5 ^{b,x}	80.4 ^{b,x}	80.4 ^{b,x}	0.50	0.001
GE intake, kcal/d	8,434 ^{b,y}	8,032 ^{c,z}	8,738 ^{ab,wx}	8,588 ^{ab,xy}	8,768 ^{a,w}	781.5	0.001
GE in feces, kcal/d	1,689 ^{a,w}	786 ^{b,x}	1,684 ^{a,w}	1,680 ^{a,w}	1,717 ^{a,w}	138.9	0.001
GE in urine, kcal/d	369.0	349.6	413.7	398.9	450.9	67.79	0.231
DE, kcal/kg DM ²	4,054 ^{c,y}	4,310 ^{a,w}	4,153 ^{b,x}	4,130 ^{bc,x}	4,140 ^{bc,x}	25.5	0.001
ME, kcal/kg DM	3,828 ^{b,y}	4,113 ^{a,w}	3,911 ^{b,x}	3,896 ^{b,x}	3,878 ^{b,xy}	36.2	0.001

^{a-c}Means within a row lacking a common superscript letter are different ($P < 0.05$).

^{w-z}Means within a row lacking a common superscript letter tend to be different ($P < 0.15$).

¹N = 10.

²DM= Dry matter.

Table 3.5. Nitrogen balance of pigs fed experimental diets

Item	Negative Control	Positive Control	Myristic acid	Benzoic acid	<i>Aspergillus niger</i>	SEM	<i>P</i> <
Feed intake, g/d	1,896	1,918	1,934	1,904	1,948	176.2	0.232
N intake, g/d ¹	60.59 ^b	46.33 ^c	60.41 ^b	61.89 ^b	66.68 ^a	5.376	0.001
Fecal N, g/d ²	9.33 ^a	4.67 ^b	9.44 ^a	9.88 ^a	9.64 ^a	0.651	0.001
Urinary N, g/d ³	13.95 ^{ab}	8.24 ^b	12.89 ^{ab}	13.55 ^{ab}	17.53 ^a	2.216	0.014
N retained, g/d ⁴	37.30	33.43	38.08	38.46	39.51	4.106	0.195
N retention, % of intake ⁵	61.1 ^{ab}	71.9 ^a	62.3 ^{ab}	62.2 ^{ab}	59.6 ^b	3.07	0.036
ATTD of N, %	84.6 ^b	89.9 ^a	84.2 ^b	83.9 ^b	85.4 ^b	0.64	0.001

^{a-c}Means within a row lacking a common superscript letter are different ($P < 0.05$).

¹N intake, g/d = feed intake during collection period, g/d × (feed N content, % ÷ 100)

²Fecal N, g/d = feces weight, g/d, air-dry basis × (feces N content, % ÷ 100)

³Urinary N, g/d = urine weight, g/d × (urine N content, % ÷ 100)

⁴N retained, g/d = N intake, g/d – Fecal N, g/d – Urinary N, g/d

⁵N retention, % of intake = (N retained g/d ÷ N intake, g/d) × 100

Chapter 4 - Effect of the inclusion of exogenous protease on the ileal and total tract digestibility of energy and nutrients, concentrations of digestible and metabolizable energy, and N retention in commercial diets fed to swine

ABSTRACT

Two experiments were conducted to determine the effect of increasing inclusion of protease on nutrient and energy digestibility in diets fed to growing pigs. In Exp. 1, a corn soybean meal, distillers dried grain with solubles, and wheat middlings-based control diet was formulated. Dietary treatments consisted of the control diet and the control diet with protease added to achieve 4,000, 8,000, and 12,000 U/kg of feed. Twelve barrows with an initial average body weight of 36.1 ± 1.62 kg were allotted to a triplicated 4×4 Latin square design with 4 treatments and 4 experiment periods and a 7-d washout period after each experiment period. Animals were individually housed in metabolism crates and each period consisted of 5 days of collection and 5 days of collection of feces and urine. In Exp. 2, the same control diet was utilized. Treatments consisted of the control diet and the control diet with protease added to achieve 4,000 and 8,000 U/kg of feed. A N-free diet was also formulated for a total of 4 dietary treatments. Titanium dioxide was added to all diets as an indigestible marker. Twelve pigs with an initial average body weight of 32.6 ± 1.81 kg were surgically equipped with a t-canula in the distal ileum and allotted to a 4×5 Latin square designed with 4 dietary treatments and 5 experimental periods. Each period consisted of 5 days of adaptation and 2 days of collection followed by a 7-day wash-out period. Pigs were individually housed in pens equipped with smooth side walls. Inclusion of protease resulted in an increase (quadratic; $P < 0.05$) in apparent total tract digestibility (ATTD)

of dry matter (**DM**), neutral detergent fiber (**NDF**), and organic matter (**OM**) as the concentration of protease in the diet increased. Increasing the concentrations of protease increased (quadratic; $P < 0.05$) the apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of crude protein (**CP**) and most amino acids (**AA**) excluding Leu and Ala, with the greatest value of digestibility observed with protease added at 4,000 U/kg of feed. In conclusion, the ATTD of NDF and SID of CP and AA increased when protease was included at 4,000 U/kg of feed; however, the ATTD of Ca, P, gross energy (**GE**), acid detergent fiber (**ADF**), and fat was unaffected due to the inclusion of protease.

INTRODUCTION

Supplementation of exogenous enzymes can contribute to improvements in nutrient utilization (Adeola and Cowieson, 2011). Similar to the function of endogenous enzymes, exogenous enzymes have specific actions that are determined by their structure, which allows for the interaction with their substrate to break down the bonds between nutrients present in the feed (Ferket, 1993; Bhutia and Ganapathy, 2018). To improve protein and amino acids (**AA**) utilization in monogastric diets, proteases have been used to support the breakdown of peptide bonds, or to neutralize antinutritional factors that decrease nutrient digestibility (Chen et al., 2017; Lee et al., 2018; Duarte et al., 2019; Chen et al., 2020; Park et al., 2020; Aderibigbe et al., 2021). Due to the differences between types of proteases that vary in origin, optimum pH for action, or substrate, the results of using proteases to increase nutrient availability are variable and usually depend on the dietary formulation including ingredient composition and formulated nutrient profile (Cowieson and Roos, 2014; Lee et al., 2018).

A key factor to take into consideration when supplementing diets with protease is the level of inclusion. Previously published data suggests that greater inclusions of protease resulted in a plateau or decreasing effect on apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of crude protein (**CP**) and AA (Zhu et al., 2022). This suggests that supplementation with protease needs to be an accurate exercise in order to obtain the most benefit and possibly avoid detrimental effects. Additionally, because of the presence of complexes between proteins and other nutrients, protease supplementation might result in an indirect positive effect on the digestibility of other nutrients (Duodu et al., 2003). Which raises the question about the optimum inclusion of protease to not only increase AID and SID of CP and AA, but also to potentially support the digestibility of other nutrients.

However, due to the specificity of each source of protease and the limited research using increasing concentrations of protease in the diet, research is warranted to further understand the effect of proteases and their level of inclusion on digestibility of energy and nutrients. Therefore, the objective of this experiment is to determine the effect of increasing inclusions of protease on the apparent total tract digestibility (**ATTD**), AID, and SID of nutrients, nitrogen retention, and concentration of digestible energy (**DE**) and metabolizable energy (**ME**) in a commercial diet fed to growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Kansas State University approved the protocol for two experiments. Barrows (Line 241 × 600; DNA Genetics, Columbus, NE, USA) were used in both experiments.

Diet preparation and feeding

A corn, soybean meal, distillers dried grains with solubles and wheat middlings-based diet was formulated to meet or exceed the recommended nutrient requirements for pigs between 35 and 100 kg (NRC, 2012). Phytase and xylanase were added to the diet and accounted for using a nutrient release matrix adjustment. The diets were mixed at the O.H. Kruse Feed Technology and Innovation Center in Manhattan, KS, USA, following standard procedures and using a twin-shaft counterpoise mixer (Hayes and Stolz, model TRDB63-0152, Fort Worth, TX, USA). After mixing, the feed was divided into 7 aliquots of 500 kg. One aliquot remained unchanged to be used as a control diet. Three more dietary treatments were formulated by adding protease (Aextra PRO; Danisco Animal Nutrition and Health [IFF], Cedar Rapids, IA, USA) at 4,000, 8,000, and 12,000 U/kg of feed to each of the remaining 3 aliquots. Titanium dioxide was added to the remaining 3 aliquots, keeping one of them as a control diet, and the remaining 2 aliquots were used to mixed dietary treatments by adding 4,000 and 8,000 U of protease per kg of feed. Therefore 4 dietary treatments without titanium dioxide were used to determine the total track digestibility of nutrients and energy and 3 dietary treatments with titanium dioxide were used to determine the AID and SID of CP and AA. Lastly, a N-free diet was formulated, and 430 kg were mixed using a paddle mixer (Davis, model 2014197-SS-S1, Bonner Springs, KS, USA) to allow for the calculation of the endogenous losses of AA and CP. After mixing, ingredients and diet samples were collected and properly stored for analysis.

Experiment 1: Energy and Nutrients

A total of 12 barrows (average initial body weight: 36.07 ± 1.57 kg) were individually housed in metabolism crates and allotted to a 4×4 triplicated Latin squared design with 4 treatments and 4 periods of 12 days, for a total of 12 replicate pigs per dietary treatment. Dietary treatments used in this experiment included the control diet, and the diets containing 4,000,

8,000, and 12,000 U/kg of feed of protease. Each experimental period consisted of 5 days of adaptation, followed by 5 days of collection of feces and urine. Pigs were given a washout period in between experimental periods where they were fed a common diet that with no protease inclusion. Pigs were fed at 3.0 times the estimated maintenance requirement for ME (i.e. 197 kcal/kg body weight^{0.60}; NRC, 2012) and had ad libitum access to water throughout the experiment. The daily allowance of feed was divided into 2 equal meals fed at 0700 and 1600 h. Fecal and urine samples were collected using the marker-to-marker approach (Adeola, 2001). In short, an indigestible marker (iron oxide) was added to the meal fed in the morning of day 6 to signal the start of the collection of fecal samples once the marker was spotted in the feces. The marker was also added to the meal fed in the morning of day 11 to signal the end of the collection of fecal samples once the marker was spotted again in the feces. During the collection, a urine pan and a screen were installed underneath the crates to allow the total but separate collection of fecal and urine samples. Additionally, a bucket with 50 mL of 6 N HCl to prevent volatilization of N, was placed underneath the urine pan under each crate and covered with cheesecloth. Feces and orts were collected twice daily, and urine buckets were weighed daily and 10% of the content was subsampled and stored. After urine subsampling was done, the remaining content of the buckets was discarded and HCl was added again to the bucket. All samples were stored at -20°C.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet. A subsample was collected for chemical analysis. Fecal samples were dried in a forced-air oven at 55°C (Jacobs et al., 2011), finely ground, and subsampled. Urine samples were filtered and lyophilized in preparation for analysis. Feed, ingredient, urine, and fecal samples were analyzed for gross energy (GE) using bomb calorimetry (Parr 6200, Parr Instruments

Company, Moline, IL, USA) and N using the combustion method in a LECO analyzer (method 990.03; AOAC, 2019). Crude protein was calculated as $N \times 6.25$. Ingredient, diet, and fecal samples were analyzed for AEE (method 954.02; AOAC, 2019), calcium and phosphorus (method 2011.14; AOAC, 2019), acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**) using an ANKOM fiber analyzer (ANKOM technology, Macedon, NY, USA; method 14 and 15), and DM (method 930.15; AOAC, 2019). Ingredient and diet samples were analyzed for ash (method 942.05; AOAC, 2019), crude fiber (method 978.10; AOAC, 2019), and AA (method 982.30 E [a, b, c]; AOAC, 2019). Additionally, ingredients and diet samples were analyzed for particle size according to the dry sieving methodology (ASAE S319.2; ASAE, 1995) using a 13-sieve stack and a dispersing agent (silicon dioxide, model SSA-58, Gilson, Lewis Center, OH, USA). Diets samples were analyzed (Eurofins Scientific, Inc. Des Moines, IA, USA) for protease, phytase, and xylanase activity. Organic matter (**OM**) was calculated as DM – ash for diets and fecal samples. Following analysis of samples, ATTD of GE, DM, Ca, P, NDF, ADF, OM, and fat was calculated and DE and ME were calculated for each diet (Adeola, 2001).

Experiment 2: Amino Acid Digestibility

A total of 15 barrows (average initial body weight: 32.6 ± 1.81 kg) were surgically equipped with a T-cannula in the distal ileum, following the procedures of Stein et al. (1998) and housed individually in pens (1.2×1.2 m) at KSU Swine Teaching and Research Center. Pigs were allotted to a replicated 4×5 Latin square design with four dietary treatments and four periods of 7 d. Each experimental period consisted of 5 days of adaptation to the diet (Adeola, 2001), and a collection period of 2 d where samples were collected for 8 h each day. Digesta was collected by removing the plastic lid of the cannulas and attaching a plastic bag to the end of the barrel and then bags were changed every 30 minutes or when the bag was full, whichever

happened first. Samples were immediately stored at -20°C. Dietary treatments used in this experiment contained titanium dioxide and consisted of a N-free diet, a control diet, and diets supplemented with 4,000 and 8,000 U/kg of feed of protease. Pigs were fed at 3.0 times the estimated maintenance requirement for ME (i.e. 197 kcal/kg body weight^{0.60}; NRC, 2012) divided into 2 equal meals provided at 0800 and 1400 h and had ad libitum access to water throughout the experiment. Pigs were given a washout period in between experimental periods where they were fed a common diet that with no protease inclusion.

At the conclusion of the experiment, digesta samples were thawed, subsampled, lyophilized, and ground in preparation for analysis. Ingredients, diets, and ileal samples were analyzed for titanium following the method described by Myers et al. (2004) and for DM, AEE, AA, and nitrogen as previously described. Crude protein was calculated as nitrogen $\times$ 6.25. Ingredients and diet samples were analyzed for GE, crude fiber, calcium and phosphorus, ADF and NDF, ash, and particle size as previously described. Basal endogenous losses of individual AA and CP, AID of DM, CP, AA, and fat, and SID of CP and AA were calculated for dietary treatments as described by Stein et al. (2007).

Statistical Analysis

Data were analyzed using the GLIMIX procedure of SAS (SAS Instituto Inc., Cary, NC, USA) using pig as the experimental unit. Diet was considered the fixed effect, and pig and period were random effects. Contrast statements were used to test linear and quadratic effects of the increasing inclusion of protease. Observations with a studentized residual outside of ± 3 were considered outliers and removed. Results were considered significant at $P \leq 0.05$ and a tendency at $> 0.05 P \leq 0.10$.

RESULTS

Analyzed concentrations of GE and nutrients in feed ingredients (Table 1) are within expected ranges (NRC, 2012), except for the GE and crude fiber of wheat middlings, which are marginally greater than expected, and the concentration of trypsin inhibitor units (TIU) in soybean meal, that was considerably greater than expected values (Lagos and Stein, 2017; Gaffield et al., 2024). Furthermore, small variations were observed between calculated composition (Table 2) and analyzed composition (Table 3) of diets for Exp. 1. Similarly, small variations were observed between calculated composition (Table 4) and analyzed composition (Table 5) of diets for Exp. 2. Additionally, small to no variation was observed in the analyzed compositions among Exp. 1 and Exp. 2 diets, except for the N-free diet.

No differences were observed on the concentration of DE or ATTD of GE, Ca, P, ADF, and Fat (Table 6). However, the ATTD of DM and NDF increased (quadratic, $P < 0.05$) when including 4,000 or 8,000 U/kg of protease and decreased back to similar to that of the control when 12,000 U/kg of protease was added. Additionally, there was a tendency (quadratic, $P < 0.10$) for the concentration of ME and the ATTD of OM to increase when up to 8,000 U/kg of protease was added and decreased back to similar to the control when 12,000 U/kg of protease was added. No evidence of differences were observed for N retention or ATTD of N when increasing protease in the diet (Table 7).

There was no evidence of difference in the AID of DM and AEE when increasing concentrations of protease in the diet (Table 8). The AID of CP and all AA increased (quadratic, $P < 0.05$) with protease inclusion up to 4,000 U/kg, but 8,000 U/kg resulted in similar values compared with the control, except for Leu and Ala. Similarly, the SID of CP and all AA increased (quadratic, $P < 0.05$) with protease inclusion up to 4,000 U/kg, but 8,000 U/kg resulted

in similar values compared with the control, except for Leu and Ala. For both AID and SID of Leu, no significant differences were observed, whereas AID and SID Ala increased numerically at 4,000 U/kg of protease, and decreased at 8,000 U/kg.

DISCUSSION

The use of exogenous proteases in diets for pigs has become very common, especially when incorporated into enzyme cocktails that aim to improve nutrient utilization (Cowieson and Adeola, 2011; Lee et al., 2018). Most modern proteases that are available commercially have a bacterial origin and an optimum pH of activity ranging from neutral to alkali, including the exogenous protease used in the work herein presented (Danisco Animal Nutrition & Health [IFF], 2025). However, due to the diversity of combinations of AA bound by peptide bonds, the differences in cleaving sites of proteases can lead to differences in their effect on growth performance and digestibility of nutrients (Lee et al., 2018). Nevertheless, an increase in the AID or SID of CP and AA has been observed in most studies supplementing diets with exogenous proteases (Cowieson and Roos, 2014). It is noteworthy that because of the lack of a standard unit measurement for protease activity, observations of positive effects on growth performance and digestibility of nutrients might be difficult to compare (Lee et al., 2018) and is usually the manufacturer of the exogenous enzyme that gives a recommendation on their optimal inclusion.

The observed increase in ATTD of DM when protease was included at 4,000 and 8,000 U/kg of feed is in agreement with previous data (Park et al., 2020; Zhang et al., 2024) where diets supplemented with protease showed a greater ATTD of DM compared with control diets with no inclusion of protease. The effect observed on ATTD of DM may be explained by the increased ATTD of NDF, which showed similar results. The main storage of protein in grains is

in the form of prolamins (e.g. zein [corn], kafirin [sorghum]) which are located in the endosperm of the grains. In ruminants fed corn, the capacity for the bacteria to access the starch and fiber in the grain for fermentation, depends on the proteolysis of the hydrophobic prolamin zein (Hoffman and Shaver, 2011). Therefore, it is possible that the activity of the supplemented protease could contribute to the breakdown of the content of prolamins in the experimental diets, which in turn could have increased the ATTD of NDF. However, lack of differences by the supplementation of protease on the ATTD of DM have also been observed in previous data (Zuo et al., 2015). Additionally, previous data also indicates that the use of protease in an enzyme cocktail did not have an effect in the ATTD of DM of a corn-soybean meal diet (Kim et al., 2017). The discrepancies in the observed effects of the supplementation of proteases could be explained by the inherent differences between their origin, optimum pH for action, or even site of action (Razzaq et al., 2019). However, because most proteases produced commercially have a bacterial origin, which leads to similar optimum pH of action, the cleaving site in the proteins is likely a possible reason for the differences in responses.

Previous data has reported a positive effect of the inclusion of protease on nitrogen retention in diets of monogastric (Xu et al., 2017). However, in the present experiment, the diets were formulated to meet 100% of the requirements of AA of the pigs while considering a matrix of nutrient release. Therefore, the lack of differences in nitrogen retention can be explained by the concentrations of SID AA provided to the pigs, regardless of the increase in the digestibility of the AA in the diet. Although numerical differences were observed in the ATTD of N, the differences were too small to be significant, which is in agreement with previous data (Ling et al., 2012; Zuo et al., 2015; Ma et al., 2020; Park et al., 2020). However, supplementing protease in diets that have a lower inherent ATTD of N has been shown to be more effective (Munezero et

al., 2022; Zhang et al., 2024). Therefore, a possible explanation for the lack of differences observed in the ATTD of N in the present experiment could be due to an inherent high ATTD of N in the diet, which limited the effect of protease. Additionally, because the determination of digestibility of CP in the total tract can be confounded by the fermentation of AA in the hindgut that result in further breakdown of some AA but that are not available for the pig (Nyachoti et al., 1996), the results of SID of CP presented in this experiment should be considered more accurate.

The increase in AID and SID of CP and all AA with the protease supplementation of 4,000 U/kg of feed is in agreement with published data (Cowieson and Roos, 2014; Lee et al., 2018). Similar to the effects on ATTD, the positive results observed in AID and SID of CP and AA from the supplementation of protease in monogastric diets seem to be influenced by the inherent digestibility of the diets that are being supplemented (Cowieson and Roos, 2014). Previous research indicates that diets with lower inherent digestibility of CP and AA showed a greater response to the supplementation of exogenous proteases (Lee et al., 2018) estimating that diets with 10% less inherent digestibility of CP and AA nearly doubled the improvement in AID and SID of CP and AA following protease supplementation.

The indirect effect of other exogenous enzymes, such as phytase and xylanase on the AID and SID of CP and AA has also been described in previous literature (Fan et al., 2005; Cowieson and Roos, 2014). It has been suggested that this observation might be the result of xylanase and phytase ameliorating the possible negative effects of their substrates on AID and SID of CP and AA (Selle et al., 2009). However, there is no evidence of a synergetic effect by the inclusion of enzyme cocktails on the AID or SID of CP and AA (Lee et al., 2018) but rather a decrease in the perceived effect of the inclusion of protease. Nevertheless, it has been suggested that the

decrease in the effect of protease when used in enzyme cocktails might be a result of the overlap of the effect of inherent digestibility of the diet (Cowieson and Roos, 2014). Similarly to protease, inherent greater digestibility of the diet might decrease the magnitude of the effect of other exogenous enzymes on nutrient digestibility. Therefore, the use of protease in combination with other exogenous enzymes might render its effect negligible. However, it is noteworthy that this observation is not due to shared substrates among enzymes.

In the present experiment, protease supplementation at 4,000 U/kg increased the AID and SID of CP and AA by 1.12 to 5.41 %. In contrast, previously published data indicates an increase of AID of AA 1.95 to 4.75% in diets supplemented only with protease, whereas diets supplemented with proteases and other exogenous enzyme showed no increase in AID of AA due to the supplementation of protease (Lee et al., 2018). The observed increase in AID and SID in the present experiment indicates that the supplementation with protease in combination with phytase and xylanase did not have a diminishing effect on the effect of the supplementation of protease. It is theorized that the composition of the diet might partially explain the positive reaction to protease supplementation regardless of the inclusion of other exogenous enzymes. High-fiber ingredients offered a greater amount of substrate for xylanase and possible for phytase to increase digestibility of fiber and phosphorus respectively while also supporting the increase in AID and SID of CP and AA. Another possible explanation is the high concentration of TIU in soybean meal used in the work herein presented. Because AID of AA can decrease in the presence of high TIU concentrations (Herkelman et al., 1992), it is possible that the supplementation with protease resulted in the deactivation of this antinutritional factor and therefore, increasing the AID and SID of CP and AA more than expected for a diet containing other exogenous enzymes.

The decrease on the AID and SID of CP and AA due to the protease supplementation at 8,000 U/kg is in agreement with previous data (Zhu et al., 2022). The authors indicated that supplementation of protease needs to be carefully considered due to possible reduction in digestibility of nutrients and consequently, growth performance of animals. It has been theorized that the tight regulation of digestion and absorption of nutrients, which includes the synthesis and secretion of enzymes, can be affected by high doses of exogenous enzymes (Yuan et al., 2015; Walk et al., 2019). However, because enzyme activity was not measured in this experiment, it is not possible to confirm that the activity of endogenous enzymes in the gastro intestinal tract was reduced as the inclusion of protease increased, or if it differs between pigs fed the control diet and the diet containing 4,000 U/kg.

In conclusion, the inclusion of protease used in this study increased the digestibility of NDF, CP, and most AA at an inclusion of 4,000 U/kg of feed. However, greater inclusion decreased the digestibility of nutrients in high-fiber diets fed to growing pigs. Therefore, the supplementation of protease needs to be carefully considered to take full advantage of its positive effects on nutrient digestion and absorption.

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Table 4.1. Analyzed composition of feed ingredients for Exp. 1 and 2(as-fed basis)

Item,	Corn	SBM	Corn DDGS	Wheat middlings
Dgw, μm ¹	523	958	391	465
Sgw ²	3.39	2.01	1.84	2.46
Gross energy, kcal/kg	4,085	4,693	4,690	4,413
Dry matter, %	88.14	88.61	89.47	88.45
Crude protein, %	7.93	48.09	28.54	16.35
Fat, %	3.06	1.49	4.51	3.73
Ash, %	1.37	6.41	4.67	5.49
Crude fiber, %	1.75	3.42	7.66	8.99
Trypsin, TIU/mg	-	13.8	-	-
Indispensable AA, %				
Arginine	0.36	3.55	1.17	1.18
Histidine	0.23	1.35	0.76	0.49
Isoleucine	0.30	2.36	1.10	0.60
Leucine	0.91	3.70	3.25	1.09
Lysine	0.31	3.13	0.80	0.75
Methionine	0.17	0.69	0.49	0.27
Phenylalanine	0.38	2.49	1.43	0.71
Threonine	0.29	1.85	1.17	0.58
Tryptophan	0.06	0.73	0.18	0.20
Valine	0.40	2.44	1.44	0.85
Dispensable AA, %				
Alanine	0.58	2.06	1.91	0.85
Aspartic acid	0.61	5.52	1.58	1.27
Cysteine	0.17	0.73	0.53	0.39
Glutamic acid	1.46	8.84	4.05	3.36
Glycine	0.32	2.02	1.03	0.94
Serine	0.35	1.99	1.16	0.64
Tyrosine	0.18	1.73	1.02	0.42

¹Dgw = Geometric mean diameter.²Sgw = Geometric standard deviation.

Table 4.2. Ingredient composition of dietary treatments for Exp. 1

Item, %	Basal diet	Protease, U/kg		
		4,000	8,000	12,000
Corn	40.03	40.00	39.98	39.95
Soybean meal	8.89	8.89	8.89	8.89
Distillers dried grains with solubles	30.00	30.00	30.00	30.00
Wheat middlings	19.00	19.00	19.00	19.00
Calcium carbonate	1.00	1.00	1.00	1.00
Sodium chloride	0.40	0.40	0.40	0.40
L-Lysine-HCl	0.20	0.20	0.20	0.20
Trace mineral premix ¹	0.15	0.15	0.15	0.15
Vitamin premix ²	0.25	0.25	0.25	0.25
Phytase ³	0.01	0.01	0.01	0.01
Xylanase ⁴	0.03	0.03	0.03	0.03
Protease ⁵	-	0.03	0.05	0.08
Total	100.00	100.00	100.00	100.00
Calculated composition				
Standardized ileal digestible AA ⁶ , %				
Lysine	0.80	0.80	0.80	0.80
Isoleucine:lysine	80	80	80	80
Leucine:lysine	204	204	204	204
Methionine:lysine	36	36	36	36
Methionine and cysteine:lysine	74	74	74	74
Threonine:lysine	74	74	74	74
Tryptophan:lysine	20	20	20	20
Valine:lysine	99	99	99	99
Histidine:lysine	56	56	56	56
Total lysine, %	0.94	0.94	0.94	0.94
Metabolizable energy, kcal/kg	3,236	3,235	3,235	3,234
Crude protein, %	20.00	20.00	20.00	20.00
Ca, %	0.71	0.71	0.71	0.71
STTD P, %	0.46	0.46	0.46	0.46
Phytase, FTU/kg	750	750	750	750
Xylanase, U/kg	4,000	4,000	4,000	4,000
Protease, U/kg	0	4,000	8,000	12,000

¹Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate.

²Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D₃; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B₁₂; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.

³Axtra®Phy Gold 5 T, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

⁴Danisco®Xylanase 15,000 G, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

⁵Axtra®Pro 302 TPT, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

⁶AA = amino acids.

Table 4.3. Analyzed composition of dietary treatments for Exp. 1 (as-fed basis)

Item,	Basal diet	Protease, U/kg		
		4,000	8,000	12,000
Dgw, μm ¹	513	584	575	589
Sgw ²	2.67	2.56	2.55	2.67
Gross energy, kcal/kg	4,091	4,078	4,074	4,080
Dry matter, %	89.75	88.42	89.03	89.17
Crude protein, %	20.68	20.69	21.59	20.89
AEE, % ³	6.37	6.72	6.68	6.95
Ash, %	5.70	5.27	5.18	5.04
Ca, %	0.62	0.64	0.64	0.64
P, %	0.65	0.68	0.66	0.68
NDF, % ⁴	19.90	18.30	19.40	18.50
ADF, % ⁵	6.30	6.00	5.60	6.10
Phytase, FTU/kg	1,740	1,720	1,480	1,640
Xylanase, U/kg	5,770	7,240	5,750	5,610
Protease, U/kg	ND ⁶	4,190	11,500	14,600

¹Dgw = Geometric mean diameter.²Sgw = Geometric standard deviation.³AEE = Acid hydrolyzed ether extract.⁴NDF = Neutral detergent fiber.⁵ADF = Acid detergent fiber.⁶ND = Non detectable.

Table 4.4. Ingredient composition of dietary treatments for Exp. 2

Item, %	Basal diet	Protease, U/kg		N-free diet
		4,000	8,000	
Corn	39.53	39.50	39.48	-
Soybean meal	8.89	8.89	8.89	-
Distillers dried grains with solubles	30.00	30.00	30.00	-
Wheat middlings	19.00	19.00	19.00	-
Calcium carbonate	1.00	1.00	1.00	0.50
Sodium chloride	0.40	0.40	0.40	0.40
L-Lysine-HCl	0.20	0.20	0.20	-
Trace mineral premix ¹	0.15	0.15	0.15	0.15
Vitamin premix ²	0.25	0.25	0.25	0.25
Phytase ³	0.01	0.01	0.01	-
Xylanase ⁴	0.03	0.03	0.03	-
Protease ⁵	-	0.03	0.05	-
Titanium dioxide	0.50	0.50	0.50	0.50
Corn Starch	-	-	-	68.25
Sucrose	-	-	-	20.00
Solka Floc	-	-	-	4.00
Monocalcium phosphate	-	-	-	2.45
Magnesium oxide	-	-	-	0.10
Potassium carbonate	-	-	-	0.40
Soybean oil	-	-	-	3.00
Total	100.00	100.00	100.00	100.00
Standardized ileal digestible (SID) AA, %				
Lysine	0.80	0.80	0.80	-
Isoleucine:lysine	80	80	80	-
Leucine:lysine	203	203	203	-
Methionine:lysine	36	36	36	-
Methionine and Cysteine:lysine	74	74	74	-
Threonine:lysine	74	74	74	-
Tryptophan:lysine	20	20	20	-
Valine:lysine	99	99	99	-
Histidine:lysine	56	56	56	-
Total lysine, %	0.94	0.94	0.94	-
Metabolizable energy, kcal/kg	3,219	3,218	3,218	3,736
Crude protein, %	20.00	20.00	20.00	-
Ca, %	0.71	0.71	0.71	0.59
STTD P, %	0.46	0.46	0.46	0.53
Phytase, FTU/kg	750	750	750	-
Xylanase, U/kg	4,000	4,000	4,000	-
Protease, U/kg	0	4,000	8,000	-

¹Provided per kg of diet: 110 mg of Zn from zinc sulfate; 110 mg of Fe from iron sulfate; 33 mg of Mn from manganese oxide; 17 mg of Cu from copper sulfate; 0.30 mg of Se from sodium selenite; 0.30 mg of I from calcium iodate.

²Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D₃; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B₁₂; 50 mg of niacin; 28 mg pantothenic acid; 8 mg riboflavin.

³Axtra® Phy Gold 5 T, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

⁴Danisco® Xylanase 15,000 G, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

⁵Axtra® Pro 302 TPT, Danisco Animal Nutrition & Health (IFF), Cedar Rapids, IA, USA.

Table 4.5. Analyzed composition of dietary treatments for Exp. 2 (as-fed basis)^{1,2}

Item,	Basal diet	Protease, U/kg		N-free diet
		4,000	8,000	
Dgw, μm^3	485	432	507	-
Sgw ⁴	2.75	2.80	2.68	-
Dry matter, %	87.53	87.83	87.66	91.21
Crude protein, %	19.19	19.78	19.55	0.45
AEE, %	4.52	4.52	4.31	1.85
Ash, %	5.40	5.28	5.35	3.68
Crude fiber, %	5.45	5.31	5.57	1.11
Phytase, FTU/kg	1,530	1,780	1,520	-
Xylanase, U/kg	5,680	6,220	5,330	-
Protease, U/kg	ND	4,430	9,910	-
Indispensable AA, %				
Arginine	1.04	1.14	1.11	0.01
Histidine	0.53	0.56	0.56	ND
Isoleucine	0.77	0.81	0.80	0.01
Leucine	1.92	1.93	1.96	0.03
Lysine	0.99	1.06	1.03	0.02
Methionine	0.35	0.37	0.35	0.01
Phenylalanine	0.93	0.97	0.96	0.02
Threonine	0.71	0.75	0.75	0.01
Tryptophan	0.17	0.18	0.17	<0.02
Valine	0.99	1.03	1.03	0.02
Dispensable AA, %				
Alanine	1.20	1.21	1.23	0.02
Aspartic acid	1.51	1.64	1.60	0.02
Cysteine	0.40	0.44	0.40	0.01
Glutamic acid	3.49	3.62	3.57	0.04
Glycine	0.83	0.88	0.87	0.01
Serine	0.81	0.85	0.83	0.01
Tyrosine	0.61	0.65	0.64	0.01

¹AEE = Acid hydrolyzed ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

²ND = Non detectable.

³Dgw = Geometric mean diameter.

⁴Sgw = Geometric standard deviation.

Table 4.6. Digestible energy (DE), metabolizable energy (ME), and apparent total tract digestibility (ATTD) of nutrients and gross energy (GE) in experimental diets for Exp. 1¹

Item,	Control	Protease, U/kg			SEM	<i>P</i> <	
		4,000	8,000	12,000		Linear	Quadratic
Feed intake, kg/d	2.07	2.08	2.07	2.08	0.24	0.973	0.882
GE Intake, kcal/d	8,472	8,489	8,428	8,463	971	0.779	0.891
GE Output feces kcal/d	1,906	1,878	1,749	1,965	189	0.921	0.259
GE Output urine kcal/d	464	392	394	406	76.9	0.223	0.164
DE, kcal/kg of DM ²	3,527	3,576	3,607	3,516	62.6	0.987	0.167
ME, kcal/kg of DM ⁴	3,286	3,363	3,398	3,289	61.0	0.855	0.091
ATTD of GE, % ⁵	77.4	77.5	78.8	76.8	1.37	0.944	0.329
ATTD of DM, %	78.0	79.0	78.8	77.9	0.52	0.766	0.040
ATTD of Ca, %	65.1	66.1	67.2	64.3	4.87	0.845	0.184
ATTD of P, %	59.6	60.3	59.6	56.8	4.06	0.141	0.196
ATTD of NDF, % ³	44.3	47.3	49.6	43.8	2.01	0.858	0.001
ATTD of ADF, % ⁴	26.4	32.7	28.8	30.1	4.13	0.370	0.187
ATTD of OM, % ⁵	78.2	79.1	79.0	78.2	0.49	0.927	0.066
ATTD of Fat, %	61.7	61.0	59.6	59.8	2.09	0.181	0.684

¹N = 12.

²DM= dry matter.

³NDF= neutral detergent fiber.

⁴ADF= acid detergent fiber.

⁵OM= Organic matter.

Table 4.7. Nitrogen retention and apparent total tract digestibility (ATTD) of N in dietary treatments for Exp. 1¹

Item,	Control	Protease, U/kg			SEM	<i>P</i> <	
		4,000	8,000	12,000		Linear	Quadratic
N retained, g ²	39.4	39.2	40.5	37.7	4.71	0.329	0.124
N retention, % of intake ³	56.9	56.7	56.5	54.6	1.23	0.108	0.387
ATTD of N, %	81.5	81.7	82.9	81.4	0.68	0.784	0.173

¹N = 12.

²N retained, g/d = N intake, g/d – Fecal N, g/d – Urinary N, g/d.

³N retention, % of intake = (N retained g/d ÷ N intake, g/d) × 100.

Table 4.8. Apparent ileal digestibility (AID) of dry matter (DM), acid hydrolyzed extract (AEE), crude protein (CP) and AA in dietary treatments for Exp. 2¹

Item, %	Control	Protease, U/kg		SEM	<i>P</i> <	
		4,000	8,000		Linear	Quadratic
DM	64.9	65.9	65.1	0.81	0.779	0.211
AEE	74.6	76.6	75.2	1.00	0.632	0.108
CP	73.1	75.1	72.4	0.69	0.352	0.001
Indispensable AA						
Arginine	83.0	85.1	83.8	0.66	0.064	0.001
Histidine	78.5	80.2	79.0	0.71	0.402	0.004
Isoleucine	77.6	79.3	77.5	0.60	0.940	0.001
Leucine	83.6	84.2	83.5	0.55	0.795	0.121
Lysine	78.0	80.0	78.5	0.52	0.384	0.001
Methionine	83.8	85.0	83.4	0.51	0.518	0.007
Phenylalanine	80.6	82.0	80.8	0.57	0.702	0.004
Threonine	67.3	70.1	68.6	0.77	0.125	0.003
Tryptophan	73.0	75.5	71.7	1.23	0.270	0.003
Valine	75.4	77.2	75.8	0.64	0.486	0.004
Mean	79.1	80.7	79.4	0.58	0.614	0.003
Dispensable AA						
Alanine	78.8	79.6	78.8	0.57	0.974	0.104
Aspartic acid	71.7	74.8	72.7	0.71	0.140	0.001
Cysteine	68.5	72.3	68.0	0.99	0.663	0.001
Glutamic acid	83.6	84.9	83.3	0.61	0.589	0.001
Glycine	62.5	66.3	63.5	1.36	0.377	0.002
Serine	75.2	77.3	75.5	0.67	0.669	0.002
Tyrosine	80.2	82.0	80.6	0.56	0.524	0.002
Mean	76.5	78.3	76.6	0.83	0.910	0.009
Total AA	77.7	79.4	77.9	0.70	0.779	0.004

^{a-b}Values within a row lacking a common superscript letter are different ($P < 0.05$).

^{x-y}Values within a row lacking a common superscript letter tend to be different ($P < 0.10$).

¹N = 15.

Table 4.9. Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in dietary treatments for Exp. 2^{1,2}

Item, %	Control	Protease, U/kg		SEM	<i>P</i> <	
		4,000	8,000		Linear	Quadratic
CP	80.1	81.8	79.4	0.70	0.315	0.002
Indispensable AA						
Arginine	87.4	89.1	87.9	0.66	0.219	0.001
Histidine	81.3	82.9	81.7	0.71	0.557	0.006
Isoleucine	81.0	82.6	80.1	0.60	0.761	0.001
Leucine	85.8	86.4	85.6	0.55	0.732	0.112
Lysine	81.4	83.2	81.8	0.52	0.523	0.002
Methionine	85.9	87.0	85.5	0.515	0.522	0.011
Phenylalanine	83.4	84.7	83.5	0.57	0.828	0.005
Threonine	73.6	76.2	74.6	0.77	0.250	0.005
Tryptophan	79.1	81.3	77.8	1.23	0.274	0.006
Valine	79.1	80.8	79.4	0.64	0.637	0.005
Mean	82.6	84.0	82.7	0.58	0.780	0.005
Dispensable AA						
Alanine	82.7	83.5	82.6	0.57	0.903	0.094
Aspartic acid	76.1	78.9	76.8	0.71	0.261	0.001
Cysteine	72.3	75.8	71.9	0.99	0.667	0.001
Glutamic acid	85.9	87.2	85.5	0.61	0.525	0.002
Glycine	78.5	81.4	78.8	1.36	0.779	0.008
Serine	80.4	82.3	80.6	0.67	0.800	0.004
Tyrosine	83.6	85.2	83.8	0.56	0.714	0.003
Mean	84.8	86.2	84.7	0.83	0.841	0.020
Total AA	83.8	85.2	83.8	0.70	0.976	0.009

¹N = 15.

²Values for SID were calculated with the correction of apparent ileal digestibility values for basal endogenous losses of each individual AA and CP. Basal ileal endogenous losses were determined as (g/kg DMI): CP, 15.41; Arg, 0.52; His, 0.17; Ile, 0.31; Leu, 0.48; Lys, 0.39; Met, 0.08; Phe, 0.30; Thr, 0.51; Trp, 0.12; Val, 0.42; Ala, 0.53; Asp, 0.77; Cys, 0.18; Glu, 0.92; Gly, 1.52; Ser, 0.48; and Tyr, 0.24.