The effect of pelleting parameters on phytase stability and pellet quality

by

Marut Saensukjaroenphon

B.S., Silapakorn University, 2001
M.S., Kansas State University, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science & Industry
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Abstract

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in animal feed. Temperature and moisture content have been identified as two factors that can influence enzyme inactivation. Thus, exogenous phytases were developed to tolerate the high temperature reached during pelleting and the low pH in the stomach. It is hypothesized that there are many factors that can account for phytase denaturing during the pelleting process, such as pellet mill model, die length to diameter ratio (L:D), conditioner or die retention time, and steam quality. Moreover, phytase may be further degraded in feed samples if moisture is left in the sample and the sample is not properly stored prior analysis. The objectives of this dissertation were to determine the effects of pelleting parameters, moisture content in the mash feed or sample, sample preparation, storage condition or storage time of the sample prior to analysis, and phytase analysis method on phytase stability or pellet quality. The first experiment was conducted to evaluate the effect of the following factors: cooling method, sample preparation, storage condition and storage time on phytase denaturation after steam conditioning or pelleting. The results of the first experiment indicated that freeze-drying, vacuum sealing and freezing were not required when the sample is analyzed within 3 weeks of production. The pellet temperature, pellet moisture and cooling time after the pellet die did not affect phytase stability. The second experiment investigated mash moisture content and exposure time in feed prior to pelleting and the subsequent effect on phytase denaturation post-pelleting. The results of the second experiment indicated that the stability of phytase was not affected when feed was stored in a bin up to 2 hr. prior to pelleting. The added water in the mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei*

phytase stability. Increasing moisture content of mash feed by 0.6% did not improve pellet quality. The third experiment was conducted to determine the effect of die retention time on phytase stability. The results of the third experiment indicated that the phytase was stable up to 88°C. The hot pellet temperature should be measured when enzymes are added to the feed to help monitor phytase stability. The die L:D is one of the most important factors for pellet quality. Increasing die retention time by reducing production rate improved pellet quality but may reduce phytase stability. The fourth and fifth experiments were designed to determine the effect of corn starch inclusion level, fines inclusion level, die thickness and conditioning temperature on pellet quality. The results demonstrated that increasing conditioning temperature or die thickness increased pellet quality. Increasing the ratio of corn starch to corn protein resulted in lower PDI when the diet contained more than 60% ground corn. When a diet contained less than 1.5% oil, fines returned from the sifter improved pellet quality.

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Approved by:

Major Professor
Dr. Charles R. Stark

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Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in animal feed. Temperature and moisture content have been identified as two factors that can influence enzyme inactivation. Thus, exogenous phytases were developed to tolerate the high temperature reached during pelleting and the low pH in the stomach. It is hypothesized that there are many factors that can account for phytase denaturing during the pelleting process, such as pellet mill model, die length to diameter ratio (L:D), conditioner or die retention time, and steam quality. Moreover, phytase may be further degraded in feed samples if moisture is left in the sample and the sample is not properly stored prior analysis. The objectives of this dissertation were to determine the effects of pelleting parameters, moisture content in the mash feed or sample, sample preparation, storage condition or storage time of the sample prior to analysis, and phytase analysis method on phytase stability or pellet quality. The first experiment was conducted to evaluate the effect of the following factors: cooling method, sample preparation, storage condition and storage time on phytase denaturation after steam conditioning or pelleting. The results of the first experiment indicated that freeze-drying, vacuum sealing and freezing were not required when the sample is analyzed within 3 weeks of production. The pellet temperature, pellet moisture and cooling time after the pellet die did not affect phytase stability. The second experiment investigated mash moisture content and exposure time in feed prior to pelleting and the subsequent effect on phytase denaturation post-pelleting. The results of the second experiment indicated that the stability of phytase was not affected when feed was stored in a bin up to 2 hr. prior to pelleting. The added water in the mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei*

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Table of Contents

List of Figures xii
List of Tables xiii
Acknowledgements xvi
Chapter 1 - Effect of Cooling Method, Sample Preparation, Storage Condition and Storage Time on Phytase Activity of a Corn-Soy Swine Diet 1
Abstract 1
Introduction 3
Materials and Methods 4
Experiment 1 4
Experiment 2 4
Experiment 3 5
Data collection 6
Statistical Analysis 7
Results and Discussion 8
Conclusion 11
References 12
Figures and Tables 13
Chapter 2 - The Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality 20
Abstract 20
Introduction 22
Materials and Methods 23
Data collection 24
Statistical Analysis 25
Results and Discussion 26
Conclusion 30
References 31
Tables 33

Chapter 3 - Effect of Die Retention Time on Pellet Quality and Phytase Stability of A Corn-Soy Swine Diet 40
Abstract 40
Introduction 42
Materials and Methods 43
Data collection 43
Statistical analysis 44
Results and Discussion 45
Conclusion 47
References 49
Tables 50
Chapter 4 - The Effect of Different Inclusion Levels of Corn Starch and Fine Ground Corn with Different Conditioning Temperature or Die Thickness on Pellet Quality 54
Abstract 54
Introduction 56
Materials and Methods 57
Experiment 1 57
Experiment 2 57
Data collection 58
Statistical Analysis 58
Results and Discussion 59
Experiment 1 59
Experiment 2 60
Conclusion 61
References 62
Tables 63
Chapter 5 - The Impact of Fines Inclusion Level and Conditioning Temperature on Pellet Quality and Energy Consumption 69
Abstract 69
Introduction 71
Materials and Methods 72

Experiment 1 72
Experiment 2 72
Data collection 73
Statistical Analysis 74
Results and Discussion 75
Experiment 1 75
Experiment 2 75
Conclusion 78
References 79
Figures and Tables 80
Chapter 6 - Effect of Feed Form, Corn Particle Size and Extrusion of Corn on Broiler Performance 88
Abstract 88
Introduction 90
Materials and Methods 91
Ingredient and Extrusion Conditions 91
Diet 91
Feeding Trial 92
Data collection 92
Statistical Analysis 93
Results 94
Corn analysis 94
Feed analysis 94
Corn type effect 95
Live performance 95
The relative organ weights 96
Feed form effect 96
Live performance 96
The relative organ weights 98
Discussion 98
Corn type effect 98

Feed form effect 100
Conclusion 102
References 103
Tables 105
Chapter 7 - Summary of Findings and Future Research 119

List of Figures

Figure 1.1 Illustration of treatment arrangement of Experiment 2 13
Figure 5.1 The effect of fines inclusion level and conditioning temperature on pellet mill specific energy consumption (SEC) 80

List of Tables

Table 1.1 Diet composition of finishing swine diet 14
Table 1.2 The effect of the drying process and storage condition on phytase activity in the mash sample (Exp. 1) 15
Table 1.3 The effect of cooling method and sample preparation on phytase activity, and phytase stability of conditioned mash samples (Exp.2) 16
Table 1.4 The effect of cooling method and sample preparation on moisture content of conditioned mash samples (Exp. 2) 17
Table 1.5 The effect of cooling method, storage condition and storage period on phytase activity, and phytase stability of cooled pellet samples (Exp. 3) 18
Table 1.6 The effect of cooling method on moisture content of pelleted samples (Exp. 3) 19
Table 2.1 Diet composition of broiler starter diet 33
Table 2.2 Pellet mill processing parameters 34
Table 2.3 The effect of sample type, added water level and holding time on moisture content .. 35
Table 2.4 The effect of added water level, holding time and phytase analysis method on phytase activity of mash samples 36
Table 2.5 The effect of added water level, holding time and phytase analysis method on phytase activity and phytase stability of conditioned mash samples 37
Table 2.6 The effect of added water level, holding time and phytase analysis method on phytase activity and phytase stability of cooled pellet samples 38
Table 2.7 The effect of added water level and holding time on pellet durability index (PDI) as determined by the Holmen method with 120 sec. analytical time 39
Table 3.1 Diet composition of swine finishing diet 50
Table 3.2 Actual die retention time for each retreatment 51
Table 3.3 Pellet mill processing parameters 52
Table 3.4 The effect of combination of pellet mill model, die L:D ratio, and die retention time on phytase activity and phytase stability of cooled pellet samples. 53
Table 4.1 Diet compositions of swine grower diet 63
Table 4.2. Diet compositions of swine grower diet 64
Table 4.3 Pellet mill processing parameters of Exp. 1 65

Table 4.4 The effect of corn starch inclusion level and die thickness on pellet durability index (PDI) as determined by the Holmen method for 30 s, PDI and modified PDI (Exp. 1)..... 66
Table 4.5 Pellet mill processing parameters of Exp. 2 67
Table 4.6 The effect of fine ground corn inclusion level and conditioning temperature on pellet durability index (PDI) as determined by the PDI and modified PDI (Exp. 2)..... 68
Table 5.1 Diet composition of swine grower diet..... 81
Table 5.2 Diet composition of swine finishing diet..... 82
Table 5.3 Pellet mill processing parameters of Exp. 1 83
Table 5.4 The effect of fines inclusion level and conditioning temperature on pellet durability index (PDI) as determined by the PDI and modified PDI (Exp. 1)..... 84
Table 5.5 Pellet mill processing parameters of Exp. 2 85
Table 5.6 The effect of fines inclusion level and conditioning temperature on pellet durability index (PDI) and gelatinized starch as determined by the PDI, modified PDI, total starch, gelatinized starch and cooked starch (Exp. 2)..... 86
Table 5.7 The effect of fines inclusion level and conditioning temperature on energy consumption as determined by specific energy consumption (SEC) and standard deviation of SEC (Exp. 2)..... 87
Table 6.1 Extrusion processing parameters of extruded corn..... 105
Table 6.2 Diet compositions of broiler starter diet 106
Table 6.3 Pellet mill processing parameters of crumble feed..... 107
Table 6.4 Starch analysis of raw corn, extruded corn and diets and pellet quality of pellets before crumble 108
Table 6.5 Effect of corn type and corn particle size on body weight (BW) of male broilers..... 109
Table 6.6 Effect of corn type and corn particle size on average daily feed intake of male broilers 110
Table 6.7 Effect of corn type and corn particle size on average daily gain of male broilers..... 111
Table 6.8 Effect of corn type and corn particle size on the feed conversion ratio (FCR) of male broilers 112
Table 6.9 Effect of corn type and corn particle size on percentage of gizzard and pancreas of male broilers at 21d..... 113

Table 6.10 Effect of feed form and extruded corn particle size on body weight (BW) of male broilers 114
Table 6.11 Effect of feed form and extruded corn particle size on average daily feed intake of male broilers..... 115
Table 6.12 Effect of feed form and extruded corn particle size on average daily gain of male broilers 116
Table 6.13 Effect of feed form and extruded corn particle size on the feed conversion ratio (FCR) of male broilers 117
Table 6.14 Effect of feed form and extruded corn particle size on percentage of gizzard and pancreas of male broilers at 21 d. 118

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Chapter 1 - Effect of Cooling Method, Sample Preparation, Storage Condition and Storage Time on Phytase Activity of a Corn-Soy Swine Diet

Abstract

Temperature and moisture content have been identified as two factors that influence enzyme inactivation. Phytase may be further degraded in feed samples if moisture is left in the sample and the sample is not properly stored prior to analysis. Therefore, the objective of this experiment was to determine the effect of cooling method, sample preparation, storage condition and storage time on phytase stability. In experiment 1, treatments were arranged in a 2 × 2 factorial design of sample preparation (none and freeze-dried) and storage condition (ambient storage and freezer storage). In experiment 2, treatments were arranged in a 2 × 3 factorial of cooling method (counterflow cooler and freezer) and sample preparation (non-dried and freezer storage, freeze-dried and freezer storage, freeze-dried and ambient storage). In experiment 3, treatments were arranged in a 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler and freezer), storage condition (ziplock/ambient, ziplock/frozen and vacuum/frozen) and storage time (1 wk. and 3 wk.). There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. The result of Experiment 1 demonstrated that there was no interaction between drying process and storage condition ($P = 0.122$) for mash samples collected from the mixer. The sample drying process and storage condition did not impact the phytase stability ($P > 0.539$). The result of Experiment 2 demonstrated that there was no interaction between the cooling method and sample preparation ($P = 0.144$) for phytase stability of conditioned mash samples. The cooling method

and sample preparation did not affect the phytase stability ($P > 0.686$). The result of Experiment 3 demonstrated that there were no three-way and two-way interactions among cooling method, storage condition and storage time ($P > 0.686$). The cooling method, storage condition and storage time did not impact phytase stability ($P > 0.348$). Therefore, freeze-drying, vacuum sealing and freezing were not required when the feed samples were analyzed within 3 weeks of production. The pellet temperature, pellet moisture and cooling time after the pellet die did not affect phytase stability.

Keywords: freeze-dry, phytase stability, storage condition

Introduction

Enzymes are large protein molecules that have a tertiary structure, which are stabilized by van der Waals interactions, hydrogen bonds, electrostatic interactions, and configurational entropy (Woolley, 1996). The enzyme must maintain its shape to allow the substrate to adhere to the active site. There are many factors that can change the protein's three-dimensional structure by interrupting these forces such as increasing temperature, adjusting pH, and changing ionic strength (presence of heavy metal ion) (Woolley, 1996). Exogenous phytase is commonly added in non-ruminant feed to increase phosphorus releasing from plant-based ingredients, which reduces the amount of phosphorus in the manure. Exogenous phytases were developed to tolerate high temperatures during pelleting and low pH in the stomach. *Trichoderma reesei* phytase is considered to be one of the more heat-tolerant phytases on the market. However, the research conducted on the stability of phytases after conditioning and pelleting at a similar temperature is highly variable. For instance, Wilkinson et al. (2013) and Pope (2019) reported when the feed containing *Trichoderma reesei* phytase was steam conditioned at 85-86°C then pelleted, the phytase stability was 74% and 34%, respectively. There are additional factors which may account for the differences in stability, such as pellet mill size, die length to diameter ratio (L:D), steam quality or residence time in the conditioner. In addition, the moisture content can influence inactivation of enzyme (Perdana et al., 2012). Water molecules around phytase may change hydrogen bonding within the three-dimensional structure of phytase, which may alter the shape of the active site. However, there is no data on how sample handling and freeze-drying could affect the stability of phytase. Phytase may be further degraded in feed samples based on sample moisture content and storage conditions during the time period prior to analysis. Therefore, the

objective of this experiment was to determine the effect of cooling method, sample preparation, storage condition and storage time on phytase stability.

Materials and Methods

Experiment 1

Treatments were arranged in a 2 × 2 factorial design of drying process (none and freeze-dried) and storage condition (ambient storage and freezer storage at -23°C) to determine the effect on phytase activity. A swine finishing feed was used for the experiment (Table 1.1). The ingredients were added to a 0.056 m^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX). The feed was mixed for 3 min. Two 400-g samples were collected from the mixer discharge. Diets were mixed 3 separate times to provide 3 replicates per treatment. Each sample was randomly assigned into two different drying processes. A sample was dried using a freeze-dryer (Labconco model FreeZone 12, Kansas City, MO) for 8 hrs. and another was kept at room temperature. After that the samples were split into 2 samples and placed in a plastic bag, then randomly assigned to either ambient storage or freezer storage for 1 week before being sent to the laboratory for phytase activity. The sample from each drying process was analyzed for moisture content.

Experiment 2

Treatments were arranged in a 2 × 3 factorial design of cooling method (counterflow cooler and freezer) and sample preparation (non-dried and freezer storage, freeze-dried and freezer storage, freeze-dried and ambient storage) to determine the effect on phytase activity. A swine finishing feed was used for the experiment (Table 1.1). The ingredients were added to a 0.056 m^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX) and mixed for 3 min. The diet was steam conditioned for approximately 45 sec. at 85°C using a 13 × 91 cm

single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) at a production rate of 1 kg/min by holding the feeder at a constant speed setting. The sample was collected at the end of the conditioner and did not pass through the pellet die. An 800-g sample was collected from the mixer discharge and four 1-kg samples were collected after conditioning. The conditioner was run 3 separate times to provide 3 replicates for each treatment. Each sample was randomly assigned to two different cooling methods. Two samples were cooled using the counterflow experimental cooler for 10 min, while the other two samples were cooled in the freezer (Criterion model CCF50M2W, Medley, FL) for one hour. Each set of two samples was randomly assigned to two drying processes: non-dried [a cooled sample of conditioned mash was placed in a plastic bag] and freeze-dried [a cooled sample of conditioned mash was dried using a freeze-dryer (Labconco model FreeZone 12, Kansas City, MO) for 8 hr]. After that the freeze-dried samples were split into 2 samples and placed in a plastic bag then were randomly assigned to either ambient storage or freezer storage. For the non-dried treatment, the samples from both cooling methods were stored in the freezer. After 1-week storage, the samples from three sample preparation methods: non-dried and freezer storage, freeze-dried and freezer storage and freeze-dried and ambient storage were sent to the laboratory for phytase activity. The treatment arrangement is illustrated in Figure 1.1. Both non-dried and freeze-dried samples from each cooling method were analyzed for moisture content.

Experiment 3

Treatments were arranged in a 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler and freezer), storage condition (ziplock/ambient, ziplock/frozen and vacuum/frozen) and storage time (1 week and 3 weeks) to determine the effect on phytase activity. A swine finishing feed was used for the

experiment (Table 1.1). The ingredients were added to a 0.056 m^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX) and mixed for 3 min. The diet was steam conditioned for approximately 45 sec. at 85°C and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with a 4.0 mm × 12.7 mm die. Diets were pelleted at a production rate of 1 kg/min by holding the feeder at a constant speed setting. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. A 250 g sample was collected from the mixer discharge and thirty 250 g samples were collected after pelleting. Each sample was randomly assigned into five different temperature reduction methods: none [sample placed directly in a sample bag], heat diffusion [sample placed on 30 cm paper plate for 30 min.], experimental fan cooler or counterflow cooler [sample cooled with a 153 mm axial fan or a counterflow cooler for 10 min], and freezer [sample placed in a freezer (Criterion model CCF50M2W, Medley, FL) for one hour]. Six cooled samples from each method were randomly assigned to three different storage conditions: ziplock/ambient-placed in a 11.7 cm × 18.8 cm Ziploc® seal top plastic bag and ambient storage, ziplock/frozen-placed in a 11.7 cm × 18.8 cm Ziploc® seal top plastic bag and freezer storage, and vacuum/frozen- vacuum sealed by a vacuum sealer (Ziploc® model V203, Racine, WI) and freezer storage. Two packed samples from each cooling method and storage condition were randomly assigned to two different storage times: 1 or 3 weeks. The samples were analyzed for phytase activity. The sample from each cooling method were analyzed for moisture content.

Data collection

Phytase

Both mash and pellet samples were analyzed by using the QuantiPlate™ Kit for Quantum Blue®. The color reaction was measured by a plate reader at 450/630 nm. The color was used to

evaluate the phytase activity based on a calibration curve. The phytase results were reported as FTU/kg and percent phytase stability. The percent phytase stability of the conditioned mash sample or cooled pellets was calculated by dividing the phytase activity of the conditioned mash sample or cooled pellets by the average phytase activity of the mash samples then multiplying by 100. The theoretical phytase activity was calculated by multiplying the actual phytase unit (FTU/g) in the phytase premix by the inclusion rate (g/tonne) then dividing by 1,000 (kg/tonne).

Moisture (AOAC 930.15, 1990)

An aluminum tray weight was recorded then a 2-g sample was placed on the tray. The sample was dried in the oven at 135°C for 2 hrs. then the sample tray was placed in the desiccator for 30 min. The moisture content was calculated by dividing the difference between the sample tray and the empty tray by sample weight then multiplying by 100.

Statistical Analysis

Data were analyzed as a completely randomized design for the three experiments. Experiment 1 treatments were arranged in a 2 × 2 factorial design of drying process (none and freeze-dried) and storage condition (ambient storage and freezer storage) to determine the effect on phytase activity. Experiment 2 treatments were arranged in a 2 × 3 factorial of cooling method (counterflow cooler and freezer) and sample preparation (non-dried and freezer storage, freeze-dried and freezer storage, freeze-dried and ambient storage) to determine the effect on phytase activity. For moisture content in Exp. 2, treatments were arranged in a 2 × 2 factorial of cooling method (counterflow cooler and freezer) and sample preparation (non-dried and freeze-dried) to determine the effect on moisture content. Experiment 3 treatments were arranged in a 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler and freezer), storage condition (ziplock/ambient, ziplock/frozen

and vacuum/frozen) and storage time (1 week and 3 weeks) to determine the effect on phytase activity. For moisture content in Exp. 3, treatments were arranged to determine the effect of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler and freezer) on moisture content. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$

Results and Discussion

The *Trichoderma reesei* phytase activity in the phytase premix was 5,023 FTU/g, which was above guaranteed level (5,000 FTU/g). Based on the phytase activity of phytase premix, the theoretical phytase activity in the mash sample was 1,005 FTU/kg. The results of Experiment 1 demonstrated that there was no interaction between drying process and storage condition (P = 0.122) for the mash samples collected from the mixer. The sample drying process and storage condition did not impact the phytase activity ($P > 0.539$) (Table 1.2). The phytase activity was similar between samples that were stored under room temperature and in a freezer at -23°C (903 and 879 FTU/kg, respectively) for 1 week before they were sent to the laboratory. The phytase activity was 921 and 862 FTU/kg for non-dried and freeze-dried samples, respectively. The average phytase activity of the mash feed collected from the mixer was 902 FTU/kg. The phytase activity of the mash samples were 2 to 23% lower than the theoretical phytase activity, which was probably due to the laboratory variation. The European Union Reference Laboratory, EURL (2013) reported the percent phytase recovery in feed that contained 500-1,500 FTU/kg was between 77 and 108%. The moisture content was 9.38 and 9.28% for non-dried and freeze-dried samples, respectively. The freeze-dryer pulled out only 0.1% moisture from the mash sample after 8 hrs of operation. The lower moisture content of the initial sample (9.38%) may have

reduced the efficiency of the drying process. Thus, when the feed moisture was lower than 9.4% and stored for one week, drying or freezing the samples before sending the samples for phytase analysis did not affect the degradation of *Trichoderma reesei* phytase.

The result of Experiment 2 demonstrated that there was no interaction between the cooling method and sample preparation ($P = 0.144$) for phytase stability of the conditioned mash samples. The cooling method and sample preparation did not affect the phytase stability of the conditioned mash samples ($P > 0.686$) (Table 1.3). The phytase stability was similar among the three sample preparation methods regardless of cooling method. The phytase stability was 81.4% when the sample was freeze-dried then stored at room temperature for 1 week. There was no difference for phytase stability between non-dried sample (76.7%) and freeze-dried sample (77.7%) when they were stored in a freezer at -23°C for 1 week. The conditioned mash sample that was cooled by the counterflow experimental cooler for 10 min (76.4%) had a similar phytase stability as compared to the samples that were cooled in the freezer at -23°C for one hour (79.9%). The phytase activity decreased between 7 and 31% from the mash sample when the mash feed was conditioned at 85.6° ± 0.6°C for 46 ± 4 sec. and then pelleted at a production rate of 1.2 kg/min. De Jong et al. (2017) reported that the phytase activity in feed (350 FTU/kg of *Trichoderma reesei* phytase) dropped 62.1% after the feed was conditioned at 85°C. The percent loss of phytase from De Jong's study was between 31 and 55% greater than the current experiment. However, the results of the current experiment demonstrated that using different sample handling procedures after the feed was conditioned was not a major factor in the difference in results observed between De Jong's and the current study results. There was an interaction between cooling method and sample preparation ($P = 0.012$) for moisture content of the conditioned mash samples. The freeze-dried sample had significantly lower moisture content

as compared to the non-dried sample when they were cooled in the freezer at -23°C for one hour. However, there was no significant difference in moisture content between the freeze-dried sample and non-dried sample when they were cooled by the experimental counter flow cooler for 10 min. The freeze-dryer pulled out 2% moisture from the sample cooled in the freezer at -23°C but only 0.3% moisture from the sample that was cooled by a counterflow cooler (Table 1.4). The counterflow cooler decreased the sample temperature and took away moisture while the freezer only cooled the samples. Thus, when the moisture of the sample was lower than 11% and stored for one week, freezing the sample before sending them for phytase analysis did not affect the degradation of *Trichoderma reesei* phytase.

The result of Experiment 3 demonstrated that there was no three-way and two-way interaction among cooling method, storage condition and storage time ($P > 0.686$). The cooling method, storage condition and storage time did not impact phytase stability in P:M ($P > 0.348$, respectively) (Table 1.5). The phytase stability was similar among the five different cooling methods regardless of storage condition and storage time (48.4, 51.3, 54.8, 53.5, and 46.9% for fan cooler, freezer, heat diffusion, counterflow cooler, and none, respectively). The phytase stability was not affected when the moisture was not removed from the hot pellets. For storage condition, the phytase stability was 48.5% when the sample was vacuum sealed then stored in a freezer at -23°C. There was no difference for phytase stability between the samples stored at room temperature and -23°C, (51.1% and 53.4%, respectively) when they were packed in a 11.7 cm × 18.8 cm Ziploc® seal top plastic bag. For storage time, the phytase stability was similar between the samples stored for 1-week and 3-weeks (52.9% and 49.0%, respectively) regardless of cooling method and storage condition. The phytase activity was between 39 and 63% lower from the mash sample when the mash feed was conditioned at 85.6° ± 1.6°C for 39 ± 3 sec. and

pelleted at a production rate of 0.88 kg/min. Wilkinson et al. (2013) reported the percent loss of *Trichoderma reesei* phytase after pelleting at 85°C was 26%. The results of this experiment demonstrated that the cooling method, storage condition, and storage time was not a major contributing factor for the differences observed in phytase stability in Wilkinson's study and the current experiment. The cooling method resulted in different moisture contents in the cooled pellets ($P < 0.0001$) (Table 1.6). The pellet moisture content of each cooling method was 16.28, 15.43, 14.75, 14.38, and 13.97% for non-dried, freezer, heat diffusion, cooling fan, and counterflow cooler, respectively. There was no significant difference in pellet moisture content between the experimental fan cooler and experimental counterflow cooler. Therefore, when the moisture of the sample was lower than 16.3% and stored up to 3 weeks, vacuum sealing, and freezing did not prevent the degradation of *Trichoderma reesei* phytase. There was no evidence that the added moisture from the conditioning step, the efficiency of the cooling step, and a 3-weeks holding period prior to phytase analysis affected the phytase stability.

Conclusion

The results of this experiment suggest that freeze-drying, vacuum sealing and freezing were not required when the sample is analyzed within 3 weeks of production. The pellet temperature, pellet moisture and cooling time after the pellet die did not affect phytase stability.

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Figures and Tables

Figure 1.1 Illustration of treatment arrangement of Experiment 2

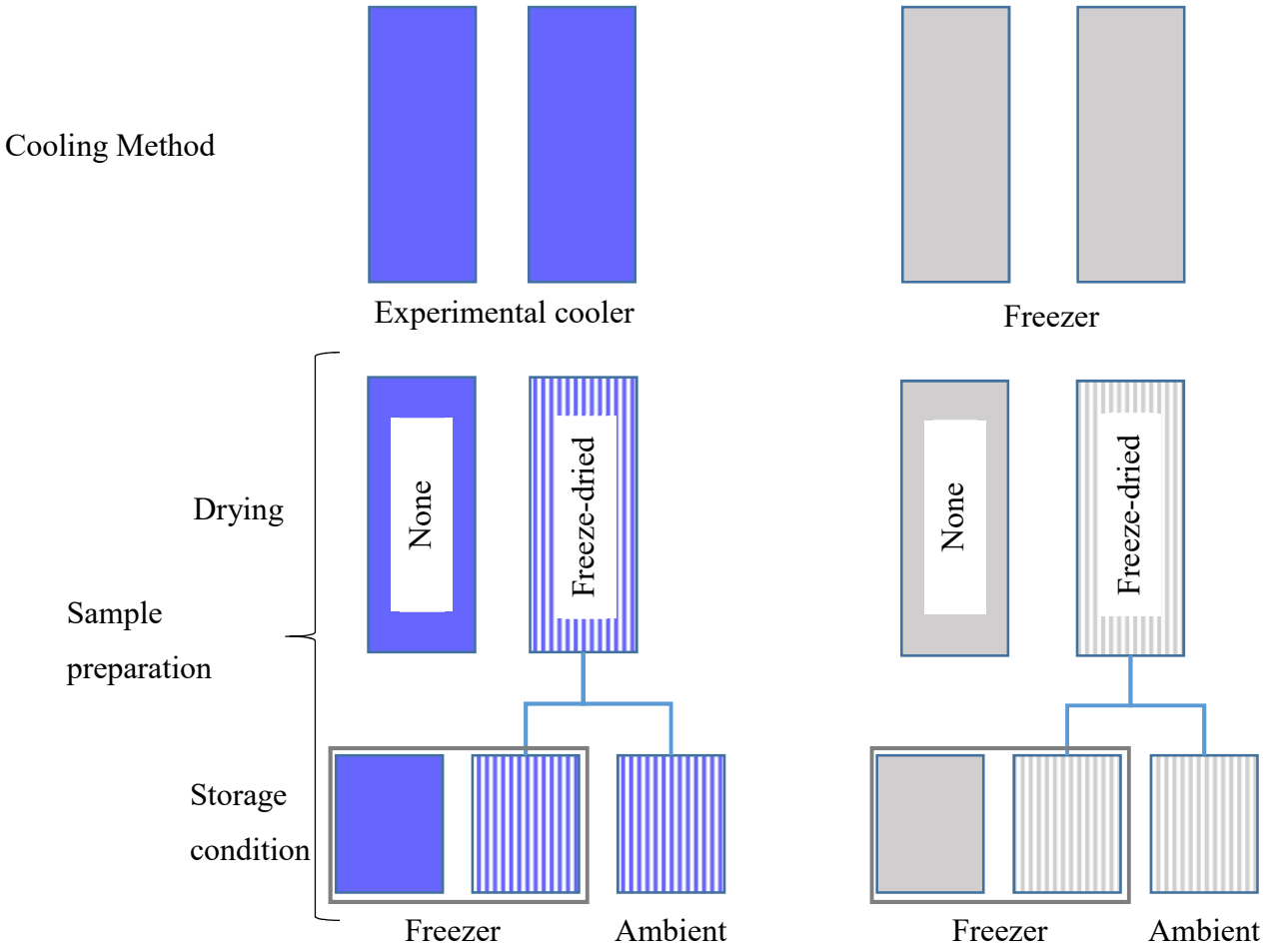

Table 1.1 Diet composition of finishing swine diet

Ingredients	Percent
Corn	78.42
Soybean meal	19.20
Mono-calcium phosphate, 21% P	0.33
Limestone	1.10
Swine vitamin premix[1]	0.13
Swine trace mineral premix[2]	0.13
L-Lysine HCl	0.25
DL-Methionine	0.02
L-Threonine	0.05
Salt	0.35
Phytase[3]	0.02
Total	100.00

[1]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[2]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[3]Quantum® Blue 5G (AB Vista Inc., Plantation, FL) provided 1,000 phytase units (FTU)/kg with a release of 0.195% available P.

Table 1.2 The effect of the drying process and storage condition on phytase activity in the mash sample (Exp. 1)

Drying process	Storage condition	n	Phytase activity, FTU/kg
Interaction effects			
None	Ambient storage	3	852
None	Freezer	3	989
Freeze-dried[1]	Ambient storage	3	954
Freeze-dried	Freezer	2	989
SEM			105.5
Main effect			
None		6	921
Freeze-dried		5	862
SEM			68.1
	Ambient storage	6	903
	Freezer	5	879
	SEM		68.1
Source of variation			*P-value*
Drying process × Storage condition			0.122
Drying process			0.539
Storage condition			0.798

[1]Sample was dried with freeze-dryer for 8 hrs.

Table 1.3 The effect of cooling method and sample preparation on phytase activity, and phytase stability of conditioned mash samples (Exp.2)

Cooling Method[1]	Sample preparation[2]	n	Phytase activity, FTU/kg	Phytase stability, %
Interaction effects				
Experimental cooler	Non-dried and freezer storage	3	673	74.6
Experimental cooler	Freeze-dried and ambient storage	3	631	70.0
Experimental cooler	Freeze-dried and freezer storage	3	778	86.2
Freezer	Non-dried and freezer storage	2	711	78.8
Freezer	Freeze-dried and ambient storage	3	836	92.7
Freezer	Freeze-dried and freezer storage	3	624	69.1
SEM			102.1	11.32
Main effect				
Experimental Cooler		9	689	76.4
Freezer		8	721	79.9
SEM			59.0	6.54
	Non-dried and freezer storage	5	692	76.7
	Freeze-dried and ambient storage	6	734	81.4
	Freeze-dried and freezer storage	6	701	77.7
	SEM		65.9	7.31
Source of variation			*P*-value	
Cooling method × Sample preparation			0.144	0.144
Sample preparation			0.879	0.879
Cooling method			0.686	0.686

[1]Counterflow cooler - sample was cooled with a counterflow cooler for 10 min and freezer - sample was placed in a freezer for one hour.
[2]Freeze-dried – sample was dried with a freeze-dryer for 8 hrs and freezer storage - sample was placed in a freezer at -23°C.

Table 1.4 The effect of cooling method and sample preparation on moisture content of conditioned mash samples (Exp. 2)

Cooling Method	Sample preparation	n	Moisture, %
Interaction effects			
Cooler	None	3	9.84[c]
Cooler	Freeze-dried	3	9.54[c]
Freezer	None	3	13.02[a]
Freezer	Freeze-dried	3	11.01[b]
SEM			0.263
Main effect			
Cooler		6	9.69[l]
Freezer		6	12.02[k]
SEM			0.186
	None	6	11.43[x]
	Freeze-dried	6	10.28[y]
	SEM		0.186
Source of variation			*P-value*
Cooling method × Sample preparation			0.012
Sample preparation			0.002
Cooling method			<0.0001

[a-c]Means within an interaction effect between cooling method and sample preparation followed by a different letter are significantly different ($P \leq 0.05$).
[k-l]Means within a main effect of cooling method followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means within a main effect of sample preparation followed by a different letter are significantly different ($P \leq 0.05$).

Table 1.5 The effect of cooling method, storage condition and storage period on phytase activity, and phytase stability of cooled pellet samples (Exp. 3)

Cooling method[1]	Storage condition	Storage time, wk	n	Phytase activity, ftu/kg	Phytase stability, %
Interaction effects					
Fan cooler	Ziplock/ambient	1	3	537	52.7
Fan cooler	Ziplock/ambient	3	3	437	42.9
Fan cooler	Vacuum/frozen	1	2	420	41.1
Fan cooler	Vacuum/frozen	3	3	498	48.8
Fan cooler	Ziplock/frozen	1	3	467	45.8
Fan cooler	Ziplock/frozen	3	3	604	59.2
Freezer	Ziplock/ambient	1	3	505	49.5
Freezer	Ziplock/ambient	3	3	503	49.3
Freezer	Vacuum/frozen	1	3	508	49.8
Freezer	Vacuum/frozen	3	3	459	45.0
Freezer	Ziplock/frozen	1	3	622	61.0
Freezer	Ziplock/frozen	3	3	543	53.2
Heat diffusion	Ziplock/ambient	1	3	626	61.4
Heat diffusion	Ziplock/ambient	3	3	509	49.9
Heat diffusion	Vacuum/frozen	1	3	563	55.2
Heat diffusion	Vacuum/frozen	3	3	542	53.1
Heat diffusion	Ziplock/frozen	1	3	597	58.5
Heat diffusion	Ziplock/frozen	3	3	514	50.4
None	Ziplock/ambient	1	3	531	52.0
None	Ziplock/ambient	3	3	477	46.7
None	Vacuum/frozen	1	3	515	50.5
None	Vacuum/frozen	3	3	375	36.8
None	Ziplock/frozen	1	3	598	58.6
None	Ziplock/frozen	3	3	374	36.6
Counterflow cooler	Ziplock/ambient	1	3	552	54.1
Counterflow cooler	Ziplock/ambient	3	3	532	52.2
Counterflow cooler	Vacuum/frozen	1	3	557	54.6
Counterflow cooler	Vacuum/frozen	3	3	506	49.6
Counterflow cooler	Ziplock/frozen	1	3	500	49.1
Counterflow cooler	Ziplock/frozen	3	2	624	61.1
SEM				90.1	13.68
Main effect					
Fan cooler			17	494	48.4
Freezer			18	523	51.3
Heat diffusion			18	559	54.8
None			18	478	46.9
Counterflow cooler			17	545	53.5
SEM				48.4	4.75
	Ziplock/ambient		30	521	51.1
	Vacuum/frozen		29	494	48.5
	Ziplock/frozen		29	544	53.4
	SEM			36.9	3.62
		1	44	540	52.9
		3	44	500	49.0
		SEM		29.9	2.93
Source of variation				*P*-value	
Cooling method × Storage condition × Storage time				0.958	0.958
Storage condition × Storage time				0.948	0.948
Cooling method × Storage time				0.686	0.686
Cooling method × Storage condition				0.999	0.999
Storage time				0.348	0.348
Storage condition				0.636	0.636
Cooling method				0.725	0.725

[1]None- sample was placed directly in a sample bag, heat diffusion- sample was placed on 30 cm paper plate for 30 min., fan cooler or counterflow cooler- sample was cooled with a 153 mm axial fan or a counterflow cooler for 10 min, and freezer-sample was placed in a freezer for one hour.

Table 1.6 The effect of cooling method on moisture content of pelleted samples (Exp. 3)

Cooling Method	n	Moisture, %
None	12	16.28[a]
Freezer	12	15.43[b]
Heat diffusion	12	14.75[b,c]
Fan cooler	12	14.38[c,d]
Counterflow cooler	12	13.97[d]
SEM		0.349
Source of variation		*P*-value
Cooling method		<0.0001

[a-d]Means within a column followed by a different letter are significantly different ($P \leq 0.05$).

Chapter 2 - The Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality

Abstract

The addition of water to the mixer prior to pelleting is sometimes necessary to reach the target moisture content at the end of the conditioning process. However, there is limited data to demonstrate the impact of water addition in the mixer on phytase stability during the pelleting process. In addition, the variation of phytase analysis method may lead to incorrect or biased conclusions for research and industrial phytase stability. Therefore, the objective of this experiment was to determine the effect of water added in the mixer, feed holding time, and phytase analysis method on phytase stability and pellet quality. Treatments were arranged in a 2 × 2 × 2 factorial of added water (0% and 1%), holding time (0 and 2 hr.), and phytase analysis method (ELISA and EN ISO), respectively. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. The results demonstrated that there were no three-way and two-way interactions among added water, holding time, and analysis method ($P > 0.05$) on phytase stability for mash samples, conditioned mash samples, and pellets. The added water and holding time did not impact phytase stability ($P > 0.05$) on phytase stability for mash samples, conditioned mash samples, and pellets. The ELISA method had significantly greater phytase activity than the EN ISO method for the pellet samples. The phytase activity was similar between the two analytical methods for mash samples and conditioned mash samples. For pellet quality, there was no interaction between added water and holding time (P=0.163). Both added water and holding time did not impact pellet durability index (PDI) ($P > 0.05$). Therefore, the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when feed was

stored in a bin up to 2 hr. prior to pelleting. The added water in mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei* phytase stability. Finally, increasing moisture content of mash feed by 0.6% did not improve pellet quality.

Keywords: added water, phytase stability, holding

Introduction

Previous research demonstrated that increasing mash feed moisture up to 15% improved pellet quality and decreased energy consumption (Greer, 2013). This is often accomplished by adding water to the mixer or conditioner in addition to the water added by steam in the conditioning process. The added water and steam have the potential to reduce enzyme stability. Temperature and moisture content are two factors that influence enzyme inactivation kinetics (Perdana et al., 2012). Bychkov et al. (2011) demonstrated that the presence of water during the heating process increased cellulosolytic enzyme denaturation. The reduced enzyme stability could lead to nutritional deficiencies in animals as more feed mills increase the number of enzymes (phytase, xylanase, and protease) added to the feed to reduce the cost of the feed by increasing nutrient digestibility. There is limited data to demonstrate the impact of moisture addition in the mixer on phytase denaturation during the pelleting process. Increasing mash feed moisture and the interaction time between water and phytase may increase phytase degradation during the pelleting process. European Union Feed Additives and Premixtures Association (FEFANA) developed a method to determine phytase activity regardless of phytase sources, so meaningful comparisons could be made across products (Gizzi et al., 2008). Each phytase supplier typically has a rapid phytase method to provide a quick turnaround time for their customers to help ensure the phytase activity is at the correct concentration in their diets. However, when the quick method is used for research projects there may be an unexplained bias that is created due to the variation within the rapid method that could lead to a misinterpretation of the data. Therefore, the objectives of this experiment were to determine the effect of adding water in the mixer, mash holding time, and phytase analysis method on phytase stability and to determine the effect of adding water in the mixer and mash holding time pellet quality.

Materials and Methods

A broiler starter feed was used for the experiment (Table 2.1). The basal diet was mixed in a 1.78 m^3 counterpoise mixer (Hayes & Stolz, model TRDB63-0152, Fort Worth, TX) for 3 min. The basal diet and phytase produced by a strain of *Trichoderma reesei* were added to a 0.170 m^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Springs, KS). For the 0% added water treatment, a 95.27-kg basal feed and 0.014-kg phytase were mixed for 5 min. For the 1% added water treatments, a 94.31-kg basal feed and 0.014-kg phytase were mixed for 120 sec. followed by the addition of 0.95-kg water and then the mixture was mixed for 180 sec. wet mix time. The water was applied to dry feed in the mixer using a hand-held sprayer (Country Tuff model 26329, Sedalia, MO) with a flat spray tip nozzle (TeeJet model TP11006, Glendale Heights, IL). After the diets were mixed, treatments were immediately pelleted or held in a closed container for 2 hr. before pelleting. Treatments were steam conditioned at 85°C for approximately 30 sec. and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 4.0 mm × 22.2 mm die. The feeder setting was held constant at approximately 1 kg/min. There were 3 replicates for each treatment. Samples were collected during discharge of the mixer, after conditioning and after pelleting. The conditioned mash and pelleted samples were cooled for 10 min using an experimental counterflow cooler. All the samples were analyzed for moisture content then kept at room temperature before being sent to the laboratory for analysis. For moisture content, treatments were arranged in a 3 × 2 × 2 factorial of sample type (mixer, conditioned mash, and cooled pellet), percent added water (0% and 1%) and holding time (0 and 2 hrs.) to determine the effect on sample moisture. Phytase activity was determined by ELISA and EN ISO method. For phytase, treatments were arranged in a 2 × 2 × 2 factorial of added water (0% and 1%), feed

holding time (0 and 2 hr.), and phytase analysis method (ELISA and EN ISO) to determine the effect on phytase stability of the mash, conditioned mash, and pellet samples. The pelleted samples from each pellet run were analyzed for pellet durability index (PDI) using the Holmen NHP 100 for 120 sec. at 60 bars. Treatments were arranged in a 2 × 2 factorial of added water (0% and 1%) and holding time (0 and 2 hrs.) to determine the effect on pellet quality.

Data collection

Moisture (AOAC 930.15, 1990)

An aluminum tray weight was recorded then a 2-g sample was placed on the tray. The sample was dried in the oven at 135°C for 2 hrs. then the sample tray was placed in the desiccator for 30 min. The moisture was calculated by dividing the difference between the sample tray and the empty tray by the sample weight and then multiplying by 100.

Phytase

The supplier's ELISA QuantiPlate^{TM} Kit for Quantum Blue® was used for both mash and pellet samples. The color reaction was measured by a plate reader at 450/630 nm. The amount of color was used to evaluate the phytase activity based on a calibration curve. For the EN ISO method (European Standard and International Organization for Standardization, EN ISO, 2009), a feed sample was extracted with 0.01% polysorbate 20 then incubated with 5 mM phytate substrate in pH 5.5 acetate buffer at 37°C for 30 min. A 0.8-ml stop agent (molybdate/vanadate) was added, resulting in the formation of phosphomolybdate-vanadate, which produced a yellow complex. The yellow complex was measured at 415 nm then compared to a phosphate standard curve. The phytase results were reported as FTU/kg and percent phytase stability. The percent phytase stability of the conditioned mash sample or cooled pellets was calculated by dividing phytase activity of the conditioned mashed sample of cooled pellets by the

average phytase activity of the mash samples then multiplying by 100. The theoretical phytase activity was calculated by multiplying the actual phytase unit (FTU/g) in the phytase premix by the inclusion rate (g/tonne) then dividing by 1,000 (kg/tonne). The coefficient of variation (CV) was calculated by dividing the standard deviation of mash, conditioned mash or cooled pellet samples by its average and then multiplying by 100 for each phytase method.

Pellet Durability Index (PDI)

The sample was sifted with a U.S. No. 6 (4.0 mm) sieve. A 100-g sample of sifted pellets was placed in the Holmen NHP 100 chamber. The Holmen NHP 100 was run for 120 sec. The PDI was calculated by dividing the remaining sample weight by initial weight and then multiplying by 100 (Stark and Fahrenholz, 2015).

Statistical Analysis

Data were analyzed as a completely randomized design. For phytase stability, treatments were arranged in a 2 × 2 × 2 factorial of percent added water (0% and 1%), holding time (0 and 2 hrs.), and phytase analysis method (ELISA and EN ISO) to determine the effect on phytase stability of mash, conditioned mash, and cooled pellet samples. For moisture, treatments were arranged in a 3 × 2 × 2 factorial of sample type (mixer, conditioned mash, and cooled pellet), percent added water (0% and 1%) and holding time (0 and 2 hrs.) to determine the effect on moisture. For PDI, treatments were arranged in a 2 × 2 factorial of added water (0% and 1%) and holding time (0 and 2 hrs.) to determine the effect on pellet quality. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

The phytase activity was 6,140, 6,220, and 6040 FTU/g for the *Trichoderma reesei* phytase enzyme source used in replication 1, 2 and 3, respectively. Based on the inclusion of 0.015% phytase premix, the theoretical phytase level in the mash sample should be 921, 933 and 906 FTU/kg for replication 1, 2 and 3, respectively. The pellet mill throughput and retention times were similar across treatments (Table 2.2). For the moisture content of the three sample types (Table 2.3), there were no three-way and two-way interactions among sample type, added water level and holding time ($P > 0.300$). There was significant difference for moisture content among sample types ($P < 0.0001$). The conditioned mash sample (17.26%) had the highest moisture content followed by cooled pellets (13.81%) and mash sample (12.51%). The feed with 1% added water had significantly greater moisture than feed without added water ($P = 0.001$) as expected. The holding time did not affect moisture content of the feed ($P = 0.567$). For the mash samples (Table 2.4), there were no three-way and two-way interactions among added water, holding time, and analysis method ($P = 0.147$). The added water, holding time, and analysis method did not impact phytase stability ($P = 0.233$) in mash feed prior to conditioning and pelleting. The phytase activity was from 824 to 1061 FTU/kg for the ELISA method and from 884 to 1,100 FTU/kg for the EN ISO method. The coefficient of variation (CV) of the mash samples from all treatments were 19.49 and 16.69% for the ELISA and EN ISO method, respectively. The European Food Safety Authority, EFSA (2013) reported that the ELISA method had a CV of 18% when *Trichoderma reesei* phytase was mixed in mash poultry feed. For conditioned mash samples (Table 2.5), there were no three-way and two-way interactions among added water, holding time, and analysis method for phytase activity and phytase stability ($P > 0.614$). The added water, holding time and analysis method did not impact phytase activity and

phytase stability ($P > 0.086$). The phytase stability was from 81.11 to 93.25% for the ELISA method and from 69.29 to 84.00% for the EN ISO method when the samples were steam conditioned at 85°C for 31 ± 6.2 sec. retention time. The CVs of the conditioned mash samples from all treatments were 13.38 and 18.66% for the ELISA and EN ISO method. The results were similar to those reported by the European Union Reference Laboratory, EURL, (2013) in which the variation of the EN ISO method was greater than the ELISA method. EURL (2013) reported that the relative standard deviation for intermediate precision (RSD_{ip}), which is the variation of the method when a sample is analyzed at the same laboratory on different days, was 2.3% for the ELISA method when feedstuffs contained 1,464 FTU/kg and was from 3.3 to 12.7% for the EN ISO method when feedstuffs contained 500-1,500 FTU/kg. The phytase stability of conditioned mash was similar between the mash feed with no-added and 1% added water (79.2 and 87.1%, respectively). De Jong et al. (2017) reported that the phytase stability in feed (350 FTU/kg of *Trichoderma reesei* phytase) was 37.9% after the feed was conditioned at 85°C. The difference in phytase stability between the current study and De Jong's study was not clear. However, the results of the current experiment demonstrated that the moisture of mash diets prior to conditioning was not a major factor in the difference in results observed between De Jong's and the current study results. EFSA (2013) reported the phytase stability of *Trichoderma reesei* phytase premix was between 21 to 50% when it was exposed to 40°C and 75% humidity for one month. The EFSA's result demonstrated that the phytase could lose its activity when exposed to water molecules. Samborska et al. (2005) concluded that increasing moisture content decreased thermostability of *Aspergillus oryzae* α-amylase. The moisture content may change the hydrogen bonding in the tertiary structure which may result in changes in enzyme activity. As enzymes maintain their active site structure by stabilizing the van der Waals interactions, hydrogen bonds,

electrostatic interactions, and configurational entropy (Woolley, 1996). In the current study, when the mash diets containing 0.015% phytase premix were exposed to 85°C conditioning temperature for 31 ± 6.2 sec., the phytase activity was decreased only 20.8 and 12.9% for the 12.2% and 12.8% mash feed moisture treatments, respectively. For the cooled pellets (Table 2.6), there were no three-way and two-way interactions among added water, holding time and analysis method for phytase activity and phytase stability ($P > 0.059$). The added water and holding time did not impact phytase activity and phytase stability ($P > 0.570$). When the cooled pellets were analyzed by two methods, the EN ISO method had significantly lower phytase activity and phytase stability compared to the ELISA method ($P < 0.023$). The phytase stability of cooled pellets was between 60.38 and 71.08% for the ELISA method and between 50.35 and 62.10% for the EN ISO method when the samples were pelleted with a 5.6 L:D at 85°C for 31 ± 6.2 sec. retention time. The CVs of cooled pellets from all treatments were 18.86 and 18.32% for the ELISA and EN ISO methods, which was similar to percent CV of uniformity test of EFSA's findings. This data indicated that there was no evidence of a difference between the ELISA method and EN ISO method in mash and conditioned mash samples. However, there was a difference in phytase activity when analyzing cooled pellets. The standard error of phytase activity between methods was 55.6, 47.4, and 25.9 FTU/kg for mash, conditioned mash, and cooled pellet samples, respectively. The standard error of the cooled pellet samples was dramatically decreased when compared to the mash and condition mash samples due to twice the number of cooled pellet samples analyzed. There were no added water × holding time × method, added water × method, and holding time × method interactions, indicating that the differences observed in analytical method did not impact the phytase responses of other variables. Thus, both methods could be used in the laboratory to determine *Trichoderma reesei* phytase activity. In the

current study, adding 1% water or increasing the holding time by 2 hrs. resulted in a similar phytase activity as compared to feed that had no water addition or was immediately pelleted. Pope (2019) reported that the phytase stability was similar when 0 to 2% water was added in the mixer to the mash feed and then pelleted at 86°C, which agreed with the current study.

For pellet quality (Table 2.7), there was no interaction between added water and holding time ($P = 0.830$). Both added water and holding time did not impact PDI ($P > 0.163$). Greer (2013) reported that when the moisture of the mash feed increased from 12.2 to 13.1%, PDI significantly increased from 77.6 to 80.0%. The result of this experiment demonstrated that adding 1% water in the mixer increased the moisture of mash feed from 12.2 to 12.8%. When the mash feed was pelleted with a 5.6 L:D die at 85°C, the PDI was similar between mash feed with 12.2 and 12.8% moisture content (77.5 and 80.0%, respectively). The results of the current experiment contrasted with those reported by Greer (2013). Though the PDI of the treatments were similar between experiments, the standard error within treatment was lower for Greer versus the current experiments. Increasing moisture content decreased the glass transition temperature of feed ingredient protein sources (Verbeek and van den Berg, 2010) and corn starch (Liu et al., 2009). Behnke (2001) stated that the adhesion mechanism of pellets occurs when protein and starch ingredients are transitioned to a glassy or rubbery state, which appears after temperatures are above their T_g. Thus, increasing mash feed moisture content should reduce T_g of the feed mixture and could improve pellet quality. Adding water to mash feed in the mixer may have a greater benefit on pellet quality when the moisture content of the mash feed is less than 12.0%.

Conclusion

The results of this study indicated that the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when feed was stored in a bin up to 2 hr. prior to pelleting. The added water in mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei* phytase stability. Finally, increasing moisture content of mash feed by 0.6% did not improve pellet quality.

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Tables

Table 2.1 Diet composition of broiler starter diet

Ingredients	Percent
Corn	54.24
Soybean meal	36.00
Soy oil	4.50
Defluorinated phosphate	0.95
Limestone	1.55
Poultry mineral and vitamin premix[1]	0.25
Choline Chloride	0.10
L-Lysine HCl	0.20
DL-Methionine	0.20
L-Threonine	0.10
Salt	0.50
Econase (Xylanase)	0.01
Inert filler[2]	1.40
Total	100.00

[1]Composition per kilogram: 20 g Iron, 40 g Zinc, 40 g Manganese, 4.5 g Copper, 0.6 g Iodine, and 0.06 g Selenium. 3,080,000 IU Vitamin A, 1,100,000 IU Vitamin D3, 6,600 IU Vitamin E, 4.4 mg Vitamin B12, 330 mg Menadione, 2,640 mg Riboflavin, 2,640 mg d-Pantothenic Acid, and 11,000 mg Niacin.
[2]Silicon dioxide

Table 2.2 Pellet mill processing parameters

Treatment		Production rate, kg/hr.	Retention time, sec.	Condition mash temperature, °C
Added water level, %	Holding time, hr.			
0	0	63.3 ± 4.2	29.1 ± 2.0	84.2 ± 0.9
0	2	62.2 ± 3.7	28.7 ± 3.9	84.8 ± 0.9
1	0	62.9 ± 4.3	31.0 ± 4.8	83.9 ± 1.7
1	2	61.3 ± 6.0	31.6 ± 5.6	83.6 ± 1.4

Table 2.3 The effect of sample type, added water level and holding time on moisture content

Sample type	Added water level, %	Holding time, hr.	n	Moisture, %
Interaction effects				
Condition mash	0	0	3	16.95
Condition mash	0	2	3	17.16
Condition mash	1	0	3	17.72
Condition mash	1	2	3	17.19
Cooled pellet	0	0	6	13.45
Cooled pellet	0	2	5	13.33
Cooled pellet	1	0	6	14.27
Cooled pellet	1	2	6	14.18
Mash	0	0	3	12.15
Mash	0	2	3	12.28
Mash	1	0	3	12.89
Mash	1	2	3	12.71
SEM				0.31
Main effect				
Condition mash			12	17.26 [a]
Cooled pellet			23	13.81 [b]
Mash			12	12.51 [c]
SEM				0.15
	0		23	14.22 [x]
	1		24	14.83 [y]
	SEM			0.12
		0	24	14.57
		2	23	14.48
		SEM		0.12
Source of variation				*P*-value
Sample type × Water level × Holding time				0.602
Water level × Holding time				0.300
Sample type × Holding time				0.951
Sample type × Water level				0.509
Holding time				0.567
Water level				0.001
Sample type				<0.0001

[a-c]Means within a main effect of sample type followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means within a main effect of added water level followed by a different letter are significantly different ($P \leq 0.05$).

Table 2.4 The effect of added water level, holding time and phytase analysis method on phytase activity of mash samples

Added water level, %	Holding time, hr.	Method	n	Phytase activity, FTU/kg
Interaction effects				
0	0	ELISA[1]	3	1,061
0	0	EN ISO[2]	3	1,084
0	2	ELISA	2	824
0	2	EN ISO	3	884
1	0	ELISA	3	897
1	0	EN ISO	2	1,100
1	2	ELISA	3	1,036
1	2	EN ISO	3	1,006
SEM				128.5
Main effect				
0			11	963
1			11	1,010
SEM				55.6
	0		11	1,036
	2		11	937
	SEM			55.6
		ELISA	11	955
		EN ISO	11	1,018
		SEM		55.6
Source of variation				*P*-value
Water level × Holding time× Method				0.405
Water level × Holding time				0.147
Water level × Method				0.779
Method × Holding time				0.546
Water level				0.560
Holding time				0.233
Method				0.431

[1]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.
[2]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity".

Table 2.5 The effect of added water level, holding time and phytase analysis method on phytase activity and phytase stability of conditioned mash samples

Added water level, %	Holding time, hr.	Method	n	Phytase activity, FTU/kg	Phytase stability, %
Interaction effects					
0	0	ELISA[1]	2	784	81.1
0	0	EN ISO[2]	2	701	69.3
0	2	ELISA	3	901	93.3
0	2	EN ISO	3	740	73.2
1	0	ELISA	3	888	91.9
1	0	EN ISO	2	818	80.9
1	2	ELISA	2	886	91.6
1	2	EN ISO	3	849	84.0
SEM				103.9	10.47
Main effect					
0			10	781	79.2
1			10	860	87.1
SEM				47.4	4.78
	0		9	798	80.8
	2		11	844	85.5
	SEM			49.7	5.01
		ELISA	10	865	89.5
		EN ISO	10	777	76.8
		SEM		47.4	4.78
Source of variation				*P*-value	
Water level × Holding time× Method				0.684	0.675
Water level × Holding time				0.643	0.635
Water level × Method				0.614	0.630
Method × Holding time				0.872	0.859
Water level				0.263	0.265
Holding time				0.503	0.499
Method				0.215	0.086

[1]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.
[2]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity".

Table 2.6 The effect of added water level, holding time and phytase analysis method on phytase activity and phytase stability of cooled pellet samples

Added water level, %	Holding time, hr.	Method	n	Phytase activity, FTU/kg	Phytase stability, %
Interaction effects					
0	0	ELISA[1]	4	676	70.0
0	0	EN ISO[2]	4	628	62.1
0	2	ELISA	5	667	69.0
0	2	EN ISO	5	509	50.4
1	0	ELISA	5	584	60.4
1	0	EN ISO	5	535	52.9
1	2	ELISA	6	687	71.1
1	2	EN ISO	6	591	58.4
SEM				55.61	5.66
Main effect					
0			18	620	62.8
1			22	599	60.7
SEM				27.8	2.83
	0		18	606	61.3
	2		22	613	62.2
	SEM			26.4	2.69
		ELISA	20	653[a]	67.6[a]
		EN ISO	20	566[b]	56.0[b]
		SEM		25.9	2.64
Source of variation				*P*-value	
Water level × Holding time × Method				0.677	0.711
Water level × Holding time				0.059	0.062
Water level × Method				0.680	0.673
Method × Holding time				0.292	0.292
Water level				0.573	0.570
Holding time				0.835	0.817
Method				0.023	0.004

[1]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.

[2]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity".

[a-b]Means within a column followed by a different letter are significantly different ($P \leq 0.05$).

Table 2.7 The effect of added water level and holding time on pellet durability index (PDI) as determined by the Holmen method with 120 sec. analytical time

Added water level, %	Holding time, hr.	n	PDI, %
Interaction effects			
0	0	6	78.4
0	2	5	76.7
1	0	6	80.5
1	2	6	79.5
SEM			2.20
Main effect			
0		11	77.5
1		12	80.0
SEM			1.49
	0	12	79.4
	2	11	78.1
	SEM		1.22
Source of variation			*P*-value
Water level × Holding time			0.830
Holding time			0.430
Water level			0.163

Chapter 3 - Effect of Die Retention Time on Pellet Quality and Phytase Stability of A Corn-Soy Swine Diet

Abstract

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in animal feed. However, the pelleting process is a thermal process that can denature phytase. There are many factors that can account for phytase denaturing in the pelleting process such as pellet mill (PM) model, die length to hole diameter ratio (L:D), production rate, residence time in the conditioner, and die retention time (DRT). Therefore, the objective of this experiment was to determine the effect of die retention time on pellet quality and phytase stability. Treatments were arranged as a completely randomized design to determine the effect of die retention time. The DRT treatments were created by changing the L:D and production rate of the PM, (10.6 L:D for 4.2 DRT, 10.6 L:D for 2.6 DRT, 9.3 L:D for 1.6 DRT, 9.3 L:D for 1.1 DRT, 6.6 L:D for 2.6 DRT, and 6.6 L:D for 1.6 DRT). The pellet mills were run 3 separate times to provide 3 replicates for each treatment. Data were analyzed using the GLIMMIX procedure of SAS. There was an overall effect ($P < 0.001$) of treatment on phytase stability in cooled pellets. When using the 1012 PM, phytase was more stable regardless of die retention time when diets were manufactured using the 6.6 L:D die compared to the 10.6 L:D die ($P \leq 0.05$). The hot pellet temperature of 10.6 L:D die was 95.0 ± 4.4°C, while the 6.6 L:D die was 85.8 ± 1.4°C. However, the phytase stability was similar between the feed pelleted with the 1012 PM equipped with the 6.6 L:D die and the 3016 PM equipped with the 9.3 L:D die regardless of die retention time ($P > 0.05$). The hot pellet temperature of feed pelleted with the 1012 PM equipped with the 6.6 L:D die was 85.8 ± 1.4°C, while the feed pelleted with the 3016 PM equipped with the 9.3 L:D die was 83.1 ± 0.9°C. There was also a quadratic decrease in phytase stability as the die L:D

increased ($P < 0.0001$). Therefore, the pellet mill model or die retention time did not affect phytase stability when the hot pellet temperature was less than 88°C. Pellet quality increased (linear; $P < 0.0001$ for pellet durability index (PDI) or quadratic; $P < 0.0001$ for modified PDI) as die L:D increased. The die L:D had a greater effect on both PDI methods than the die retention time. However, increasing the die retention time improved ($P \leq 0.05$) pellet quality when the feed was pelleted with the 6.6 L:D die, but not when the feed was pelleted using the 9.3 or 10.6 L:D die. In conclusion, the phytase that was produced by the *Trichoderma reesei* strain could tolerate hot pellet temperatures up to 88°C, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures were above 91°C. Therefore, hot pellet temperatures should be measured to monitor phytase stability. In addition, increasing the die L:D resulted in the greatest improvement in pellet quality.

Keywords: retention time, phytase stability, pellet quality

Introduction

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in cereal grains, which is a main ingredient in animal feed. This enzyme can be used to reduce phosphorus content in manure by breaking down the phytate found in cereal grains to make the phosphorus available to the animal. Super-dosing phytase in the diet can improve animal performance. Birds fed a starter diet with 1,000 FTU/kg *Trichoderma reesei* phytase from d 0 to d 21 had improved body weight gain, feed conversion ratio, and percent tibia ash as compared to control diet (Wilkinson et al., 2013). Weaned pigs that were fed a nursery diet with 2,500 FTU/kg *Trichoderma reesei* phytase from d 0 to d 35 had significantly higher average daily gain and gain per feed ratio as compared to the diet without phytase (Holloway et al., 2019). Feeding pelleted feed also improves the handling characteristics of the feed and improves feed conversion ratio and average daily gain (Stark, 2012). However, the pelleting process is a thermal process that can denature phytase. This has led to the development of several thermostable phytases: *Trichoderma reesei* phytase, *Aspergillus niger* phytase, *Pichia pastoris* phytase, and *Aspergillus oryzae* phytase. Researchers have reported different responses on the stability of thermostable phytases when the feed was pelleted at 80-85°C. De Jong et al. (2017) reported that when the *Trichoderma reesei* phytase was included in the feed and then steam conditioned at 85°C, phytase stability was 37.9%. In contrast, Wilkinson et al.'s result (2013) reported phytase stability of *Trichoderma reesei* phytase after pelleting at 85°C was 74%. There are limited explanations for the differences in the literature. There are many factors that may account for the differences such as pellet mill model, die length to diameter ratio (L:D), production rate, residence time in the conditioner, and die retention time. The objective of this experiment was to determine the effect of die retention time on pellet quality and phytase stability.

Materials and Methods

Treatments were arranged as a completely randomized design to determine the effect of die retention time (DRT). The DRT treatments were created by changing the length to die hole diameter ratio (L:D) and production rate of the pellet mills (PM) (10.6, L:D for 4.2 DRT, 10.6 LD for 2.6 DRT, 9.3 L:D for 1.6 DRT, 9.3 L:D for 1.1 DRT, 6.6 L:D for 2.6 DRT, and 6.6 L:D for 1.6 DRT) on pellet quality and phytase (*Trichoderma reesei* derived phytase, Quantum Blue 5G, AB Vista, Plantation, FL) stability. A corn-soybean meal swine finishing diet was used for the experiment (Table 3.1). The ingredients were added to a 1.64 m^3 twin counterpoise mixer (Hayes and Stolz model TRDB63-0152, Fort Worth, TX) and mixed for 1 min dry mix and 2 min wet mix time. The mixture was then pelleted using either a 3016-4 California Pellet Mill (CPM) master model equipped with a 4.8 mm × 44.5 mm (9.3 L:D) die or a 1012-2 CPM master model equipped with a 4.8 mm × 50.8 mm (10.6 L:D) die or a 4.8 mm × 31.8 mm (6.6 L:D) die. The conditioner conditioning time was held constant an approximately 30 sec. at 85 °C with each designated production rate (Table 3.2). The PM were run 3 separate times to provide 3 replicates for each treatment. Samples were collected during the discharge of the mixer, after conditioning, and after pelleting. The conditioned mash samples were cooled using an experimental 153 mm axial fan and the pellet samples were cooled using an experimental counterflow cooler for 10 min. The mash samples were analyzed for phytase activity and the pellet samples were analyzed for phytase activity, pellet durability index, and modified pellet durability index.

Data collection

Measuring die retention time

The DRT was estimated using a modified calculation proposed by Hu (2001) for calculating the residence time in the extruder by multiplying the extruder's volume by the degree

of fill divided by volumetric throughput. Die retention time is defined as the amount of material in the effective length of the die divided by the material flow rate through the die.

$$DRT\ (sec.) = \frac{Amount\ of\ material\ in\ the\ effective\ length\ of\ the\ die\ (AMD)^1\ (kg)}{Mass\ flow\ rate\ (kg/sec)}$$

[1] AMD = internal die surface area (cm^2) × number of holes per cm^2 × effective volume per hole (cm^3) × material density (kg/cm^3)

Phytase

Mash, conditioned mash, and pellet samples were analyzed by using the QuantiPlate™ Kit for Quantum Blue®. The color reaction was measured by a plate reader at 450/630 nm. The color was used to evaluate the phytase activity based on a calibration curve. The phytase results were reported as FTU/kg and percent phytase stability. The percent phytase stability of the conditioned mash sample or cooled pellets was calculated by dividing the phytase activity of the conditioned mash sample or cooled pellets by the average phytase activity of the mash samples then multiplying by 100.

Pellet durability index (PDI)

The PDI was determined by ASAE S269.5. The sample was sifted with a U.S. No. 5 (4.8 mm) sieve. A 500-g sample of sifted pellets was placed in the tumble box for 10 min. The sample was sifted again with the same sieve. The PDI was calculated by dividing the whole pellets after tumbling by the initial sample weight and then multiplying by100 (ASAE, 2012). The PDI procedure was modified by adding three 19-mm hex nuts to the tumble box and tumbling for the 10 min.

Statistical analysis

Data were analyzed as a completely randomized design to determine the effect of die retention time on phytase stability and pellet quality. There were 3 replicates per treatment. Data

were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

The average phytase concentration was 6,495 FTU/g in the *Trichoderma reesei* phytase premix and 1,252 FTU/kg in the mash samples. The conditioned mash samples were steam conditioned for 34 ± 5 sec. in the conditioner at 84.5° ± 2.5°C as reported (Table 3.3). There was no significant difference in the phytase activity of the conditioned mash samples between the 1012 and 3016 PM ($P = 0.481$). The average phytase activities of the conditioned mash samples were 1,089 and 1,146 FTU/kg for 1012 and 3016 PM, respectively. The phytase stability of the conditioned mash samples were 86.96% and 91.50% for 1012 and 3016 PM, respectively. The average loss of phytase during conditioning process was 12.39%. There was an overall effect ($P < 0.0001$) of treatment on phytase stability in the cooled pellets. When using the 1012 PM, phytase was more stable regardless of the die retention time when diets were manufactured using the 6.6 L:D die compared to the 10.6 L:D die ($P < 0.05$; Table 3.4). The hot pellet temperature (HPT) of feed pellets from the 10.6 L:D die was 95.0 ± 4.4°C, while those produced using the 6.6 L:D die was 85.8 ± 1.4°C. However, the phytase stability was similar between the feed pelleted with the 1012 PM equipped with the 6.6 L:D die and pellets produced using the 3016 PM equipped with the 9.3 L:D die regardless of die retention time ($P > 0.05$). The HPT of feed pelleted with the 1012 PM equipped with the 6.6 L:D die was 85.8 ± 1.4°C, while the feed pelleted with the 3016 PM equipped with the 9.3 L:D die was 83.1 ± 0.9°C. There was a quadratic decrease in phytase stability as the die L:D increased ($P < 0.0001$). The pellet mill model or die retention time did not affect ($P > 0.05$) the phytase stability when the HPT was less than 88°C. In addition, when the die retention time was approximately 2.6 sec, the 6.6 L:D

treatment with an HPT of 85.8 ± 1.4°C had greater phytase stability as compared to 10.6 L:D treatment with an HPT of 95.0 ± 4.4°C. However, there was no significant difference for phytase stability between the 6.6 L:D and 9.3 L:D treatment when the die retention time was approximately 1.6 sec. The HPT of feed pelleted with the 6.6 L:D die was 85.6 ± 0.6°C and the 9.3 L:D die was 83.1 ± 0.9°C. Moreover, there was an additional loss of phytase in the pellet die between 7% and 15% after conditioner (the average loss of phytase activity in the conditioner was 12.4%) when the HPT was less than 88°C. The percent loss increased up to 86% when the HPT was over 90°C. Pope (2019) reported that when feed was steamed conditioned at 86°C for 30 sec. then pelleted with an 8.0 L:D die, phytase stability was 13.5, 44.7, 73.7, and 111.0% for 227, 454, 908 and 1,816 kg/hr production rate, respectively. Additionally, based on the DRT calculation of this study, the DRT of each production rate was 7.1, 3.6, 1.8, and 0.9 sec. for 227, 454, 908, and 1,816 kg/hr. Decreasing DRT from 7.1 to 0.9 sec. significantly improved phytase stability when the HPT was between 86.8° and 89.7°C. When the HPT was lower than 90°C, while there was no significant difference on phytase stability when the DRT increased from 1.1 to 2.6 sec. regardless of pellet mill model and die L:D for the current study, which disagreed with the results reported by Pope (2019). The different response on phytase stability was probably due to the different range of DRT treatments between experiments and laboratory variation of the phytase assay. The European Union Reference Laboratory, EURL, (2013) reported that the variation of percent phytase recovery in feed that contained 500 to1,500 FTU/kg was between 77 and 108%. European Food Safety Authority, EFSA, (2013) reported that the stability of phytase produced by the *Trichoderma reesei* stain was greater than 90% when wheat-soybean mixtures contained 5,800 FTU/kg of phytase were conditioned between 70° and 90°C and then pelleted.

The results in the current study were similar to the EFSA report for pelleted feed when the conditioning temperature was less than 90°C.

Pellet quality increased (linear; $P < 0.0001$ for PDI or quadratic; $P < 0.0001$ for modified PDI) as die L:D increased (Table 3.4). The die L:D had a greater effect on both PDI methods than the die retention time. However, increased die retention time improved ($P \leq 0.05$) pellet quality when the feed was pelleted with the 6.6 L:D die, but not when pelleted using the 9.3 or 10.6 L:D die. Based on the resulted reported by Pope (2019), feed that was steamed conditioned at 86°C for 30 sec. and then pelleted with an 8.0 L:D die resulted in a decrease in the DRT from 7.1 to 0.9 sec. as the production rated increased from 227 to 1,816 kg/hr (based on the DRT calculation of this study). The decreased DTR resulted in a decrease in PDI from 96.3 to 81.0%. The results of the current experiment were similar, in which the PDI decreased as the DRT decreased when the feed was pelleted using the 1012 PM equipped with the 6.6 L:D die or the 3016 PM equipped with the 9.3 L:D die. The 1012 PM equipped with the 10.6 L:D die was unable to operate as originally planned production rate of 0.25 kg/sec. The planned production rate caused the pellet mill die to plug multiple times during the production run. Thus, the production rate had to be reduced to 0.23 kg/sec. resulting in a die retention time of 2.9 sec. instead of the planned DRT of 2.6 sec. Increasing die L:D can improve the pellet quality but the production rate may need to be decreased.

Conclusion

The phytase that was produced by the *Trichoderma reesei* strain could tolerate hot pellet temperatures up to 88°C, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures were above

91°C. Therefore, hot pellet temperatures should be measured to monitor phytase stability. In addition, increasing the die L:D resulted in the greatest improvement in pellet quality.

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Tables

Table 3.1 Diet composition of swine finishing diet

Ingredients	Percent
Corn	81.760
Soybean meal	14.247
Soy oil	1.500
Mono-calcium phosphate 21% P	0.800
Limestone	0.850
Salt	0.350
L-Lysine HCl	0.220
L-Threonine	0.050
Swine vitamin premix[1]	0.100
Swine trace mineral premix[2]	0.100
L-Tryptophan	0.003
Phytase[3]	0.020
Total	100.000

[1]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[2]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[3]Quantum® Blue 5G (AB Vista Inc., Plantation, FL) provided 1,000 phytase units (FTU)/kg with a release of 0.195% available P.

Table 3.2 Actual die retention time for each retreatment

Pellet mill model	1012	1012	3016	3016	1012	1012
Pellet die length:diameter	6.6	6.6	9.3	9.3	10.6	10.6
Planned die retention time[1], sec.	1.6	2.6	1.1	1.6	2.6	4.2
Actual die retention time[1], sec.	1.6	2.6	1.1	1.7	2.9	4.3
Die hole diameter, mm (D)	4.8	4.8	4.8	4.8	4.8	4.8
Internal die surface area, cm^2	547.0	547.0	1458.6	1458.6	547.0	547.0
Effective length, mm (L)	31.8	31.8	44.5	44.5	50.8	50.8
Holes per cm^2	2.17	2.17	2.27	2.27	2.17	2.17
Effective Volume per hole, cm^3 ($\pi D^2L/4$)	0.57	0.57	0.80	0.80	0.92	0.92
Density, kg/cm^3	0.609	0.609	0.609	0.609	0.609	0.609
Feed per die in effective length[2], kg	0.42	0.42	1.62	1.62	0.66	0.66
Actual production rate, kg/sec.	0.25	0.16	1.47	0.94	0.23	0.16

[1]Die retention time (DRT) was calculated by dividing the amount of material in the die (kg) by the production rate (kg/sec).

[2]The amount of material in the effective length of die (kg) equals multiplication of the internal die surface area (cm^2), number of holes per cm^2, effective volume per hole (cm^3), and material density (kg/cm^3).

Table 3.3 Pellet mill processing parameters

Treatment[1]	Die hole diameter, mm	Die effective length, mm	Production rate, ton/hr	Conditioned mash temperature, °C	Hot pellet temperature, °C
6.6 L:D, 1012 PM[2], 1.6 sec.	4.8	31.8	0.92	85.0 ± 1.1	85.6 ± 0.6
6.6 L:D, 1012 PM, 2.6 sec.	4.8	31.8	0.57	85.6 ± 1.2	85.8 ± 1.4
9.3 L:D, 3016 PM[3], 1.1 sec.	4.8	44.5	5.31	82.8 ± 1.1	83.1 ± 0.9
9.3 L:D, 3016 PM, 1.7 sec.	4.8	44.5	3.38	82.8 ± 1.1	83.1 ± 0.9
10.6 L:D, 1012 PM, 2.9 sec.	4.8	50.8	0.82	85.6 ± 1.2	95.0 ± 4.4
10.6 L:D, 1012 PM, 4.3 sec.	4.8	50.8	0.56	85.6 ± 1.2	95.0 ± 2.8

[1]Die specification, Pellet mill model, Actual die retention time.
[2]1012 PM equipped with a 30.5 cm internal diameter die.
[3]3016 PM equipped with a 40.6 cm internal diameter die.

Table 3.4 The effect of combination of pellet mill model, die L:D ratio, and die retention time on phytase activity and phytase stability of cooled pellet samples.

Treatment[1]	n	Phytase activity, FTU/kg	Phytase stability[2], %	PDI, %	Modified PDI, %
Main effect					
6.6 L:D, 1012 PM, 1.6 sec.	8	1,010[a]	80.7[a]	62.9[e]	36.7[d]
6.6 L:D, 1012 PM, 2.6 sec.	8	916[a]	73.1[a]	74.8[d]	52.2[c]
9.3 L:D, 3016 PM, 1.1 sec.	9	1,021[a]	81.5[a]	85.7[c]	68.3[b]
9.3 L:D, 3016 PM, 1.7 sec.	9	938[a]	74.9[a]	87.5[b]	70.2[b]
10.6 L:D, 1012 PM, 2.9 sec.	9	123[b]	9.9[b]	95.1[a]	89.8[a]
10.6 L:D, 1012 PM, 4.3 sec.	9	17[b]	1.3[b]	95.4[a]	90.8[a]
SEM		65.0	5.19	0.57	1.19
Source of variation			*P*-value		
Treatment		<0.0001	<0.0001	<0.0001	<0.0001
Linear of die L:D		<0.0001	<0.0001	<0.0001	<0.0001
Quadratic of die L:D		<0.0001	<0.0001	0.863	<0.0001

[1]Die specification, Pellet mill model, Actual die retention time.

[2]Phytase stability was calculated by dividing phytase activity of cooled pellet sample by average phytase activity of mash samples and then multiplied by 100.

[a-e]Means within a column followed by a different letter are significantly different ($P \leq 0.05$).

Chapter 4 - The Effect of Different Inclusion Levels of Corn Starch and Fine Ground Corn with Different Conditioning Temperature or Die Thickness on Pellet Quality

Abstract

Pelleted diets improve animal performance. In addition to feeding pellets, there is additional value in feeding a greater percentage of whole pellets. Pellet binders are commonly used in commercial feed mills but the added cost has limited their use in poultry and swine feed mills. However, in commercial feed mills where pellet quality is a key objective, they may be used in the diets that do not make an acceptable quality pellet. Corn starch could be a potential natural binder for feed as it is for a biomass pellet. The objective of this experiment was to determine the effect of different inclusion levels of corn starch and fine ground corn with different conditioning temperatures or die thicknesses on pellet quality. Experiment 1 treatments were arranged in a 3 × 2 factorial design of corn starch inclusion level (0%, 5% and 10%) and die thickness (12.7 and 22.2 mm). Experiment 2 treatments were arranged in a 3 × 2 factorial design of fine ground corn inclusion level (0%, 10% and 20%) and conditioning temperature (80° and 85°C). The result of Experiment 1 demonstrated that there was no interaction between corn starch inclusion level and die thickness on modified pellet durability index (PDI) (P = 0.636). Increasing die thickness from 12.7 mm to 22.2 mm significantly increased the modified PDI (P = 0.001). There was a linear decrease in modified PDI as the corn starch inclusion level increased (P < 0.001). The result of Experiment 2 demonstrated that there was no interaction between fine ground corn inclusion level and conditioning temperature on PDIs (P > 0.541). The

fine ground corn inclusion level did not impact PDIs ($P > 0.238$). Increasing conditioning temperature from 80° to 85°C significantly increased PDIs by 0.4 and 9.4% for standard and modified methods, respectively ($P < 0.0001$). Therefore, the use of pure corn starch was not an effective binding agent in the feed when the diet contains at least 60% ground corn. Increasing the ratio of starch to protein decreased PDI. The ratio of small corn particles to large corn particles in the diet did not impact pellet quality when the diets were conditioned above 80°C for 35 sec. and then pelleted with a 5.6 L:D die. Increasing die thickness and conditioning temperature improved pellet quality.

Introduction

Pelleted feed is commonly fed to nursery pigs. The advantages of pelleted feed are improvement of feed flowability, animal performance and storage capacity. Groesbeck et al. (2009) reported nursery pigs that were fed a pelleted diet had significantly greater gain to feed ratio (G:F) as compared to when they were fed a mash diet. There are factors that influence pellet quality such as ingredient characteristics, grinding, process uniformity and pellet mill parameters (Stark et al., 2014). The commercial feed industry uses pellet binders in diets that contain ingredients that are difficult to pellet or contain a high percentage of oils or large particles. Wood (1987) reported that pre-gelatinized tapioca starch improved pellet quality as compared to native starch. PelletBond^{TM} is a commercial pellet binder that is manufactured from modified starch, which has a recommended inclusion rate between 0.4 to 0.7%. Tumuluru et al. (2016) reported that adding 2 to 4% pure corn starch acted as a binder in ground corn stover to increase pellet durability when the mixture was pelleted with a flat die pellet mill. In addition, Wondra et al. (1995) reported that decreasing corn particle size in a swine finishing diet from 1,000 µm to 400 µm increased pellet durability index (PDI) from 78.8 to 86.4%. Small particles have more surface area to absorb steam during pelleting and also have more potential contact points with other particles. Increasing the percentage of very fine corn particle may improve the pellet quality of the pelleted feed. However, there is limited data on the impact of replacing a portion of the ground corn in a diet with corn starch and fine ground corn on pellet quality in corn-soybean meal diet. Therefore, the objective of this experiment was to determine the effect of different inclusion levels of corn starch and fine ground corn with different conditioning temperature or die thickness on pellet quality.

Materials and Methods

Experiment 1

Treatments were arranged in a 3 × 2 factorial design of corn starch inclusion level (0%, 5% and 10%) and die thickness (12.7 and 22.2 mm) to determine the effect on pellet quality. A swine grower feed was used for the experiment (Table 4.1). The ingredients were added to a 0.170 m^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Spring, KS) and were mixed for 5 min. The mixtures were steam conditioned for approximately 30 sec. at 85°C and pelleted using a pellet mill (California Pellet Mill (CPM) model CL-5, Crawfordsville, IN) equipped with 2 different pellet dies (4.0 mm × 12.7 mm and 4.0 mm × 22.2 mm). The feeder setting was held constant at approximately 1 kg/min. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. The pelleted samples were cooled for 10 min. using a counterflow cooler. The PDI of the cooled pellets was determined according to the ASAE S269.5 standard and modified methods.

Experiment 2

Treatments were arranged in a 3 × 2 factorial design of fine ground corn inclusion level (0%, 10% and 20%) and conditioning temperature (80° and 85°C) to determine the effect on pellet quality. A swine grower feed was used for the experiment (Table 4.2). Corn was ground using a Fitz® Mill (Fitzpatrick, model DAS06, Elmhust, IL) equipped with screen no.1532-0050 (1.27 mm) which was used as fine ground corn. The ingredients were added to a 0.170 m^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Spring, KS) and were mixed for 5 min. The mixtures were steam conditioned for approximately 30 sec. at 80° or 85°C and pelleted using a pellet mill (CPM, model CL-5, Crawfordsville, IN) equipped with 4.0 mm × 22.2 mm (length to die diameter, L:D, of 5.6). The feeder setting was held constant at approximately 1 kg/min. The

pellet mill was run 3 separate times to provide 3 replicates for each treatment. The pelleted samples were cooled for 10 min. using a counterflow cooler. The PDI of the cooled pellets was determined according to the ASAE S269.5 standard and modified methods.

Data collection

Pellet durability index (PDI)

The PDI was determined by ASAE S269.5. The sample was sifted with a U.S. No. 6 (4.0 mm) sieve. A 500-g sample of sifted pellets was placed in the tumble box for 10 min. The sample was sifted again with the same sieve. The PDI was calculated by dividing the whole pellets after tumbling by the initial sample weight and then multiplying by100 (ASAE, 2012). The PDI procedure was modified by adding three 19-mm hex nuts to the tumble box and tumbling for the 10 minutes.

Particle size

Particle size was determined with a Ro-Tap model RX-29 (W.S. Tyler Industrial Group, Mentor, OH) using the method of determining and expressing fineness of feed materials by sieving (ASAE S319.2) with 0.5 g of flow agent for 10 min.

Statistical Analysis

Data were analyzed as a completely randomized design. Experiment 1, treatments were arranged in a 3 × 2 factorial design of corn starch inclusion level (0%, 5% and 10%) and die thickness (12.7 and 22.2 mm) to determine the effect on pellet quality. Experiment 2, treatments were arranged in a 3 × 2 factorial design of fine ground corn inclusion level (0%, 10% and 20%) and conditioning temperature (80° and 85°C) to determine the effect on pellet quality. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Experiment 1

The pelleting parameters were similar across treatments (Table 4.3). The pellet quality of the diets that were pelleted with the 22.2 mm die were between 87.05 to 92.52% for the standard method and between 61.88 to 76.74% for the modified method (Table 4.4). The standard PDIs were all above 87% with only a 5% difference across corn starch inclusion levels. The modified PDI appeared to be a better method for determining the effect of corn starch inclusion level and die thickness on pellet quality. There was no interaction between corn starch inclusion level and die thickness ($P = 0.636$). Both corn starch inclusion level and die thickness affected the modified PDI ($P < 0.001$). Increasing die thickness from 12.7 mm to 22.2 mm significantly increased the modified PDI. There was a linear decrease in the modified PDI as the corn starch inclusion level increased ($P < 0.001$). Behnke (2001) suggested that natural polymers such as starch and protein found in feed ingredients melted when the conditioned ingredients exceeded their glass transition temperature (T_g). The adhesion mechanism of pelleted feed occurs when the conditioned mixture, which contains melted ingredients whose temperature is above their T_g, is forced through the die. The friction between the die and rough surface of the melted materials shapes and diffuses the melted materials on the pellet surface. Simultaneously, the melted materials also interact with each other by ionic, covalent, and hydrogen bonding, dipole-dipole interactions, and Van der Waal forces. During the cooling process, the temperature of the pellets drops below the glass transition temperature, which causes the melted materials to become a solid. The T_g of protein ingredients at 15% moisture are 55°, 60° and 40°C for corn gluten meal, soya and wheat gluten, respectively (Verbeek and van den Berg, 2010). The T_g of corn starch at 13.3% moisture is 59.2°C (Liu et al., 2009). Therefore, at 85°C, corn protein and starch, and soy

protein should be transitioned to a rubbery state. However, in the current study, increasing the corn starch in the diet from 0 to 10% changed the starch to protein ratio from 2.38 to 2.65%, which decreased PDIs by 11 and 18% for standard and modified methods, respectively. As the calculated crude protein decreased from 18.3 to 17.5%, the PDIs decreased. Wood (1987) reported that protein content in the diet had a greater impact on pellet quality than starch, which is in agreement with the result of the current experiment. In addition, increasing the die thickness increased the temperature of feed on the surface of the pellet. The friction between the die and feed that is generated while forming the pellet may also enhance the interaction of melted materials, resulting in stronger bonds between natural polymers. The results of the current experiment demonstrated that increasing die thickness from 12.7 to 22.2 mm increased the modified PDI by 27%. Stark (2009) reported similar improvement in pellet quality (28%) when the die thickness was increased from 29 to 44 mm.

Experiment 2

The pelleting parameters were similar across treatments (Table 4.5). There were similar responses on PDI between standard and modified methods. The particle size of diets was 574, 557 and 544 for diets with 0, 10, and 20% fine ground corn (209 μm), respectively. For both PDI methods (Table 4.6), there was no interaction between fine ground corn inclusion level and conditioning temperature ($P > 0.541$). When the diets were conditioned above 80°C for approximately 35 sec., increasing the fine ground corn from 0 to 20% did not increase the PDIs ($P > 0.238$), even though there was an increased surface area for steam penetration that should have decreased the time for the ingredients to achieve their T_g. Soya protein has the highest T_g (60°C) of all the ingredients in the mixture. Therefore, the large and small particles should have reached their T_g when the mixture was conditioned above 80°C within 35 sec. Moreover,

increasing conditioning temperature from 80° to 85°C significantly increased PDIs by 0.4% and 9.4% for the standard and modified methods, respectively ($P < 0.0001$). Pfost (1964) reported that an increase in conditioning temperature improved pellet quality, which supported the results of the current study.

Conclusion

The results of this experiment suggest that pure corn starch was not an effective binding agent in the feed when the diet contained at least 60% ground corn. Increasing the ratio of starch to protein decreased PDI. The ratio of small corn particles to large corn particles in the diet did not impact pellet quality when the diets were conditioned above 80°C for 35 sec. and then pelleted with a 5.6 L:D die. Increasing die thickness and conditioning temperature improved pellet quality.

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Tables

Table 4.1 Diet compositions of swine grower diet

	Corn Starch, %		
Ingredients	0%	5%	10%
Corn	69.19	64.19	59.19
Corn starch	0.00	5.00	10.00
Soybean meal	26.50	26.50	26.50
Soy oil	1.50	1.50	1.50
Mono-calcium phosphate 21% P	0.55	0.55	0.55
Limestone	1.13	1.13	1.13
Swine vitamin premix[1]	0.15	0.15	0.15
Swine trace mineral premix[2]	0.15	0.15	0.15
L-Lysine HCl	0.31	0.31	0.31
DL-Methionine	0.07	0.07	0.07
L-Threonine	0.09	0.09	0.09
Phytase[3]	0.02	0.02	0.02
Salt	0.35	0.35	0.35
Calculated analysis			
Crude protein	18.26	17.87	17.49
Starch	43.40	44.85	46.30
Starch:Protein	2.38	2.51	2.65

[1]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[2]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[3]Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.3 phytase units (FTU)/kg with a release of 0.10% available P.

Table 4.2. Diet compositions of swine grower diet

	Fine ground corn, %		
Ingredients	0%	10%	20%
Corn	69.18	59.18	49.18
Fine ground corn[1]	0.00	10.00	20.00
Soybean meal (SBM)	26.50	26.50	26.50
Soy oil	1.50	1.50	1.50
Mono-calcium phosphate 21% P	0.55	0.55	0.55
Limestone	1.13	1.13	1.13
Swine vitamin premix[2]	0.15	0.15	0.15
Swine trace mineral premix[3]	0.15	0.15	0.15
L-Lysine HCl	0.31	0.31	0.31
DL-Methionine	0.07	0.07	0.07
L-Threonine	0.09	0.09	0.09
Phytase[4]	0.02	0.02	0.02
Salt	0.35	0.35	0.35
Total	100.00	100.00	100.00

[1]The fine ground corn particle was 209 μm.
[2]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[3]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[4]Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.3 phytase units (FTU)/kg with a release of 0.10% available P.

Table 4.3 Pellet mill processing parameters of Exp. 1

Treatment		Production rate, kg/hr	Retention time, sec.	Condition mash temperature, °C
Corn starch inclusion level, %	Die thickness, mm			
0	12.7	59.1 ± 1.4	31.3 ± 1.6	84.7 ± 0.9
5	12.7	51.1 ± 3.2	31.6 ± 0.5	85.0 ± 1.1
10	12.7	49.9 ± 0.8	30.8 ± 4.0	85.3 ± 1.4
0	22.2	61.0 ± 3.6	31.2 ± 1.6	85.0 ± 1.1
5	22.2	56.5 ± 4.1	30.5 ± 2.0	85.0 ± 0.6
10	22.2	51.1 ± 2.0	30.2 ± 0.5	85.3 ± 1.4

Table 4.4 The effect of corn starch inclusion level and die thickness on pellet durability index (PDI) as determined by the Holmen method for 30 s, PDI and modified PDI (Exp. 1)

Corn starch inclusion level, %	Die thickness, mm	n	PDI, %	Modified PDI, %
Interaction effects				
0	12.7	3	76.1[b]	50.6
0	22.2	3	92.5[a]	76.7
5	12.7	3	72.9[b]	47.0
5	22.2	3	90.6[a]	72.2
10	12.7	3	58.3[c]	30.2
10	22.2	3	87.1[a]	61.9
SEM			2.24	3.64
Main effect				
0		6	84.3	63.7
5		6	81.8	59.6
10		6	72.7	46.0
SEM			1.58	2.57
	12.7	9	69.1[l]	42.6[l]
	22.2	9	90.1[k]	70.3[k]
	SEM		1.29	2.10
Source of variation			*P*-value	
Corn starch × Die thickness			0.033	0.636
Die thickness			<0.0001	<0.0001
Corn starch			0.001	0.001
Linear			<0.001	<0.001
Quadratic			0.119	0.159

[a-c]Means in a column within an interaction effect followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means in a column within a main effect of corn starch inclusion level followed by a different letter are significantly different ($P \leq 0.05$).
[k-l]Means in a column within a main effect of die thickness followed by a different letter are significantly different ($P \leq 0.05$).

Table 4.5 Pellet mill processing parameters of Exp. 2

Treatment		Die size (diameter, mm × thickness, mm)	Production rate, kg/hr	Retention time, sec.	Condition mash temperature, °C
Fine ground corn, %	Conditioning temperature, °C				
0	80	4.0 × 22.2	47.0 ± 0.5	35.7 ± 0.9	80.0 ± 1.1
10	80	4.0 × 22.2	48.6 ± 1.2	34.9 ± 0.8	79.7 ± 0.9
20	80	4.0 × 22.2	48.0 ± 1.5	35.3 ± 1.0	80.3 ± 0.9
0	85	4.0 × 22.2	48.1 ± 2.4	35.0 ± 2.2	84.7 ± 0.9
10	85	4.0 × 22.2	45.9 ± 0.7	37.3 ± 0.8	84.7 ± 0.9
20	85	4.0 × 22.2	48.5 ± 2.5	35.6 ± 1.3	84.7 ± 0.9

Table 4.6 The effect of fine ground corn inclusion level and conditioning temperature on pellet durability index (PDI) as determined by the PDI and modified PDI (Exp. 2)

Fine ground corn, %	Conditioning temperature, °C	n	PDI, %	Modified PDI, %
Interaction effects				
0	80	3	94.5	74.5
0	85	3	95.9	84.6
10	80	3	94.5	75.4
10	85	3	96.0	85.1
20	80	3	94.7	76.8
20	85	3	96.1	85.1
SEM			0.18	1.53
Main effect				
0		6	95.2	79.5
10		6	95.2	80.2
20		6	95.4	81.0
SEM			0.12	0.89
	80	9	94.6[b]	75.5[b]
	85	9	96.0[a]	84.9[a]
	SEM		0.09	0.64
Source of variation			*P*-value	
Fine ground corn × Temperature			0.879	0.541
Temperature			<0.0001	<0.0001
Fine ground corn			0.238	0.298

[a-b]Means in a column within a main effect of conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).

Chapter 5 - The Impact of Fines Inclusion Level and Conditioning Temperature on Pellet Quality and Energy Consumption

Abstract

Nursery diets are typically pelleted to improve growth performance. In addition to feeding pellets, there is an additional value in feeding whole pellets. Commercial feed mills typically remove fines with a screener after cooling in order to provide a consistent product to customers. There is limited data on the effect of returning pellet fines back to the pellet mill on pellet quality and pellet mill efficiency. The objective of this experiment was to determine the effect of fines inclusion level and conditioning temperature on pellet quality and energy consumption. Experiment 1 treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10, and 20%) and conditioning temperature (77° and 82°C). Experiment 2 treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10, and 20%) and conditioning temperature (80° and 85°C). The results of Experiment 1 demonstrated there was no interaction between fines inclusion level and conditioning temperature on pellet durability index (PDI) ($P > 0.348$). Increasing conditioning temperature from 77° to 82°C significantly increased PDI by 0.6 and 4.3% for both the standard and modified methods, respectively ($P < 0.003$). There was a linear decrease in PDI as the fines inclusion level increased ($P < 0.032$). The results of Experiment 2 demonstrated that there was an interaction between fines inclusion level and conditioning temperature for modified PDI ($P < 0.001$). When the diets were pelleted at 85°C, increasing the fines inclusion level increased modified PDI. However, there was no significant difference for modified PDI of the diets with 0, 10, and 20% fines inclusion level when they were pelleted at 80°C. For starch analysis, there was no interaction between fines

inclusion level and conditioning temperature on total starch ($P = 0.562$). There was no significant difference on total starch between the diets that were pelleted at 80° and 85°C ($P = 0.715$). The total starch was the lowest in the diet with 0% fines (54.11%) followed by the diet with 20% and 10% fines (56.42% and 57.90%), respectively ($P = 0.013$). For gelatinized starch and cooked starch, there was no interaction between the fines inclusion level and conditioning temperature ($P > 0.276$). Both fines inclusion level and conditioning temperature did not affect gelatinized starch ($P > 0.067$). For energy consumption, there was an interaction between fines inclusion level and conditioning temperature ($P < 0.0001$). When the diets were pelleted at 85°C conditioning temperature, the diet with 20% fines required significantly more energy during the pelleting process as compared to the diets with 0 and 10%. However, there was no significant difference in energy consumption for diets containing 0, 10, and 20% fines when the diets were pelleted at 80°C conditioning temperature. Therefore, increasing conditioning temperature increased pellet quality. When a diet contained less than 1.5% oil, recirculating fines through the conditioner and pellet die improved pellet quality. However, the 20% inclusion of fines led to occasional roll slips, decreased pellet mill stability, and increased energy usage when the diet was pelleted.

Introduction

The advantages of pelleted feed are improved flowability, animal performance and storage capacity. Nursery diets are typically pelleted to improve growth performance. Groesbeck et al. (2009) reported nursery pigs that were fed a pelleted diet from d 0 to 22 had significantly greater gain to feed ratio (G:F) as compared to when they were fed a mash diet. In addition to feeding pellets, there is additional value in feeding a greater percentage of whole pellets. Stark (1994) reported a tendency for poorer feed conversion when the fines in nursery diets increased from 0 to 25% in a 28-day feeding study. Commercial feed mills typically use a mechanical screener to remove the fines from the pellets after cooling in order to provide a consistent product to customers. The screened fines are returned to the pellet mill and can be up to 30% when the pellet mill is manufacturing poor quality pellets (California Pellet Mill Co., 2019). Winowiski (2009) and Bortone (2019) reported that the addition of fines with a higher fat content than the mash decreased pellet quality. However, Wood (1987) reported that pre-gelatinized tapioca starch improved pellet quality as compared to native tapioca starch. Pellet fines that are returned to the pellet mill may have some starch granules that are pre-gelatinized, which may improve pellet quality. There is limited data on the effect of returning pellet fines back to the pellet mill on pellet quality and pellet mill efficiency. Therefore, the objective of this experiment was to determine the effect of fines inclusion level and conditioning temperature on pellet quality and energy consumption.

Materials and Methods

Experiment 1

Treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10, and 20%) and conditioning temperature (77° and 82°C) to determine the effect on pellet quality. A swine grower feed was used for the experiment (Table 5.1). The ingredients were added to a 1.64 m^3 counterpoise mixer (Hayes and Stolz model TRDB63-0152, Fort Worth, TX) and were mixed for 1 min dry mix and 2 min wet mix time. The mixtures were steam conditioned for approximately 30 sec. at 77° or 82°C and pelleted using a pellet mill (CPM, model CL-5, Crawfordsville, IN) equipped with a 4.0 mm × 31.8 mm die. The feeder setting was held constant at approximately 1 kg/min. The diets pelleted at each temperature were ground using a single pair crumble roll (Colorado Mill Equipment model EcoRoll 7, Canon City, CO) with a 1 mm gap to produce the fines. The mash swine grower diet and pellet fines mixtures were produced by combining 85.72-kg of mash swine grower diet and 9.53-kg of pellet fines or 76.20-kg of mash swine grower diet and 19.05-kg of pellet fines for the 10 and 20% fines treatments, respectively. The mixture was mixed in a 0.170 m^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Spring, KS) for 5 min. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. The pelleted samples were cooled for 10 min. using a counterflow cooler. The PDI of the cooled pellets was determined according to the ASAE S269.5 standard and modified methods.

Experiment 2

Treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10, and 20%) and conditioning temperature (80° and 85°C) to determine the effect on pellet quality, gelatinized starch and energy consumption. A swine finishing feed was used for the experiment

(Table 5.2). The ingredients were added to a 1.64 m^3 counterpoise mixer (Hayes and Stolz model TRDB63-0152, Fort Worth, TX) and were mixed for 1 min dry mix and 2 min wet mix time. The mixtures were steam conditioned for approximately 30 sec. at 80° or 85°C and pelleted using a 1-ton 30-horsepower pellet mill (CPM, model 1012-2 HD Master, Crawfordsville, IN) equipped with a 4.8 mm × 50.8 mm die. The feeder setting was held constant at approximately 15 kg/min. The diets pelleted at each temperature were ground using a single pair crumble roll (Colorado Mill Equipment model EcoRoll 7, Canon City, CO) with a 1 mm gap to produce the fines. The mash swine grower diet and pellet fines mixtures were produced by combining 204.12-kg of mash swine grower diet and 22.68-kg of pellet fines or 181.44-kg of mash swine grower diet and 45.36-kg of pellet fines for the 10 and 20% fines treatments, respectively. The mixture was mixed in the 1.64 m^3 counterpoise mixer for 3 min. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. The pelleted samples were cooled for 10 min. using a counterflow cooler. The cooled samples were analyzed for total starch, gelatinized starch and cooked starch. The PDI of the cooled pellets was determined according to the ASAE S269.5 standard and modified methods. The energy consumption was recorded during pelleting process.

Data collection

Pellet durability index (PDI)

The PDI was determined by ASAE S269.5. The sample was sifted with a U.S. No. 5 (4.8 mm) sieve. A 500-g sample of sifted pellets was placed in the tumble box for 10 min. The sample was sifted again with the same sieve. The PDI was calculated by dividing the whole pellets after tumbling by the initial sample weight and then multiplying by100 (ASAE, 2012). The PDI procedure was modified by adding three 19-mm hex nuts to the tumble box and tumbling for the 10 min.

Energy consumption

Specific energy consumption (SEC) was calculated by dividing energy used (kW) by production rate (ton/hr) (Lawrence et al., 2019). The energy consumption was recorded with a Data View Current and Voltage Data Logger (Supco model DVCV, Allenwood, NJ).

Starch analysis (as described by Lewis et al., 2015)

Total starch was determined by the amount of D-glucose that was released from a mixture of 0.5-g sample and 25 ml distilled water after boiling for 20 min. followed by 70 min. of incubation at 40°C with glucoamylase. Gelatinized starch was determined by the amount of D-glucose that was released from a mixture of 0.5-g sample and 25 ml distilled water after resting for 20 min. followed by 70 min. incubation at 40°C with glucoamylase. Cooked starch was determined by dividing gelatinized starch by total starch then multiplying by 100.

Statistical Analysis

Data were analyzed as a completely randomized design. Experiment 1 treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10. and 20%) and conditioning temperature (77° and 82°C) to determine the effect on pellet quality. Experiment 2 treatments were arranged in a 3 × 2 factorial design of fines inclusion level (0, 10, and 20%) and conditioning temperature (80° and 85°C) to determine the effect on pellet quality, gelatinized starch and energy consumption. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Experiment 1

The pellet mill throughput and retentions time were similar across treatments (Table 5.3). There were similar PDI responses for the standard and modified methods. For both methods, there was no interaction between fines inclusion level and conditioning temperature ($P > 0.348$) (Table 5.4). Both fines inclusion level and conditioning temperature had an effect on PDIs ($P < 0.028$). Increasing conditioning temperature from 77° to 82°C significantly increased PDIs by 0.6 and 4.3% for standard and modified methods, respectively. There was a linear decrease in PDIs as the fines inclusion level increased ($P < 0.032$). Increasing the conditioning temperature and percent fines improved pellet quality. The improvement may be due to increased gelatinization of the starch each time the recirculated fines passed through the conditioner and pellet die. Wood (1987) reported that pre-gelatinized tapioca starch improved pellet quality when compared to native tapioca starch. However, Winowiski (2009) suggested that adding back fines that contain a higher percentage of fat would have a negative effect on pellet quality. In this current experiment, the diet contained only 1.5% soybean oil, with no oil added post-pelleting, thus the level of fat in the mixture of mash and fines was constant.

Experiment 2

The pellet mill throughput and retentions time were similar across treatments (Table 5.5). The PDI of the six treatments was between 86 and to 93% for the standard method and between 68 and 85% for the modified method (Table 5.6). There were different PDI responses for the standard and modified methods. Differences between treatments were not observed when the standard method was used to measure PDI due to the high PDI results for all of the treatments. The modified PDI provided a better explanation of the effect of fines inclusion level and

conditioning temperature on pellet quality for this experiment. There was an interaction between fines inclusion level and conditioning temperature for modified PDI ($P < 0.001$). There was an increased in the modified PDI when the diets were pelleted at 85°C conditioning temperature and the fines inclusion level was increased. However, there was no significant difference for modified PDI of the diets with 0, 10, and 20% fines inclusion level when all diets were pelleted at 80°C conditioning temperature. Both fines inclusion level and conditioning temperature impacted modified PDI ($P < 0.0001$). Increasing conditioning temperature or fines inclusion level improved pellet quality.

There was no interaction between fines inclusion level and conditioning temperature on total starch (Table 5.6) ($P = 0.562$). There was no difference in the total starch between the diets that were pelleted at 80° and 85°C conditioning temperatures ($P = 0.715$). The total starch was the lowest in the diet with 0% fines (54.11%) followed by the diet with 20% and 10% fines (56.42 and 57.90%, respectively) ($P = 0.013$). There was no interaction between fines inclusion level and conditioning temperature ($P > 0.276$) for gelatinized and cooked starch results. Both fines inclusion level and conditioning temperature did not affect gelatinized and cooked starch results ($P > 0.067$). However, the cooked starch was marginal higher when the diets were pelleted at 85°C versus 80°C conditioning temperature. Recirculated fines that passed through the conditioner and pellet die had a marginal higher percentage of cooked starch.

For energy consumption (Table 5.7), there was an interaction between fines inclusion level and conditioning temperature ($P < 0.0001$) (Figure 5.1). When the diets were pelleted at 85°C conditioning temperature, the diet with 20% fines (25.2 kWh/ton) required significantly more energy during the pelleting process as compared to the diets with 0 and 10% (17.0 and 16.6 kWh/ton, respectively). However, there was no significant difference in energy consumption for

the diets that contained 0, 10, and 20% fines (16.7, 16.8, and 16.5 kWh/ton, respectively) when the diets were pelleted at 80°C conditioning temperature. The pellet mill required more energy per ton when the diet was pelleted at 85°C versus 80°C conditioning temperature ($P < 0.001$). CPM (2019) stated that increasing the conditioning temperature with steam increases the moisture of the conditioned mash feed and reduces the friction of feed as it passes through the die hole, which lowers the energy cost. The result of the current experiment demonstrated that when the diet was steam conditioned above an optimum temperature, more energy was required to produce the pellets. There was a quadratic increase in energy consumption per ton as the fines inclusion level increased ($P < 0.001$). There was no interaction between fines inclusion level and conditioning temperature ($P = 0.075$) for variation in energy consumption, as measured by the standard deviation of the specific energy consumption (SEC). There was a linear increase in variation of energy consumption as the fines inclusion level increased ($P = 0.021$). The pellet mill was less consistent when the diets were pelleted at 85°C conditioning temperature as compared to 80°C conditioning temperature ($P = 0.019$). Zhengchang (2018) noted that uneven moisture of the conditioned mixture can result in roll-slip and die blockage. The higher energy consumption and inconsistent energy consumption that was observed when pelleting the diet that contained 20% fines may have occurred as a result of nonuniform conditioning of the mixtures. The different particle sizes and steam absorbance rates of mash and fines could have created the variation observed during pelleting at 85°C. Additionally, there were occasional roll-slips during the pelleting process. Diets that were pelleted at 85°C conditioning temperature with 20% fines had 3.8% more gelatinized starch, 10.6% higher modified PDI, 8 kWh/ton SEC, and 1.6 kWh/ton higher standard deviation of SEC as compared to the diet with no fines. When the rolls slip, feed is not being pushed into the die hole, which allows feed to build up in the gap between rolls and

die, which creates more friction. This results in a higher energy requirement to turn the die at the same speed. Additionally, the material in the pellet die hole is cooked longer especially on the surface of the pellet, which results in more gelatinized starch and higher PDI. Therefore, the pellet mill should be operated at the temperature that results in the most consistent operation to reduce energy consumption.

Conclusion

The results of this experiment suggest that increasing conditioning temperature increased pellet quality. When a diet contained less than 1.5% oil, recirculating fines through the conditioner and pellet die improved pellet quality. However, 20% fines inclusion led to occasional roll slips, decreased pellet mill stability, and increased energy usage when the diet was pelleted.

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Figures and Tables

Figure 5.1 The effect of fines inclusion level and conditioning temperature on pellet mill specific energy consumption (SEC)

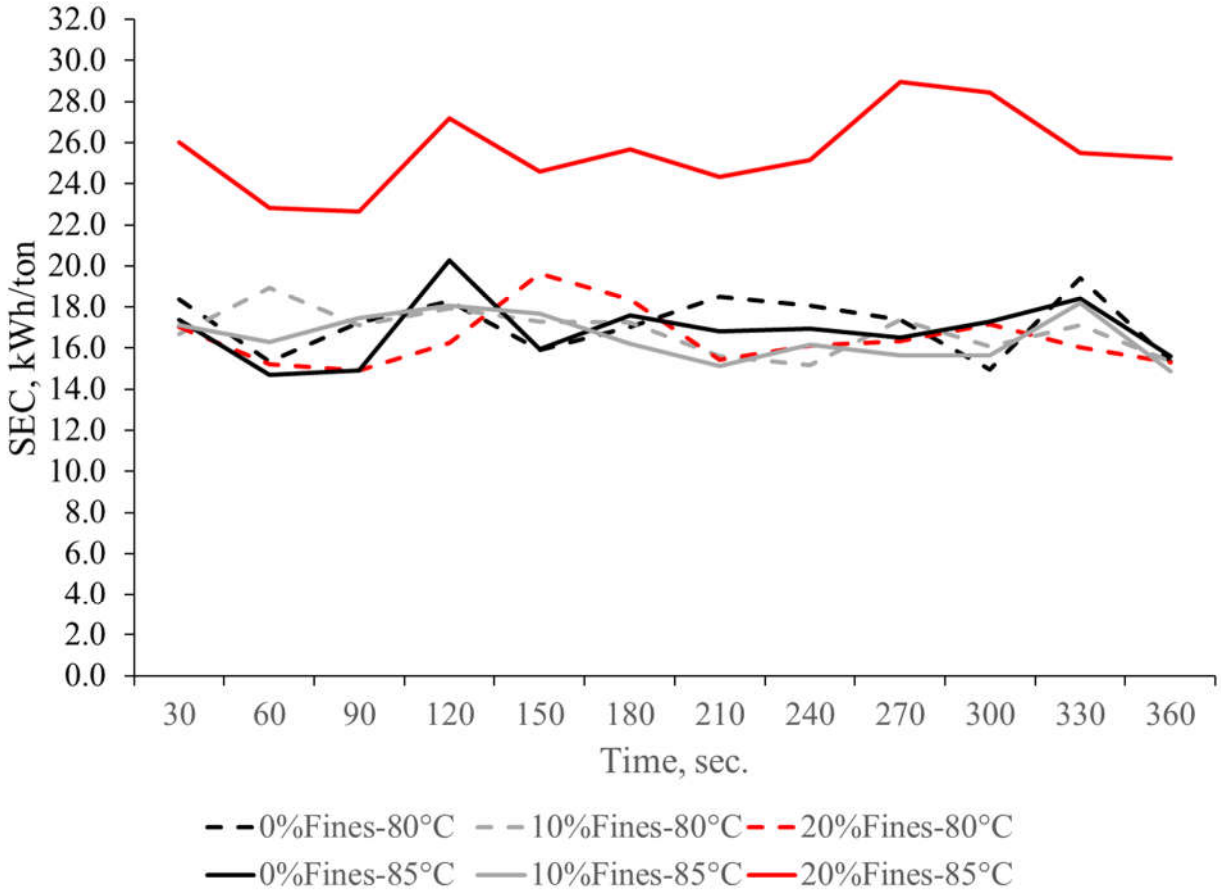

Table 5.1 Diet composition of swine grower diet

Ingredients	Percent
Corn	69.19
Soybean meal (SBM)	26.50
Soy oil	1.50
Mono-calcium phosphate 21%	0.55
Limestone	1.13
Swine vitamin premix[1]	0.15
Swine trace mineral premix[2]	0.15
L-Lysine HCl	0.31
DL-Methionine	0.07
L-Threonine	0.09
Phytase[3]	0.02
Salt	0.35
Total	100.00

[1]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[2]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[3]Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.3 phytase units (FTU)/kg with a release of 0.10% available P.

Table 5.2 Diet composition of swine finishing diet

Ingredients	Percent
Corn	76.06
Soybean meal (SBM)	20.05
Soy oil	1.50
Mono-calcium phosphate 21% P	0.32
Limestone	1.10
Swine vitamin premix[1]	0.12
Swine trace mineral premix[2]	0.12
L-Lysine HCl	0.26
DL-Methionine	0.02
L-Threonine	0.05
Phytase[3]	0.02
Salt	0.35
Total	100.00

[1]Composition per kilogram: 73 g Iron, 73 g Zinc, 22 g Manganese, 11 g Copper, 0.2 g Iodine and 0.2 g Selenium.
[2]Composition per kilogram: 1,653,439 IU Vitamin A, 661,376 IU Vitamin D3, 17,637 IU Vitamin E, 13.3 mg Vitamin B12, 1,323 mg Menadione, 3,307 mg Riboflavin, 11,023 mg d-Pantothenic Acid and 19,841 mg Niacin.
[3]Ronozyme HiPhos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ) provided 476.3 phytase units (FTU)/kg with a release of 0.10% available P.

Table 5.3 Pellet mill processing parameters of Exp. 1

Treatment		Die size, mm (diameter × thickness)	Production rate, kg/hr	Retention time, sec.	Condition mash temperature, °C
Fines inclusion level, %	Conditioning temperature, °C				
0	77	4.0 × 31.8	55.1 ± 4.5	31.7 ± 4.6	76.7 ± 1.1
10	77	4.0 × 31.8	59.1 ± 3.8	29.3 ± 3.5	76.7 ± 1.1
20	77	4.0 × 31.8	58.0 ± 5.7	30.2 ± 2.7	76.7 ± 1.1
0	82	4.0 × 31.8	55.7 ± 5.3	30.8 ± 4.9	81.9 ± 0.9
10	82	4.0 × 31.8	53.5 ± 1.5	33.7 ± 1.3	81.9 ± 0.9
20	82	4.0 × 31.8	56.1 ± 1.1	32.1 ± 1.4	82.2 ± 0.6

Table 5.4 The effect of fines inclusion level and conditioning temperature on pellet durability index (PDI) as determined by the PDI and modified PDI (Exp. 1)

Fines, %	Conditioning temperature, °C	n	PDI, %	Modified PDI, %
Interaction effects				
0	77	3	94.5	77.1
0	82	3	94.9	80.1
10	77	3	94.4	76.2
10	82	3	95.0	79.9
20	77	3	94.9	78.2
20	82	3	95.8	84.5
SEM			0.21	1.13
Main effect				
0		6	94.7	78.6
10		6	94.7	78.0
20		6	95.3	81.3
SEM			0.15	0.80
	77	9	94.6[y]	77.2[y]
	82	9	95.2[x]	81.5[x]
	SEM		0.12	0.65
Source of variation			*P*-value	
Fines × Temperature			0.419	0.348
Temperature			0.003	0.001
Fines			0.017	0.028
Linear			0.011	0.032
Quadratic			0.119	0.072

[a-b]Means in a column within a main effect of fines inclusion level followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means in a column within a main effect of conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).

Table 5.5 Pellet mill processing parameters of Exp. 2

Treatment		Die size, mm (diameter × thickness)	Production rate, ton/hr	Retention time, sec.	Condition mash temperature, °C
Fines inclusion level, %	Conditioning temperature, °C				
0	80	4.8 × 50.8	0.925 ± 0.005	27.2 ± 1.1	80.0 ± 0.6
10	80	4.8 × 50.8	0.925 ± 0.005	26.9 ± 0.8	79.4 ± 0.6
20	80	4.8 × 50.8	0.925 ± 0.005	26.3 ± 1.7	79.7 ± 0.9
0	85	4.8 × 50.8	0.925 ± 0.005	25.0 ± 0.9	85.3 ± 0.9
10	85	4.8 × 50.8	0.925 ± 0.005	25.9 ± 2.1	85.3 ± 0.9
20	85	4.8 × 50.8	0.925 ± 0.005	26.6 ± 1.2	85.3 ± 0.9

Table 5.6 The effect of fines inclusion level and conditioning temperature on pellet durability index (PDI) and gelatinized starch as determined by the PDI, modified PDI, total starch, gelatinized starch and cooked starch (Exp. 2)

Fines, %	Conditioning temperature, °C	n	PDI, %	Modified PDI, %	Total starch, %	Gelatinized starch, %	Cooked starch, %
Interaction effects							
0	80	3	86.2	68.1^{d}	53.60	12.66	23.63
0	85	3	89.3	74.7^{c}	54.62	13.56	24.80
10	80	3	87.3	69.7^{d}	58.49	13.61	23.27
10	85	3	90.2	78.1^{b}	57.31	14.28	24.93
20	80	3	87.1	69.0^{d}	56.83	13.11	23.10
20	85	3	93.2	85.3^{a}	56.00	17.32	30.73
SEM			1.14	1.18	1.07	1.40	2.12
Main effect							
0		6	87.8	71.4	54.11^{y}	13.11	24.22
10		6	88.8	73.9	57.90^{x}	13.95	24.10
20		6	90.1	77.1	56.42^{xy}	15.22	26.92
SEM			0.81	0.83	0.76	0.99	1.50
	80	9	86.9^{l}	68.9^{l}	56.31	13.13	23.33
	85	9	90.9^{k}	79.4^{k}	55.98	15.05	26.82
	SEM		0.12	0.68	0.62	0.81	1.22
Source of variation					*P*-value		
Fines × Temperature			0.329	<0.001	0.562	0.396	0.276
Temperature			0.001	<0.0001	0.715	0.118	0.067
Fines			0.151	<0.0001	0.013	0.349	0.356
Linear			0.058	<0.0001	0.052	0.158	0.228
Quadratic			0.857	0.718	0.015	0.861	0.440

[a-d]Means in a column within an interaction effect of fines inclusion level and conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).

[x-z]Means in a column within a main effect of fines inclusion level followed by a different letter are significantly different ($P \leq 0.05$).

[k-l]Means in a column within a main effect of conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).

Table 5.7 The effect of fines inclusion level and conditioning temperature on energy consumption as determined by specific energy consumption (SEC) and standard deviation of SEC (Exp. 2)

Fines, %	Conditioning temperature, °C	n	SEC, kWh/ton	Standard deviation of SEC, kWh/ton
Interaction effects				
0	80	2	16.65[b]	1.99
0	85	2	17.00[b]	2.23
10	80	3	16.82[b]	1.98
10	85	3	16.63[b]	2.23
20	80	3	16.49[b]	2.18
20	85	3	25.19[a]	3.79
SEM			1.93	0.41
Main effect				
0		4	16.83	2.11
10		6	16.72	2.10
20		6	20.84	2.99
SEM			0.96	0.29
	80	8	16.65[l]	2.05[l]
	85	8	19.61[k]	2.75[k]
	SEM		0.64	0.21
Source of variation			*P*-value	
Fines × Temperature			<0.0001	0.075
Temperature			<0.001	0.019
Fines			<0.0001	0.015
Linear			<0.0001	0.021
Quadratic			0.007	0.139

[a-b]Means in a column within an interaction effect of fines inclusion level and conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means in a column within a main effect of fines inclusion level followed by a different letter are significantly different ($P \leq 0.05$).
[k-l]Means in a column within a main effect of conditioning temperature followed by a different letter are significantly different ($P \leq 0.05$).

Chapter 6 - Effect of Feed Form, Corn Particle Size and Extrusion of Corn on Broiler Performance

Abstract

The pelleting and extrusion processes use both thermal and mechanical energies to alter the crystalline structure of the starch granule, which makes it more digestible than raw starch. A greater percentage of the starch is gelatinized in the extrusion process due to the higher mechanical shear, as well as higher temperature and moisture content. The particle size of the ground corn particle in the diet also effects the rate at which gelatinization occurs in the extrusion process. Previous researchers have reported different growth performance when birds are fed a diet containing extruded corn. There is limited data on broiler performance when feeding diets containing different particle sizes of extruded corn. The object of this study was to determine the effect of feed form, corn particle size, and the extrusion of corn on growth performance of 21 d old broiler chicks. For corn type effect, the treatments were arranged in a 2 × 3 factorial of corn type (raw corn and extruded corn) and corn particle size (400, 800, and 1200 μm) to determine the effect of growth performance. There was no interaction between corn type and corn particle size on BW from d 0 to 21 ($P > 0.164$). The raw corn diet had significantly greater BW from d 7 to 21 as compared to the extruded corn diet ($P < 0.0001$). There was no significant difference in BW at d 21 among diets with the three different corn particle sizes ($P = 0.215$). There was an interaction between corn type and corn particle size on both the relative gizzard and pancreas weights ($P < 0.039$). For feed form effect, treatments were arranged in a 2 × 2 factorial of feed form (mash and crumble feed) and corn particle size (400 and 800 μm) to determine the effect of growth performance. There was no interaction between feed form and

corn particle size on BW from d 0 to 21 ($P > 0.253$). The crumble extruded corn diet had significantly greater BW from d 7 to 21 as compared to the mash extruded corn diet ($P < 0.0001$). There was no significant difference in BW at d 21 ($P = 0.996$) between extruded diets containing 400 µm corn and 800 µm corn regardless of feed form. There was no interaction between feed form and corn particle size on relative gizzard and pancreas weights ($P > 0.528$). The mash extruded corn diet had significantly greater relative gizzard and pancreas weights as compared to the crumble extruded corn diet ($P < 0.001$). The relative gizzard weight was greater for the extruded corn diet containing 800 µm versus 400 µm corn ($P = 0.002$), while there was no significant difference in relative pancreas weight between the extruded corn diets containing 400 and 800 µm corn ($P = 0.263$). Therefore, increasing the amount of gelatinized starch in the feed by replacing ground corn with extruded corn in a broiler starter diet did not improve growth performance. Increasing corn particle size led to improved gizzard development. The corn particle size in the starter diet should be between 400 and 800 µm to optimize broiler performance from d 0 to 21.

Introduction

The pelleting and extrusion processes use both thermal and mechanical energies to alter the crystalline structure of the starch granule, which makes it more digestible than raw starch. A greater percentage of the starch is gelatinized in the extrusion process due to the higher mechanical shear, as well as higher temperature and moisture content. Starch gelatinization is the process of breaking down the crystal structure of starch granules. The first step in the gelatinization process is to loosen the hydrogen bonds. The starch granule swells as water is absorbed and then thermal energy is added, which causes a loss of birefringence (Buleon and Colonna, 2010). Moritz et al. (2005) reported 29 and 92% gelatinized starch for pelleted and extruded ground corn, respectively. He reported that adding either pelleted corn or extruded corn in a mash diet significantly improved apparent metabolizable energy (AME) as compared to feeding the mash diet containing ground corn. However, only the mash diet that contained extruded corn had a significantly higher BW at 21 d as compared to the mash diet that contained ground corn. However, the diet with extruded corn had a poorer FCR as compared to ground corn treatment (Moritz et al., 2005). Amornthewaphat et al. (2005) reported no significant difference in BW at d 21 between the crumbled diet that contained ground corn and the crumbled diet that contained both ground corn and extruded corn in a 1:1.1 ratio, which disagreed with Moritz's study. Bazolli et al. (2015) showed that increasing the particle size of the diet fed to dogs decreased the starch gelatinization in the final product. Jacobs et al. (2010) also demonstrated that corn particle size in the diet impacted growth performance. Birds had the highest BW and relative gizzard weight when fed a diet that contained 858 µm corn. The authors reported a difference in growth performance when the birds were fed a diet that contained extruded corn, however there was only one corn particle size in the study. There is limited data

on broiler performance when birds are fed diets that contain different original particle sizes of extruded corn. The object of this study was to determine the effect of feed form, corn particle size, and extrusion of corn on growth performance of 21 d old broiler chicks.

Materials and Methods

Ingredient and Extrusion Conditions

Corn was ground with a 3-high roller mill (RMS Roller-Grinder model 924, Harrisburg, SD) to three target particle sizes: 400, 800, and 1,200 μm. The three ground corn particle sizes were then extruded at 130-140°C using a single screw extruder (Wenger Manufacturing model X20, Sabetha, KS) equipped with a 6.5 mm die. The length-diameter ratio of the screw was 10:1. Additional production parameters are reported in Table 6.1. The extruded corn was dried at 104°C for 18 min. and then cooled for 7 min. using a belt dryer (Wenger Manufacturing model 4800, Sabetha, KS). Ground corn and extruded corn were analyzed for particle size, total starch (TS), gelatinized starch (GS), and cooked starch (CS).

Diet

A corn-soy broiler starter diet was formulated to meet or exceed the nutritional requirements of Cobb 500 nutrient recommendation (Cobb-Vantress, 2015) (Table 6.2). Formulation was the same across treatments. Three corn particle sizes (400, 800, and 1,200 μm) were fed as unprocessed or extruded. Prior to mixing, the three extruded corn particles size treatments were reground using a single pair crumble roll (Colorado Mill Equipment model EcoRoll 7, Canon City, CO). Diets were mixed for 5 minutes in a 0.170 m^3 paddle mixer (Davis model 2014197-SS-S1, Bonner Spring, KS). Diets containing the raw corn were steam conditioned at approximately 79°C for 18 sec. and pelleted using a pellet mill (California Pellet Mill model CL-5, Crawfordsville, IN) equipped with a 4.0 mm × 22.2 mm die. Diets containing

the extruded corn were steam conditioned at 69°C for approximately 18 sec. and pelleted using the same pellet mill equipped with a 4.0 mm × 12.7 mm die. The feeder setting was held constant at approximately 1 kg/min. Die thickness and conditioning temperature were chosen to achieve good pellet quality, while facilitating smooth operation. The pellet mill process parameters of each treatment are reported in Table 6.3. All pelleted diets were crumbled using the single pair crumble roll. There was a total of 8 treatments that consisted of six crumble diets with 400, 800 or 1200 µm either raw corn (CRC) or extruded corn (CEC) and two mash diets with 400 or 800 µm extruded corn (MEC) were yield a total of eight dietary treatments. The pelleted complete diets were analyzed for particle size, TS, GS, and CS.

Feeding Trial

All handling procedures and euthanasia protocols were carried out in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Kansas State University. A 21-day battery trial was conducted utilizing 270 male Cobb-500 broiler chicks. Birds were randomly allotted to one of eight dietary treatments and placed with 5 birds per cage, totaling 8 replicate cages per treatment. As described previously, treatments were formulated with 400 µm, 800 µm or 1,200 µm ground corn and 400 µm, 600 µm, or 1,200 µm extruded corn. There were eight dietary treatments: three CRC diets, three CEC diets and two MEC dies. The BW and feed disappearance were collected 7, 14, and 21 d. Average daily gain and feed conversion ratio were calculated and adjusted for mortality. At d 21, two birds of the approximated weight of the cage were euthanized via cervical dislocation to collect gizzard and pancreas weights.

Data collection

Pellet durability index (PDI)

The PDI was determined by ASAE S269.5. The sample was sifted with a U.S. No. 6 (4.0 mm) sieve. A 500-g sample of sifted pellets was placed in the tumble box for 10 min. The sample was sifted again with the same sieve. The PDI was calculated by dividing the whole pellets after tumbling by the initial sample weight and then multiplying by 100 (ASAE, 2012). The PDI procedure was modified by adding three 19-mm hex nuts to the tumble box and tumbling for the 10 min.

Starch analysis (as described by Lewis et al., 2015)

Total starch was determined by the amount of D-glucose released from a mixture of 0.5-g sample and 25 ml distilled water. The sample mixture was boiled for 20 min. followed by 70 min. incubated at 40°C with glucoamylase. Gelatinized starch was determined by the amount of D-glucose released from a mixture of 0.5-g sample and 25 ml distilled water. The sample mixture was allowed to rest for 20 min. followed by 70 min. incubated at 40°C with glucoamylase. Cooked starch was determined by dividing gelatinized starch by total starch then multiplying by 100.

Particle size

Particle size was determined with a Ro-Tap model RX-29 (W.S. Tyler Industrial Group, Mentor, OH) using the method of determining and expressing fineness of feed materials by sieving (ASAE S319.2) with 0.5 g of flow agent for 10 min.

Statistical Analysis

Data were analyzed as a completely randomized design. For corn type effect, treatments were arranged in a 2 × 3 factorial of corn type (raw corn and extruded corn) and corn particle size (400, 800, and 1200 μm) to determine the effect of growth performance. For feed form effect, treatments were arranged in a 2 × 2 factorial of feed form (mash feed and crumble feed)

and corn particle size (400 and 800 µm) to determine the effect of growth performance. There were 8 replicates per treatment for both corn type and feed form effects. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results

Corn analysis

The raw ground corn particle sizes were 396, 795, and 1,204 µm for 400, 800, and 1200 µm treatments, respectively. The reground extruded corn particle sizes were 1,343, 1,668, and 1,732 µm for 400, 800, and 1200 µm treatments, respectively. The gap of the crumble roll was the same across all extruded treatments. However, the difference in the reground extruded corn particle sizes may be due to the difference in the amount of gelatinized starch in the extruded corn. The amount of gelatinized starch was higher in the extruded corn compared to the raw corn by 46 to 58% (Table 6.4). Decreasing the initial corn particle size increased the percent gelatinized starch. This was to be expected since steam and heat penetrations were better with smaller corn particles than larger corn particles. Bazolli et al. (2015) reported similar results in dog food that contained corn of different particles sizes.

Feed analysis

The particle size of the mash feed was 497, 722, 943, 1,157, 1,195, and 1,363 µm for crumble with 400 µm raw corn (400RC), crumble with 800 µm raw corn (800RC), crumble with 1200 µm raw corn (1200RC), crumble with 400 µm extruded corn (400EC), crumble with 800 µm extruded corn (800EC), and crumble with 1200 µm extruded corn (1200EC), respectively. Both the PDI and modified PDI of the pelleted feed before crumbling was above 91% for all crumble feed. The gelatinized starch was 15.3, 14.5, 14.4, 39.2, 37.5, 42.9, 40.6, and 33.3% for

the crumble with 400RC, crumble with 800RC, crumble with 1200RC, mash feed with 400EC, mash feed with 800EC, crumble with 400EC, crumble with 800EC, and crumble with 1200EC, respectively (Table 6.4). The crumble feed with raw corn had greater gelatinized starch than the actual raw corn regardless of particle size. The percent cooked starch between the extruded corn and diets containing extruded corn were similar. The percent cooked starch should be a better parameter to compare between diets or ingredients containing different total starch levels. The pelleting process increased the percent cooked starch by 14% as compared to mash diets with raw corn while the pelleted diets with extruded corn had similar percent cooked starch to their mash diets.

Corn type effect

Live performance

The mortality rate for the experiment was 5.8%. There was no interaction between corn type and corn particle size on BW from d 0 to 21 ($P > 0.164$) (Table 6.5). The raw corn diet had significantly greater BW from d 7 to 21 as compared to the extruded corn diet ($P < 0.0001$). Overall the BW was 860 and 788 g for the birds fed the raw corn diet and the extruded corn diet, respectively. There was no significant difference in BW at d 21 among diets with the three different corn particle sizes ($P = 0.215$). There was no interaction between corn type and corn particle size on average daily feed intake (ADFI) in each period from d 0 to 21 ($P > 0.291$) (Table 6.6). The raw corn diet had significantly greater ADFI in each period from d 0 to 21 as compared to the extruded corn diet ($P < 0.0001$). Overall the ADFI was 50.5 and 46.1 g for the birds fed the raw corn diet and the extruded corn diet, respectively. There was no significant difference in overall ADFI among diets with the three different corn particle sizes ($P = 0.258$). There was no interaction between corn type and corn particle size on average daily gain (ADG)

in each period from d 0 to 21 ($P > 0.230$) (Table 6.7). The raw corn diet had significantly greater ADG in each period from d 0 to 21 as compared to the extruded corn diet ($P < 0.0001$). Overall the ADG was 38.9 and 35.0 g for the birds fed the raw corn diet and the extruded corn diet, respectively. There was no significant difference in overall ADG among diets with the three different corn particle sizes ($P = 0.583$). There was an interaction between corn type and corn particle size on feed conversion ratio (FCR) from d 0 to 7 ($P = 0.001$) (Table 6.8). However, the interaction disappeared by d 14 and 21. There was no significant difference in overall FCR between diet with two different corn types ($P = 0.075$). There was a linear increase in overall FCR as corn particle size increased ($P = 0.015$). Overall the FCR was 1.30, 1.32, and 1.33 for the birds fed the diets with 400, 800 and 1200 μm corn, respectively.

The relative organ weights

There was an interaction between corn type and corn particle size on gizzard size ($P = 0.006$) (Table 6.9). The raw corn diet had significantly larger gizzard weight as compared to the extruded corn diet when the corn particle sizes were 400 and 800 μm. However, there was no significant difference in relative gizzard weight between the raw corn diet and extruded corn diet when the corn particle size was 1200 μm. There was an interaction between corn type and corn particle size on relative pancreas weight ($P = 0.039$). The birds fed the extruded corn diet with 1200 μm corn had significantly larger pancreases as compared to the extruded corn diets with 400 and 800 μm corn. However, there was no significant difference in relative pancreas weight among the raw corn diet with the three different corn particle sizes.

Feed form effect

Live performance

The mortality rate for the experiment was 5.0%. There was no interaction between feed form and corn particle size on BW from d 0 to 21 ($P > 0.253$) (Table 6.10). The birds fed the crumbled diet with extruded corn (CEC) diet had a significantly greater BW from d 7 to 21 as compared to the mash with extruded corn (MEC) diet ($P < 0.001$). The overall BW was 792 and 707 g for the birds fed the CEC and MEC diets, respectively. There was no significant difference in BW from d 7 to 21 between the extruded corn diets containing 400 and 800 µm corn regardless of feed form ($P > 0.190$). There was no interaction between feed form and corn particle size on ADFI in each period from 0 to 21 ($P > 0.067$) (Table 6.11). The CEC diet had significantly greater ADFI in each period from d 0 to 21 as compared to the MEC diet ($P < 0.0001$). The overall ADFI was 46.0 and 41.7 g for the birds fed the CEC and MEC diets, respectively. There was no significant difference in overall ADFI between the extruded corn diets containing 400 and 800 µm corn regardless of feed form ($P = 0.189$). There was no interaction between feed form and corn particle size on ADG in each period from 0 to 21 ($P > 0.183$) (Table 6.12). The CEC diet had significantly greater ADG in each period from d 0 to 21 as compared to the MEC diet ($P < 0.0001$). The overall ADG was 35.0 and 31.5 g for the birds fed the CEC and the MEC diets, respectively. There was no significant difference in ADG for each period from d 0 to 21 between the extruded corn diets containing 400 and 800 µm corn regardless of feed form ($P > 0.193$). There was no interaction between feed form and corn particle size on FCR in each period from 0 to 21 ($P > 0.179$) (Table 6.13). The FCR from d 0 to 7 was lower for the CEC diet than the MEC diet ($P < 0.0001$) while there was no significant difference in overall FCR between CEC diet and MEC diet ($P = 0.179$). There was no significant difference in FCR for each period from d 0 to 21 between extruded corn diet containing 400 and 800 µm corn regardless of feed form ($P > 0.277$).

The relative organ weights

There was no interaction between feed form and corn particle size on relative gizzard and pancreas weights ($P > 0.528$) (Table 6.14). The MEC diet had significantly greater relative gizzard and pancreas weights as compared to the CEC diet ($P < 0.001$). Regardless of feed form, the relative gizzard weight was greater for the extruded corn diet containing 800 μm corn than 400 μm corn ($P = 0.002$) while there was no significant difference in relative pancreas weight between the extruded corn diets containing 400 and 800 μm corn ($P = 0.263$).

Discussion

Corn type effect

The overall BW from d 0 to 21 was significantly lower for the broilers fed the extruded corn diets as compared to the raw corn diets primarily due to the lower feed intake of the broilers that were fed the extruded corn diets. Ljubojevic et al. (2011) reported birds fed a pelleted extruded corn diet had similar BW and feed intake at 14 and 28 d compared to birds fed a pelleted raw corn diet in contrast to the results of the current study. In the current study, birds fed the crumble diet containing the extruded corn appeared to have a larger crumb size, which may have reduced feed intake resulting in a lower BW at 7 d. The average crumble particle size of the feed samples was 1.6 and 1.8 mm for raw corn and extruded corn diets, respectively. Nabi et al. (2017) reported there was no significant difference in feed intake between birds fed 1.0 and 1.5 mm crumble diets in contrast to the current study. However, the crumble sizes of the current study were larger compared to Nabi's study. A possible explanation for the lower feed intake at the first 7-days was that the extruded corn diet had a slower passage rate because the gizzard was not fully developed. Agboola et al. (2016) reported that a corn-soybean meal diet without

exogenous enzymes had a higher intestinal viscosity and slower passage rate than the diet with exogenous enzymes. In the current study, starch in the extruded corn was gelatinized, which would make it more digestible and could produce the same effect as enzymes resulting in a lower pancreatic enzyme request and higher intestinal viscosity. Furthermore, the birds fed the extruded corn diet in the current study had smaller pancreases, which could indicate the feed was more digestible. Therefore, an extruded corn diet could increase the intestinal viscosity thus slowing down digesta passage rate, which may lead to lower feed intake. At d 7, the BW of birds fed the extruded corn were 14 g lower than birds fed the raw corn, this occurred regardless of corn particle size. Lower feed intake could also be due to the palatability of the extruded diets that went through two heat treatments. The extruded corn in the diet was cooked through the extruder at 130°C and then reheated again in the pelleting process to 69°C during the conditioning and pelleting processes. Broilers fed the extruded corn or raw corn diets with 400 and 800 μm corn had similar FCR. The extruded corn diet with 1200 μm had the poorest FCR at d 7, which may have resulted from the poor digestibility of the large corn. The cooked starch of the extruded corn was 87, 79, and 74% cook for the 400, 800, and 1200 um, respectively. After d 7, there was no significant difference in FCR between the broilers fed extruded corn diet and raw corn diet. However, the slower growth performance of bird fed extruded corn diets during the first 7-day period resulted in an overall lower body weight at 21 days. The extrusion process increased percent gelatinized starch in the ground corn, but addition of extruded corn to the diet did not improve FCR. Amornthewaphat et al. (2005) reported the BW and FCR of broilers fed a crumbled diet containing 25% extruded corn (52% of total corn in the diet) was similar at d 21. Additionally, broilers fed the diet contained partially extruded corn diet had a similar FCR as broilers fed the extruded corn diet in the current experiment. However, the extruded corn diet

resulted in a lighter bird at 21-d in the current study, in contrast to Amornthewaphat et al.'s findings. In the current study, the diet with 400 µm corn had an improved FCR as compared to the diets with 800 and 1200 µm corn. However, there was no significant difference in BW when the broilers were fed three different corn particle sizes regardless of corn type. The BW were 874, 865, and 840 g for the raw corn diets with 400, 800, and 1200 µm corn, respectively. Increasing corn particle size from 400 to 1200 µm for the raw corn diet increased the relative gizzard weight from 1.54 to 1.71%. However, the relative gizzard weight increased by 0.02% when the corn particle size in the diet increased from 800 to 1200 µm. Jacobs et al. (2010) reported that increasing corn particle size from 557 to 1,387 µm did not improve BW and FCR but increased relative gizzard weight from 1.50 to 2.20%. In the current study, when the 400 and 800 µm corn particle sizes were used in the diet, the birds fed extruded corn diets had significantly smaller gizzard weight compared to birds fed raw corn diets which may have been due to reduce gizzard development resulting from under stimulation. Moreover, the relative pancreatic weight at d 21 was significantly smaller in broilers fed the extruded corn diets with 400 and 800 µm corn compared to the raw corn diets, which could indicate the feed was more digestible. Wang et al. (2005) suggested that highly digestible feed, which contains a supplement enzyme, may result in smaller pancreas. Therefore, based on the results of this experiment, the corn particle size for a starter broiler should be between 400 to 800 µm. Pre-gelatinized starch did not improve growth performance.

Feed form effect

The overall BW from d 0 to 21 was significantly greater when the broilers were fed the CEC diets as compared to the MEC diets because the broilers consumed more feed when they were fed the crumble diets as compared to the mash diets. Amornthewaphat et al. (2005)

reported that birds fed a crumble diet containing 25% extruded corn had significantly higher BW than the birds fed the mash diet containing 25% extruded corn which was similar to the results of the current experiment. Since broilers consume feed based on gastrointestinal tract volume, the lower density diets may result in a lower feed intake. The density observed in the current study was 468, 462, 552, and 541 g/l for the MEC diet with 400 µm corn, MEC diet with 800 µm corn, CEC diet with 400 µm corn and CEC diet with 800 µm corn, respectively. Feeding crumbles from d 0 to 7 improved FCR. However, the overall FCR was similar between the crumble and mash diets. The difference in FCR at d 0 to 7 and d 0 to 21 between the feed forms may be due to selective feeding of pieces of extruded corn or soybean meal from the mash diet, which cannot occur within a crumble diet. The birds fed the mash diets had significantly larger gizzards and pancreases as compared to the crumble diets. Xu et al. (2015) reported the birds fed crumbled diets had significantly smaller gizzard weight compared to birds fed mash diet regardless of coarse ground corn inclusion, which was similar to the result of current study. The final particle size of the CEC diets as compared to the MEC diets could have been reduced during the pelleting and crumbling processes, which resulted in smaller gizzard. Additionally, the relative gizzard weight was greater when broilers were fed the extruded diet with 800 µm corn versus 400 µm corn which was similar to Jacobs' findings. Jacobs et al. (2010) reported that increasing corn particle size increased gizzard size. Svihus et al. (2004) reported that the particle size distribution in the pelleted diet was reduced after the pelleting process. Moreover, the relative pancreas weight was lower for the CEC diets as compared to the MEC diet, which could indicate the feed was more digestible from the higher percent gelatinized starch and smaller final particle size of the CEC diet.

Conclusion

The results of this study indicated that increasing the amount of gelatinized starch in the feed by replacing ground corn with extruded corn in a broiler starter diet did not improve growth performance. Increasing corn particle size led to improved gizzard development. The corn particle size in the starter diet should be between 400 and 800 μm to optimize broiler performance from d 0 to 21.

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Tables

Table 6.1 Extrusion processing parameters of extruded corn

Corn particle size, μm	Preconditioning			Extrusion							
	Discharge temperature sitting, °C	Steam flow, kg/hr.	Water flow, kg/hr.	Shaft speed, RPM	Motor load, %	Water flow, kg/hr.	Steam flow, kg/hr.	Die temperature, °C	Die pressure, PSIG	Knife speed, RPM	SME[1], kJ/kg
400	90	9.8	4.1	386	48	5.4	0.0	140	140	915	135
800	90	9.8	4.2	373	51	5.3	0.0	130	200	915	155
1200	90	9.8	4.1	375	45	5.0	0.0	135	200	915	107

[1]Specific mechanical energy in the extruder.

Table 6.2 Diet compositions of broiler starter diet

	Raw ground corn			Extruded corn[1]		
Ingredients	400 µm	800 µm	1200 µm	400 µm	800 µm	1200 µm
Corn	59.71	59.71	59.71	59.71	59.71	59.71
Soybean meal	34.60	34.60	34.60	34.60	34.60	34.60
Soy oil	1.00	1.00	1.00	1.00	1.00	1.00
Mono-calcium phosphate, 21% P	2.10	2.10	2.10	2.10	2.10	2.10
Limestone	1.40	1.40	1.40	1.40	1.40	1.40
Poultry vitamin and mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.28	0.28	0.28	0.28	0.28	0.28
L-Threonine	0.08	0.08	0.08	0.08	0.08	0.08
Sodium bicarbonate	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.23	0.23	0.23	0.23	0.23	0.23
Calculated analysis						
Crude protein, %	22.10	22.10	22.10	22.10	22.10	22.10
Crude fat, %	3.37	3.37	3.37	3.37	3.37	3.37
ME, kcal/kg	3040	3040	3040	3040	3040	3040

[1]Three ground corn particle sizes were extruded at 130-140 °C using a single screw extruder equipped with 6.5 mm die then reground using a single pair crumble roll.
[2]Composition per kilogram: 20 g Iron, 40 g Zinc, 40 g Manganese, 4.5 g Copper, 0.6 g Iodine, and 0.06 g Selenium. 3,080,000 IU Vitamin A, 1,100,000 IU Vitamin D3, 6,600 IU Vitamin E, 4.4 mg Vitamin B12, 330 mg Menadione, 2,640 mg Riboflavin, 2,640 mg d-Pantothenic Acid, and 11,000 mg Niacin.

Table 6.3 Pellet mill processing parameters of crumble feed

Treatment		Production rate, kg/hr	Retention time, sec.	Condition mash temperature, °C
Corn type	Corn particle size[1], µm			
Raw corn	400	51.2	18.2	80.3 ± 0.9
Raw corn	800	53.9	17.3	78.6 ± 0.8
Raw corn	1200	62.6	14.9	78.4 ± 0.6
Extruded corn	400	62.6	16.4	68.6 ± 1.4
Extruded corn	800	59.9	17.2	69.7 ± 1.4
Extruded corn	1200	61.2	16.8	69.7 ± 1.4

[1]The particle sizes were initial ground corn particle sizes.

Table 6.4 Starch analysis of raw corn, extruded corn and diets and pellet quality of pellets before crumble

Item	Particle size[1], µm		Total starch, %	Gelatinized starch, %	Cooked starch, %	PDI[2], %	Modified PDI[3], %
	d_{gw}	S_{gw}					
400 µm ground raw corn	396	2.22	77.17	8.76	11.4		
800 µm ground raw corn	795	2.08	71.91	6.95	9.70		
1200 µm ground raw corn	1,204	2.18	70.46	8.12	11.5		
Mash Feed with 400 µm raw corn	497	2.63	44.70	7.50	16.8		
Mash Feed with 800 µm raw corn	722	2.57	46.23	7.15	15.5		
Mash Feed with 1200 µm raw corn	943	2.48	43.45	6.97	16.0		
Crumble Feed with 400 µm raw corn			48.62	15.27	31.4	97.5	93.5
Crumble Feed with 800 µm raw corn			49.78	14.51	29.1	97.5	93.0
Crumble Feed with 1200 µm raw corn			46.24	14.42	31.2	97.4	93.0
400 µm Extruded Corn[4]	1,343	2.24	76.71	66.56	86.8		
800 µm Extruded Corn[4]	1,668	2.06	75.72	60.13	79.4		
1200 µm Extruded Corn[4]	1,732	2.25	73.68	54.59	74.1		
Mash Feed with 400 µm extruded corn	1,157	2.22	45.90	39.23	85.5		
Mash Feed with 800 µm extruded corn	1,195	2.26	44.29	37.47	84.6		
Mash Feed with 1200 µm extruded corn	1,363	2.18	44.96	32.25	71.7		
Crumble Feed with 400 µm extruded corn			49.22	42.94	87.3	97.2	94.7
Crumble Feed with 800 µm extruded corn			48.84	40.63	83.2	95.1	91.3
Crumble Feed with 1200 µm extruded corn			47.35	33.30	70.4	95.8	92.8

[1]Particle size was determined by ASAE S319.2 with a 0.5 g flow agent for 10 min running time and reported in geometric mean diameter (d_{gw}) and geometric standard deviation (S_{gw}).
[2]PDI was determined by ASAE S269.5.
[3]The PDI procedure was modified by adding three 19-mm hex nuts to the tumble box and tumbling for the 10 minutes.
[4]The particle sizes were analyzed after extruded corn products were reground using a single pair crumble roll.

Table 6.5 Effect of corn type and corn particle size on body weight (BW) of male broilers

Corn type	Corn particle size[1], µm	n	Body weight (BW), g		
			7-d	14-d	21-d
Interaction effects					
Raw	400	8	146	414	874
Raw	800	8	146	421	865
Raw	1200	8	142	406	840
Extruded	400	8	132	377	800
Extruded	800	8	138	385	783
Extruded	1200	8	124	367	781
SEM			2.7	12.9	26.6
Main effect					
Raw		24	144[a]	413[a]	860[a]
Extruded		24	131[b]	376[b]	788[b]
SEM			1.53	11.2	22.4
	400	16	139	395	837
	800	16	142	403	824
	1200	16	133	386	811
	SEM		1.9	11.8	23.4
Source of variation				*P*-value	
Corn type × Corn particle size			0.164	0.975	0.742
Corn type			<0.0001	<0.0001	<0.0001
Corn particle size			0.007	0.088	0.215
Linear for corn particle size			0.037	0.225	0.081
Quadratic for corn particle size			0.014	0.066	0.976

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of corn type followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.6 Effect of corn type and corn particle size on average daily feed intake of male broilers

Corn type	Corn particle size[1], µm	n	Average Daily Feed Intake, g		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Raw	400	8	17.3	32.2	49.6
Raw	800	8	17.7	33.5	51.6
Raw	1200	8	17.2	32.4	50.3
Extruded	400	8	15.4	29.1	45.5
Extruded	800	8	16.9	30.7	46.6
Extruded	1200	8	15.9	29.6	46.1
SEM			0.36	1.04	1.00
Main effect					
Raw		24	17.4[a]	32.7[a]	50.5[a]
Extruded		24	16.1[b]	29.8[b]	46.1[b]
SEM			0.21	0.91	0.53
	400	16	16.4[y]	30.6[y]	47.6
	800	16	17.3[x]	32.1[x]	49.1
	1200	16	16.5[y]	31.0[x,y]	48.2
	SEM		0.25	0.96	0.66
Source of variation			*P*-value		
Corn type × Corn particle size			0.291	0.952	0.858
Corn type			<0.0001	<0.0001	<0.0001
Corn particle size			0.039	0.045	0.258
Linear for corn particle size			0.673	0.499	0.478
Quadratic for corn particle size			0.012	0.016	0.135

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of corn type followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.7 Effect of corn type and corn particle size on average daily gain of male broilers

Corn type	Corn particle size[1], µm	n	Average Daily Gain, g		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Raw	400	8	15.5	26.8	38.8
Raw	800	8	15.6	27.5	39.6
Raw	1200	8	14.8	26.2	38.1
Extruded	400	8	13.4	23.8	34.9
Extruded	800	8	14.4	25.0	35.2
Extruded	1200	8	12.4	23.4	35.0
SEM			0.38	0.93	1.34
Main effect					
Raw		24	15.3[a]	26.9[a]	38.9[a]
Extruded		24	13.4[b]	24.1[b]	35.0[b]
SEM			0.22	0.80	1.11
	400	16	14.4	25.3[x,y]	36.9
	800	16	15.0	26.3[x]	37.4
	1200	16	13.6	24.8[y]	36.6
	SEM		0.27	0.85	1.16
Source of variation				*P*-value	
Corn type × Corn particle size			0.230	0.933	0.694
Corn type			<0.0001	<0.0001	<0.0001
Corn particle size			0.003	0.0400	0.583
Linear for corn particle size			0.034	0.373	0.706
Quadratic for corn particle size			0.006	0.018	0.339

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of corn type followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.8 Effect of corn type and corn particle size on the feed conversion ratio (FCR) of male broilers

Corn type	Corn particle size[1], µm	n	FCR, g feed intake/g body weight gain		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Raw	400	8	1.12[b]	1.21	1.28
Raw	800	8	1.14[b]	1.22	1.31
Raw	1200	8	1.16[b]	1.24	1.33
Extruded	400	8	1.15[b]	1.23	1.31
Extruded	800	8	1.17[b]	1.24	1.34
Extruded	1200	8	1.29[a]	1.27	1.32
SEM			0.014	0.010	0.018
Main effect					
Raw		24	1.14[y]	1.23[y]	1.31
Extruded		24	1.20[x]	1.24[x]	1.32
SEM			0.008	0.005	0.015
	400	16	1.14	1.22	1.30
	800	16	1.15	1.23	1.32
	1200	16	1.22	1.26	1.33
	SEM		0.010	0.007	0.016
Source of variation			*P*-value		
Corn type × Corn particle size			0.001	0.638	0.371
Corn type			<0.0001	0.016	0.075
Corn particle size			<0.0001	<0.001	0.026
Linear for corn particle size			<0.0001	<0.0001	0.015
Quadratic for corn particle size			0.033	0.267	0.210

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within an interaction effect followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of corn type followed by a different letter are significantly different ($P \leq 0.05$).

[k-l]Means in a column within a main effect of corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.9 Effect of corn type and corn particle size on percentage of gizzard and pancreas of male broilers at 21d.

Corn type	Corn particle size[1], µm	n	Relative weight, % of BW	
			Gizzard	Pancreas
Interaction effects				
Raw	400	8	1.535[b,c]	0.279[a]
Raw	800	8	1.692[a,b]	0.285[a]
Raw	1200	8	1.712[a]	0.284[a]
Extruded	400	8	1.332[d]	0.240[b]
Extruded	800	8	1.499[c]	0.244[b]
Extruded	1200	8	1.823[a]	0.285[a]
SEM			0.0824	0.0162
Main effect				
Raw		24	1.646[x]	0.283[x]
Extruded		24	1.551[y]	0.256[y]
SEM			0.0625	0.0130
	400	16	1.434	0.259
	800	16	1.595	0.265
	1200	16	1.767	0.285
	SEM		0.0675	0.0138
Source of variation			*P*-value	
Corn type × Corn particle size			0.006	0.039
Corn type			0.035	0.001
Corn particle size			<0.0001	0.018
Linear for corn particle size			<0.0001	0.008
Quadratic for corn particle size			0.917	0.366

[1]The particle sizes were initial ground corn particle sizes.

[a-d]Means in a column within an interaction effect followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of corn type followed by a different letter are significantly different ($P \leq 0.05$).

[k-m]Means in a column within a main effect of corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.10 Effect of feed form and extruded corn particle size on body weight (BW) of male broilers

Feed form	Extruded corn particle size[1], μm	n	Body weight (BW), g		
			7-d	14-d	21-d
Interaction effects					
Crumble	400	8	132	377	800
Crumble	800	8	138	385	783
Mash	400	8	114	347	698
Mash	800	8	115	348	715
SEM			2.7	12.9	25.2
Main effect					
Crumble		16	135[a]	381[a]	792[a]
Mash		16	115[b]	347[b]	707[b]
SEM			1.9	11.8	23.0
	400	16	123	362	749
	800	16	126	366	749
	SEM		1.9	11.8	23.0
Source of variation				*P*-value	
Feed form × Extruded corn particle size			0.362	0.668	0.253
Feed form			<0.0001	<0.0001	<0.0001
Extruded corn particle size			0.190	0.537	0.996

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of feed form followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.11 Effect of feed form and extruded corn particle size on average daily feed intake of male broilers

Feed form	Extruded corn particle size[1], µm	n	Average Daily Feed Intake, g		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Crumble	400	8	15.4	29.1	45.5
Crumble	800	8	16.9	30.7	46.6
Mash	400	8	13.9	26.8	41.1
Mash	800	8	14.0	27.2	42.4
SEM			0.36	1.04	0.92
Main effect					
Crumble		16	16.2[a]	29.9[a]	46.0[a]
Mash		16	14.0[b]	27.0[b]	41.7[b]
SEM			0.25	0.96	0.63
	400	16	14.7[y]	27.9	43.3
	800	16	15.5[x]	28.9	44.5
	SEM		0.25	0.96	0.63
Source of variation			*P*-value		
Feed form × Extruded corn particle size			0.067	0.313	0.891
Feed form			<0.0001	<0.0001	<0.0001
Extruded corn particle size			0.035	0.083	0.189

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of feed form followed by a different letter are significantly different ($P \leq 0.05$).

[x-y]Means in a column within a main effect of extruded corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.12 Effect of feed form and extruded corn particle size on average daily gain of male broilers

Feed form	Extruded corn particle size[1], µm	n	Average Daily Gain, g		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Crumble	400	8	13.4	23.8	34.9
Crumble	800	8	14.4	25.0	35.2
Mash	400	8	11.0	21.8	31.0
Mash	800	8	11.0	22.1	32.0
SEM			0.38	0.93	1.26
Main effect					
Crumble		16	13.9[a]	24.4[a]	35.0[a]
Mash		16	11.0[b]	21.9[b]	31.5[b]
SEM			0.27	0.85	1.14
	400	16	12.2	22.8	32.9
	800	16	12.7	23.5	33.6
	SEM		0.27	0.85	1.14
Source of variation			*P*-value		
Feed form × Extruded corn particle size			0.183	0.435	0.632
Feed form			<0.0001	<0.0001	<0.0001
Extruded corn particle size			0.193	0.206	0.397

[1]The particle sizes were initial ground corn particle sizes.

[a-b]Means in a column within a main effect of feed form followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.13 Effect of feed form and extruded corn particle size on the feed conversion ratio (FCR) of male broilers

Feed form	Extruded corn particle size[1], µm	n	FCR, g feed intake/g body weight gain		
			0 - 7 d	0 - 14 d	0 - 21 d
Interaction effects					
Crumble	400	8	1.15	1.23	1.31
Crumble	800	8	1.17	1.24	1.34
Mash	400	8	1.27	1.24	1.34
Mash	800	8	1.28	1.24	1.34
SEM			0.014	0.010	0.017
Main effect					
Crumble		16	1.16[b]	1.23	1.32
Mash		16	1.28[a]	1.24	1.34
SEM			0.010	0.007	0.015
	400	16	1.21	1.23	1.33
	800	16	1.23	1.24	1.34
	SEM		0.010	0.007	0.015
Source of variation			*P*-value		
Feed form × Extruded corn particle size			0.785	0.665	0.180
Feed form			<0.0001	0.271	0.179
Extruded corn particle size			0.277	0.571	0.371

[1]The particle sizes were initial ground corn particle sizes.
[a-b]Means in a column within a main effect of feed form followed by a different letter are significantly different ($P \leq 0.05$).

Table 6.14 Effect of feed form and extruded corn particle size on percentage of gizzard and pancreas of male broilers at 21 d.

Feed form	Extruded corn particle size[1], µm	n	Relative weight, % of BW	
			Gizzard	Pancreas
Interaction effects				
Crumble	400	8	1.332	0.240
Crumble	800	8	1.499	0.244
Mash	400	8	1.669	0.270
Mash	800	8	1.857	0.286
SEM			0.0748	0.0146
Main effect				
Crumble		16	1.415[b]	0.242[b]
Mash		16	1.763[a]	0.278[a]
SEM			0.0642	0.0132
	400	16	1.501[y]	0.255
	800	16	1.678[x]	0.265
	SEM		0.0642	0.0132
Source of variation			*P*-value	
Feed form × Extruded corn particle size			0.842	0.528
Feed form			<0.0001	<0.001
Extruded corn particle size			0.002	0.263

[1]The particle sizes were initial ground corn particle sizes.
[a-b]Means in a column within a main effect of feed form followed by a different letter are significantly different ($P \leq 0.05$).
[x-y]Means in a column within a main effect of extruded corn particle size followed by a different letter are significantly different ($P \leq 0.05$).

Chapter 7 - Summary of Findings and Future Research

In general, the thermostability of commercial phytase products has improved over time; however, feed mills continue to report different responses on the phytase stability after pelleting even when using the same product. Thus, it is hypothesized that there are additional factors that may affect phytase denaturing during the pelleting process or when handling samples prior to analysis. These factors may include pellet mill model, die length to diameter ratio (L:D), steam quality, conditioner or die retention time, moisture in the feed, or the sample storage condition. Ultimately the authors believe that the impact of these factors on phytase stability is a consequence of changes in temperature, moisture content, and exposure time during the pelleting process. The effect of these factors could be different between phytase products. The focus of the current studies was on the stability of the *Trichoderma reesei* phytase.

The results of Chapter 1 suggested that freeze-drying, vacuum sealing, and freezing were not required when the sample is analyzed within 3 weeks of production. The pellet temperature, pellet moisture, and cooling time after the pellet die did not affect phytase stability. Application of the current study's results are limited by the ambient environmental conditions, and care should be taken for application in high temperature and humidity areas. Pellet samples that had less than 16% moisture did not impact the phytase stability of the uncoated phytase. However, if using a coated phytase then the impact of moisture content on phytase stability should be reevaluated.

The results of Chapter 2 indicated that the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when feed was stored in a bin up to 2 hr. prior to pelleting. The added water in the mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could

be used in the laboratory to determine *Trichoderma reesei* phytase stability. However, it is unclear what method commercial phytase companies utilize when determining their product release values. An animal growth experiment may clarify the best method of phytase analysis for creating the phosphorous release curve of a given product. In addition, the number of samples analyzed per treatment should be considered when evaluating phytase stability. Increasing the number of samples could reduce standard error within the treatment results. Finally, in the current study, increasing moisture content of mash feed by 0.6% did not improve pellet quality. The response of added water in the mixer on pellet quality may be amplified by low initial moisture content in the mash feed, a lower target conditioning temperature or the use of a thinner die thickness.

The results of Chapter 3 indicated the phytase that was produced by the *Trichoderma reesei* strain could tolerate hot pellet temperatures up to 88°C, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures were above 91°C. Therefore, hot pellet temperatures should be measured to monitor phytase stability. In addition, increasing the die L:D resulted in the greatest improvement in pellet quality. The accuracy of a phytase stability experiment could be improved by measuring the die temperature. However, a practical method for measuring die temperature during pelleting has not been developed. Decreasing die thickness and increasing production rate resulted in improved phytase stability and decreased in pellet quality. Decreasing temperature increments at the die during pelleting should increase the phytase stability. Future researchers should focus on improving pellet quality with a high production rate or when using a thinner die, as well as methods to decrease friction between the feed and die to reduce die temperature.

The results of Chapter 4 and 5 suggested that pure corn starch was not an effective binding agent in the feed when the diet contained at least 60% ground corn. Increasing the ratio of starch to protein decreased PDI. The ratio of small corn particles to large corn particles in the diet did not impact pellet quality when the diets were conditioned above 80°C for 35 sec. and then pelleted with 5.6 L:D die. Moreover, when a diet contained less than 1.5% oil, recirculating fines through the conditioner and pellet die increased cooked starch and improved pellet quality. However, 20% fines inclusion led to occasional roll slips, decreased pellet mill stability, and increased energy usage when the diet was pelleted. Increasing die thickness and conditioning temperature improved pellet quality. Based on the results of these chapters, the pelleting and regrinding of corn should result in increased percent cooked starch. Thus, the replacement of ground corn with the pelleted ground corn in the diet may have the potential to improve pellet quality.

The results of Chapter 6 indicated that increasing the amount of gelatinized starch in the feed by replacing ground corn with extruded corn in a broiler starter diet did not improve growth performance. Increasing corn particle size led to improved gizzard development. The corn particle size in the starter diet should be between 400 and 800 μm to optimize broiler performance from d 0 to 21.