Influence of exogenous enzymes and pelleting on feed manufacturing and broiler performance

by

Courtney Nicole Truelock

B.S., Texas A&M University, 2015
M.S., Texas A&M University, 2017

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

A multitude of exogenous enzymes, such as phytase, amylase, and protease, have been developed to improve nutrient digestion in monogastrics. The majority of monogastric feed is pelleted, and the efficacy of these enzymes in pelleted diets requires some investigation. Heat, moisture, and mechanical pressure associated with pelleting are known to alter enzyme structure and activity. Thus, it is probable any one of the pelleting variables could inactivate exogenous feed enzymes and reduce their feeding value. The objectives of this dissertation were to determine the effects of pelleting parameters and phytase sources on phytase stability during pelleting, evaluate the influence of corn type on starch gelatinization of pelleted diets and subsequent broiler performance, and examine the efficacy of protease in poultry diets. The first set of experiments evaluated the effect of phytase source, conditioning temperature, conditioner retention time, steam pressure, and die thickness on phytase stability and pellet quality. These results indicated a negative linear relationship between conditioning temperature and phytase stability that is influenced by phytase source. Additionally, increasing conditioning temperature increases pellet durability and hot pellet temperature. Even at the lowest conditioning temperature of 74°C, maximum phytase stability averaged 63% in pellets. There was no evidence that conditioner retention time or die L:D affected phytase stability, and increasing steam pressure tended to improve phytase recovery by 18% in pellets. The second experiment determined the effects of die thickness and conditioning temperature on pelleting and starch characteristics in diets containing conventional or Enogen® Feed corn, a high amylase corn variety. It was concluded that starch gelatinization increased with increasing conditioning temperature, and Enogen® Feed corn diets resulted in greater starch gelatinization than conventional corn diets. Furthermore, die thickness had no effect on starch characteristics,

improved pellet durability, and increased pellet mill energy consumption. The third experiment evaluated the effects of corn type and conditioner retention time on pelleting characteristics of a poultry diet and subsequent broiler growth performance and carcass traits. The results demonstrated broilers fed Enogen® Feed corn consumed more feed, had heavier body weights, and heavier carcasses than broilers fed conventional corn, however there was no difference in carcass feed efficiency between treatments. Moreover, pelleting of Enogen® Feed corn resulted in greater starch solubility in cooled pellets compared to pelleted conventional corn diets, and broiler performance was not affected by conditioner retention time. Lastly, the fourth experiment examined the effects of dietary Lys concentration and exogenous protease inclusion on growth performance and amino acid digestibility in poultry. It was concluded that broilers fed 1.12 and 1.21% digestible Lys diets with added protease had a 2-point improvement in FCR compared to chicks fed these diets without protease. Increasing digestible Lys concentration improved FCR in broilers and poults and improved BW, ADG, and ADFI in poults. There was no evidence that added protease had an effect on BW, ADG, or ADFI in broilers or poults. Finally, ileal amino acid digestibility was not affected by digestible Lys or protease inclusion for either 20-d old broiler chicks or 42-d old turkey poults.

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Approved by:

Major Professor
Dr. Chad B. Paulk

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Abstract

A multitude of exogenous enzymes, such as phytase, amylase, and protease, have been developed to improve nutrient digestion in monogastrics. The majority of monogastric feed is pelleted, and the efficacy of these enzymes in pelleted diets requires some investigation. Heat, moisture, and mechanical pressure associated with pelleting are known to alter enzyme structure and activity. Thus, it is probable any one of the pelleting variables could inactivate exogenous feed enzymes and reduce their feeding value. The objectives of this dissertation were to determine the effects of pelleting parameters and phytase sources on phytase stability during pelleting, evaluate the influence of corn type on starch gelatinization of pelleted diets and subsequent broiler performance, and examine the efficacy of protease in poultry diets. The first set of experiments evaluated the effect of phytase source, conditioning temperature, conditioner retention time, steam pressure, and die thickness on phytase stability and pellet quality. These results indicated a negative linear relationship between conditioning temperature and phytase stability that is influenced by phytase source. Additionally, increasing conditioning temperature increases pellet durability and hot pellet temperature. Even at the lowest conditioning temperature of 74°C, maximum phytase stability averaged 63% in pellets. There was no evidence that conditioner retention time or die L:D affected phytase stability, and increasing steam pressure tended to improve phytase recovery by 18% in pellets. The second experiment determined the effects of die thickness and conditioning temperature on pelleting and starch characteristics in diets containing conventional or Enogen® Feed corn, a high amylase corn variety. It was concluded that starch gelatinization increased with increasing conditioning temperature, and Enogen® Feed corn diets resulted in greater starch gelatinization than conventional corn diets. Furthermore, die thickness had no effect on starch characteristics,

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Dedication

To my husband who has walked every step of this journey with me.

Chapter 1: Factors affecting phytase stability during the pelleting process

ABSTRACT

Three experiments were conducted to determine the effect of various pelleting parameters and phytase sources on phytase stability during pelleting. In experiment 1, treatments were arranged in a 4 × 3 × 2 factorial with phytase source (A, B, C, and D), conditioning temperature (82, 88, and 93°C), and conditioner retention time (30 and 60 s). In experiment 2, treatments were arranged as a 2 × 3 factorial of steam pressure (1.7 and 3 bar) and conditioning temperature (77, 82, and 88°C). In experiment 3, treatments were arranged as a 2 × 3 factorial of die thickness (lengh:diameter [L:D] 5.6 and 8.0) and conditioning temperature (74, 79, and 85°C). Treatment diets for all three experiments were mixed using a horizontal counterpoise mixer. Phytase was added to the mixer via a hand-add port after being hand mixed for 3 min with 2.3 kg of ground corn. Diets were steam conditioned and pelleted using a 30-horsepower pellet mill. Initial mash, conditioned mash, and pellet samples were collected for analysis of phytase, moisture, and pellet durability (PDI). Conditioning temperature, hot pellet temperature (HPT), and production rate were also recorded for each treatment, and each treatment was replicated 3 times. For Exp. 1, there was a source × conditioning temperature interaction for phytase stability. At conditioning temperatures of 82 and 88°C, phytase A had greater stability compared to all other phytase sources. At 93°C, phytase A stability was not different than phytase C, but greater than phytase B and D. At 82°C conditioning temperature, phytase stability of phytase B was less than that of the other phytase sources and not different than the stability of phytase C or D at 88 and 93°C. For all 3 experiments, phytase stability decreased and PDI and HPT increased with increasing conditioning temperature. Even at the lowest conditioning temperature of 74°C, maximum phytase stability averaged 63% in pellets. There was no evidence that conditioner retention time

or die L:D affected phytase stability, and increasing steam pressure tended to improve phytase recovery by 18% in pellets. There is a complex matrix of pelleting factors that influence phytase stability with most of the factors affecting HPT and enzyme recovery accordingly, perhaps making HPT the best determinant for phytase stability.

Keywords: conditioning temperature, die thickness, pelleting, phytase stability, retention time, steam pressure

INTRODUCTION

Phosphorus is stored in plant tissues as phytic acid, a cyclic structure that is not easily digested by animals that lack endogenous production of the phytase enzyme. Limited bioavailability of phytate-bound phosphorus requires nutritionists to over-formulate for phosphorus. However, microbial phytase can be used to release phytate-bound phosphorus within plant-based feed ingredients, resulting in increased phosphorus digestion and decreased phosphorus excretion (Selle and Ravindran, 2007). Improved phosphorus utilization requires less phosphorus to be added to diets (Yan et al., 2001), simultaneously decreasing feed costs and improving performance.

The majority of broiler diets are pelleted to capture the benefits of thermal processing, such as decreased ingredient segregation, improved handling characteristics, decreased feed wastage, increased bulk density, and pathogen reduction (Behnke, 2001). Therefore, due to the prominence of phytase in pelleted diets, it is important to evaluate the effect of thermal processing on phytase stability. It is known that enzyme activity is inhibited by heat (Spring et al., 1996), suggesting that steam pelleting at high conditioning temperatures may damage phytase. Previous research in this area has demonstrated the potential problems incurred when corn, soybean meal diets supplemented with various microbial phytases are conditioned at increasing conditioning temperatures (De Jong et al., 2017). Authors reported a 1.9% decrease in phytase activity with every 1°C increase in conditioning temperature. However, these authors only observed the effects of conditioning temperature on phytase activity and did not evaluate the potential effects sustained through the pellet die. Additionally, this experiment was conducted using a lab-scale pellet mill with maximum production rates of 90 kg/h. Heat generated by steam in the conditioner is not the only factor of concern when discussing phytase

destruction during pelleting. Moisture is also a variable of concern, as well as frictional heat generated across the pellet die. Therefore, the experiments herein were conducted to evaluate the effects of various pelleting conditions such as conditioning temperature, steam pressure, retention time, die thickness, and microbial phytase source on phytase stability.

MATERIALS AND METHODS

Diet Manufacture

Treatment diets for all three experiments were mixed using a 907-kg horizontal counterpoise mixer (Hayes & Stolz, Fort Worth, TX). In instances where a single treatment diet was larger than 907 kg, multiple batches of the same treatment were blended together to ensure uniform composition across the entire treatment. Phytase was added to the mixer via a hand-add port after being hand mixed for 3 min with 2.3 kg of ground corn. Diets were steam conditioned (twin staff pre-conditioner, Model 150, Wenger) and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill). Conditioner retention time was calculated by adjusting the conditioner screw speed and dividing the amount of feed in the conditioner by the production rate. For each experiment, a conventional corn, soybean meal flush diet without phytase was used to warm up the pellet mill to the appropriate conditioning temperature. Additionally, a similar flush diet was pelleted between treatments to minimize any carryover between diets. For all 3 experiments, treatments were repeated on 3 separate days, thus achieving 3 replications per treatment.

Experiment 1

Treatments were arranged in a 4 × 3 × 2 factorial with phytase source (A, B, C, and D), conditioning temperature (82, 88, and 93°C), and conditioner retention time (30 and 60 s). Each phytase source was added to the diet to release 0.15% phosphorus (Table 1.1). Diets were steam

conditioned for 30 or 60 s at 82, 88, or 93°C and pelleted using a 4.8 mm × 50.8 mm pellet die (length-to-diameter ratio [L:D] 10.6). Production rate was set at 272 kg/h, approximately 30% of the rated throughput for the pellet mill. Retention time was randomized within day to ensure there were no effects of time due to environmental temperature or humidity. Phytase sources were randomized within retention time to ensure no effects of pelleting order. Conditioning temperature, hot pellet temperature (HPT), ambient temperature, ambient humidity, and production rate were recorded at 3 time points during each run (Table 1.5). Conditioning temperatures were as expected for each treatment. Average ambient temperature and humidity were 86°F and 42%, respectively.

Prior to pelleting, a total of 10 mash samples (500 g each) per treatment were collected. During each processing run, pellet samples were collected throughout the run and immediately placed in an experimental counter-flow cooler for 10 min. Once pellets were cool, 10 pellet samples (500 g each) per treatment were collected for analysis of phytase. Prior to analysis, mash and pellet samples were composited such that there were 5 mash and 5 pellet samples per treatment analyzed in duplicate for phytase activity according to the official AOAC method by incubation with sodium phytate (AOAC, 2001). Phytase stability was calculated as the percentage of phytase remaining in pelleted diets averaged across 5 pelleted samples compared to the initial phytase (FYT) averaged across 5 mash samples (Table 1.2).

Data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC), with pelleting run as the experimental unit and day as the blocking factor. Main effects included phytase source, conditioning temperature, and retention time. Linear and quadratic contrasts were used to evaluate the effect conditioning temperature. Results were

considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

Experiment 2

Treatments were arranged as a 2 × 3 factorial of steam pressure (1.7 and 3 bar) and conditioning temperature (77, 82, and 88°C). Phytase was added to a corn, soybean meal-based diet at 1.4 kg/ton (Table 2). Prior to pelleting, initial mash samples of the diet were collected for phytase analysis. Diets were steam conditioned for 30 sec at 77, 82, or 88°C and subsequently pelleted using a 4.8 mm × 31.8 mm pellet die (L:D 6.7). Production rate was set at 900 kg/h, approximately 100% of the rated throughput for the pellet mill. Steam pressure was randomized across day to minimize the effects of pelleting order and was adjusted via the cospect valve at the steam harness prior to the conditioner. Conditioning temperature, HPT, and production rate were recorded at 5 time points during each run (Table 1.8).

For each treatment, 3 conditioned mash samples were collected as feed exited the conditioner prior to the pellet die, and immediately cooled in an experimental counter-flow cooler for 15 min. Conditioned mash samples were then composited into 2 samples for phytase analysis. Likewise, 5 pellet samples per treatment were collected as feed exited the pellet die, cooled for 15 min, and composited into 2 samples for phytase analysis. All samples were analyzed similar to Experiment 1.

Composite samples of initial mash, conditioned mash, and cooled pellets were analyzed for moisture and pellet durability index (PDI). Samples for moisture analysis were ground with a mortar and pestle, weighed to 1 g, and placed in a forced air oven for 24 h at 105°C. Moisture was calculated as the percentage of weight loss after drying. For analysis of PDI, fines were sifted off from cooled pellets using a U.S. #5 (4 mm) sieve. One hundred g of the sifted pellets

were placed into a Holmen 100 pellet tester and agitated with air for 60 sec. Following agitation, the sample was again sifted through a No. 5 sieve and the remaining pellets were weighed. Pellet durability index was calculated as the percentage of the initial pellet sample remaining after agitation with air.

Data were analyzed using the GLIMMIX procedure in SAS v. 9.4, with pelleting run as the experimental unit and day as the blocking factor. Main effects included steam pressure and conditioning temperature. Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature. Results were considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

Experiment 3

Treatments were arranged as a 2 × 3 factorial of die thickness (L:D 5.6 and 8.0) and conditioning temperature (74, 79, and 85°C). Phytase was added to a corn, soybean meal-based diet (Table 3) and mash samples were collected for phytase analysis. Diets were steam conditioned for 30 s at 74, 79, or 85°C, and pelleted using a 4 mm × 22.2 mm (L:D 5.6) or 4 mm × 31.8 mm (L:D 8) pellet die. Production rate was set at 900 kg/h, and steam pressure was 1.7 bar. Die thickness was randomized across day to minimize the effects of pelleting order. Conditioning temperature, HPT, and production rate were recorded at 3 time points during each run (Table 1.9).

For each treatment, 3 conditioned mash samples were collected as feed exited the conditioner prior to the pellet die and immediately cooled in an experimental counter-flow cooler for 15 min. Cooled conditioned mash samples were then composited into 2 samples for phytase analysis. Likewise, 3 pellet samples per treatment were collected as feed exited the pellet die, cooled for 15 min, and composited into 3 samples, 2 for phytase analysis and 1 for analysis of

pellet durability index (PDI). Phytase and PDI analysis was conducted according to the methods outlined in Experiments 1 and 2.

Data were analyzed using the GLIMMIX procedure in SAS v. 9.4, with pelleting run as the experimental unit and day as the blocking factor. Main effects included die thickness and conditioning temperature. Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature. Results were considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Experiment 1

There was no evidence for a source × conditioning temperature × retention time interaction for hot pellet temperature or phytase stability (Table 1.5). A source × conditioning temperature interaction did not exist ($P > 0.16$) for hot pellet temperature.

Hot pellet temperature increased (quadratic, $P = 0.01$) with increasing conditioning temperature (Table 1.7). At conditioning temperatures of 82, 88, and 93°C, hot pellet temperatures were 94.6, 97.7, and 99.4°C, respectively. There was no evidence of difference in hot pellet temperature due to retention time (Table 1.7) or phytase source (Table 1.6).

There was a source × conditioning temperature interaction ($P = 0.01$; Figure 1.1) for phytase stability. At conditioning temperatures of 82 and 88°C, phytase A had greater stability compared to all other phytase sources (Table 1.5). At 93°C, phytase A stability was not different than phytase C, but greater than phytase B and D. At 82°C conditioning temperature, phytase stability of phytase B was less than that of the other phytase sources and not different than the stability of phytase C or D at 88 and 93°C. As conditioning temperature increased, phytase stability across all phytase sources decreased (quadratic, $P < 0.01$; Table 1.7), with a majority of

the decrease occurring from 82 to 88°C. Main effect of phytase source was significant ($P < 0.01$) for phytase stability. Across all conditioning temperatures and retention times, phytase stability was greatest for phytase A (22.5%) and least for phytase B (10.1%), with phytase C and D being intermediate (15.7 and 13.9%, respectively; Table 1.6). There was no evidence of difference in phytase stability ($P = 0.11$) due to retention time (Table 1.7).

Experiment 2

There was no evidence ($P > 0.17$) for a steam pressure × conditioning temperature interaction for HPT, phytase stability, moisture, or PDI (Table 8). Production rate and conditioning temperature were as expected for each treatment. Increasing conditioning temperature from 77 to 88°C increased (linear, $P < 0.01$) HPT, and there was no evidence of difference ($P = 0.80$) in HPT between steam pressures.

Increasing conditioning temperature from 77 to 88°C decreased (linear, $P < 0.01$) phytase stability of conditioned mash. In cooled pellets, phytase stability was also decreased (linear, $P < 0.01$) with increasing conditioning temperature. Cooled pellets tended ($P = 0.08$) to have greater phytase stability when steam pressure was set at 3 bar compared to 1.7 bar.

Average moisture of initial mash prior to pelleting was 13.6%. Differences in moisture between the two steam pressure treatments were not observed ($P > 0.35$) in conditioned mash, hot pellets, or cooled pellets. Moisture of conditioned mash and pellets increased (linear, $P \leq 0.05$) with increasing conditioning temperature.

Pellet durability index tended to increase (linear, $P = 0.06$) with increasing conditioning temperature. There was no evidence for difference ($P = 0.48$) in PDI when feed was steam conditioned at 1.7 or 3 bar.

Experiment 3

There was no evidence ($P > 0.14$) for a die thickness × conditioning temperature interaction for any of the pelleting or phytase stability responses analyzed in this study (Table 1.9). Production rate and conditioning temperature were as expected for each treatment. Hot pellet temperature was increased by 1% when pelleting with a thicker die ($P < 0.01$) and increased with increasing conditioning temperature (linear, $P < 0.01$). Pellet durability index was greater ($P < 0.01$) for diets pelleted using the thicker die with a L:D of 8 compared to the die with a L:D of 5.6. Additionally, PDI increased (linear, $P = 0.03$) with increasing conditioning temperature.

Increasing conditioning temperature from 74 to 85°C decreased (linear, $P < 0.01$) phytase stability of conditioned mash and cooled pellets, with no difference ($P > 0.72$) in stability due to die thickness.

DISCUSSION

In all 3 experiments, phytase stability was reduced by increasing conditioning temperature, and in experiment 1, the amount of reduction was dependent on commercial phytase source as illustrated in Figure 1. It is important to note that all treatments were exposed to a HPT ≥ 93.7°C, which resulted in stabilities ≤ 33.7%. Similar results have been reported by De Jong et al. (2017) and Pope (2019). In the conditioner, not only is heat generated in the form of steam, but there is an approximate 1% moisture addition for every 14°C increase in conditioning temperature. These increases in heat and moisture are responsible for a portion of the phytase inactivity as evidenced by decreased stability in conditioned mash with increasing conditioning temperatures. In experiments 2 and 3, a single source of phytase was exposed to a range of conditioning temperatures (74 to 88°C) and HPT (79 to 89°C). Across the 2

experiments, phytase stability in conditioned mash was ≥ 91% when conditioned at 74°C but declined as conditioning temperatures exceeded 77°C. Phytase stability in cooled pellets was 59 and 63% when pellets achieved a HPT of 79°C and continued to decline when HPT increased. It is important to note that HPT of 79°C was the lowest achieved in these experiments.

It is important to consider that conditioning temperature does not equal final pellet temperature. The process of conditioned mash being forced through the holes in the pellet die generates frictional heat, the extent of which will depend on several factors including die L:D, dietary fat percentage, production rate, and conditioning temperature. Estimates of frictional heat across the pellet die can be derived by comparing HPT to conditioning temperature. The larger the difference between HPT and conditioning temperature (otherwise known as the change in temperature across the pellet die), the greater the frictional heat. Thus, in instances of little lubrication, such as low conditioning temperatures, low dietary fat, and slow production rates, the final pelleted diet can reach temperatures much higher than that of the actual conditioning temperature. The findings of the 3 experiments herein have led to the hypothesis that HPT is a greater determining factor for phytase recovery than conditioning temperature. This is demonstrated by the difference in phytase stabilities between conditioned mash and cooled pellets after exposure to a similar conditioning temperature. Therefore, HPT is the best indicator of the highest temperature to which phytase will be exposed.

Pelleted diets in experiment 1 had the largest reductions in phytase stability, which most likely can be attributed to the HPT temperature achieved (> 93.7°C) when pelleting these diets. This HPT can be attributed to the higher die L:D that was used to pellet the diets as well as the less than 1% fat that was added to the diet. The influence of each of these factors during pelleting on enzyme recovery has been examined previously. Using similar equipment and pelleting

parameters as the current experiment, Pope (2019) reported a quadratic decrease in xylanase recovery in pellets compared to conditioned mash when mixer added fat decreased from 5% to 1%. Furthermore, xylanase recovery in pellets compared to initial mash decreased from 13.9% to 6% when pellet die L:D increased from 8 to 10. Thus, together a higher die L:D and lower added fat could greatly increase the amount of frictional heat that was generated using the parameters in experiment 1. This conclusion is supported by the large change in temperature across the pellet die at conditioning temperatures of 82 and 88°C, which averaged 12.6°C and 9.7°C in the first experiment and averaged 3.6°C and 1.1°C in the second, respectively.

Conversely, there was no evidence that increasing die L:D from 5.6 to 8 had an effect on phytase stability in experiment 3. This is in opposition to Pope (2019) who observed a 57% decrease in xylanase recovery when die L:D increased from 8 to 10. Hot pellet temperatures in the Pope (2019) study averaged 86.5°C and 88.7°C for the L:D 8 and 10 dies, respectively. Hot pellet temperatures in experiment 3 averaged 83.2°C and 84.2°C for the L:D 5.6 and 8 dies, respectively. Differences in enzymes as well as the larger increase in HPT between dies compared to experiment 3 may explain why there was a larger effect observed on xylanase phytase stability in Pope (2019). Additionally, these results may indicate the negative effects of dies with an L:D higher than 8 as mixer added fat and average conditioning temperature were slightly lower in experiment 3 than Pope (2019), and theoretically, should have potentially increased frictional heat creating harsher conditions for effective enzyme recovery.

There was a tendency for steam pressure to affect phytase stability in pellets but not conditioned mash in experiment 2. During conditioning, steam pressure can be manipulated to enhance the efficiency of pelleting as variations in steam pressure can produce variations in product quality and pellet mill capacity (Robinson, 1976). Thus, it was hypothesized that

changes in steam pressure may affect moisture content in the conditioner, thus altering phytase stability. Although changes in steam pressure are applied to the conditioner, the effect of such changes were not observed in the conditioner, but rather across the pellet die. Increasing the pressure of steam entering the conditioner from 1.7 to 3 bar resulted in an approximate 18% increase in phytase stability in cooled pellets. Furthermore, steam pressure did not influence HPT. If increases in steam pressure lead to less moisture entering the conditioner, this could explain the potential benefit for phytase stability. However, less moisture in the conditioner would also correspond to less die lubrication and greater frictional heat. This should increase HPT, which has previously been attributed to greater phytase inactivity. Further, there were no differences in moisture content observed in conditioned mash or pellets between the two steam pressure treatments. Therefore, these results are perplexing and the reasoning for increased steam pressure to enhance enzyme recovery while having no effect on HPT should be investigated further.

Pelleting parameters that reduced phytase stability were the same ones that improved pellet quality. This inverse relationship has previously been described by Pope (2019) and Sansukjaroenphon (2019). Thus, pellet mill operators must consider the delicate balance between producing good quality pellets and preserving enzyme activity.

Overall, it seems there is a complex matrix of pelleting factors that influence phytase stability with most of the factors affecting both HPT and enzyme recovery accordingly. Results of these experiments suggest that HPT may be the best parameter for determining phytase stability. Additionally, poor phytase recoveries observed in the present experiments are consistent with previous research using similar equipment, which is smaller than that found in

commercial feed mills. More research is needed to further evaluate the ability to compare between commercial- and experimental-sized pellet mills and their effects on enzyme stability.

REFERENCES

AOAC. 2001. AOAC Official Method 2000.12 Phytase Activity in Feed. J. AOAC Int. 84:629.

Behnke, K. C. 2001. Factors influencing pellet quality. Feed Technol. 5:19-22.

De Jong, J. A., J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, M. D. Tokach, S. S. Dritz, C. R. Stark, and C. K. Jones. 2017. Stability of four commercial phytase products under increasing thermal conditioning temperatures. Transl. Anim. Sci. 1:255-260.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of Microbial Phytase in Broiler Diets. Poult. Sci. 83:962-970.

DuPont Animal Nutrition. 2020. Phytase feed enzyme solutions. http://animalnutrition.dupont.com/productsservices/feed-enzymes/feed-phytase-solutions/. Accessed June 16, 2020.

Pieniazek, J., K. A. Smith, M. P. Williams, M. K. Manangi, M. Vasquez-Anon, A. Solbak, M. Miller, and J. T. Lee. 2017. Evaluations of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 96:370-382.

Pope, J. T. 2019. Non-conditioning Factors Affecting Enzyme Thermostability during Feed Processing. Ph.D. Diss. North Carolina State University.

Robinson, R. 1976. Pelleting – Introduction and General Definitions. In: *Feed Manufacturing Technology*. Ed. H. B. Pfost. American Feed Manufacturers Association, Inc., Arlington, VA.

Saensukjaroenphon, M. 2019. The effect of pelleting parameters on phytase stability and pellet quality. Ph.D. Diss. Kansas State University.

Sebastian S., S. P. Touchburn, E. R. Chavez, and P. C. Laguë. 1997. Apparent Digestibility of Protein and Amino Acids in Broiler Chickens Fed a Corn-Soybean Diet Supplemented with Microbial Phytase. Poult. Sci. 76:1760-1769.

Selle, P. H. and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1-41.

Spring, P. K., E. Newman, C. Wenk, R. Messikommer, and M. Vukic Vranies. 1996. Effect of pelleting temperature on the activity of different enzymes. Poult. Sci. 75:357-361.

Yan, F. J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus Requirements of Broiler Chicks to Six Weeks of Age as Influenced by Phytase Supplementation. Poult. Sci. 80:455-459.

TABLES AND FIGURES

Table 1.1 Composition (as-fed basis) of pelleted diets in Experiment 1[1]

Ingredient	g/kg, as-is
Corn	549.9
Soybean meal, 47% crude protein	376.0
DDGS[2]	40.0
Choice white grease	7.5
Defluorinated phosphate	6.6
Limestone	9.5
Salt	3.5
L-Lysine HCl	1.9
DL-Methionine	1.7
Choline chloride	1.0
Vitamin trace mineral premix[3]	2.5

[1]Phytase sources were added to the diet in place of corn and formulated to release 0.15% nPP. Phytase A was added at 20.6 g/ton, phytase B at 45.9 g/ton, phytase C at 9.5 g/ton, and phytase D at 41.2 g/ton.
[2]DDGS = distillers dried grains with solubles.
[3]Provided per lb of diet; vitamin A, 1,400,000 IU; vitamin D_3, 500,000 IU; vitamin E, 3,000 IU; vitamin B_{12}, 2 mg; menadione, 150 mg; riboflavin, 1,200 mg; thiamine, 200 mg; D-pantothenic acid, 1,200 mg; niacin, 5,000 mg; vitamin B_6, 250 mg; folic acid, 125 mg; choline, 70,000 mg; biotin, 6 mg.

Table 1.2 Composition (as-fed basis) of pelleted diets in Experiment 2[1]

Ingredient	g/kg, as-is
Ground corn	690.7
Soybean meal, 47% crude protein	264.8
Choice white grease	15.0
Monocalcium phosphate	5.5
Limestone	11.3
Salt	3.5
L-Lysine HCl	3.1
DL-Methionine	0.7
L-Threonine	0.9
Trace mineral premix	1.5
Vitamin premix	1.5
Phytase[2]	1.5

[1]Diets were mixed in a 1-ton Hayes & Stolz (Fort Worth, TX) horizontal counterpoise mixer.
[2]HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) was superdosed in the diet to minimize analytical variation due to small inclusion levels.

Table 1.3 Composition (as-fed basis) of pelleted diets in Experiment 3[1]

Ingredient	g/kg, as-is
Ground corn	758.8
Soybean meal, 47% crude protein	200.7
Soybean oil	15.0
Monocalcium phosphate	5.0
Limestone	11.0
Salt	3.5
L-Lysine HCl	2.6
DL-Methionine	0.2
L-Threonine	0.5
Trace mineral premix	1.3
Vitamin premix	1.3
Phytase[2]	0.3

[1]Diets were mixed in a 1-ton Hayes & Stolz (Fort Worth, TX) horizontal counterpoise mixer.
[2]HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) was added to the experimental diets at 809 FYT/kg.

Table 1.4 Analyzed phytase levels in mash samples of diets containing 4 commercially available microbial phytase sources prior to pelleting (Exp. 1)[1]

Retention time:	30 s				60 s			
Phytase source:	A	B	C	D	A	B	C	D
Initial phytase,[2] FYT/kg	426	1,217	1,058	854	453	1,045	820	725
CV,[3] %	17.6	27.3	14.0	43.4	17.4	26.1	19.8	43.5

[1]Four commercial microbial phytase sources (A, B, C, and D) were added to a corn, soybean meal diet. Diets were mixed as 8 separate 1,800 lb batches.

[2]Phytase sources were added to the diet in place of corn and formulated to release 0.15% nPP.

[3]A total of 30 mash samples (500 g each) were collected within each batch. Pairs of mash samples were combined such that 15 separate mash samples for each batch were analyzed for initial phytase levels according to the official AOAC method. Phytase results were averaged within source × retention time treatments (total of 15 samples). CV was then calculated using the following equation, CV = (FYT standard deviation of 15 samples ÷ mean FYT of 15 samples) × 100.

Table 1.5 Phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 3 conditioning temperatures (Exp. 1)[1]

	Phytase source					Probability, $P <$
Item	A	B	C	D	SEM[2]	Source × Temp
Hot pellet temperature, °C						
82°C	95.3	94.5	95.0	93.7	0.45	0.16
88°C	97.2	98.0	97.8	97.8		
93°C	99.3	99.7	99.0	99.5		
Phytase stability,[3] %						
82°C	33.7^{a}	13.0^{def}	24.2^{b}	20.7^{bc}	1.86	0.01
88°C	17.5^{cd}	8.3^{f}	11.2^{f}	11.5^{ef}		
93°C	16.3^{cde}	9.0^{f}	11.8^{ef}	9.7^{f}		

[1]Four commercial microbial phytase sources (A, B, C, and D) were added to a corn, soybean meal diet and steam conditioned (twin staff pre-conditioner, Model 150, Wenger) for 30 or 60 s at 82, 88, or 93°C and pelleted (1012-2 HD Master Model, California Pellet Mill) with a 4.8 mm × 50.8 mm pellet die over 3 days of replication.

[2]Pooled standard error of least squares means ($n = 3$).

[3]Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

[abcdef]Means within a row and column without a common superscript differ ($P < 0.05$).

Table 1.6 Main effects of phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 2 retention times and 3 conditioning temperatures (Exp. 1)[1]

	Phytase source					Probability, $P <$
Item	A	B	C	D	SEM[2]	Source
Hot pellet temperature, °C	97.4	97.3	97.3	97.0	0.26	0.75
Phytase stability,[3] %	22.5[a]	10.1[c]	15.7[b]	13.9[b]	1.21	0.01

[1]Four commercial microbial phytase sources (A, B, C, and D) were added to a corn, soybean meal diet and steam conditioned (twin staff pre-conditioner, Model 150, Wenger) for 30 or 60 s at 82, 88, or 93°C and pelleted (1012-2 HD Master Model, California Pellet Mill) with a 4.8 mm × 50.8 mm pellet die over 3 days of replication.

[2]Pooled standard error of least squares means ($n = 3$).

[3]Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

[abcd]Means within a row without a common superscript differ ($P < 0.05$).

Table 1.7 Main effects of retention time and conditioning temperature on phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 2 retention times and 3 conditioning temperatures (Exp. 1)[1]

	Retention time, s			Conditioning temp, °F				Probability, $P <$		
Item	30	60	SEM[2]	82	88	93	SEM	Retention	Linear	Quadratic
Hot pellet temperature, °C	97.2	97.3	0.19	94.6	97.7	99.4	0.23	0.92	0.01	0.01
Phytase stability,[3] %	16.4	14.8	0.98	22.9	12.1	11.7	1.10	0.11	0.01	0.01

[1]Four commercial microbial phytase sources (A, B, C, and D) were added to a corn, soybean meal diet and steam conditioned (twin staff pre-conditioner, Model 150, Wenger) for 30 or 60 s at 82, 88, or 93°C and pelleted (1012-2 HD Master Model, California Pellet Mill) with a 4.8 mm × 50.8 mm pellet die over 3 days of replication.
[2]Pooled standard error of least squares means ($n = 3$).
[3]Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.
[ab]Means within a row without a common superscript differ ($P < 0.05$).

Table 1.8 Effect of steam pressure and conditioning temperature on hot pellet temperature, phytase stability, and pellet durability index of a corn, soybean meal diet (Exp. 2)[1]

	Steam pressure, bar:										
	1.7			3				Probability, <			
Target conditioning temp, °C:	77	82	88	77	82	88	SEM[2]	Bar	Linear[3]	Quadratic	Bar × Temp
Production rate, kg/min	15.4	15.5	15.4	15.3	15.4	15.5	0.09	-	-	-	-
Conditioning temp, °C	76.8	82.4	87.9	76.8	82.7	88.1	0.11	-	-	-	-
Hot pellet temp, °C	81.8	85.7	88.9	81.8	85.5	89.3	0.26	0.17	0.01	0.42	0.54
Phytase stability,[4] %											
Conditioned mash	106.0	64.2	31.5	113.1	71.4	32.7	8.66	0.38	0.01	0.62	0.88
Cooled pellet	52.4	20.3	4.7	51.2	29.0	11.4	4.09	0.08	0.01	0.07	0.26
Moisture,[5] %											
Conditioned mash	15.9	17.5	18.2	17.5	16.6	18.1	0.63	0.65	0.05	0.48	0.17
Cooled pellet	14.3	14.5	14.8	14.5	14.6	14.7	0.20	0.35	0.03	0.96	0.61
Pellet durability index, %	78.7	83.3	84.1	80.9	83.3	86.5	2.58	0.48	0.06	0.74	0.87

[1]A corn, soybean meal finishing swine diet was mixed in a 1-ton Hayes & Stolz horizontal counterpoise mixer. HiPhos 2700 was added to the diet at 3 lb/ton and steam pelleted (10 in width × 55 in length Wenger twin staff pre-conditioner, Model 150) for approximately 30 sec at 2 steam pressures (24 and 44 psi) and 3 conditioning temperatures (170, 180, and 190°F) on a 1-ton, 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 × 1 1/4 in pellet die (L:D 6.7).
[2]Pooled standard error of least squares means (n = 3).
[3]Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.
[4]Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.
[5]Average initial moisture of mash feed was 13.6%.

Table 1.9 Effect of die thickness and conditioning temperature on pelleting characteristics and phytase stability of a corn, soybean meal diet (Exp. 3)[1]

Die L:D:	5.6			8				Probability, <			
Conditioning temp, °C:	74	79	85	74	79	85	SEM[2]	Die	Linear[3]	Quadratic	Die × Temp
Production rate, kg/min	15.3	15.4	15.4	15.3	15.3	15.4	0.08	-	-	-	-
Conditioning temp, °C	74.2	79.6	85.1	74.1	79.7	84.7	0.16	-	-	-	-
Hot pellet temp, °C	79.3	83.0	87.3	80.8	84.2	87.6	0.56	0.01	0.01	0.15	0.73
Phytase stability,[4] %											
Conditioned mash	102.8	61.0	35.4	91.4	77.5	36.2	8.14	0.72	0.01	0.64	0.14
Cooled pellet	63.0	38.1	23.7	58.6	35.6	28.1	7.09	0.85	0.01	0.19	0.70
Pellet durability index, %	80.0	80.9	84.7	88.5	88.9	91.7	1.71	0.01	0.03	0.39	0.91

[1]Diets were steam-conditioned (10 × 55 in Wenger twin staff pre-conditioner, Model 150) for 30 sec at 165, 175, or 185°F and pelleted (CPM, 1012-2 HD Master Model) using a 5/32 × 7/8 in (L:D 5.6) or 5/32 × 1 1/4 in (L:D 8.0) pellet die.
[2]Pooled standard error of least squares means (n = 3).
[3]Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.
[4]Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

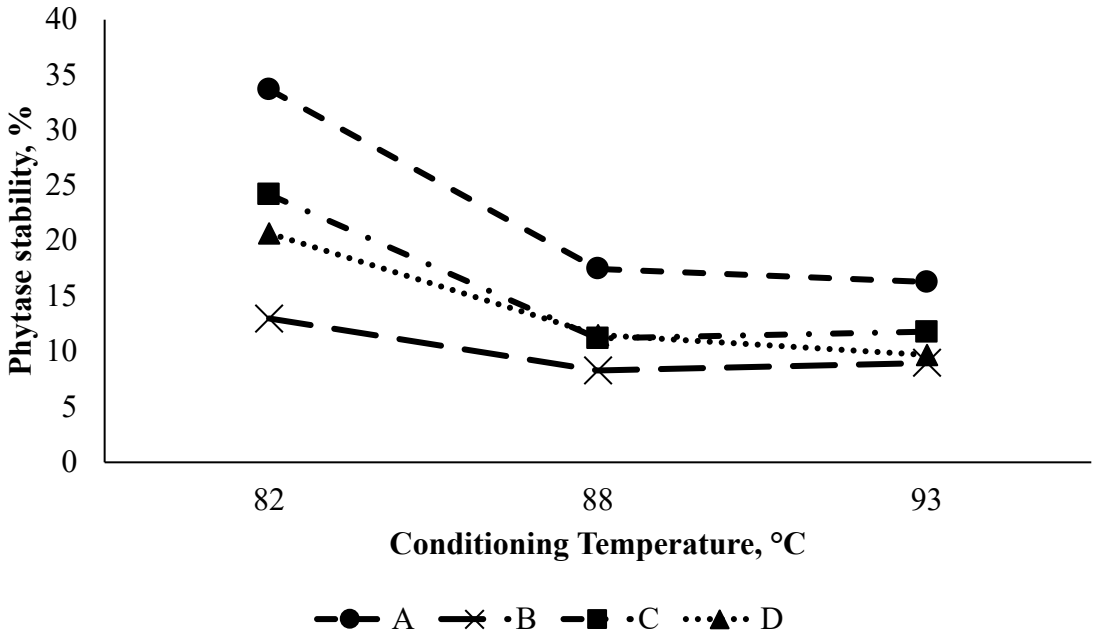

Figure 1.1 Interaction between phytase source and conditioning temperature in a corn, soybean meal diet containing 4 commercially available microbial phytase sources pelleted at 3 conditioning temperatures and 2 retention times ($P = 0.01$).

Chapter 2: Pelleting and starch characteristics of diets containing different corn varieties

ABSTRACT

This experiment determined the effects of die thickness and conditioning temperature on pelleting and starch characteristics in diets containing conventional or Enogen® Feed corn (Syngenta Seeds, LLC). Treatments were arranged as a 2 × 2 × 3 factorial of corn type (conventional [CON] and Enogen® Feed corn [EFC]), die thickness (5.6 and 8 effective thickness:hole diameter [L:D]), and conditioning temperature (74, 79, and 85°C). Diets were steam conditioned (Wenger twin staff pre-conditioner, Model 150) and pelleted (CPM, Model 1012-2) with a 4 × 22.2 mm (L:D 5.6) or 4 × 31.8 mm (L:D 8) pellet die. Conditioner retention time was set at 30 s and production rate was set at 15 kg/min. All treatments were represented within 3 replicate days. Pellets were composited and analyzed for gelatinized starch and pellet durability index (PDI). Conditioning temperature, hot pellet temperature (HPT), production rate, and pellet mill energy consumption were recorded throughout each processing run. Data were analyzed using the GLIMMIX procedure in SAS (v. 9.4, SAS Institute Inc., Cary, NC) with pelleting run as the experimental unit and day as the blocking factor. Pelleting with a larger die L:D improved PDI ($P = 0.01$) and increased ($P = 0.02$) pellet mill energy consumption. Increasing conditioning temperature from 74 to 85°C increased (linear, $P < 0.03$) PDI and tended to decrease energy consumption (quadratic, $P = 0.07$). There was a corn × conditioning temperature interaction ($P = 0.01$) for gelatinized starch in conditioned mash. Enogen® Feed corn diets steam conditioned at 85°C had the greatest amount of gelatinized starch. Cooked starch in conditioned mash and pellets was greater ($P < 0.01$) for EFC diets compared to CON diets and increased (linear, $P < 0.01$) with increasing conditioning temperature in conditioned mash.

Similarly, starch gelatinization was greater ($P < 0.01$) in pelleted EFC diets compared to CON diets and was increased (linear, $P = 0.05$) by increasing conditioning temperature from 74 to 85°C. In conclusion, increasing die L:D and increasing conditioning temperature improved PDI. Starch gelatinization was increased when diets were pelleted at the highest conditioning temperature of 85°C, and EFC diets resulted in greater starch gelatinization than conventional corn.

Keywords: conditioning temperature, die thickness, Enogen® Feed corn, pelleting, starch gelatinization

INTRODUCTION

Starch is the primary energy source in livestock diets and constitutes up to 50% of monogastric diets. Starch is largely supplied by grains, and in the U. S., specifically corn. Much research has been dedicated to evaluating grain processing methods that increase starch availability, such as grinding and thermal processing (Rowe et al., 1999; Hancock and Behnke, 2001; Lundblad et al., 2011). For pelleting in particular, the process by which starch availability is increased is through a process known as starch gelatinization. Gelatinization is a four-step process that irreversibly solubilizes raw starch granules through the addition of heat and moisture. In the conditioner, mash feed is mixed with heat and moisture in the form of steam which begins a conformational change in the dietary components of the diet, and starch granules begin to swell. As feed exits the conditioner, it is passed through the pellet mill die to form pellets. This process generates frictional heat which drives starch gelatinization. Gelatinized starch values range from 6 to 7% in conditioned mash, similar to unconditioned mash, and ranges from 11 to 12% in pellets (Lewis et al., 2015). Gelatinized starch increases starch availability in the animal, and Rojas et al. (2016) reported an increase in apparent ileal digestibility of starch from 96.4 in mash diets to 97.7% in pelleted swine diets.

Starch digestion is largely driven by amylase, a glycolytic enzyme that degrades starch into sugars which are more readily available for absorption in the small intestine. Recent advancements in corn breeding have led to the development of corn varieties that naturally express enzyme activity. One such variety is Enogen® Feed corn (Syngenta Seeds, LLC), a high amylase corn originally developed for use in the ethanol industry (Syngenta Crop Protection, LLC, 2019). This variety utilizes in-seed technology that produces an α-amylase enzyme within the kernels. Increased amylase activity of Enogen® Feed corn is designed to assist in the rapid

degradation of starch to sugars, thereby providing more available energy for growth. The enzyme is active across a broad range of temperatures and pH conditions when moisture is adequate. Similar conditions are prevalent in the pelleting process, yet there has been no research to date evaluating the effect of a high amylase corn variety in the pelleting process. Therefore, this experiment was designed to evaluate the effects of corn type, die thickness, and conditioning temperature on pelleting and starch characteristics of a swine diet.

MATERIALS AND METHODS

Treatments were arranged as a 2 × 2 × 3 factorial of corn type (conventional [CON] and Enogen® Feed corn [EFC]), die thickness (5.6 and 8 effective thickness:hole diameter [L:D]), and conditioning temperature (74, 79, and 85°C). Conventional and Enogen® Feed corn were ground to approximately 600 μm using a 3-high roller mill (RMS, Model 924). For the EFC treatments, ground Enogen® Feed corn replaced conventional ground corn on a kg:kg basis (Table 2.1). Diets were mixed in a 907-kg horizontal counterpoise mixer (Hayes & Stolz, Fort Worth, TX), steam conditioned (25 × 140 cm twin staff pre-conditioner, Model 150, Wenger, Sabetha, KS) for approximately 30 s at 74, 79, or 85°C, and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill, Crawfordsville, IN) equipped with a 4 × 22.2 mm (L:D 5.6) or 4 × 31.8 mm (L:D 8) pellet die. Conditioner retention time was calculated by adjusting the conditioner shaft speed and dividing the amount of feed in the conditioner by the production rate. Production rate was set at 15 kg/min, approximately 100% of the rated throughput for the pellet mill, and steam pressure was set at 1.65 bar. All treatments were replicated on 3 separate days, thus achieving 3 replications of each of the 12 treatments. Thus, there were 12 to 18 replications of each main effect and a total of 36 experimental units. Die thickness was randomized across day and corn type was randomized

within die to minimize the effects of pelleting order. A conventional corn, soybean meal flush diet was used to warm the mill up to 74°C before each day of pelleting. Two hundred seventy-two kg of the first corn type diet was pelleted on the first die, according to randomization, at all 3 conditioning temperatures in ascending order. The pellet mill was flushed with a diet not containing Enogen® Feed corn and the second corn type diet was pelleted in the same manner. Once completed, the pellet mill was shut down and the die was changed. A conventional corn, soybean meal flush was again used to warm the mill up to 74°C before pelleting on the second die, and pelleting procedures followed the pattern previously mentioned.

Data collection

Conditioning temperature, hot pellet temperature (HPT), production rate, and pellet mill energy consumption were recorded at 3 time points during each treatment run (Table 2.2). Within each replicate, 3 conditioned mash and 3 pellet samples were collected per treatment. Conditioned mash samples were collected as feed exited the conditioner prior to the pellet die and immediately frozen. Frozen conditioned mash samples were composited into a single sample for starch analysis. Pellet samples were collected as feed exited the pellet die, cooled for 15 min in an experimental counter-flow cooler, and composited into 2 samples. Composite pellet samples were analyzed for total starch, gelatinized starch, cooked starch, and pellet durability index (PDI). Samples for starch analysis were sent to the Wenger Technical Center Laboratory (Sabetha, KS) and analyzed by methods outlined by Mason et al (1982). Briefly, one 0.5-g subsample was hydrolyzed in distilled water at room temperature while a second 0.5-g subsample was boiled with distilled water. Samples were incubated with glucoamylase, and free D-glucose was measured. The quantity of free glucose analyzed in the room temperature sample represents the percentage of starch that was gelatinized during processing, and the quantity of

free glucose in the boiled sample represents the percentage of total starch in the sample. Cooked starch was then calculated as the percentage of gelatinized starch divided by the percentage of total starch multiplied by 100.

For analysis of PDI, fines were sifted off from cooled pellets using a U.S. No. 5 (4 mm) sieve. One hundred g of the sifted pellets were placed into a Holmen 100 pellet tester and agitated with air for 60 sec. Following agitation, the sample was again sifted through a No. 5 sieve and the remaining pellets were weighed. Pellet durability index was calculated as the percentage of the initial pellet sample remaining after agitation with air.

Statistical analysis

Data were analyzed using the GLIMMIX procedure in SAS (v. 9.4, SAS Institute Inc., Cary, NC), with pelleting run as the experimental unit and day as the blocking factor. Hot pellet temperature, PDI, energy consumption, and starch characteristics of pellets were analyzed with main effects of corn type, die thickness, and conditioning temperature. Conditioned mash starch data was analyzed with main effects of corn type and conditioning temperature. For all data, linear and quadratic contrasts were used to evaluate the effect of conditioning temperature. Results were considered significant if $P \leq 0.05$ and were considered marginally significant if $P > 0.05$ and $P \leq 0.10$.

RESULTS

Production rate and conditioning temperature were as expected for each treatment. There was no evidence ($P > 0.21$) for a corn type × die thickness × conditioning temperature interaction for any of the pelleting or starch responses analyzed in this study (Table 2.2). Additionally, there was no evidence ($P > 0.14$) for a corn type × conditioning temperature interaction for HPT, PDI, or pellet mill energy consumption.

There was no evidence for a corn type × die thickness or die thickness × conditioning temperature interaction for HPT. Inclusion of EFC ($P < 0.01$), a thicker pellet die ($P < 0.01$), and increasing conditioning temperature from 74 to 85°C (linear, $P < 0.01$) resulted in increased HPT.

There was a tendency ($P = 0.08$) for a corn type × die thickness interaction for PDI. Pellet durability index for CON and EFC treatments were similar when diets were pelleted using the L:D 8 die. However, PDI for CON diets was greater than EFC diets when pelleted using the L:D 5.6 die. Pelleting diets with the L:D 8 die improved ($P < 0.01$) PDI compared to the L:D 5.6 die. Additionally, PDI increased (linear, $P = 0.03$) with increasing conditioning temperature.

Pellet mill energy consumption was greater for the L:D 8 pellet die ($P = 0.02$), and tended to decrease (quadratic, $P = 0.07$) with increasing conditioning temperature. There was no evidence of difference ($P = 0.12$) in energy consumption due to corn type.

There was no evidence for a corn type × conditioning temperature interaction ($P > 0.12$) for average moisture, total starch, or cooked starch in conditioned mash (Table 2.3). Corn type had no effect on average moisture in conditioned mash ($P > 0.82$), however, there was an increase (linear, $P < 0.01$) in moisture of conditioned mash with increasing conditioning temperature. Additionally, there was no evidence of difference ($P > 0.42$) in total starch due to corn type or conditioning temperature. There was a corn type × conditioning temperature interaction ($P = 0.01$) for gelatinized starch in conditioned mash. Enogen® Feed corn diets steam conditioned at 85ºC had greater gelatinized starch than all other corn type × conditioning temperature treatments. Cooked starch of conditioned mash was greater for diets containing EFC compared to CON and increased (linear, $P < 0.01$) with increasing conditioning temperature.

There were no significant interactions ($P > 0.15$) for average moisture, gelatinized starch, or cooked starch in pelleted diets (Table 2.4). There was no evidence of difference ($P > 0.40$) in pellet moisture due to corn type, die thickness, or conditioning temperature. There was a corn type × die thickness interaction ($P < 0.01$) for total starch in pellets. Total starch was greater for EFC diets pelleted using the L:D 8 compared to the L:D 5.6 die, but not different from the CON diets pelleted using either the L:D 5.6 or 8 die. Pelleted EFC diets had greater ($P < 0.01$) gelatinized starch than CON diets, and gelatinized starch increased (linear, $P = 0.05$) with increasing conditioning temperature. Similarly, cooked starch was greatest ($P < 0.01$) for the EFC diets and tended to increase (linear, $P = 0.06$) with increasing conditioning temperature.

DISCUSSION

Results of this experiment demonstrated that increasing die L:D from 5.6 to 8 improved pellet quality but increased pellet mill energy consumption. Behnke (2001) described the same positive correlation between die L:D and pellet durability and attributed this 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. Die L:D has also shown to be positively correlated with pellet mill energy consumption. An experiment examining the effect of 7 different dietary and pelleting factors on pellet durability and energy consumption demonstrated an increase in pellet mill energy consumption with increasing die L:D (Fahrenholz, 2012). In fact, among the 6 other variables examined, including corn particle size, percent fat, percent DDGS, production rate, conditioning temperature, and conditioning retention time, die L:D ratio was one of the most influential factors affecting energy consumption, second only to conditioning temperature. The reasoning behind the negative effect

of die L:D on pellet mill efficiency when pelleting corn, soybean meal-based diets has not been explained. However, it is hypothesized that additional energy is needed to move mash feed through a 32 mm die compared to a 22 mm die due to the added pressure that is generated from greater feed to die hole wall contact when using a thicker pellet die.

Increasing conditioning temperature from 74 to 85°C linearly improved PDI without increasing energy consumption. Once again, these results compare to those of Fahrenholz (2012) who reported greater pellet durability and lower pellet mill energy consumption at higher conditioning temperatures. A general rule of thumb for steam conditioning livestock diets is that moisture content of the mash feed increases by 1% for every 14°C increase in conditioning temperature. Therefore, an improvement in PDI with increasing conditioning temperature is likely due to the increase in moisture which acts as a binding agent, plasticizing the soluble fractions of the diet, and increasing the agglomeration of dietary components. The effect of conditioning temperature on pellet mill energy consumption may also be explained by the increase in moisture content of conditioned feed. Additional moisture at higher conditioning temperatures helps to lubricate the feed as it passes through the pellet die, thus lowering the amount of friction that may influence energy consumption.

Diets containing EFC had poorer PDI compared to CON when feed was pelleted using the 5.6 L:D die, but PDI was similar when pelleted using the 8 L:D die, with corn type not affecting pellet mill energy consumption. The EFC diets had poorer pellet durability compared to CON, which was only observed when pelleting with a thinner pellet die. No differences in processing parameters were observed that provide an explanation for the observed response. Pelleting with a thicker pellet die improved PDI enough to ameliorate any differences between the corn types. Moritz et al. (2002) described a positive correlation between PDI and starch

gelatinization, concluding that gelatinized starch acts as a binding agent to improve PDI. In the present study, the inclusion of EFC in pelleted diets increased gelatinized starch compared to pelleted CON diets, but there was no evidence for improvement in PDI. In addition, there was no evidence for difference that pellet die L:D affected starch gelatinization, while improving PDI. Thus, the interaction of corn type and die L:D and the possible influence of starch gelatinization on PDI needs to be further investigated.

Starch gelatinization was increased when diets were pelleted at the highest conditioning temperature of 85°C, and EFC diets resulted in greater gelatinized starch than CON diets. The relationship between conditioning temperature and starch gelatinization is in agreement with the findings of Lewis and others (2015) who reported a 19% increase in gelatinized starch when conditioning temperature increased from 77°C to 88°C. Although gelatinized starch values of conditioned mash in the experiment herein were lower than that observed previously (4.5% vs. 7.3%, respectively; Lewis et al., 2015), pelleted CON diets averaged 11.7% gelatinized starch, similar to previous work. A significant finding of Lewis et al. (2015) was the predominant increase in gelatinized starch that occurred across the pellet die rather than in the conditioner. Lewis et al. (2015) reported that gelatinized starch of conditioned mash samples was statistically similar to that of cold mash, whereas gelatinized starch in pellets was greater than both the cold mash and conditioned mash. These findings support those of the current experiment which demonstrated an increase in gelatinized starch in pellets compared to conditioned mash. Because there is no available research on the effect of EFC in the pelleting process, it is hypothesized that the high amylase activity in the corn is responsible for the increase in gelatinized starch in pelleted EFC diets compared to pelleted CON diets. Vasanthan et al. (2001) evaluated the effect of α-amylase, extrusion temperature, and moisture on the degree of starch hydrolysis in extruded

barley flours. Not only was starch hydrolysis increased with increasing temperature and moisture, but the authors also reported an increase in the degree of starch hydrolysis when exogenous α-amylase concentration increased from 2% to 4%. It has been previously discussed that starch gelatinization increases through heat processing, and it is known that gelatinization of starch enhances the rate of starch hydrolysis (Holm et al., 1988); therefore, starch hydrolysis via amylase could complement starch gelatinization during pelleting, and vice versa.

In conclusion, the results of this experiment indicate when using the pelleting parameters outlined herein there are not any significant effects on pellet mill energy consumption and only slight increases in HPT when EFC is added in place of CON in monogastric diets. Although pelleting with a larger die L:D improves pellet quality, caution should be used as thicker pellet dies can dramatically increase HPT and pellet mill energy consumption. Additionally, pelleting can be used as a means of increasing starch gelatinization in corn, soybean meal-based diets with the majority of gelatinization occurring across the pellet die. Finally, diets containing EFC may have an even greater potential of increasing gelatinized starch during pelleting compared to CON due to the high amylase activity present in EFC.

REFERENCES

Behnke, K. C. 2001. Factors influencing pellet quality. Feed Technol. 5:19-22.

Fahrenholz, A. C. 2012. Evaluating factors affecting pellet durability and energy consumption in a pilot feed mill and comparing methods for evaluating pellet durability. Ph. D. Dissertation. Kansas State University, Manhattan, KS.

Hancock, J. D. and K. C. Behnke. 2001. Use of ingredient and diet processing technologies (grinding, mixing, pelleting, and extruding) to produce quality feeds for pigs. In: Swine Nutrition. Boca Raton, Florida, USA. pp. 469-498.

Holm, J., I. Lundquist, I. Björck, A. C. Eliasson, and N. G. Asp. 1988. Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. Am. J. Clin. Nutr. 47:1010-1016.

Lewis, L. L., C. R. Stark, A. C. Fahrenholz, J. R. Bergstrom, and C. K. Jones. 2015. Evaluation of conditioning time and temperature on gelatinized starch and vitamin retention in a pelleted swine diet. J. Anim. Sci. 93:615-619.

Lundblad, K. K., S. Issa, J. D. Hancock, K. C. Behnke, L. J. McKinney, S. Alavi, E. Prestløkken, J. Fledderus, and M. Sørensen. 2011. Effects of steam conditioning at low and high temperature: expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. Anim. Feed Sci. Technol. 169:208-218.

Moritz, J. S., K. J. Wilson, K. R. Cramer, R. S. Beyer, L. J. McKinney, W. B. Cavalcanti, and X. Mo. 2002. Effect of formulation density, moisture and surfactant on feed manufacturing, pellet quality and broiler performance. J. Appl. Poult. Res. 11:155-163.

Rojas, O. J., E. Vinyeta, and H. H. Stein. 2016. Effects of pelleting, extrusion, or extrusion and pelleting on energy and nutrient digestibility in diets containing different levels of fiber and fed to growing pigs. J. Anim. Sci. 94:1951-1960.

Rowe, J. B., M. Choct, and D. W. Pethick. 1999. Processing cereal grains for animal feeding. Aust. J. Agric. Res. 50:721-736.

Syngenta Crop Protection, LLC. 2019. Minnetonka, MN. http://www.syngenta-us.com/corn/enogen.

Vasanthan, T., J. Yeung, and R. Hoover. 2001. Dextrinization of Starch in Barley Flours with Thermostable alpha-Amylase by Extrusion Cooking. Starch/Stärke. 53:616-622.

TABLES

Table 2.1 Composition (as-fed basis) of a pelleted corn, soybean meal-based swine diet[1]

Ingredient	%, as-is
Ground corn[2]	75.88
Soybean meal, 47% crude protein	20.07
Soybean oil	1.50
Monocalcium phosphate	0.50
Limestone	1.10
Salt	0.35
L-Lysine HCl	0.26
DL-Methionine	0.02
L-Threonine	0.05
Trace mineral premix	0.13
Vitamin premix	0.13
HiPhos 2700[3]	0.03

[1]Diets were mixed in a 907-kg Hayes & Stolz horizontal counterpoise mixer with a 60-s dry mix time and 120-s wet mix time.
[2]Enogen® Feed corn (Syngenta Seeds, LLC) replaced conventional yellow dent ground corn on a kg:kg basis.
[3]DSM Nutritional Products, Parsippany, NJ.

Table 2.2 Pelleting characteristics of swine diets containing either conventional or Enogen® Feed corn[1]

Die L:D:	5.6			8				Probability,[2] <					
Conditioning temp, °C:	74	79	85	74	79	85	SEM[3]	Corn	Die	Linear[4]	Quadratic[4]	Corn × die	Die × temp
Production rate, kg/min													
CON[5]	15.3	15.4	15.4	15.3	15.3	15.4	0.08	-	-	-	-	-	-
EFC[6]	15.3	15.5	15.3	15.4	15.3	15.5							
Condition temp, °C													
CON	74.2	79.6	85.1	74.1	79.7	84.7	0.16	-	-	-	-	-	-
EFC	73.8	79.6	85.1	74.1	79.8	84.6							
Hot pellet temp, °C													
CON	79.3	83.0	87.3	80.8	84.2	87.6	0.56	0.01	0.01	0.01	0.15	0.79	0.73
EFC	81.3	83.4	87.3	82.1	84.8	88.6							
Pellet durability index, %													
CON	81.2	83.7	87.9	87.9	89.0	92.0	2.42	0.11	0.01	0.03	0.39	0.08	0.91
EFC	78.8	78.1	81.4	89.1	88.9	91.4							
Energy consumption, kWh/ton													
CON	13.4	12.9	13.1	14.1	13.7	13.7	0.45	0.12	0.02	0.38	0.07	0.36	0.70
EFC	14.0	13.1	14.0	14.2	13.8	14.0							

[1]Diets were steam-conditioned (25 cm × 140 cm Wenger twin staff pre-conditioner, Model 150) for 30 s at 74, 79, or 85°C and pelleted (CPM, 1012-2 HD Master Model) using a 4 mm × 22.2 mm (L:D 5.6) or 4 mm × 31.8 mm (L:D 8) pellet die.

[2]There was no evidence for a corn type × die thickness × conditioning temperature interaction ($P > 0.46$) or corn type × conditioning temperature interaction ($P > 0.14$) for hot pellet temperature, pellet durability index, or energy consumption.

[3]Pooled standard error of least squares means ($n = 3$).

[4]Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.

[5]CON = conventional yellow dent corn.

[6]EFC = Enogen® Feed corn (Syngenta Seeds, LLC).

Table 2.3 Starch characteristics of steam conditioned swine diets containing either conventional or Enogen® Feed corn[1]

	Conditioning temperature, °C				Probability, <			
Item	74	79	85	SEM[2]	Corn	Linear[3]	Quadratic[3]	Corn × temp
Moisture, %								
CON[4]	17.3	17.7	18.0	0.26	0.93	<0.01	0.96	0.42
EFC[5]	17.3	17.8	17.7					
Total starch,[6] %								
CON	59.2	59.2	58.0	1.86	0.42	0.98	0.58	0.35
EFC	59.1	58.7	61.6					
Gelatinized starch,[7] %								
CON	3.9	4.4	4.0	0.22	<0.01	<0.01	0.93	0.01
EFC	4.4	4.4	5.6					
Cook,[8] %								
CON	6.4	7.3	6.9	0.41	<0.01	<0.01	0.64	0.12
EFC	7.3	7.4	9.1					

[1]Diets were steam-conditioned (25 cm × 140 cm Wenger twin staff pre-conditioner, Model 150) for 30 s at 74, 79, or 85°C and pelleted (CPM, 1012-2 HD Master Model) using a 4 mm × 22.2 mm (L:D 5.6) or 4 mm × 31.8 mm (L:D 8) pellet die.
[2]Pooled standard error of least squares means (n = 3).
[3]Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.
[4]CON = conventional yellow dent corn.
[5]EFC = Enogen® Feed corn (Syngenta Seeds, LLC).
[6]Total starch was calculated as the percentage of free D-glucose in a 0.5-g subsample after hydrolysis in distilled water at room temperature and incubation with glucoamylase.
[7]Gelatinized starch was calculated as the percentage of free D-glucose in a 0.5-g subsample after boiling with distilled water and incubation with glucoamylase.
[8]Cooked starch was calculated as the percentage of gelatinized starch divided by the percentage of total starch multiplied by 100.

Table 2.4 Starch characteristics of pelleted swine diets containing either conventional or Enogen® Feed corn[1]

Die L:D:	5.6			8				Probability,[2] <				
Conditioning temp, °C:	74	79	85	74	79	85	SEM[3]	Corn	Die	Linear[4]	Quadratic[4]	Corn × die
Moisture, %												
CON[5]	12.0	12.5	12.8	12.2	12.7	12.9	0.67	0.41	0.60	0.40	0.81	0.88
EFC[6]	12.1	12.2	12.0	12.4	12.3	12.4						
Total starch,[7] %												
CON	59.4	58.2	58.1	57.3	57.3	57.4	1.44	0.34	0.19	0.18	0.94	<0.01
EFC	53.2	55.6	57.4	57.5	58.9	60.2						
Gelatinized starch,[8] %												
CON	10.7	11.2	12.0	11.8	11.7	12.6	0.99	<0.01	0.14	0.05	0.37	0.85
EFC	12.2	12.2	14.1	13.1	13.5	15.0						
Cook,[9] %												
CON	18.0	19.2	20.7	20.6	20.4	22.0	1.36	<0.01	0.18	0.06	0.27	0.41
EFC	23.0	21.9	24.4	22.8	22.9	24.9						

[1]Diets were steam-conditioned (25 cm × 140 cm Wenger twin staff pre-conditioner, Model 150) for 30 s at 74, 79, or 85°C and pelleted (CPM, 1012-2 HD Master Model) using a 4 mm × 22.2 mm (L:D 5.6) or 4 mm × 31.8 mm (L:D 8) pellet die.
[2]There was no evidence for a corn type × die thickness × conditioning temperature interaction, a corn type × conditioning temperature interaction, or a die thickness × conditioning temperature interaction ($P > 0.15$) for starch responses of pelleted diets analyzed in this study.
[3]Pooled standard error of least squares means ($n = 3$).
[4]Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.
[5]CON = conventional yellow dent corn.
[6]EFC = Enogen® Feed corn (Syngenta Seeds, LLC).
[7]Total starch was calculated as the percentage of free D-glucose in a 0.5-g subsample after hydrolysis in distilled water at room temperature and incubation with glucoamylase.
[8]Gelatinized starch was calculated as the percentage of free D-glucose in a 0.5-g subsample after boiling with distilled water and incubation with glucoamylase.
[9]Cooked starch was calculated as the percentage of gelatinized starch divided by the percentage of total starch multiplied by 100.

Chapter 3: Effect of Enogen® Feed corn on pelleting characteristics of a poultry diet and subsequent broiler growth performance and carcass traits

ABSTRACT

This experiment evaluated the effects of corn type and conditioner retention time on pelleting characteristics and broiler growth and carcass traits. Twelve hundred male broiler chicks (Cobb-Vantress, Siloam Springs, AR) were used in a 45-d experiment with a 2 × 2 factorial treatment structure of corn source (conventional [CON] and Enogen® Feed corn [EFC; Syngenta Crop Protection, Inc.]) and conditioner retention time (30 or 80 s). Conventional corn was replaced by EFC on a kg:kg basis. Pelleting and starch characteristics of the diets were collected and analyzed. Chicks were randomly allocated to groups of 15 and assigned to 1 of 80 floor pens. Chicks received experimental treatments beginning on d 5 of age. A starter diet was fed from d 0 to 10 of the study, a grower diet from d 11 to 24, and a finisher diet from d 25 to 45 of the experiment. Pen weights and feed consumption were measured on d 11, 25, 39, and 45 for calculation of body weight gain, feed intake, and feed efficiency. Half of the chicks from each treatment were harvested on d 39 and the remaining half were harvested on d 45 for determination of carcass weight and dressing percentage. Pelleting of EFC resulted in greater starch solubility in cooled pellets compared to pelleted CON diets. Broiler performance was not affected by conditioner retention time. Broilers fed EFC consumed more feed, had heavier body weights, and heavier carcasses than broilers fed CON; however, there was no difference in carcass feed efficiency among treatments.

Keywords: broilers, carcass weight, Enogen® Feed corn, growth performance, pelleting

INTRODUCTION

Starch is the primary energy source in livestock diets and constitutes up to 50% of poultry diets. Starch is largely supplied by cereal grains, and in the U. S., specifically corn. Much research has been dedicated to evaluating grain processing methods that increase starch availability, such as grinding and thermal processing (Rowe et al., 1999; Hancock and Behnke, 2001; Lundblad et al., 2011; Al-Rabadi et al., 2017). The purpose of these processing methods is to disrupt the outer shell of the corn kernel and expose the starch endosperm to make it more accessible for enzymatic digestion in the animal. Starch digestion is largely driven by amylase, a glycolytic enzyme that degrades starch into sugars. Therefore, grain processing can improve starch digestion by increasing the surface area of starch molecules and providing more substrate for amylase to bind.

Enogen Feed corn (EFC) is a corn variety which contains a bacterial transgene that produces an α-amylase enzyme (Syngenta Crop Protection, Inc., 2019). Originally developed to improve the efficiency of ethanol production, recent studies have revealed potential benefits in animal performance when EFC is included in livestock diets. Increased amylase activity of EFC corn is designed to assist in the rapid degradation of starch to sugars, thereby providing more available energy for growth. Research has shown a 5% increase in feed efficiency and a 4.1% increase in starch digestion when conventional corn is replaced by EFC in the diets of stocker and finishing cattle (Syngenta Crop Protection, Inc., 2019). An experiment in swine revealed a tendency for improved average daily gain for pigs consuming EFC compared to conventional corn during the last 82 days of the finishing period (Ochonski et al., 2019). Evaluation of EFC in poultry diets has not yet occurred.

Effects of EFC on feed processing characteristics are also of interest. It is currently unknown how a corn variety with high amylase may react in the pelleting process. Pelleting requires addition of heat and moisture in the form of steam. Steam is typically mixed with mash feed in the steam conditioner for 15 to 30 s but can be retained up to 120 s depending on equipment and processing parameters. Pressure is then applied to the steamed feed mixture as it is pressed through the pellet die. The combination of moisture and frictional heat increases starch gelatinization, which is an irreversible process that leads to greater starch availability (Lewis et al., 2015); thus, the amylase activity in EFC would be expected to further increase the degree of starch gelatinization in pelleted feed.

This experiment was designed evaluate the effects of corn type and conditioner retention time on pelleting characteristics of a poultry diet and subsequent broiler growth performance and carcass traits.

MATERIALS AND METHODS

This research was conducted according to the experimental protocols approved by the Institutional Animal Care and Use Committee at Kansas State University.

Twelve hundred male broiler chicks (Cobb-Vantress, Siloam Springs, AR) were used in a 45-d randomized complete block experiment with treatments arranged as a 2 × 2 factorial, with factors consisting of corn source (conventional [CON] and EFC) and conditioner retention time (30 and 80 s). Corn was ground to 700 μm in the starter phase and 900 μm in the grower and finisher phases using a hammermill (Bliss, Model 22115). Starter, grower, and finisher broiler diets were mixed in a 907-kg Hayes & Stolz horizontal counterpoise mixer (Table 1). For treatments containing EFC, conventional corn was replaced by EFC on a kg:kg basis.

For the pelleting trial, diets were steam conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 4 mm × 32 mm pellet die. Production rate was set at 10 kg per min, approximately 65% of the rated throughput for the pellet mill. Pelleting of the starter, grower, and finisher diets provided three replications for each treatment; therefore, diet served as the blocking factor. Conditioner retention times were calculated by adjusting the conditioner screw speed and dividing the amount of feed in the conditioner by the production rate. For each run, a conventional corn-soybean meal flush diet was used to warm the mill up to 75ºC, the first treatment was pelleted, and the mill was shut down to allow the pelleted feed to cool and to adjust conditioner screw settings for the next treatment. Conditioning temperature, hot pellet temperature (HPT), and production rate were recorded at 3 time points during each run (Table 2). Pellet mill amps and volts were also monitored throughout each run to calculate energy consumption.

Prior to pelleting, a total of 10 mash samples per treatment were collected for analysis of soluble starch. During each processing run, 3 conditioned mash and 3 pellet samples were collected throughout the run. Conditioned mash samples were immediately analyzed for soluble starch and pellets were immediately placed in an experimental counter-flow cooler for 10 min. Once pellets were cool, they were analyzed for soluble starch and pellet durability index (PDI). Data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) with pelleting run as the experimental unit and diet as the blocking factor. Main effects included corn type and conditioner retention time. Results were considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

For the performance trial, chicks were maintained on a 24-h lighting schedule in a thermostatically controlled room with *ad libitum* access to feed and water. Chicks were housed in 1.2 m × 2.4 m floor pens with 7 to 10 cm of pine shavings. Each pen was fitted with single hanging feeder and 4 nipple waterers. Chicks were randomly allocated to groups of 15, weighed, and randomly assigned to 1 of 80 floor pens. Treatments were randomly assigned to pens and blocked by location for a total of 20 replications per treatment. Chicks were fed a common diet from 0 to 4 days of age before initiation of the experiment. Therefore, d 0 of the experiment corresponded with 5 days of age for the chicks. A starter diet was fed from study d 0 to d 10, a grower diet from d 11 to d 24, and a finisher diet from d 25 to d 45. In the case of mortality, chick weight, feeder weight, treatment, and pen number were recorded. Pen weights and feed consumption were measured on d 11, 25, 39, and 45 for calculation of body weight gain, feed intake, and feed efficiency (Table 3). Additionally, half of the chicks from each treatment were harvested on d 39 and the remaining half were harvested on d 45 for determination of carcass weight and dressing percentage (Table 4). Data were analyzed using the GLIMMIX procedure in SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. Main effects included corn type and conditioner retention time. Results were considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS

There was no evidence ($P > 0.23$) for a corn type × conditioner retention time interaction for any responses in the pelleting trial. Production rate and conditioning temperature were as expected for each treatment, averaging 10.4 kg/min and 75.3ºC, respectively (Table 2). Hot pellet temperature increased ($P = 0.03$) when conditioner retention time increased from 30 to 80 s (84.3 vs 84.9ºC, respectively). There was no evidence of difference ($P = 0.54$) in HPT due to

corn type. Percentage of starch solubles present in cooled pellets was greater ($P = 0.03$) in EFC diets (6.1%) compared to CON diets (5.4%), but there was no evidence of difference ($P > 0.15$) in starch solubles in the initial mash or conditioned mash due to corn type or retention time. Pellet durability index and pellet mill energy consumption were also not different ($P < 0.30$) between treatments.

There was no evidence ($P > 0.31$) for a corn type × conditioner retention time interaction for any responses in the performance trial. Additionally, there were no main effects of conditioner retention time observed ($P > 0.18$) for body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), or feed conversion ratio (FCR) (Table 3). From d 0 to d 11, FCR was improved ($P < 0.01$) in chicks consuming EFC, but there was no evidence of differences ($P > 0.16$) in BW, ADG, or ADFI among treatments. From d 11 to d 25, ADG and ADFI were greater ($P <0.01$) for chicks fed EFC compared to those fed CON. Body weight was also greater ($P < 0.01$) for chicks consuming EFC compared to CON on d 25 (2.00 vs 1.98 kg, respectively). For the chicks that were harvested on d 39, ADFI was greater ($P < 0.01$) in the finishing phase for chicks fed EFC diets (185.1 g/d) compared to those fed CON diets (179.8 g/d). Additionally, final BW on d 39 was greater ($P < 0.04$) for chicks consuming the EFC treatments compared to those consuming the CON treatments (3.66 vs 3.60 kg, for EFC and CON, respectively). For the second group of chicks harvested on d 45, there was no evidence of difference ($P =0.31$) in final BW, but ADFI on d 45 was greater ($P = 0.05$) and, contrary to the starter phase, FCR was poorer ($P = 0.03$) in chicks fed EFC. From d 0 to 39, BW, ADG, and ADFI were increased ($P < 0.04$) for chicks consuming EFC diets compared to CON diets. From d 0 to 45, however, greater ADFI ($P = 0.05$) and poorer FCR ($P = 0.03$) were observed for chicks receiving EFC.

Chicks consuming EFC diets had greater ($P < 0.03$) carcass weights than chicks consuming CON diets at d 39 (2.57 vs 2.50 kg, respectively) and d 45 (3.11 vs 3.06 kg, respectively; Table 5). Furthermore, carcass dressing percentage was also greater ($P < 0.02$) for chicks on the EFC treatments than the CON treatments at d 39 (70.2 vs 69.5%, respectively) and d 45 (71.0 vs 70.3%, respectively). When feed efficiency was calculated on a carcass basis, there was no evidence of difference ($P > 0.79$) between treatments (Table 5).

DISCUSSION

Results of this experiment show no evidence of difference in pelleting parameters, such as HPT, PDI, or pellet mill energy consumption, when pelleting EFC or CON diets. This is similar to the findings of Truelock et al. (2019), who observed only minor increases in HPT and no differences in PDI or energy consumption when EFC replaced CON in pelleted diets. In the current trial, an increase in soluble starch was observed in cooled pellets of EFC diets compared to CON pellets. Pelleting of corn-soybean meal-based diets is known to increase starch gelatinization (Lewis et al., 2015), an irreversible process potentiated by moisture and heat in the pelleting process. Furthermore, previous research has indicated that pelleted diets containing EFC have potential for greater gelatinized starch than pelleted CON diets (Truelock et al., 2019), possibly due to increased amylase activity in EFC. These observations likely explain the increase in soluble starch detected in the experiment herein.

Broiler diets containing EFC resulted in greater ADFI in the grower and finisher phases and increased ADG from 5 to 43 days of age compared to CON. Improvements in FCR when feeding EFC were only observed in the starter phase. There has been no previous research on the effects of EFC in poultry; however, studies in cattle and swine have revealed improvements in ADG of varying magnitudes. An experiment evaluating steam-flaked EFC in finishing cattle

diets revealed a 5.6% increase in ADG (Horton et al., 2017) while EFC in the diets of finishing pigs only tended to improve ADG by 1.2% (Ochonski et al., 2019). Although the current study is the first of its kind using EFC to supply exogenous α-amylase in broiler diets, there has been previous research in poultry examining effects of α-amylase enzyme preparations derived from various *Bacillus* species. In these experiments, exogenous α-amylase derived from *Bacillus amyloliquefaciens* or *Bacillus stearothermophilus* improved ADG in broilers consuming corn-soy diets by 4.5 to 9.4% and FCR by 3 to 4.2% for birds up to 42 d of age (Gracia et al., 2003; Onderci et al., 2006). Meanwhile, another study has reported no differences in ADG and poorer FCR when a *Bacillus licheniformis*-derived amylase was fed to broilers up to 42 d of age (Amerah et al., 2017).

For the experiment conduct herein, EFC increased broiler carcass weight and dressing percentage. There was no evidence that EFC influenced carcass characteristics in swine (Ochonski et al., 2019), although hot carcass weights of finishing cattle were 1.6% greater when steam-flaked EFC replaced CON in the diet (Horton et al., 2017). Supplementation of a bacterial derived α-amylase in broiler diets has revealed no effect on carcass weight (Amerah et al., 2017); however, these authors also reported no significant effects of an exogenous α-amylase on ADG or feed intake in 42-d-old broilers. In the present study, the increase in carcass weight observed in broilers consuming EFC compared to CON is likely explained by the associated increase in ADFI and ADG. The mechanism by which EFC affects carcass dressing percentage is not fully understood. It should be noted that aside from improving gut morphology and nutrient digestion (Gracia et al., 2003; Onderci et al., 2006) bacterial-derived exogenous α-amylase supplementation has also been reported to increase intestinal enzyme activity in 21-d-old broilers (Jiang et al., 2008). Specifically, the authors described a quadratic increase in intestinal protease

and trypsin activity with increasing amylase supplementation and no effect on the activity of intestinal lipase. This revelation may provide evidence for the ability of exogenous α-amylase to enhance protein digestion in relation to lipolysis such that dressing percentage is improved with amylase supplementation. Nonetheless, broiler feed efficiency calculated on a carcass basis was not different between treatments in the current experiment. Body composition was not determined in the present study, therefore it is not possible to determine if differences in energy deposition may have influenced results.

Finally, there was no evidence that conditioner retention time affected processing of EFC differently than CON nor did it have an impact on broiler performance. Increased conditioner retention time is known to improve pellet quality (Behnke, 2001), which in turn has been shown to influence broiler performance (Lilly et al., 2011). There was no evidence, however, that increasing conditioner retention time from 30 to 80 s had an effect on pellet quality, soluble starch, or broiler performance in the current study.

Overall, the results from this experiment suggest that replacing CON with EFC in poultry diets should have little effect on the efficacy of the pelleting process or the quality of pellets produced. The increased amylase content of EFC appears to enhance starch gelatinization during pelleting, leading to greater soluble starch in the pelleted diet and, theoretically, greater starch availability in the animal. Furthermore, there were improvements in ADG and carcass weight when EFC replaced CON in pelleted broiler diets. However, this did not translate to improved feed efficiency, as feed intake also increased.

REFERENCES

Al-Rabadi, G. J., B. J. Hosking, P. J. Torley, B. A. Williams, W. L. Bryden, S. G. Nielsen, J. L. Black, and M. J. Gidley. 2017. Regrinding large particles from milled grains improves growth performance of pigs. Anim. Feed Sci. Technol. 233:53-63.

Amerah, A. M., L. F. Romero, A. Awati, and V. Ravindran. 2017. Effect of exogenous xylanase, amylase, and protease, as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. Poult. Sci. 96:807-816.

Behnke, K. C. 2001. Factors influencing pellet quality. Feed Technol. 5:19-22.

Gracia, M. I., M. J. Araníbar, R. Lázaro, P. Medel, and G. G. Mateos. 2003. α-Amylase Supplementation of Broiler Diets Based on Corn. Poult. Sci. 82:436-442.

Hancock, J. D. and K. C. Behnke. 2001. Use of ingredient and diet processing technologies (grinding, mixing, pelleting, and extruding) to produce quality feeds for pigs. In: Swine Nutrition. Boca Raton, Florida, USA. pp. 469-498.

Horton, L. M., C. L. Van Bibber-Krueger, H. C. Muller, S. L. Katulski, T. J. Ellerman, and J. S. Drouillard. 2017. In vitro and in situ digestion characteristics and feedlot performance of cattle fed steam-flaked Enogen (high-amylase) feed corn. J. Anim. Sci. Suppl. 4:372-373.

Jiang, Z., Y. Zhou, F. Lu, Z. Han, and T. Wang. 2008. Effects of Different Levels of Supplementary Alpha-amylase on Digestive Enzyme Activities and Pancreatic Amylase mRNA Expression of Young Broilers. Asian-Aust. J. Anim. Sci. 21:97-102.

Lewis, L. L., C. R. Stark, A. C. Fahrenholz, J. R. Bergstrom, and C. K. Jones. 2015. Evaluation of conditioning time and temperature on gelatinized starch and vitamin retention in a pelleted swine diet. J. Anim. Sci. 93:615-619.

Lilly, K. G. S., C. K. Gehring, K. R. Beaman, P. J. Turk, M. Sperow, and J. S. Moritz. 2011. Examining the relationship between pellet quality, broiler performance, and bird sex. J. Appl. Poult. Res. 20:231-239.

Lundblad, K. K., S. Issa, J. D. Hancock, K. C. Behnke, L. J. McKinney, S. Alavi, E. Prestløkken, J. Fledderus, and M. Sørensen. 2011. Effects of steam conditioning at low and high temperature: expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. Anim. Feed Sci. Technol. 169:208-218.

Ochonski, P., F. Wu, E. Arkfeld, J. M. Lattimer, J. M. DeRouchey, S. S. Dritz, R. D. Goodband, J. C. Woodworth, and M. D. Tokach. 2019. Evaluation of High Amylase Corn on Growth Performance and Carcass Characteristics of Finishing Pigs. Kansas Agricultural Experiment Station Research Reports. Vol. 5: Iss. 8.

Onderci, M., N. Sahin, K. Sahin, G. Cikim, A. Aydín, I. Ozercan, and S. Aydín. 2006. Efficacy of Supplementation of α-Amylase-Producing Bacterial Culture on the Performance, Nutrient Use, and Gut Morphology of Broiler Chickens Fed a Corn-Based Diet. Poult. Sci. 85:505-510.

Rowe, J. B., M. Choct, and D. W. Pethick. 1999. Processing cereal grains for animal feeding. Aust. J. Agric. Res. 50:721-736.

Syngenta Crop Protection, LLC. 2019. Minnetonka, MN. http://www.syngenta-us.com/corn/enogen.

Truelock, C. N., M. D. Tokach, C. R. Stark, and C. B. Paulk. 2019. Pelleting and starch characteristics of diets containing high amylase corn. Kansas Agricultural Experiment Station Research Reports. Vol. 5: Iss. 8.

TABLES

Table 3.1 Ingredient and nutrient composition (as-is basis) of broiler diets containing either conventional or Enogen® Feed corn[1]

Phase:	Starter		Grower		Finisher	
Corn:[2]	CON	EFC	CON	EFC	CON	EFC
Ingredient, %						
Ground corn[1]	61.49	61.49	66.21	66.21	67.66	67.66
Soybean meal	32.75	32.75	28.00	28.00	25.40	25.40
Choice white grease	1.45	1.45	2.10	2.10	3.20	3.20
L-lysine HCl	0.22	0.22	0.17	0.17	0.19	0.19
DL-methionine	0.29	0.29	0.26	0.26	0.25	0.25
L-threonine	0.11	0.11	0.06	0.06	0.07	0.07
Monocalcium phosphate	1.42	1.42	1.12	1.12	1.14	1.14
Limestone	1.47	1.47	1.27	1.27	1.28	1.28
Salt	0.23	0.23	0.23	0.23	0.23	0.23
Vitamin/Mineral	0.25	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.23	0.23	0.23	0.23	0.23	0.23
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Analyzed nutrients, %						
Crude protein	21.4	20.6	18.9	18.7	17.3	18.4
Ether extract	3.2	3.3	4.2	3.8	5.0	4.5
ADF	3.4	3.7	3.3	2.7	3.2	3.0
Starch	38.86	39.1	43.5	45.1	42.6	42.9
Calcium	1.00	0.91	0.85	0.85	0.70	0.82
Phosphorous	0.68	0.67	0.60	0.61	0.54	0.55
Potassium	0.98	0.95	0.86	0.87	0.77	0.75
Sodium	0.18	0.17	0.17	0.17	0.15	0.18
Lysine	1.39	1.32	1.21	1.18	1.10	1.15
Methionine	0.57	0.53	0.51	0.52	0.45	0.49
Threonine	0.90	0.86	0.77	0.75	0.71	0.74
Tryptophan	0.28	0.26	0.24	0.24	0.21	0.22
Arginine	1.38	1.33	1.22	1.20	1.11	1.15
Valine	1.04	1.00	0.91	0.90	0.86	0.91
Isoleucine	0.95	0.91	0.82	0.81	0.77	0.83

[1] Enogen® Feed corn (Syngenta Crop Protection, Inc.) replaced conventional yellow dent corn on a kg:kg basis.
[2] CON = conventional yellow dent corn; EFC = Enogen® Feed corn.

Table 3.2 Effect of conventional or Enogen® Feed corn diets steam-conditioned for 30 or 80 s on hot pellet temperature, starch solubility, pellet durability index, and pellet mill energy consumption[1]

Corn type:	CON		EFC			Probability, <		
Conditioner retention time,[2] s:	30	80	30	80	SEM[3]	Corn type	Retention time	Corn × Retention
Production rate, kg/min	10.3	10.5	10.3	10.3	0.09	0.28	0.35	0.23
Conditioning temp, ºC	75.3	75.3	75.1	75.3	0.22	0.51	0.73	0.56
Hot pellet temp, ºC	84.4	84.9	84.1	84.9	0.48	0.54	0.03	0.72
Starch solubles, %								
Initial mash	2.9	3.0	2.7	2.8	0.19	0.15	0.41	0.90
Conditioned mash	2.7	2.7	2.8	2.7	0.14	0.73	0.73	0.57
Cooled pellets	5.5	5.3	6.0	6.1	0.26	0.01	0.67	0.52
Pellet durability index, %	84.6	85.1	84.4	83.9	5.67	0.39	0.99	0.58
Energy consumption, kWh/ton	23.0	22.2	22.6	22.4	0.55	0.79	0.30	0.49

[1] A corn-soy based poultry diet was mixed in a 907 kg Hayes & Stolz counterpoise mixer. The corn fraction of the diet was made up of conventional (CON) or Enogen® Feed corn (EFC) with EFC replacing CON on a kg:kg basis. Diets were steam-conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4 mm × 32 mm pellet die (L:D 8).

[2] The screw feeding the steam conditioner was set to achieve a production rate of 10 kg/min. Conditioner retention times were calculated by adjusting the conditioner screw speed and dividing the amount of feed in the conditioner by the production rate.

[3] Pooled standard error of least squares means (n = 3).

Table 3.3 Effect of conventional or Enogen® Feed corn diets steam-conditioned for 30 or 80 s on broiler performance[1]

Corn type:	CON		EFC			Probability, <		
Conditioner retention time,[2] s:	30	80	30	80	SEM[3]	Corn type	Retention time	Corn × Retention
BW, kg								
d 0	0.18	0.17	0.17	0.17	0.001	0.12	0.22	0.07
d 11	0.57	0.58	0.58	0.58	0.004	0.32	0.92	0.68
d 25	1.97	1.98	2.00	2.00	0.016	0.01	0.63	0.81
d 39	3.60	3.59	3.65	3.66	0.031	0.04	0.91	0.69
d 45	4.37	4.34	4.39	4.38	0.034	0.31	0.53	0.87
d 0 to 11								
ADG, g/d	36.25	36.46	36.96	36.65	0.333	0.16	0.89	0.41
ADFI, g/d	56.54	56.78	56.37	56.06	0.401	0.27	0.93	0.50
FCR	1.56	1.56	1.53	1.53	0.009	0.01	0.99	0.68
d 11 to 25								
ADG, g/d	99.14	99.64	101.28	101.62	0.941	0.01	0.57	0.91
ADFI, g/d	126.40	127.90	130.30	131.19	1.316	0.01	0.25	0.77
FCR	1.27	1.28	1.28	1.29	0.005	0.07	0.18	0.88
d 25 to 39								
ADG, g/d	116.22	115.17	117.17	118.23	1.336	0.14	1.00	0.43
ADFI, g/d	179.74	179.92	184.19	185.98	1.845	0.01	0.54	0.61
FCR	1.56	1.57	1.58	1.59	0.015	0.22	0.51	0.66
d 0 to 39								
ADG, g/d	87.76	87.57	89.06	89.44	0.779	0.03	0.90	0.68
ADFI, g/d	126.08	127.03	129.03	129.27	1.253	0.02	0.58	0.74
FCR	1.44	1.45	1.46	1.45	0.008	0.53	0.61	0.31
d 25 to 45								
ADG, g/d	119.36	117.48	118.95	117.80	1.175	0.97	0.21	0.76
ADFI, g/d	212.51	211.69	216.77	215.43	2.076	0.06	0.61	0.90
FCR	1.78	1.80	1.82	1.83	0.013	0.01	0.31	0.64
d 0 to 45								
ADG, g/d	93.07	92.49	93.74	93.29	0.743	0.33	0.49	0.93
ADFI, g/d	147.44	147.35	150.29	150.24	1.386	0.05	0.96	0.99
FCR	1.58	1.59	1.60	1.61	0.008	0.03	0.26	0.90
Removals, %	1.2	1.2	1.1	1.1	0.27	0.79	0.99	0.93

[1] A total of 1200 male broiler chicks were used in a 45-d experiment with 15 birds per pen and 20 pens per treatment. Chicks were fed a corn-soy based diet where the corn fraction of the diet was made up of conventional (CON) or Enogen® Feed corn (EFC) and EFC replaced CON on a kg:kg basis. A starter diet was fed from d 0 to d 11, a grower diet from d 11 to d 25, and a finisher diet from d 25 to d 45. In the finishing phase, chicks were weighed and harvested in two groups; half of the birds from each treatment were weighed and harvested on d 39, and the remaining chicks were weighed and harvested on d 45.

[2] Diets were steam-conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4 mm × 32 mm pellet die (L:D 8).

[3] Pooled standard error of least squares means (d 0 to d 25, $n = 20$; d 25 to d 45, $n = 10$).

Table 3.4 Effect of conventional or Enogen® Feed corn diets steam-conditioned for 30 or 80 s on broiler carcass characterisitics[1]

Corn type:	CON		EFC			Probability, <		
Conditioner retention time,[2] s:	30	80	30	80	SEM[3]	Corn type	Retention time	Corn × Retention
Carcass weight, kg								
d 39	2.50	2.50	2.57	2.57	0.022	0.01	0.86	0.94
d 45	3.06	3.05	3.11	3.11	0.041	0.03	0.89	0.67
Dressed carcass, %								
d 39	69.50	69.50	70.36	70.05	0.283	0.02	0.58	0.59
d 45	70.26	70.37	70.80	71.24	0.445	0.01	0.24	0.49
Carcass feed efficiency	2.06	2.07	2.06	2.06	0.022	0.81	0.79	0.75

[1] A total of 1200 male broilers were used in a 45-d experiment with 15 birds per pen and 20 pens per treatment. Chicks were fed a corn-soy based diet where the corn fraction of the diet was made up of conventional (CON) or Enogen® Feed corn (EFC) and EFC replaced CON on a kg:kg basis. Half of the birds from each treatment were harvested on d 39 and the remaining chicks were harvested on d 45.

[2] Diets were steam-conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4 mm × 32 mm pellet die (L:D 8).

[3] Pooled standard error of least squares means (n = 10).

Chapter 4: Effects of dietary lysine and exogenous protease inclusion on growth performance and apparent ileal amino acid digestibility in poultry

ABSTRACT

Two experiments were conducted to evaluate the effects of dietary Lys concentration and exogenous protease inclusion on growth performance and amino acid digestibility in poultry. In experiment 1, Cobb 500 broiler chicks (n = 480) were fed different concentrations of digestible Lys which corresponded to approximately 95, 97.5, 100, or 102.5% of the digestible Lys requirement based on breeder recommendations, and each diet was fed with or without an exogenous protease. In experiment 2, Hybrid turkey poults (n = 780) were fed diets formulated to provide low or adequate digestible Lys (approximately 91 and 100% of the NRC recommended requirement, respectively) with each diet being fed with or without an exogenous protease. Growth performance metrics were calculated from pen weights and feed consumption recorded throughout each experiment, and digestibility data was obtained from analysis of ileal contents. Data were analyzed using SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. Results of these experiments demonstrated broilers fed 1.12 and 1.21% digestible Lys diets with added protease had a 2-point improvement in FCR compared to chicks fed these diets without protease. Increasing digestible Lys concentration improved FCR in broilers and poults and improved BW, ADG, and ADFI in poults. There was no evidence that added protease had an effect on BW, ADG, or ADFI in broilers or poults. Finally, ileal amino acid digestibility was not affected by digestible Lys or protease inclusion for either 20-d old broiler chicks or 42-d old turkey poults.

Keywords: amino acid, broiler, digestibility, exogenous protease, lysine, turkey

INTRODUCTION

Protein is one of the most expensive nutrients in poultry diets. In an effort to minimize feed costs, protein digestion and utilization by the animal must be carried out as efficiently as possible. Biological tissues and metabolic processes used for growth and maintenance require certain amino acids, and if not provided by the diet, must withdraw and degrade protein from body stores to maintain function (National Research Council, 1994). Commonly in poultry diets, dietary protein is supplied by solvent extracted soybean meal, not only because of its high protein content, but also because of its amino acid profile. The ratios of essential amino acids within soybean meal make it one of the most desirable proteinaceous feed ingredients as it closely matches the balance of amino acids needed by the growing chick. However, the apparent ileal digestibility of soybean meal in broilers is approximately 82% (Ravindran et al., 2005), indicating 18% of the feedstuff remains undigested and is not utilized for growth. Furthermore, ileal digestibilities of the individual amino acids in soybean meal range from 75% to 88% for broilers (Ravindran et al., 2005) and from 70% to 95% in turkeys (Firman, 1992). In diets utilizing a byproduct protein source, such as meat and bone meal or feather meal, amino acid digestibility coefficients can be as low as 65%, suggesting a great potential for improvement.

Exogenous proteases are a nutritional tool designed to enhance protein digestion in livestock diets. They are added to the diet to supplement the activity of endogenous proteases that are secreted by the animal and drive protein degradation. Combining the effects of both proteases results in the potential for greater peptide bond cleavage, and thus, more efficient proteolysis. Improvements in protein digestion through the use of an exogenous protease may allow the quantity of protein in the diet to be lowered without impairing animal performance. Such a dietary modification could ultimately lower production expenses by reducing feed costs.

However, available research concerning exogenous protease use is inconsistent and work specifically in turkeys is limited. Thus, the following experiments were designed to determine the effect of exogenous protease inclusion on growth performance and amino acid digestibility in broiler and turkey diets.

MATERIALS AND METHODS

This research was conducted according to the experimental protocols approved by the Institutional Animal Care and Use Committee at Kansas State University.

Experiment 1

A total of 480 1-d-old male Cobb 500 broilers (Cobb-Vantress, Siloam Springs, AR) were used in a 20-d study to determine the effects of adding protease to diets with varying concentrations of digestible Lys on growth performance and digestion. Treatments consisted of a 2 × 4 factorial design with main effects of commercial protease (with or without) and digestible Lys (1.12, 1.15, 1.18, or 1.21%). Chicks were housed in 4 Petersime batteries, and treatments were randomly assigned to 80 cages within location block, resulting in 10 cages per treatment with 6 chicks per cage at placement. A commercial enzyme complex with 3 proteolytic activities was added to the protease diets at 125 g/tonne and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and Lys:amino acid ratios. Titanium dioxide was included in the diets at 0.5% as an indigestible marker.

Whole corn was ground to approximately 700 μm using a 3-high roller mill (RMS, Model 924). Experimental diets were mixed in a 907 kg horizontal counterpoise mixer (Hayes and Stolz, Fort Worth, TX) at the O. H. Kruse Feed Technology Innovation Center in Manhattan, KS (Table 4.1). To ensure uniform diet composition across treatments, the 1.12% and 1.21% digestible Lys treatments were each mixed in a single batch. Feed from each treatment was then

blended to create the intermediate (1.15% and 1.18%) treatments. Half of each treatment batch was mixed with the exogenous protease and half was mixed with sand to form the Lys treatments with protease and Lys treatments without protease, respectively. All diets were steam conditioned (Wenger twin staff pre-conditioner, Model 150) for approximately 30 s at 85ºC and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 4 mm × 25.4 cm pellet die. Pellets were cooled to room temperature in an experimental counter-flow cooler and crumbled (CME, EcoRoll 7).

Chicks were maintained on a 24-h lighting schedule in an environmentally controlled room with *ad libitum* access to feed and water. Each cage measured approximately 100 cm × 73 cm and was fitted with a single bulk feeder and waterer. In the case of mortality, chick weight, treatment, cage number, and date of mortality were recorded. Initial cage BW was measured at placement of chicks on d 1. Pen weights and feed consumption were measured on d 7, 14, and 20 for calculation of body weight gain, feed intake, and feed efficiency. Diet samples were collected for analysis of titanium dioxide and amino acid profile. On d 20, ileal contents from 2 chicks per pen were collected, composited by pen, and immediately frozen. Frozen ileal samples were subsequently freeze-dried and ground for analysis of titanium dioxide, crude protein, and amino acid profile for calculation of apparent ileal amino acid digestibility (AIAAD; Stein et al., 2007).

Experiment 2

A 42-d experiment was conducted to evaluate the effects of dietary Lys level and exogenous protease inclusion on growth performance and AIAAD in turkeys. A total of 780 1-d-old female poults (Hybrid Genetics, Ag Forte LLC, Aurora, MO) were weighed and randomly assigned to 1 of 32 floor pens with 24 or 25 poults per pen. Treatments were arranged in a 2 × 2 factorial of digestible Lys (low or adequate) and commercial protease (with or without).

Treatments were randomly assigned to floor pens within location block for a total of 8 replications per treatment. Poults were fed a starter diet from 1 to 28 d and a grower diet from 29 to 42 d (Table 4.2). Starter diets were formulated to provide 1.52% and 1.62% digestible Lys for the low and adequate digestible Lys treatments, respectively. Grower diets were formulated to provide 1.39% and 1.49% digestible Lys, respectively. A commercial enzyme complex with 3 proteolytic activities was added to the protease diets at 125 g/tonne, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and had equal amino acid ratios. Titanium dioxide was included in the grower diets at 0.5% as an indigestible marker for determination of AIAAD.

Whole corn was ground to approximately 700 μm in the starter phase and 900 μm in the grower phase using a hammermill (Bliss Industries, Model 22115). Experimental mash diets were mixed in a 907-kg horizontal counterpoise mixer (Hayes and Stolz, Fort Worth, TX) at the O. H. Kruse Feed Technology Innovation Center in Manhattan, KS. To ensure uniform diet composition across low digestible Lys treatments, a single batch of the low digestible Lys starter phase formulation was mixed and split in half. Half of the batch was mixed with the exogenous protease and half was mixed with sand to form the low Lys with protease and low Lys without protease treatments, respectively. For the protease diets, exogenous protease was hand-mixed with 2.5 kg of ground corn for 60 s before mixing with all other ingredients to facilitate uniform dispersion of the protease throughout the batch. The same method was followed for the addition of sand in diets not containing protease. These methods were repeated to form the adequate Lys treatments with and without protease for the starter phase, as well as the treatments for the grower phase.

Poults were maintained on a lighting schedule with 21 h of light and 3 h of dark in an environmentally controlled room with *ad libitum* access to feed and water. Poults were housed in 1.5 × 4.3 m floor pens with new pine shavings. Each pen was fitted with a single hanging metal feeder and a Lubing© EasyLine 4-cup drinker system. Feeders were shaken twice a day to ensure adequate feed flow to the feeder pan. In the case of mortality, poult weight, treatment, pen number, and date of mortality were recorded. Pen weights and feed consumption were measured on d 14, 28, and 42 for calculation of body weight gain, feed intake, and feed efficiency. Diet samples were collected from each pen during the grower phase and composited by treatment for analysis of titanium dioxide and amino acid profile. On d 42, ileal contents from 2 poults per pen were collected, composited by pen, and immediately frozen. Ileal samples were subsequently freeze-dried and ground for analysis of titanium dioxide, gross energy, crude protein, and amino acid profile for calculation of AIAAD (Stein et al., 2007).

Statistical Analysis

Experiment 1. Data were analyzed as a randomized complete block design using the MIXED procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) to evaluate interactive and main effects of Lys and protease. Increasing Lys level linear and quadratic polynomials were used for main effects of Lys. Results were considered significant at $P \leq 0.05$.

Experiment 2. Data were analyzed as a 2 × 2 factorial using the GLIMMIX procedure in SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. Main effects included dietary Lys and protease inclusion. Results were considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Experiment 1

There was no evidence of a Lys × protease interaction ($P > 0.05$) for BW, ADG or ADFI (Table 4.3). There was a Lys × protease interaction (quadratic, $P < 0.05$) for FCR from d 1 to 20. Chicks fed 1.12 and 1.21% digestible Lys diets with added protease had a 2-point improvement in FCR compared to chicks fed these diets without protease. There was no difference in FCR between birds consuming diets with or without protease when fed 1.15 and 1.18% digestible Lys diets. There was no evidence of difference ($P > 0.10$) in ADG or ADFI due to dietary Lys concentration throughout the feeding period. However, from d 1 to 20, broiler FCR was improved (linear, $P < 0.01$) by increasing dietary digestible Lys from 1.12 to 1.21%. There was no evidence ($P > 0.10$) that added protease had an effect on BW, ADG, ADFI, or FCR.

There was no evidence of a Lys × protease interaction ($P > 0.09$) or main effect of dietary digestible Lys concentration or protease inclusion ($P > 0.12$) on AIAAD of Lys, Arg, Met, Cys, Thr, Ile, Leu, Val, or Trp (Table 4.4).

Experiment 2

There was no evidence of a Lys × protease interaction ($P > 0.12$) for BW, ADG, ADFI, or FCR (Table 4.5). On d 28 and 42, poults fed adequate digestible Lys had greater ($P < 0.01$) BW than those fed low digestible Lys diets. From d 1 to 28, poults fed diets containing adequate digestible Lys concentrations had improved ($P < 0.01$) ADG, ADFI, and FCR compared to those fed the low Lys diets. From d 1 to 28, there was no evidence of difference ($P > 0.43$) in poult performance due to protease inclusion. From d 29 to 42, poults fed diets containing adequate digestible Lys concentrations had improved ($P < 0.04$) ADG, ADFI, and FCR compared to those fed the low Lys diets. Poults had increased ($P < 0.03$) ADG and ADFI when fed an exogenous

protease compared to those fed diets without added protease. There was no evidence of difference in final BW at d 42 ($P = 0.06$) or FCR in the grower phase ($P = 0.53$) due to protease inclusion. For the overall experiment (d 0 to 42), poults fed diets containing adequate digestible Lys concentrations had improved ($P < 0.01$) ADG, ADFI, and FCR compared to those fed the low Lys diets. There was no evidence of difference ($P > 0.14$) in poult performance due to protease inclusion.

There was a Lys × protease interaction ($P = 0.01$) for AIAAD of Trp (Table 4.6). There was no evidence of difference in AIAAD coefficients of Trp in the low digestible Lys diets with or without protease. However, in the adequate digestible Lys diets, poults not receiving protease had greater AIAAD of Trp than those consuming protease. There was no evidence of difference ($P > 0.09$) in AIAAD coefficients of Arg, Met, Cys, Thr, Ile, Leu, Lys or Val due to dietary digestible Lys or protease inclusion.

Improvements in BW and FCR with increasing dietary digestible Lys observed for both broiler chicks and turkey poults was expected and pairs with previous work (Kidd et al., 1998; Selle et al., 2007). In the current study, there was no response in broiler ADG to increasing digestible Lys up to 1.21%. This is different from previous research which has reported a 1.43% and 1.34% increase in ADG and gain:feed ratio, respectively, with increasing digestible Lys concentrations fed to 10-d-old Ross 308 broilers consuming an average of 14.5 g/d (Lee et al., 2018). A digestible Lys requirement of 1.62% in the starter phase was used in the turkey experiment per breeder recommendations. However, Boling and Firman (1998) and Firman and Boling (1998) suggest the digestible Lys requirement during this phase is much lower, approximately 1.32 to 1.34%. The present research would dispute this claim since a performance response to increased digestible Lys concentrations above 1.32% was observed. It should be

noted that feed intake of poults in the present study was 3 to 7 g/d lower than in the cited literature, which could help explain the increase in response to lysine in the current trial.

Available literature suggests the efficacy of exogenous protease supplementation in poultry diets is variable. Several researchers have reported a beneficial effect of protease on feed efficiency (Angel et al., 2011; Freitas et al., 2011; Vieira et al., 2013) and increases in BW gain and FI (Angel et al., 2011), while others have found no effect of protease on growth performance (Ding et al., 2016). While there are known differences in protease efficacy due to protease type (Ghazi et al., 2002; Cowieson and Roos, 2016), each of these studies used the same protease which was produced by fermentation of *Bacillus licheniformis* containing transcribed genes from *Nocardiopsis prasina*. The protease herein tested is a fermentation soluble with three proteolytic activities, which cuts amino acid sequences in more locations than endogenous enzymes. In the present broiler and turkey trials, BW results were similar in that no effect of protease supplementation was observed for BW in chicks or poults. These results are consistent with those of Freitas et al. (2011) and Vieira et al. (2013) who found that protease supplementation up to 1600 mg/kg was not sufficient to recover the weight gain lost from lowering the dietary CP by 4.4%, and neither was 200 mg/kg enough to overcome weight loss due to a 1% decrease in dietary CP.

Differences in FCR among treatments were observed for chicks, but not for poults in the present study. Even though protease supplementation had no effect on BW or ADFI in broilers up to 20 d of age, protease-supplemented diets containing 1.12 and 1.21% digestible Lys resulted in improved broiler FCR compared to the same diets without protease. Similarly, in a 42-d experiment with broiler chicks, Ding et al. (2016) reported a 2-point improvement in FE from d 1 to 21 when protease supplementation increased from 0 to 300 mg/kg, but observed no benefit of

protease on FCR beyond 21 d of age. The results of this study would suggest an apparent improvement in FE by way of increased nutrient availability; however, neither apparent nor standardized ileal amino acid digestion were affected by protease supplementation in broilers.

Vieira et al. (2013) found no effect of exogenous protease inclusion on BW gain or ADFI in turkeys up to 26 d of age. These results match the data in the current study in which no differences in ADG or ADFI were observed in turkeys up to 28 d of age. Vieira et al. (2013) also reported a benefit in FCR due to the addition of 200 mg/kg of an exogenous protease to a low protein diet which was not observed in the present study. Rather, in this trial, poults receiving protease-supplemented diets experienced greater ADG and ADFI in the grower phase from d 29 to 42.

It should be noted that this turkey experiment was conducted from May to July, and outdoor ambient temperatures averaged 34ºC during the 42-d experimental period with average temperatures rising to 37ºC during the last 2 weeks of the study. Thus, despite attempts to provide adequate ventilation and airflow in the barn, it is possible poults experienced heat stress over the course of the study, especially during the grower phase. Depressions in feed intake are known to occur at temperatures exceeding the poult's thermoneutral zone (National Resource Council, 1994; Quinteiro-Filho et al., 2012), which is above approximately 24ºC. The potential impacts these environmental factors may have had on the response to protease treatment is not entirely known. Nonetheless, at 28 and 42 d of age, poults' BW and feed intake were below breed estimates. Theoretically, a decrease in feed intake, and thus protein intake, would perhaps work in the favor of protease supplementation because some researchers reported an increase in protease efficacy in low protein diets or diets with lower protein digestibility (Cowieson and Roos, 2014).

Contrary to these data, a meta-analysis conducted by Cowieson and Roos (2014) reported that apparent ileal digestibility of all amino acids except Trp increased by an average of 3.74% over control diets when an exogenous protease was included in monogastric diets. The authors found this improvement in digestibility was not dependent on geographic location, diet composition, or monogastric species, but rather could be partially explained by the digestibility of amino acids in the control diet. Modeling the protease response, the authors determined protease efficacy doubles with every 10% decrease in amino acid digestibility. In the present broiler and turkey experiments, mean amino acid digestibility coefficients for the low digestible Lys diets without protease were greater than those reported for the control diets by Cowieson and Roos (2014; 0.824, 0.870, and 0.798, respectively). Thus, the lack of improvement in AIAAD in response to protease inclusion in the studies herein is in alignment with the conclusions of Cowieson and Roos (2014), as AIAAD of the control diets was already high, offering little potential for improvement.

REFERENCES

Angel, C. R., W. Saylor, S. L. Vieira, and N. Ward. 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poult. Sci. 90:2281-2286.

Boling, S. D. and J. D. Firman. 1998. Digestible lysine requirement of female turkeys during the starter period. Poult. Sci. 77:547-551.

Brito, C. O., L. F. T. Albino, H. S. Rostagno, P. C. Gomes, L. R. Nery, and E. A. Silva. 2009. Aminoacidic diet in determining ileal endogenous losses in broiler chickens: a methodological proposal. R. Bras. Zootec. 38:2161-2166.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Tech. 119:293-305.

Cowieson, A. J. and F. F. Roos. 2014. Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. J. Appl. Anim. Nutr. 2:1-8.

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

Ding, X. M., D. D. Li, Z. R. Li, J. P. Wang, Q. F. Zeng, S. P. Bai, Z. W. Su, and K. Y. Zhang. 2016. Effects of dietary crude protein levels and exogenous protease on performance, nutrient digestibility, trypsin activity and intestinal morphology in broilers. Livest. Sci. 193:26-31.

Firman, J. D. 1992. Amino acid digestibilities of soybean meal and meat meal in male and female turkeys of different ages. J. Appl. Poult. Res. 1:350-354.

Firman, J. D. and S. D. Boling. 1998. Ideal protein in turkeys. Poult. Sci. 77:105-110.

Ford, J. E. and C. Shorrock. 1971. Metabolism of heat-damaged proteins in the rat. Br. J. Nutr. 26:311.

Freitas, D. M., S. L. Vieira, C. R. Angel, A. Favero, and A. Maiorka. 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. J. Appl. Poult. Res. 20:322-334.

Ghazi, S., J. A. Rooke, H. Galbraith, and M. R. Bedford. 2002. The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. Br. Poult. Sci. 43:70-77.

Kidd, M. T., B. J. Kerr, K. M. Halpin, G. W. McWard, and C. L. Quarles. 1998. Lysine levels in starter and grower-finisher diets affect broiler performance and carcass traits. J. Appl. Poult. Sci. 7:351-358.

Lee, J., Y. Sung, and C. Kong. 2018. Standardized ileal digestible lysine requirement of male broilers at the age of 0-10 days. Anim. Feed Sci. Technol. 241:55-62.

National Research Council. 1994. Nutrient Requirement of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Quinteiro-Filho, W. M., M. V. Rodrigues, A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, L. R. M. Sá, A. J. P. Ferreira, and J. Palermo-Neto. 2012. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hypothalamic-pituitary-adrenal axis activation. J. Anim. Sci. 90:1986-1994.

Ravindran, V., L. I. Hew, G. Ravindran, and W. L. Bryden. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. J. Anim. Sci. 81:85-97.

Selle, P. H., V. Ravindran, G. Ravindran, and W. L. Bryden. 2007. Effects of dietary lysine and microbial phytase on growth performance and nutrient utilization of broiler chickens. Asian-Aust. J. Anim. Sci. 20:1100-1107.

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285.

Vieira, S. L. C. R. Angel, D. J. A. Miranda, A. Favero, R. F. A. Cruz, and J. O. B. Sorbara. 2013. Effects of a monocomponent protease on performance and protein utilization in 1- to 26-day-of-age turkey poults. J. Appl. Poult. Res. 22:680-688.

Willis, S. 2003. The use of soybean meal and full fat soybean meal by the animal feed industry. Pages 1-8 in Proc. 12th Australian Soybean Conf., Toowoomba, Australia.

TABLES

Table 4.1 Composition (as-fed basis) of broiler diets with increasing dietary Lys with and without added protease[1]

	Digestible Lys, %			
	1.12	1.15	1.18	1.21
Ingredient, %				
Ground corn	60.26	--[2]	--	56.16
Soybean meal	32.50	--	--	36.00
Choice white grease	2.00	--	--	2.60
Monocalcium phosphate	2.10	--	--	2.05
Limestone	1.40	--	--	1.40
Salt	0.23	--	--	0.23
L-Lysine HCl	0.15	--	--	0.15
DL-Methionine	0.23	--	--	0.26
L-Threonine	0.08	--	--	0.09
L-Valine	0.00	--	--	0.01
Vitamin premix[3]	0.25	--	--	0.25
Choline chloride	0.10	--	--	0.10
Sodium bicarbonate	0.20	--	--	0.20
Titanium dioxide[4]	0.50	--	--	0.50
Calculated nutrients				
ME, kcal/kg	3,001	--	--	3,001
Crude protein, %	21.10	--	--	22.48
Isoleucine, %	0.79	--	--	0.84
Leucine, %	1.63	--	--	1.70
Lysine, %	1.12	--	--	1.21
Methionine, %	0.52	--	--	0.56
Threonine, %	0.74	--	--	0.80
Tryptophan, %	0.21	--	--	0.23
Valine, %	0.86	--	--	0.93
Calcium, %	1.00	--	--	1.00
Phosphorus, %	0.83	--	--	0.83
Analyzed nutrients				
GE, kcal/g	3,835	3,856	3,870	3,838
Crude protein, %	20.50	20.80	21.20	21.40
Isoleucine, %	0.92	0.92	0.92	0.97
Leucine, %	1.74	1.75	1.76	1.83
Lysine, %	1.30	1.32	1.30	1.37
Methionine, %	0.51	0.51	0.52	0.55
Threonine, %	0.83	0.85	0.84	0.89
Tryptophan, %	0.28	0.28	0.27	0.29
Valine, %	1.00	1.01	1.00	1.06

[1]Day-old broilers were fed starter diets with increasing concentrations of dietary Lys for 20 d. Each diet was fed with and without an exogenous protease which was added to the feed at 125 g/tonne.
[2]Diets formulated to provide 1.12 and 1.21% digestible Lys were blended to create the intermediate digestible Lys diets.
[3]Provided per kg of premix: vitamin A, 3,086,440 IU; vitamin D_3, 1,102,300 IU; vitamin E, 6,614 IU; vitamin B_{12}, 4 mg; menadione, 331 mg; riboflavin, 2,646 mg; D-pantothenic acid, 2,646 mg; thiamine, 441 mg; niacin, 11,023 mg; vitamin B_6, 551 mg; folic acid, 276 mg; biotin, 13 mg.
[4]Titanium dioxide was added as an indigestible marker for determination of apparent ileal amino acid digestibility.

Table 4.2 Composition (as-fed basis) of turkey mash diets with low or adequate dietary Lys with and without added protease[1]

Diet phase:	Starter		Grower	
Digestible Lys:	Low	Adequate	Low	Adequate
Ingredient, %				
Ground corn	44.15	39.25	49.96	45.31
Soybean meal	45.00	49.20	39.00	43.00
Soybean oil	4.00	4.70	4.00	4.65
Monocalcium phosphate	3.15	3.15	3.00	3.00
Limestone	2.05	2.02	1.85	1.82
Salt	0.36	0.36	0.30	0.30
L-Lysine HCl	0.27	0.27	0.30	0.30
DL-Methionine	0.34	0.37	0.28	0.31
L-Threonine	0.08	0.08	0.08	0.08
Vitamin premix[2]	0.20	0.20	0.20	0.20
Mineral premix	0.20	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.00	0.00	0.13	0.13
Titanium dioxide[3]	0.00	0.00	0.50	0.50
Calculated nutrients				
ME, kcal/kg	2,962	2,964	2,954	2,951
Crude protein, %	26.0	27.7	25.5	27.0
Arginine, %	1.55	1.67	1.53	1.63
Isoleucine, %	0.99	1.06	0.98	1.04
Leucine, %	1.89	1.98	1.87	1.96
Lysine, %	1.52	1.62	1..39	1.49
Methionine, %	0.68	0.72	0.63	0.65
Threonine, %	0.90	0.96	0.89	0.91
Tryptophan, %	0.28	0.30	0.27	0.29
Valine, %	1.06	1.13	1.05	1.11
Calcium, %	1.45	1.45	1.33	1.34
Phosphorus, %	1.09	1.10	1.05	1.07
Analyzed nutrients				
Arginine, %	1.61	1.80	1.52	1.62
Isoleucine, %	1.13	1.23	1.04	1.10
Leucine, %	2.02	2.16	1.88	1.96
Lysine, %	1.67	1.81	1.58	1.63
Methionine, %	0.64	0.71	0.57	0.59
Threonine, %	1.00	1.06	0.95	0.97
Tryptophan, %	0.30	0.33	0.29	0.31
Valine, %	1.21	1.31	1.11	1.18

[1]Starter diets were formulated to provide 1.52% and 1.62% digestible Lys for the low and adequate Lys treatments, respectively, and grower diets were formulated to provide 1.39% and 1.49% digestible Lys, respectively. Starter diets were fed from 0 to 28 d, and grower diets were fed from 29 to 42 d.
[2]Provided per kg of premix: vitamin A, 13,200,000 IU; vitamin D_3, 3,960,000 IU; vitamin E, 66,000 IU; vitamin B_{12}, 39.6 mg; menadione, 3,960 mg; riboflavin, 13,200 mg; D-pantothenic acid, 22,000 mg; thiamine, 3,960 mg; niacin, 110,000 mg; vitamin B_6, 7,920 mg; folic acid, 2,200 mg; biotin, 253 mg.
[3]Titanium dioxide was added to grower diets as an indigestible marker for determination of apparent ileal amino acid digestibility.

Table 4.3 Effect of dietary Lys and exogenous protease inclusion on broiler growth performance[1]

										Probability, $P <$				
	No protease[2]				Protease					Lys			Lys × Protease	
Dietary Lys,[3] %:	1.12	1.15	1.18	1.21	1.12	1.15	1.18	1.21	SEM[4]	Linear	Quadratic	Protease	Linear	Quadratic
BW, g														
d 0	40.7	40.7	40.7	40.6	40.6	40.7	40.6	40.5	0.14	0.425	0.385	0.385	0.638	0.891
d 20	805.5	801.1	805.0	820.5	815.4	804.9	813.0	823.2	9.90	0.198	0.146	0.380	0.779	0.976
d 0 to 20														
ADG, g	37.9	38.0	38.2	38.2	38.4	38.1	38.6	38.7	0.52	0.456	0.796	0.350	0.939	0.742
ADFI, g	47.4	47.0	46.7	46.6	47.3	47.1	47.4	46.5	0.57	0.179	0.779	0.650	0.897	0.532
FCR	1.25[a]	1.24[ab]	1.22[b]	1.22[bc]	1.23[ab]	1.24[ab]	1.23[ab]	1.20[c]	0.007	0.001	0.226	0.181	0.925	0.035

[1]A total of 480 broilers were used in a 20-d study with 10 replicate pens per treatment and 6 broilers per pen.

[2]An exogenous protease was added to protease treatments at 125 g/tonne, and sand replaced protease at the same inclusion level in treatments not containing protease.

[3]Diets were formulated to provide 1.12, 1.15, 1.18, or 1.21% digestible Lys, approximately 95, 97.5, 100, and 102.5% of the digestible Lys requirement, respectively.

[4]Pooled standard error of least squares means (n = 10).

[abc]Means within a row without a common superscript differ ($P < 0.05$).

Table 4.4 Effect of dietary Lys and exogenous protease inclusion on apparent ileal amino acid digestibility (AIAAD) coefficients in broilers[1]

	No protease[2]		Protease			Probability, $P <$		
Dietary Lys,[3] %:	1.12	1.21	1.12	1.21	SEM[4]	Protease	Lys	Lys × Protease
Arginine	88.3	89.6	88.9	87.9	0.006	0.39	0.88	0.09
Cysteine	70.7	72.6	70.6	72.8	0.013	0.95	0.12	0.88
Isoleucine	81.8	82.9	81.6	80.7	0.009	0.19	0.95	0.28
Leucine	82.7	83.4	82.5	81.5	0.009	0.25	0.89	0.35
Lysine	85.6	86.8	85.6	84.5	0.009	0.21	1.00	0.21
Methionine	92.2	92.7	92.0	91.5	0.006	0.26	0.99	0.37
Threonine	76.2	77.3	76.4	77.2	0.010	0.97	0.35	0.86
Tryptophan	85.4	84.3	84.3	84.9	0.006	0.72	0.68	0.19
Valine	78.4	79.4	78.4	78.0	0.010	0.48	0.75	0.51

[1]A total of 64 broilers were used to determine AIAAD on d 20 with 8 replicate pens per treatment. Ileal contents from 2 broilers per pen were composited and AIAAD was calculated according to Stein et al. (2007).

[2]An exogenous protease was added to protease treatments at 125 g/tonne, and sand replaced protease at the same inclusion level in treatments not containing protease.

[3]Diets were formulated to provide 1.12, 1.15, 1.18, or 1.21% digestible Lys, approximately 95, 97.5, 100, and 102.5% of the digestible Lys requirement, respectively.

[4]Pooled standard error of least squares means (n = 8).

Table 4.5 Effect of dietary Lys and exogenous protease inclusion on turkey growth performance[1]

	No protease[2]		Protease			Probability, $P <$		
Dietary Lys:[3]	Low	Adequate	Low	Adequate	SEM[4]	Lys	Protease	Lys × Protease
BW, kg								
d 1	0.06	0.06	0.06	0.06	0.000	0.64	0.96	0.57
d 28	1.02	1.07	1.03	1.08	0.010	0.01	0.18	0.97
d 42	2.20	2.31	2.23	2.35	0.020	0.01	0.06	0.79
d 0 to 28								
ADG, g	34.03	35.86	34.14	36.34	0.364	0.01	0.43	0.62
ADFI, g	48.30	49.84	48.61	50.07	0.654	0.01	0.58	0.94
FCR	1.43	1.40	1.43	1.39	0.008	0.01	0.58	0.14
d 29 to 42								
ADG, g	84.75	88.71	85.87	90.44	0.781	0.01	0.03	0.63
ADFI, g	139.63	143.89	141.85	147.64	1.612	0.01	0.02	0.54
FCR	1.66	1.63	1.66	1.64	0.011	0.04	0.53	0.77
d 0 to 42								
ADG, g	50.98	53.51	51.26	54.40	0.479	0.01	0.17	0.47
ADFI, g	78.74	81.17	79.38	82.55	0.886	0.01	0.14	0.58
FCR	1.55	1.52	1.56	1.52	0.009	0.01	0.75	0.80

[1]A total of 780 female poults were used in a 42-d study with 24 or 25 birds per pen and 8 pens per treatment.
[2]An exogenous protease was added to protease treatments at 125 g/tonne, and sand replaced protease at the same inclusion level in treatments not containing protease.
[3]Low Lys diets were formulated to provide 1.52% digestible Lys from 0 to 28 d and 1.39% digestible Lys from 29 to 42 d. Adequate Lys diets were formulated to provide 1.62% digestible Lys from 0 to 28 d and 1.49% digestible Lys from 29 to 42 d.
[4]Pooled standard error of least squares means (n = 8).

Table 4.6 Effect of dietary Lys and exogenous protease inclusion on apparent ileal amino acid digestibility (AIAAD) coefficients in turkeys[1]

	No protease[2]		Protease			Probability, $P <$		
Dietary Lys:[3]	Low	Adequate	Low	Adequate	SEM[4]	Lys	Protease	Lys × protease
Arginine	91.3	92.0	91.0	91.6	0.006	0.24	0.59	0.97
Cysteine	77.4	79.7	77.4	75.8	0.016	0.85	0.22	0.23
Isoleucine	86.2	87.6	86.1	86.6	0.008	0.29	0.50	0.62
Leucine	87.1	88.3	86.8	87.0	0.008	0.37	0.33	0.54
Lysine	90.5	91.3	90.2	90.3	0.006	0.49	0.29	0.51
Methionine	94.4	95.2	94.8	94.4	0.003	0.59	0.54	0.09
Threonine	83.1	85.5	83.3	82.5	0.010	0.45	0.19	0.12
Tryptophan	89.3[b]	91.7[a]	89.8[ab]	88.6[b]	0.007	0.41	0.06	0.01
Valine	83.4	85.7	83.1	83.8	0.010	0.16	0.30	0.45

[1]A total of 64 poults were used to determine AIAAD on d 42 with 8 replicate pens per treatment. Ileal contents from 2 poults per pen were composited and AIAAD was calculated according to Stein et al. (2007).

[2]An exogenous protease was added to protease treatments at 125 g/tonne, and sand replaced protease at the same inclusion level in treatments not containing protease.

[3]Low Lys diets were formulated to provide 1.39% digestible Lys in the grower phase, and adequate Lys diets were formulated to provide 1.49% digestible Lys in the grower phase.

[4]Pooled standard error of least squares means (n = 8).

[abc]Means within a row without a common superscript differ ($P < 0.05$).

Chapter 5: Summary of findings

Understanding the effect of exogenous enzymes during pelleting and their subsequent effect on broiler performance is of upmost importance as enzyme use in poultry diets continues to increase. Previous research has shown how conditioning temperature during pelleting can negatively influence exogenous enzyme activity, and it was the aim of the current studies to identify further pelleting parameters capable of altering enzyme activity. An additional objective was to evaluate the response to dietary exogenous enzyme inclusion by broiler chickens and turkey poults.

Results of Chapter 1 suggest caution should be taken by feed manufacturers when pelleting diets containing phytase. The data indicated a negative linear relationship between conditioning temperature and phytase stability that is influenced by phytase source. Additionally, increasing conditioning temperature increases pellet durability and hot pellet temperature. Even at the lowest conditioning temperature of 74°C, maximum phytase stability averaged 63% in pellets. There was no evidence that conditioner retention time or die L:D affected phytase stability, and increasing steam pressure tended to improve phytase recovery by 18% in pellets.

Results of Chapter 2 indicated that pelleting can be used as a means of increasing starch gelatinization in corn, soybean meal-based diets with the majority of gelatinization occurring across the pellet die. Additionally, pelleting with a larger die L:D improves pellet quality but can dramatically increase HPT and pellet mill energy consumption. Finally, diets containing EFC may have an even greater potential of increasing gelatinized starch during pelleting compared to CON due to the high amylase activity present in EFC.

Results of Chapter 3 indicated that replacing CON with EFC in poultry diets should have little effect on the efficacy of the pelleting process or the quality of pellets produced. The

increased amylase content of EFC appears to enhance starch gelatinization during pelleting, leading to greater soluble starch in the pelleted diet and, theoretically, greater starch availability in the animal. Furthermore, there were improvements in ADG and carcass weight when EFC replaced CON in pelleted broiler diets. However, this did not translate to improved feed efficiency as feed intake also increased.

Results of Chapter 4 indicated that broilers fed 1.12 and 1.21% digestible Lys diets with added protease had a 2-point improvement in FCR compared to chicks fed these diets without protease. Increasing digestible Lys concentration improved FCR in broilers and poults and improved BW, ADG, and ADFI in poults. There was no evidence that added protease had an effect on BW, ADG, or ADFI in broilers or poults. Finally, ileal amino acid digestibility was not affected by digestible Lys or protease inclusion for either 20-d old broiler chicks or 42-d old turkey poults.

In conclusion, there are many factors present in the pelleting process that are capable of altering exogenous enzyme activity, such as phytase, and the pelleting parameters that maximize pellet quality are in direct opposition to those that maximize enzyme recovery. For other enzymes, such as amylase, pelleting reveals the potential for an improvement in nutrient availability as the enzyme works in concert with heat and moisture in the process to improve starch gelatinization. Increases in nutrient availability via pelleting can then translate to greater nutrient digestion in broiler chickens. Finally, protease inclusion in poultry diets provided minimal benefits for performance in broiler chicks or turkey poults.