FEED PROCESSING CHALLENGES FACING THE SWINE INDUSTRY

by

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B.S., South Dakota State University, 2011
M.S., Kansas State University, 2013

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Eight experiments using a total of 2,964 finishing pigs and 2,947 feed, phytase, or premix samples were used to determine the effects of: 1) wheat source, particle size and feed form on finishing pig performance; 2) feed form feeding strategies; 3) fine generation from pellets during feed manufacturing and delivery, and 4) thermal stability and shelf life of phytase products. Exp. 1 and 2 evaluated wheat sources, particle size, and diet form for finishing pigs. Fine gound hard red winter wheat fed in meal form improved G:F and nutrient digestibility, whereas wheat ground from~700 to 250 μ in pelleted diets did not influence growth or carcass traits. Feeding hard red winter wheat improved ADG and ADFI compared with feeding soft white winter wheat. In Exp. 3, pellet feeding regimens were used to evaluate finishing pig performance and stomach morphology. Feeding pelleted diets improved G:F but increased stomach ulceration and pig removals; however, rotating pellets and meal diets provided an intermediate G:F response with fewer stomach ulcers and pig removals. Experiments 4 to 6 investigated fines formation during pelleted feed manufacturing and delivery. Pellet quality worsened as pellets were transported through the feed mill post pelleting and during delivery. Unloading speed or feed line location had little effect on pellet quality. There were significant differences between the fines and pellet nutrient profiles as noted by the increased concentration of ADF, crude fiber, Ca, ether extract, and starch in the fines and decreased CP and P when compared to pellets. In Exp. 7 and 8, the thermal stability and shelf life of 4 commercial phytase products was determined. Increasing conditioning temperatures decreased phytase stability regardless of product. Phytase activity was affected by storage duration, temperature, product form, and phytase source. Pure products stored between 15 and 22˚C were the most stable and premixes were affected by longer storage times and higher temperatures.

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Eight experiments using a total of 2,964 finishing pigs and 2,947 feed, phytase, or premix samples were used to determine the effects of: 1) wheat source, particle size and feed form on finishing pig performance; 2) feed form feeding strategies; 3) fine generation from pellets during feed manufacturing and delivery, and 4) thermal stability and shelf life of phytase products. Exp. 1 and 2 evaluated wheat sources, particle size, and diet form for finishing pigs. Fine gound hard red winter wheat fed in meal form improved G:F and nutrient digestibility, whereas wheat ground from~700 to 250 µ in pelleted diets did not influence growth or carcass traits. Feeding hard red winter wheat improved ADG and ADFI compared with feeding soft white winter wheat. In Exp. 3, pellet feeding regimens were used to evaluate finishing pig performance and stomach morphology. Feeding pelleted diets improved G:F but increased stomach ulceration and pig removals; however, rotating pellets and meal diets provided an intermediate G:F response with fewer stomach ulcers and pig removals. Experiments 4 to 6 investigated fines formation during pelleted feed manufacturing and delivery. Pellet quality worsened as pellets were transported through the feed mill post pelleting and during delivery. Unloading speed or feed line location had little effect on pellet quality. There were significant differences between the fines and pellet nutrient profiles as noted by the increased concentration of ADF, crude fiber, Ca, ether extract, and starch in the fines and decreased CP and P when compared to pellets. In Exp. 7 and 8, the thermal stability and shelf life of 4 commercial phytase products was determined. Increasing conditioning temperatures decreased phytase stability regardless of product. Phytase activity was affected by storage duration, temperature, product form, and phytase source. Pure products stored between 15 and 22˚C were the most stable and premixes were affected by longer storage times and higher temperatures.

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I would like to acknowledge my wife Karis, son Brooks, and the good Lord for helping me through the last 4.5 years.

Dedication

This is dedicated to my wife and son and two mentors who started me off on the path of swine nutrition. Thanks for all that you do.

Chapter 1 - Effects of Wheat Source and Particle Size in Meal and Pelleted Diets on Finishing Pig Growth Performance, Carcass Characteristics, and Nutrient Digestibility

ABSTRACT

Two experiments were conducted to test the effects of wheat source and particle size in meal and pelleted diets on finishing pig performance, carcass characteristics, and diet digestibility. In Exp. 1, pigs (PIC 327 × 1050, n=288, initially 43.8 kg BW) were balanced by initial BW and randomly allotted to 1 of 3 treatments with 8 pigs per pen and 12 pens per treatment. The same wheat-soybean meal–based diets were used for all treatments which were fed in three phases in meal form. The 3 dietary treatments were hard red winter wheat hammer-mill ground to 728, 579, or 326 µm, respectively. From d 0 to 40, decreasing wheat particle size decreased (linear; $P < 0.033$) ADFI, but improved (quadratic; $P < 0.014$) G:F. From d 40 to 83, decreasing wheat particle size increased (quadratic; $P < 0.018$) ADG and improved (linear; $P < 0.002$) G:F. Overall from d 0 to 83, reducing wheat particle size improved (linear; $P < 0.002$) G:F. In Exp. 2, pigs (PIC 327 × 1050; n=576, initially 43.4 ± 0.02 kg BW) were used to determine the effects of wheat source and particle size of pelleted diets on finishing pig growth performance and carcass characteristics. Pigs were allotted randomly to pens and pens of pigs were balanced by initial BW and randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment and 8 pigs/pen. The experimental diets used the same wheat-soybean meal formulation, with the 6 treatments using hard red winter or soft white winter wheat that were processed to 245, 465, 693 and 258, 402, 710 µm, respectively. All diets were pelleted. Overall, feeding hard red winter wheat increased ($P < 0.05$) ADG and ADFI when compared with soft white winter wheat. There

was a tendency ($P < 0.10$) for a quadratic particle size × wheat source interaction for ADG, ADFI, and both DM and GE digestibility as they decreased for pigs fed 465-µm hard red winter wheat, and were greatest for pigs fed 402-µm soft white winter wheat. There were no main or interactive effects of particle size or wheat source on carcass characteristics. In summary, fine grinding hard red winter wheat fed in meal form improved G:F and nutrient digestibility, whereas reducing particle size of wheat from~700 to 250 µm in pelleted diets did not influence growth or carcass traits. Finally, feeding hard red winter wheat improved ADG and ADFI compared with feeding soft white winter wheat.

Key words: finishing pig, growth, hard red wheat, particle size, pellet, soft white wheat

INTRODUCTION

Cereal grains for pigs have historically been ground to reduce particle size to improve digestibility and pig growth. Grinding currently occurs through a variety of different mill types ranging from roller mills to hammermills with varying sizes of screens capable of producing a wide variety of particle sizes. Mavromichalis et al. (2000) observed that G:F was improved when wheat was ground from either 1,300 to 600 or 600 to 400 µm. Murphy et al. (2009) also ground wheat from 639 to 552 µm and found that decreasing particle size of a pelleted wheat diet tended to improve G:F. Using estimated grinding costs, McEllhiney (1986) concluded that costs associated with grinding are justified through improvements in feed efficiency. Although numerous experiments have been conducted that investigated particle size reduction of corn and sorghum, few studies have determined the effects of fine-grinding hard red or soft white wheat. In addition, little work has compared the feeding value of soft white wheat to hard red wheat.

Pelleting is another feed processing technology used throughout the swine industry. Stark (1994) and Wondra et al. (1995a) have shown that pelleting diets improved ADG and feed efficiency of finishing pigs, but data is limited in regards to the effect of reducing grain particle size in a pelleted diet.

Therefore, the objective of these experiments was to determine the effects of wheat source and its particle size in meal and pelleted diets on finishing pig performance, carcass characteristics, and nutrient digestibility.

MATERIALS AND METHODS

General

All practices and procedures used in this experiment were approved by the Kansas State University Institutional Animal Care and use Committee. Both experiments were conducted at the Kansas State University Swine Teaching and Research Center, Manhattan, KS. Each pen (2.44 m × 3.05 m) contained a 2-hole, dry self-feeder (Farmweld, Teutopolis, IL) and a nipple-cup waterer to provide ad libitum access to feed and water. Pens had concrete slatted flooring with a deep pit for manure storage. Pigs were fed a common corn-soybean meal diet for approximately 10 d upon entering the facility until pigs from both experiments reached their respective starting weights.

There were 8 pigs per pen, allowing for 0.93 m^2/pig. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts for individual pens. All diets for both experiments were manufactured at the O.H. Kruse Feed Technology Innovation Center at Kansas State University (Manhattan, KS).

Chemical Analysis

For both experiments, samples of wheat and soybean meal were collected at the feed mill. Samples of each diet were also collected from the farm, combined within phase, and sub-sampled. All ingredient and feed samples were analyzed for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), crude fiber (AOAC 978.10, 2006), ash (AOAC 942.05, 2006), Ca (AOAC 965.14/985.01, 2006), P (AOAC 965.17/985.01, 2006), ADF and NDF (Van Soest, 1963) at Ward Laboratories, Inc. (Kearney, NE). Nitrogen-free extract (NFE) was calculated with the equation: NFE = DM – CP – crude fiber – fat – ash. Initial samples of both of the wheat sources were analyzed for a complete AA profile (AOAC 994.1247, 2012) at University of Missouri Analytical Services (Columbia, MO).

Physical Diet Analysis

During both experiments, bulk density (Seedburo Model 8800, Seedburo Equipment, Chicago, IL), particle size, and angle of repose of major ingredients and all meal diets were measured. Bulk density was determined for all ingredients using methods from Clementson et al. (2010). Particle size was determined using US sieves, with numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270 and a pan. A Ro-Tap shaker (W.S. Tyler, Mentor, OH) was used to sift the 100-g samples for 10 min. Particle size was conducted without or with a flow agent (Amorphous silica powder, Gilson Company Inc., Middleton, WI), which was added at 0.5 g to 100 g of feed. A geometric mean particle size (dgw) and the log normal standard deviation (sgw) were calculated by measuring the amount of grain remaining on each screen (ASAE 2008). Angle of repose was measured by allowing feed to flow freely over a flat circular platform of a known diameter (Appel, 1994). The diameter of the platform and height of the resulting pile were used to calculate the angle of repose. For pelleted diets, pellet durability index (PDI) was

determined using a Holmen NHP100 (Tekpro Limited, Norfolk, United Kingdom) for 30 s. Percentage fines were also determined with fines characterized as material that would pass through a #6 Tyler Sieve (3,360-µm opening) during 15 sec of manual shaking (ASAE, 1987).

Animals and Diets

For both experiments, pigs were weighed approximately every 2 wk and feed disappearance was measured to determine ADG, ADFI, and G:F. On d 7 of phase 3 (d 67, Exp 1; d 61 and 59, Exp. 2), fecal samples were collected from 2 pigs per pen. Phase 3 diets contained 0.5% titanium dioxide as an indigestible marker. After collection, fecal samples were dried at 50˚C in a forced-air drying oven, and then ground for analysis of GE and titanium concentration. The digestibility values were calculated using the indirect method. Caloric efficiency was determined using dietary ingredient values for ME and NE from NRC (2012) and INRA (2004), respectively. Caloric efficiency was calculated on a pen basis by multiplying total pen feed intake by dietary energy (Mcal/kg) and dividing by total pen gain.

In Exp. 1, a total of 288 pigs (PIC 327 × 1050, initially 43.8 kg BW) were used in an 83-d study. Pigs were balanced by initial BW and randomly allotted to 1 of 3 treatments with 8 pigs per pen and 12 pens per treatment. The same wheat-soybean meal–based diets were used for all treatments. Diets were fed in three phases in meal form from d 0 to 27, 27 to 60, and 60 to 83. The 3 dietary treatments were hard red winter wheat ground to 728, 579, and 326 µm, respectively. Wheat was ground to the three particle sizes using a full-circle teardrop hammermill (Bliss 22115, Bliss Industries LLC., Ponca City, OK) equipped with either a # 12, 8, or 4 screen (4.83, 3.30, and 1.52 mm, respectively).

In Exp. 2, a total of 576 pigs (PIC 327 × 1050; initially 43.4 ± 0.02 kg BW) from 2 consecutive finishing groups were used. Pigs were allotted randomly to pens upon entry into the

finisher facility. Pens of pigs were balanced by initial BW and randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment and 8 pigs per pen. The experimental diets all had the same wheat-soybean meal formulation, with the 6 treatments formed by including the wheat from 1 of 2 sources (hard red winter vs. soft white winter) that were processed to 3 different mean particle sizes (245, 465, and 693 µm and 258, 402, and 710 µm, respectively). All diets were fed in pelleted form.

The 3 particle sizes of the hard red winter wheat were created with either a # 2, 10, or 16 screen (1.00, 4.06, 6.35 mm, respectively). The hard red wheat ground to 245 µm was first ground through a 3-high roller mill (Model 924, RMS Roller-Grinder, Harrisburg, SD) to ensure a fine enough grind was achieved through the hammermill. Soft white wheat was ground through a # 4, 12, and 16 hammer-mill screen (1.52, 4.83, and 6.35 mm, respectively). Diets were all pelleted through a 30-horsepower pellet mill (30 HD Master Model, California Pellet Mill, San Francisco). Pellets were approximately 4 mm in diameter and 15 mm in length. During the grinding and pelleting process, electrical consumption and throughput were measured.

Before marketing, all pigs were individually weighed and tattooed for carcass data collection and transported approximately 202 km to a commercial abattoir (Triumph Foods LLC., St. Joseph, MO). Standard carcass characteristics including HCW, percentage carcass yield, backfat, loin depth, and percentage lean were measured. Carcass yield was calculated by dividing the HCW at the plant by the live weight at the farm before transport to the plant. Fat depth and loin depth were measured with an optical probe inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Jowl fat samples were collected and analyzed by near infrared spectroscopy

(Bruker MPA, Breman, Germany) for iodine value (IV) using the equation of Cocciardi et al. (2009).

Statistical Analysis

Data were analyzed as a completely randomized design using PROC MIXED in SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. All treatment means were analyzed using the LSMEANS statement. For Exp. 1, linear and quadratic contrasts were completed to determine the effects of decreasing wheat particle size. For Exp. 2, linear and quadratic contrasts were completed to determine the main effects of decreasing wheat particle size as well as the interaction with wheat source. The main effect of wheat source was also determined. Linear and quadratic contrasts within wheat source for particle size were also tested. Lastly, there was no interaction between the 2 groups of pigs used and, thus, group was used as a random effect. Results were considered significant at $P \leq 0.05$ and tendencies between $P > 0.05$ and $P \leq 0.10$.

RESULTS

Chemical Analysis

Nutrient and amino acid analysis were generally similar for the hard red and soft white winter wheat (Table 1). Due to the similarities in AA analysis between sources, the values for the hard red winter wheat were used for both wheat sources in formulation using NRC (2012) SID coefficients. Analysis of the treatment diets showed that formulated values (Table 2 and 3) and nutrient analysis (Table 4 and 5) were similar.

Physical Analysis

Physical analysis of ground wheat and complete diets in Exp. 1 (Table 6) revealed that, as expected, as the wheat particle size decreased, the particle size of the diet decreased as well,

which led to an increase in the diet angle of repose. Utilizing a flow agent during particle size analysis revealed a numerical reduction of up to 40 µm when compared to analysis without a flow agent. Diet analysis from Exp. 2 (Table 7) revealed that increasing the particle size of wheat regardless of the source worsened PDI values but had no effect on bulk density or percentage fines of the diets. Grinding hard red winter wheat required more kilowatt h/tonne (kWh/tonne) than grinding soft white winter wheat (Table 8). Finely ground wheat increased kWh/tonne for hard red wheat during pelleting but did not influence kWh/tonne for soft white wheat. Throughput during pelleting was improved by increasing wheat particle size as well as by pelleting soft white wheat compared with hard red wheat.

Growth, Carcass, and Digestibility

In Exp. 1, from d 0 to 40, decreasing wheat particle size decreased (linear; $P < 0.033$) ADFI, but improved (quadratic; $P < 0.014$) G:F (Table 9). From d 40 to 83, decreasing wheat particle size increased (quadratic; $P < 0.018$) ADG and improved (linear; $P < 0.002$) G:F. Overall from d 0 to 83, reducing wheat particle size improved (linear; $P < 0.001$) G:F and CE on both an ME and NE basis, with no difference in ADG or ADFI observed. Finally, reducing wheat particle size improved (linear; $P < 0.05$) DM and GE digestibility.

In Exp. 2, feeding hard red winter wheat improved ($P < 0.05$) ADG, ADFI, and caloric efficiency on an NE basis when compared with pigs fed soft white winter wheat (Table 10). There was a tendency ($P < 0.10$) for a quadratic particle size × wheat source interaction for ADG, ADFI, and a significant interaction ($P < 0.05$) for both DM and GE digestibility because ADG, ADFI, and both DM and GE digestibility values decreased in pigs fed 465-µm hard red winter wheat, but were greatest for those fed 402-µm soft white winter wheat. There were no main effects of particle size or particle size within wheat source (Table 11). Finally, dietary

treatments did not affect any carcass characteristics; however, feed efficiency on a carcass weight basis tended to be improved ($P < 0.10$ when hard red winter wheat was fed as compared to soft white winter wheat.

DISCUSSION

While wheat production has decreased in recent years in the US, it is still used in swine diet formulations domestically and across the world. Wheat can be categorized into 5 main classifications including, hard red winter, hard red spring, soft red winter, white, and durum (USDA, 2013). Of these, hard red winter wheat represents 40% of total production in the US in a given year, making it the most common wheat used for livestock diets (USDA, 2013). White wheat, though less common, is more widely used for swine located west of the Great Plains.

Previous work has shown the concentration of energy in wheat to be 91 to 97% that of corn, and the concentration of amino acids to be greater than corn (Stein et al. 2010). In numerous studies when diets were balanced for dietary energy and AA, wheat- and corn-based diets produced similar growth performance and carcass characteristics when fed during the nursery phase (Erickson et al. 1980) or the finisher phase (Han et al. 2005). This suggests corn can be replaced with wheat in diet formulation with few or no negative effects on performance. Jha et al. (2011) fed 6 Canadian wheat varieties for 21 d to weanling pigs and found that wheat variety had no effect on ADG, ADFI, or G:F. They also noted no difference for pelleting throughput or PDI between the 6 wheat varieties. Bhatty et al. (1974) fed 17 cultivars of hard- and soft spring-wheat and found that soft white had a greater GE and less fiber then hard red wheat. In the current study, pigs fed hard red winter wheat had increased ADG and ADFI and improved NE caloric efficiency compared to those fed soft white winter wheat. The improved NE caloric efficiency of the hard red winter wheat might suggest that the NE energy values

reported by NRC (2012) for soft wheat as compared to hard wheat (2,595 vs 2,472 kcal/kg NE) may be overestimated. Values for ADF and NDF were both similar between the hard red and soft white winter wheat in the current experiment suggesting that the source of soft white winter wheat used may have been higher in fiber then what would normally be expected (INRA, 2004).

In Exp. 1, particle size reduction of hard red winter wheat from 728 to 326 µm initially resulted in reduced ADFI and numerically lower ADG. However, pigs appeared to adjust to the finely ground diets during the second half of the trial as reducing wheat particle size improved ADG, and G:F which led to similar improvements in overall G:F, and caloric efficiency on both an ME and NE basis. Seerly et al. (1988) also fed varying particle sizes of wheat to finishing pigs, but found results contrasting to those reported herein. Their results suggest that a larger particle size increased ADG in the finishing period as compared to finely ground wheat. The improvement in ADG with coarsely ground wheat is difficult to explain given G:F was also numerically improved with the coarsely ground wheat in their study. Mavromichalis et al (2000) conducted 2 separate experiments exploring particle size of wheat. In their first experiment, finishing pigs were fed wheat ground to either 1,300 or 600 µm. Pigs fed the finely ground wheat had improved G:F. In their second experiment, pigs were fed wheat ground to 600 or 400 µm. Again, pigs fed the finely ground wheat had improved G:F, but also had a greater incidence of stomach ulcers.

In the current study, as hard red winter wheat was ground from coarse to fine, pigs had improved DM and GE digestibility as well as caloric efficiency on both an ME and NE basis. This agrees with Choct et al. (2004) who conducted a weanling pig trial to determine the effect of wheat particle size on nutrient digestibility. The study used both a hammer and roller-mill to reduce wheat particle size. A fine, medium, and coarse ground treatment was created for each

respective milling apparatus. Digestibility for GE was improved as particle size was reduced regardless of the processing method. In a similar designed study by Mavromichalis et al. (2000), the apparent digestibility of DM and N was improved as wheat particle size was reduced when comparing 1,300 to 600 µm and 600 to 400 µm wheat. l'Anson et al. (2012) observed that grinding wheat from 760 to 664 µm did not affect any growth criteria but did improve GE digestibility. The 96 µm decrease in particle size may not have been large enough to elicit a growth response given the replication in the study though differences in digestibility were realized. In general, it appears that as particle size is reduced, the nutrient digestibility improves in diets fed in meal form.

Conversely, in Exp. 2, nutrient digestibility was not improved as particle size was reduced when diets were fed in pelleted form. This is in contrast to work by Healy et al. (1994) who found improved digestibility of N, DM, and GE when corn and hard and soft sorghum with decreasing particle sizes (900, 700, 500 and 300 µm) were fed to weanling pigs in pelleted form. These differing results may be a result of the different grains fed, or the different pig weight ranges used. The smaller particle sizes being in the current experiment compared to larger particle sizes being fed in the Healy et al. (1994) experiment also may have influenced the results. Thus, our data would suggest that in pelleted diets, the improvement to particle size reduction for nutrient digestibility may diminish as particle size is reduced below ~500 µm.

In tandem with improved digestibility, improvements in growth performance have been associated with decreased particle size of cereal when diets are fed in meal form grains (Hedde et al., 1985; Wu et al., 1985; Wondra et al., 1995). However, data is limited on the effect of particle size of wheat in pelleted diets with finishing pigs. In Exp. 2, reducing particle size of either wheat source in pelleted diets had no effect on performance of finishing pigs. Murphy et al.

(2009) conducted a similar experiment with wheat diets ground to either 639 or 552 µm, where again all diets were fed in pelleted form. When wheat was ground finely, it tended to improve G:F; however, this may have been a result of the improved pellet quality of the finely ground wheat diets and not a direct effect of the decreased particle size. De Jong et al. (2013) investigated the effects of particle size of pelleted diets in nursery pigs utilizing corn ground to 638 or 325 µm, respectively. When corn was finely ground to 325 µm and fed in pelleted form, there was no effect of particle size on growth performance of the nursery pigs. Pelleting has been shown to improve diet digestibility (Graham et al., 1989; Wondra et al., 1995; and Xing et al., 2004), which in addition to the current data might suggest that a diet's digestibility may reach a theoretical "ceiling" when pelleting is used and additional effects of diet particle size may not be realized. This might suggest that particle size reduction in pelleted diets may only be advantageous when grain is coarsely ground (> 500 µm) and not when grain is finely ground (< 500 µm). Lahaye et al. (2008) also found that grinding wheat from 1000 to 500 µm improved DE, OM, and DM digestibility when fed in meal form but that pelleting did not improve digestibility further. This further demonstrates that pelleting and fine grinding of wheat diets does not produce an additive improvement in G:F.

Feed processing technologies have the ability to improve feed efficiency and growth in pigs, but they also can potentially increase the feed mill's cost of production. The improved animal performance needs to be balanced with the increased cost of production and reductions in feed mill throughput in order to justify the additional grain or complete diet thermal processing. In Exp. 2, grinding hard red or soft white winter wheat from ~600 to ~200 µm increased kilowatts (kW) and kilowatt hours (kWh) required for grinding and kWh/tonne. In addition, fine grinding reduced throughput by 0.08 and 0.06 tonne/h for hard red and soft white winter wheat,

respectively. This was similar to results reported by Healy et al. (1994), who also reported increased kW usage as either corn or sorghum were ground from 900 to 300 µm. Paulk et al (2015) also reported increased electrical usage as sorghum was ground from 724 to 319 µm. Both, Paulk et al. (2015) and Healy et al. (1994) reported reduced rates of production as grains were more finely ground. The reduced production rate and increased kW usage during grinding, especially at the finer grinds, will increase the cost of manufacturing at the feed mill.

In conclusion, fine grinding hard red winter wheat improved G:F and caloric efficiency when diets were fed in meal form. When hard red winter or soft white winter wheat diets were finely ground and fed in pelleted form, no effect of particle size on growth was observed. In agreement with other literature, it appears that very fine grinding (< 500 microns) is not warranted if diets are to be pelleted, except as a means to improve pellet quality.

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Table 1-1 Chemical and AA analysis of wheat sources (as-fed basis)[1]

Item, %	Hard red winter wheat	Soft white winter wheat
DM	90.86	91.80
CP	11.8	11.2
ADF	3.2	2.8
NDF	8.1	8.6
Nitrogen free extract	72.9	74.8
Ca	0.07	0.13
P	0.38	0.40
Ether extract	1.8	1.6
Ash	1.81	1.89
Starch[2]	55.4	56.9
Lys	0.40	0.40
Ile	0.41	0.44
Met	0.21	0.20
Met + Cys	0.48	0.48
Thr	0.36	0.34
Trp	0.17	0.15
Val	0.47	0.52

[1] A composite sample consisting of 6 subsamples was used for analysis.
[2] Measured only available starch.

Table 1-2 Diet composition of experimental diets, Exp. 1 (as-fed basis)[1]

	Phase		
Item	1	2	3
Ingredient, %			
Hard red wheat	81.26	87.43	92.18
Soybean meal (46.5% CP)	15.84	10.14	4.94
Calcium phosphate (21.5% P)	0.28	0.03	---
Limestone	1.43	1.28	1.30
Salt	0.35	0.35	0.35
Vitamin and trace mineral premix[2,3]	0.30	0.25	0.20
Phytase[4]	0.08	0.08	0.05
L-Lys HCl	0.33	0.33	0.35
DL-Met	0.04	0.02	0.02
L-Thr	0.09	0.09	0.11
Titanium dioxide[5]	---	---	0.50
Total	100	100	100
Calculated analysis			
Standard ileal digestible (SID) AA, %			
Lys	0.94	0.81	0.71
Ile:Lys	64	63	61
Met:Lys	30	30	30
Met + Cys:Lys	61	63	66
Thr:Lys	62	63	66
Trp:Lys	23.1	23.7	23.7
Val:Lys	68	69	67
Total Lys, %	1.05	0.91	0.79
ME,[6] kcal/kg	3,154	3,163	3,163
NE,[7] kcal/kg	2,323	2,358	2,385
SID Lys:ME, g/Mcal	2.98	2.56	2.24
CP, %	19.7	17.9	16.2
Crude fiber, %	2.7	2.6	2.6
Ca, %	0.67	0.56	0.55
P, %	0.50	0.44	0.42
Available P, %	0.30	0.25	0.24

[1] Phase 1 diets were fed from approximately 38 to 63 kg; Phase 2 from 63 to 82 kg; and Phase 3 from 82 to 120 kg.

[2] Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.

[3] Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[4] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO.) provided 450.3 FTU/kg feed, with a release of 0.11% available P.

[5] Titanium was included in the Phase 3 diet as an indigestible marker and was fed for the first 7 d of the phase at a level of 0.5% at the expense of corn.
[6] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.
[7] INRA (Institut National de la Recherche Agronomique). 2004. Tables of composition and nutritional value of feed materials, Sauvant, D., J-M. Perez and G. Tran, Eds. Wageningen Academic Publishers, The Netherlands and INRA, Paris, France.

Table 1-3 Diet composition of experimental diets, Exp. 2 (as-fed basis)[1]

	Wheat source and dietary phase					
	Hard red winter wheat			Soft white winter wheat		
Item	1	2	3	1	2	3
Ingredient, %						
Wheat	78.44	85.01	89.43	78.44	85.01	89.43
Soybean meal (46.5% CP)	17.31	11.15	6.33	17.31	11.15	6.33
Choice white grease	1.50	1.50	1.50	1.50	1.50	1.50
Calcium phosphate (21.5% P)	0.25	---	---	0.25	---	---
Limestone	1.38	1.28	1.25	1.38	1.28	1.25
Salt	0.35	0.35	0.35	0.35	0.35	0.35
VTM premix[2,3]	0.26	0.20	0.16	0.26	0.20	0.16
Phytase[4]	0.08	0.08	0.05	0.08	0.08	0.05
L-Lys HCl	0.29	0.30	0.32	0.29	0.30	0.32
DL-Met	0.05	0.05	0.01	0.05	0.05	0.01
L-Thr	0.09	0.08	0.10	0.09	0.08	0.10
Titanium dioxide[5]	---	---	0.50	---	---	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
Standard ileal digestible (SID) amino acids, %						
Lys	0.94	0.81	0.71	0.94	0.81	0.71
Ile:Lys	66	65	63	68	67	67
Met:Lys	31	30	30	30	30	30
Met + Cys:Lys	62	63	66	62	64	66
Thr:Lys	63	63	66	62	63	66
Trp:Lys	23.5	24.0	24.3	22.1	22.1	22.1
Val:Lys	70	70	70	74	75	75
Total Lys, %	1.05	0.91	0.80	1.05	0.91	0.80
ME,[6] kcal/kg	3,233	3,240	3,240	3,286	3,299	3,302
NE,[7] kcal/kg	2,422	2,455	2,475	2,519	2,559	2,587
SID Lys:ME, g/Mcal	2.91	2.50	2.19	2.86	2.45	2.15
CP, %	17.8	15.6	13.9	17.4	15.2	13.5
Crude fiber, %	2.7	2.6	2.6	0.7	0.4	0.2
Ca, %	0.65	0.55	0.53	0.65	0.55	0.53
P, %	0.48	0.41	0.40	0.48	0.41	0.40
Available P, %	0.28	0.23	0.22	0.28	0.23	0.22

[1] Phase 1 diets were fed from approximately 38 to 63 kg; Phase 2 from 63 to 82 kg; and Phase 3 from 82 to 120 kg.

[2] Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.

[3] Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[4] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO.) provided 450.3 FTU/kg feed, with a release of 0.11% available P.
[5] Titanium was included in the Phase 3 diet as an indigestible marker and was fed for the first 7 d of the phase at a level of 0.5% at the expense of corn.
[6] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.
[7] INRA (Institut National de la Recherche Agronomique). 2004. Tables of composition and nutritional value of feed materials, Sauvant, D., J-M. Perez and G. Tran, Eds. Wageningen Academic Publishers, The Netherlands and INRA, Paris, France.

Table 1-4 Chemical analysis of diets, Exp. 1

Item, %	Phase:	1	2	3
DM		90.28	89.91	89.16
CP		19.7	18.4	16.1
ADF		2.9	2.8	2.5
NDF		9.3	8.2	7.8
Crude fiber		2.6	2.2	2.3
Nitrogen free extract		62.4	62.9	65.7
Ca		0.74	0.89	0.74
P		0.51	0.48	0.41
Ether extract		1.4	1.5	1.6
Ash		4.41	4.79	3.70
Starch		44.1	45.0	51.3

[1] A composite sample consisting of 6 subsamples was used for analysis.

Table 1-5 Chemical analysis of diets, Exp. 2[1,2]

	Hard red winter wheat			Soft white winter wheat		
Item, % Phase:	1	2	3	1	2	3
DM	89.85	90.52	90.00	91.46	91.56	90.48
CP	20.3	18.3	16.00	19.6	17.5	15.37
ADF	2.5	2.3	1.87	3.0	2.9	2.20
NDF	7.7	7.3	6.93	8.6	8.4	7.63
Nitrogen free extract	61.0	64.4	67.23	62.6	65.4	67.43
Ca	0.72	0.68	0.62	0.81	0.67	0.63
P	0.50	0.43	0.45	0.50	0.42	0.41
Ether extract	2.7	2.6	2.40	2.6	2.7	2.40
Ash	3.84	3.41	3.74	4.17	3.63	3.58
Starch	39.9	45.4	47.47	41.8	44.7	49.30

[1] A composite sample consisting of 3 subsamples was used for analysis.
[2] All values are averages of the 2 finishing groups' feed.

Table 1-6 Physical analysis of diets and wheat, Exp. 1[1,2]

	Particle size, µm		
Item	728	579	326
Wheat			
Particle size (no flow agent)[3], µm	728	579	326
Particle size (with flow agent), µm	714	554	284
Bulk density, g/L	767	767	763
Angle of repose, º	42.4	45.8	50.9
Diet[4]			
Bulk density, g/L	765	763	733
Particle size (no flow agent), µm	650	504	374
Angle of repose, ˚	44.7	46.4	50.7

[1] A composite sample consisting of 6 subsamples was used for analysis.
[2] All treatments were analyzed and values were averaged as all treatments were formulated identically
[3] Particle sizes were determined using Tyler sieves, with numbers 6, 8, 10, 14, 20, 28, 35, 48, 65, 100, 150, 200, and 270 and a pan. A Ro-Tap shaker (W.S. Tyler, Mentor, OH) was used to sift the 100-g samples for 10 min. Particle sizes were run with and without flow agent at an inclusion level of 0.5 g.
[4] Diet samples from phases were averaged; no differences existed between phases.

Table 1-7 Physical analysis of diets and wheat, Exp. 2[1,2]

	Wheat source and particle size, µm					
	Hard red winter wheat			Soft white winter wheat		
Item	693	465	245	710	402	258
Wheat						
Particle size (no flow agent)[3], µm	693	465	245	710	402	258
Particle size (with flow agent), µm	631	415	201	638	341	210
Bulk density, g/L	1,134	1,224	1,088	1,192	1,133	1,125
Angle of repose, º	45.8	49.5	50.8	43.6	58.2	58.1
Diet[4]						
Bulk density, g/L	870	860	875	853	876	883
Pellet durability index, %	74.2	81.2	88.5	48.7	50.9	54.5
Pellet fines, %	26.9	22.9	24.0	24.1	27.2	22.2

[1] A composite sample consisting of 3 subsamples was used for analysis.
[2] All values are averages of samples taken from the 2 groups.
[3] Particle sizes were determined using Tyler sieves, with numbers 6, 8, 10, 14, 20, 28, 35, 48, 65, 100, 150, 200, and 270 and a pan. A Ro-Tap shaker (W.S. Tyler, Mentor, OH) was used to sift the 100-g samples for 10 min. Particle sizes were run with and without flow agent at an inclusion level of 0.5 g.
[4] Diet samples from phases were averaged; no differences existed between phases.

Table 1-8 Feed manufacturing electrical consumption and throughput, Exp. 2[1]

	Wheat source and particle size, µm					
	Hard red winter wheat			Soft white winter wheat		
Item Particle size, µm:	693	465	245	710	402	258
Wheat grinding						
Kilowatts, kW[2]	8.37	9.33	11.04	7.59	8.47	8.58
Kilowatt hours, kWh[3]	6.98	7.00	7.88	4.43	4.94	5.00
Throughput, tonne/h	4.1	3.6	3.3	4.1	4.3	4.7
kWh/tonne	2.04	2.59	3.34	1.61	1.97	2.09
Pelleting						
Kilowatts, kW	20.99	20.57	20.64	22.68	22.80	22.56
Kilowatt hours, kWh	12.25	12.66	14.07	14.88	13.83	13.63
Throughput, tonne/h	1.2	1.1	1.0	1.2	1.2	1.2
kWh/tonne	17.49	18.70	20.64	18.90	19.00	18.80

[1] Voltage was recorded during each manufacturing run, and then averaged across the dietary phases

[2] kW was calculated by the formula kW = amperage × voltage / 1000.

[3] kWh was calculated by the formula kWh = kW × hours used.

Table 1-9 Effects of wheat particle size on finishing pig performance, Exp. 1[1]

	Wheat particle size, µm				Probability, *P* <	
Item	728	579	326	SEM	Linear	Quadratic
d 0 to 40						
ADG, kg	0.92	0.93	0.90	0.01	0.349	0.247
ADFI, kg	2.29	2.24	2.20	0.03	0.033	0.966
G:F	0.400	0.413	0.409	0.003	0.015	0.014
d 40 to 83						
ADG, kg	0.92	0.90	0.95	0.01	0.028	0.018
ADFI, kg	2.87	2.80	2.84	0.04	0.484	0.228
G:F	0.319	0.322	0.336	0.003	0.002	0.180
d 0 to 83						
ADG, kg	0.92	0.91	0.93	0.01	0.470	0.500
ADFI, kg	2.59	2.53	2.53	0.03	0.130	0.434
G:F	0.354	0.361	0.367	0.003	0.001	0.824
Caloric efficiency[2]						
ME	8.94	8.76	8.62	0.06	0.001	0.755
NE	6.67	6.53	6.43	0.05	0.001	0.746
Digestibility[3]						
DM, %	89.0	91.2	91.5	0.7	0.013	0.246
GE, %	65.5	70.3	73.5	1.9	0.004	0.685
BW, kg						
d 0	43.8	43.8	43.8	0.51	0.999	0.999
d 40	80.4	80.7	79.9	0.83	0.716	0.586
d 83	119.8	119.5	121.1	1.14	0.414	0.511

[1] A total of 288 pigs (PIC 327 ×1050) were used, with 12 pens per treatment and 8 pigs per pen.
[2] Caloric efficiency is expressed as Mcal/kg of gain and represents the d 0 to 83 data.
[3] Fecal samples were taken on d 67 of the study via rectal massage from two pigs per pen.

Table 1-10 Interactive effects of wheat source and particle size of pelleted diets on finishing pig growth performance, caloric efficiency, and carcass characteristics, Exp. 2[1]

	Wheat source and particle size, µm								
	Hard red winter			Soft white winter				Probability, $P <$	
Item	693	465	245	710	402	258	SEM	Quadratic particle size × source[2]	Source main effect
ADG, kg	1.03	1.01	1.03	0.99	1.00	0.97	0.013	0.075	0.004
ADFI, kg	2.67	2.59	2.66	2.56	2.58	2.54	0.035	0.068	0.003
G:F	0.384	0.388	0.387	0.388	0.389	0.384	0.0029	0.992	0.941
Initial BW, kg	43.4	43.4	43.4	43.4	43.4	43.4	3.75	0.983	0.978
Final BW, kg	127.3	125.6	127.9	125.3	125.7	123.2	3.19	0.179	0.074
Caloric efficiency[3]									
ME	8.08	7.97	8.35	8.10	8.10	8.21	0.436	0.628	0.998
NE	5.32	5.27	5.29	5.45	5.49	5.54	0.215	0.447	0.001
Digestibility									
DM, %	88.0	87.0	87.7	85.1	87.7	85.8	0.79	0.030	0.048
GE, %	66.3	64.5	68.3	64.9	67.5	62.3	1.95	0.053	0.360
Carcass traits									
Feed/carcass gain[4]	1.66	1.63	1.65	1.71	1.70	1.67	0.029	0.491	0.065
HCW, kg	91.77	90.31	91.36	90.44	91.10	89.42	1.439	0.331	0.479
Carcass yield, %	72.9	72.8	73	73.1	73.1	73.0	0.15	0.241	0.167
BF, mm	18.96	19.28	19.62	19.93	1959	19.17	0.434	0.941	0.431
Loin depth, mm	58.71	57.97	57.14	57.75	58.21	56.40	1.300	0.482	0.445
Percentage lean, %	52.8	52.6	52.4	52.4	52.5	52.5	0.21	0.792	0.397
Jowl iodine value, mg/100 g	68.6	69.0	69.1	68.3	68.6	68.4	0.43	0.928	0.210

[1]A total of 576 pigs (PIC 327 × 1050; initially 43.4 ± 0.02 kg BW) in 2 groups were used in a 75- and 89-d study with 8 pigs per pen and 12 replications per treatment.

[2] No source × particle size interactions, main effects of particle size, or linear or quadratic effects of particle size within wheat source, $P > 0.10$

[3] Caloric efficiency is expressed as Mcal/kg of gain.

[4] Feed/carcass gain is expressed as total intake / kg carcass gain with an assumed initial yield of 75%.

Table 1-11 Main effects of wheat source and particle size of pelleted diets on finishing pig growth performance, caloric efficiency, and carcass characteristics, Exp. 2[1]

	Wheat source		Wheat particle size, µm				Probability, $P <$	
Item	Hard red winter	Soft white winter	~200	~400	~600	SEM	Source main effect	Particle size main effect
ADG, kg	1.01	0.99	1.01	1.00	1.00	0.009	0.004	0.510
ADFI, kg	2.64	2.56	2.62	2.58	2.60	0.026	0.003	0.566
G:F	0.387	0.387	0.386	0.389	0.385	0.0020	0.941	0.819
Initial BW, kg	43.4	43.4	43.4	43.4	43.4	3.72	0.978	0.979
Final BW, kg	126.9	124.7	126.3	125.7	125.5	3.01	0.074	0.627
Caloric efficiency[2]								
ME	8.14	8.14	8.09	8.04	8.28	0.371	0.997	0.558
NE	5.30	5.50	5.40	5.38	5.42	0.213	0.001	0.463
Digestibility								
DM, %	87.84	86.22	86.55	87.37	86.73	0.565	0.048	0.818
GE, %	66.37	64.91	65.60	66.00	65.32	1.376	0.360	0.884
Carcass traits								
Feed/carcass gain[3]	1.65	1.69	1.68	1.67	1.66	0.021	0.065	0.431
HCW, kg	91.15	90.32	90.10	90.71	90.39	1.012	0.479	0.618
Carcass yield, %	72.9	73.1	73.0	73.0	73.1	0.10	0.167	0.787
BF, mm	19.29	19.56	19.44	19.44	19.44	0.306	0.431	0.912
Loin depth, mm	58.27	57.46	58.23	58.09	57.28	0.919	0.445	0.467
Percentage lean, %	52.6	52.4	52.6	52.6	52.5	0.145	0.397	0.609
Jowl iodine value, mg/100 g	68.9	68.4	68.4	68.8	68.7	0.305	0.210	0.475

[1] A total of 576 pigs (PIC 327 × 1050, initially 43.4 ± 0.02 kg BW) in 2 groups were used in a 75- and 89-d study with 8 pigs per pen and 12 replications per treatment.
[2] Caloric efficiency is expressed as mcal/kg of gain.
[3] Feed/carcass gain is expressed as total intake / kg carcass gain with an assumed initial yield of 75%.

Chapter 2 - Evaluating Pellet and Meal Feeding Regimens on Finishing Pig Performance, Stomach Morphology, and Carcass Characteristics

ABSTRACT

A total of 2,100 pigs (PIC 327 × 1050; initially 31.2 kg BW) were used in a 118-d trial to determine the effects of pellet or meal feeding regimens on finishing pig growth performance, stomach morphology, and carcass characteristics. Pens of pigs were balanced by initial BW and randomly allotted to 1 of 6 dietary treatments (14 pens/treatment with 25 pigs/pen). Pens were sorted by gender allowing for 7 barrow and 7 gilt pens/treatment. The same corn-soybean meal–based diets containing 15% dried distillers grains with solubles were used for all treatments and fed in 5 phases. Phases were fed from d 0 to 28, 28 to 56, 56 to 84, 84 to 98, and 98 to 118. The 6 treatments included a meal or pelleted diet fed from d 0 to 118, a meal diet fed from d 0 to 70 followed by pellets from d 70 to 118, a pelleted diet fed from d 0 to 70 followed by a meal diet from d 70 to 118, or pellets and meal rotated every 2 wk starting with meal or pellets. On d 110, 4 pigs from each pen were harvested and stomachs collected from which a combined ulcer and keratinization score was determined for each pig.

Overall, there were no differences in ADG across feeding regimens. Pigs fed meal throughout had the greatest ($P < 0.05$) ADFI, while pigs fed pellets throughout had the lowest ($P < 0.05$), with all other treatments intermediate ($P < 0.05$). Pigs fed pelleted diets throughout had the greatest ($P < 0.05$) G:F, while pigs fed meal throughout had the worst G:F ($P < 0.05$), with all other treatments intermediate ($P < 0.05$). When pelleted diets were fed for the last 58 d or for

the entire trial, the incidence of ulceration and keratinization increased ($P < 0.05$) while pigs fed meal for the last 58 d had a lower incidence ($P < 0.05$), with all other treatments intermediate ($P < 0.05$). Feeding pellets throughout increased ($P < 0.05$) the number of pigs removed per pen compared to all other treatments. Pig removals were determined by an on-site farm manager when pigs were at risk due to weight loss, health, or animal welfare concerns and needed to be separated from the general population. There were no differences for any carcass characteristics measured. In conclusion, feeding pelleted diets improved G:F but increased stomach ulceration and pig removals; however, rotating pellets and meal diets provided an intermediate G:F response and moderated stomach ulcerations compared to only feeding pellets.

Key words: finishing pig, growth, meal, pellet, ulcer

INTRODUCTION

To improve finishing pig feed utilization and minimize wastage, many swine producers have changed to, or are considering, feeding diets in pellet form. However, due to feed mill limitations and logistics, many producers might not be able to feed pelleted diets to all of their pigs continually. Because many commercial or producer-owned mills do not have enough capacity to pellet all diets, they are left with the option to pellet only part or none of their feed. Currently, there is little data available to determine the best regimen for maximizing pig performance when feeding a limited amount of pelleted feed during the finishing period. From a health perspective, pelleting diets has been shown to increase the incidence of ulcers in finishing pigs (Wondra et al. 1995b), which can ultimately lead to increases in mortality (Guise et al. 1997). However, the effects of feeding pelleted feed for varying lengths of time or pulse feeding (switching between pelleted and meal diets) on stomach morphology is unknown.

There are also increased feed processing costs associated with pelleting feed (Wondra et al. 1995a). These increased costs can only be deemed acceptable if growth performance is great enough to compensate for the added cost of pelleting. By determining when feeding pellets can maximize profitability, production decisions can be made when mill capacity limits feeding pelleted diets. One of the practical options for producers is the rotational feeding of pellet and meal diets to realize the benefits of pelleting without having gastrointestinal challenges associated with prolonged feeding of pellets; however, data is not available to determine the overall impact on performance. Therefore, the objective of the current trial was to determine the effects of pellet feeding regimens on finishing pig growth performance, stomach morphology, and carcass characteristics.

MATERIALS AND METHODS

General

All practices and procedures used in this experiment were approved by the Kansas State University Institutional Animal Care and use Committee. The study was conducted at a commercial research-finishing barn in Eastern, MN. The barn was double curtain-sided and pens had completely slatted flooring and deep pits for manure storage. Each pen was equipped with a 3-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer for ad libitum access to feed and water. Pigs were fed a common corn-soybean meal formulated diet in meal form upon entering the finisher until the beginning of the trial. For the duration of the trial all feeders were adjusted weekly and after diet changes to a target of 60% feed pan coverage regardless of feed form.

There were 25 pigs per pen, allowing for 0.67 m^2/pig. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN)

capable of providing and measuring feed amounts for individual pens. All diets for both experiments were manufactured at a commercial feed mill (Hubbard Feeds, Mankato, MN). Diets were pelleted with a 280-horsepower pellet mill (7800 HD Master Model, California Pellet Mill, San Francisco), using a 4 mm die. Diets were conditioned at approximately 74˚C for 45 s.

Chemical Analysis

Samples of corn, soybean meal, and distillers dried grains with solubles (DDGS) were collected at the mill along with samples of each diet between each feeding period and were blended within phase, and sub-sampled. All ingredient and feed samples were analyzed for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), Ca (AOAC 965.14/985.01, 2006), P (AOAC 965.17/985.01, 2006), starch (AOAC 996.11, 2006), ADF and NDF (Van Soest, 1963) by Ward Laboratories, Inc. (Kearney, NE).

Physical Diet Analysis

During the experiment, bulk density (Seedburo Model 8800, Seedburo Equipment, Chicago, IL) and particle size of major ingredients and all meal diets were measured. Bulk density was determined for all ingredients using methods from Clementson et al. (2010). Particle size was determined using Tyler sieves, with numbers 6, 8, 10, 14, 20, 28, 35, 48, 65, 100, 150, 200, and 270 and a pan. A Ro-Tap shaker (W.S. Tyler, Mentor, OH) was used to sift the 100-g samples for 10 min. A geometric mean particle size (dgw) and the log normal standard deviation (sgw) were calculated by measuring the amount of grain remaining on each screen (ASAE 2008). No flow agent was used for particle size analysis. For pelleted diets, pellet durability index (PDI) was determined using a Holmen NHP100 (Tekpro Limited, Norfolk, United Kingdom). Percentage fines were characterized as material that would pass through a #6 Tyler Sieve (3,360-µm opening) during 15 sec of manual shaking (ASAE, 1987).

Animals and Diets

A total of 2,100 pigs (PIC 327 × 1050; initially 31.5 ± 0.13 kg BW) were used in a 118-d trial. Pens of pigs were balanced by initial BW and randomly allotted to 1 of 6 dietary treatments (14 pens/treatment with 25 pigs/pen). Pens were sorted by gender allowing for 7 barrow and 7 gilt pens/treatment. The same corn-soybean meal–based diets containing 15% DDGS were used for all treatments and fed in 5 phases. Dried distillers grains with solubles were removed from the diet during the 5th phase. Phases were fed from d 0 to 28, 28 to 56, 56 to 84, 84 to 98, and 98 to 118. The 6 treatments included a meal or pelleted diet fed from d 0 to 118, a meal diet fed from d 0 to 70 and pellets from d 70 to 118, a pelleted diet fed from d 0 to 70 and a meal diet fed from d 70 to 118, or pellets and meal rotated every 2 wk starting with meal and ending with pellets or starting with pellets and ending with meal. Pigs were weighed approximately every 2 wk and feed disappearance was measured to determine ADG, ADFI, and G:F. Pig removals were determined by an on-site farm manager when pigs were at risk due to weight loss, health, or animal welfare concerns and needed to be separated from the general population. Pigs were weighed at the time of removal and the weight was accounted for in the growth performance from the period in which the pig was removed. This procedure was also used for pigs marketed prior to the conclusion of the trial on d 110. Pig days (no. of pigs × days on test) were used to adjust ADFI and ADG for the pen at the end of each weigh period, such that a removal's pig days were added back in to the total pig days for the pen for that weigh period.

On d 110, pens of pigs were weighed and 2 randomly selected pigs from each pen were weighed and transported to Natural Food Holdings (Sioux Center, IA). Pigs had continual access to feed except during transportation. Pigs were harvested and each stomach was collected. Stomachs were then assigned an ulcer and keratinization score, which was determined by visual

inspection. Keratinization scores were assigned on a scale from 1 to 4 with 1 being normal or no keratinization of the esophageal region (Figure 1); 2 being keratin covering < 25% of the esophageal region; 3 being keratin covering 25 to 75% of the esophageal region; and 4 being keratin covering >75% of the esophageal region (Figure 2). Ulcer scores were also assigned on a scale from 1 to 4 with 1 being no ulcers present; 2 being ulceration affecting <25% of the esophageal region; 3 ulceration affecting 25 to 75% of the esophageal region; and 4 being ulceration affecting >75% of the esophageal region (Figure 3). An index of stomach morphology was developed by adding a pig's ulcer and keratinization score. An additional score of 4 was added to each pig that had an ulceration score greater than 1. Because the keratinization and ulcer score were inversely related, this was done to differentiate pigs with a high ulcer score but low keratinization score having combined scores similar to pigs with a high keratinization score but low ulceration score. As ulcer score increased, keratinization score decreased due to tissue progressively moving from being keratinized to being ulcerated. Pigs with a high ulceration score but low keratinization score were assumed to have worse stomach morphology as ulceration is a product of keratinization and represents a stomach with a more progressed case of esophageal deterioration. Thus, this index was developed so that a high score represents a stomach with more damage present from keratinization and ulceration. Before final marketing, all pigs were individually weighed and tattooed for carcass data collection. On d 112 and 118 of the trial, all remaining pigs were transported 246 km to Tyson Foods (Waterloo, IA) for harvest. Standard carcass characteristics including HCW, yield (calculated from HCW and farm weight), BF, loin depth, and percent lean (Lean % = 48.3575 - (6.38916 * backfat, mm) + (4.424677 * loin depth, mm) were measured. Loin depth and BF were determined using longitudinal ultrasound centered on the 10^{th} rib at the P2 position.

Statistical Analysis

Data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Pairwise comparisons were used to determine differences among treatments. Results were considered significant at $P \leq 0.05$ and considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Analysis

Analysis of the corn, soybean meal, and DDGS used during the experiment revealed that nutrient values were similar to those used in formulation (Tables 1 and 2). Nutrient analysis of the treatment diets showed that all of the nutrients were also similar to formulated values (Table 3). For pelleted diets analysis, percentage fines was lowest and PDI highest during the last phase when DDGS were removed from the diet. The improvement in pellet quality when DDGS were removed from the diet was expected and is similar to observations from Fahrenholz et al. (2008) who observed that when DDGS were added at greater than 10% of the diet, pellet quality was negatively affected. Particle size of the meal diets in the current study ranged from 641 to 714 μm across all phases.

Growth and Carcass

Overall, there were no differences for ADG when pigs were fed a meal or pelleted diet (Table 4). This is similar to observations of Steidinger et al. (2000) who showed no differences in ADG between nursery pigs fed meal and pelleted diets. However, the vast majority of research comparing meal and pelleted diets in finishing pigs has shown significant or numerical improvements in ADG to pelleting (Skoch et al., 1983; Wondra et al., 1995a; and Potter et al., 2010). The lack of an improvement in ADG from pelleting may have been a result of the

worsened stomach morphology scores that were reported herein from pigs that were fed pelleted diets. The increased incidence of keratinization and ulceration may have limited the positive effects of pelleting.

Pigs fed meal throughout the experiment had the greatest ($P < 0.05$) ADFI, while pigs fed pellets throughout had the lowest ($P < 0.05$), with all other treatments intermediate ($P < 0.05$). This is similar to work by Wondra et al. (1995a) who observed that pigs fed a pelleted diet had reduced intake when compared to pigs fed a meal diet. The decrease in feed intake from feeding a pelleted diet may be a result of feed wastage being limited as noted by Hanrahan et al. (1984); or as a result of the improved digestibility of a pelleted diet (Skoch et al., 1983). Improvements in digestibility for pelleted diets would also explain why pigs fed pelleted diets throughout had the most improved ($P < 0.05$) G:F, while pigs fed meal throughout had the worst G:F ($P < 0.05$), and all other treatments were intermediate ($P < 0.05$). Improvements in G:F from pelleting diets has been widely shown throughout the literature (Stark et al., 1994; Myers et al., 2013; De Jong et al., 2013).

Paulk et al. (2011) fed pellets and meal diets to finishing pigs in a two phase study. Pigs were given either pellets or meal for the entire period, pellets for the first half of finishing (time basis) and meal for the second half, or meal for the first half of finishing (time basis) and pellets for the second half. The authors noted that pigs fed pellets for the duration of the study tended to have the most improved ADG and G:F, pigs fed meal the worst, and pigs fed pellets for only part of the grow-finish phase intermediate. In the current experiment, the timing of when pigs received pellets or switching back and forth did not influence G:F. When pellets and meal were rotated every 2-wk in the study herein, G:F was improved from 5 to 13% during each 2-wk

weigh period that pellets were fed (Figure 4) compared to those pigs fed meal during the same 2-wk period.

Pigs fed a pelleted diet throughout the trial had an increased ($P < 0.05$) number of pigs removed per pen compared to all other treatments. The removals were most likely a result of stomach morphology changes. When pelleted diets were fed for the last 58 d or for the entire trial, the incidence of ulceration and keratinization increased ($P < 0.05$) while pigs fed meal for the last 58 d had lower incidence ($P < 0.05$), with all other treatments intermediate ($P < 0.05$). This is similar to Flatlandsmo and Slagsvold (1971) and Wondra et al. (1995a) who both reported that pelleting diets increased the incidence of ulcers in finishing pigs. Elbers et al. (1995) conducted a study utilizing only pelleted diets where pigs were individually weighed and individual growth differences between pigs with or without ulcers could be determined. The incidence of ulcers of the pigs fed pellets ranged from 75 to 89%, and pigs determined to have ulcers gained 50 to 75 g/day less then pigs without ulceration. Guise et al. (1997) evaluated pigs with a historically high prevalence of ulcers (44 and 60% as previously determined at the abattoir) to determine differences in individual growth rate of pigs with or without ulcers at time of harvest. Pigs were followed to the abattoir where the prevalence of ulcers in two farms was determined to be 56 and 53%, respectively. There were no significant differences between the daily live weight gains of pigs with or without ulcers. This is in contrast to Ebers et al. (1995) and the study herein where it appears that the expected improvement in ADG from pelleting may have been negatively affected due to the increased incidence of ulceration. This would agree with work by Ayles et al. (1996) who showed that as ulcer severity increased, ADG decreased in finishing pigs.

It appears that feeding a pelleted diet continuously increased the incidence of ulcers which led to an increased number of pigs needing to be removed from the study. Reimann et al. (1968) showed that a finely ground diet increased the moisture and fluidity of the stomach contents. The increased fluidity of the stomach contents might also be caused by feeding finely ground feed in pelleted form as well. Regina et al. (1999) studied the effect of coarsely ground meal and finely ground pelleted diets on the stomach contents of finishing pigs. Pigs fed a pelleted diet had decreased DM concentration of their stomach matter suggesting an increase in the fluidity of the stomach contents. This agrees with Mösseler et al. (2012) who also showed pigs fed pellets had a more liquid chyme and increased ulceration. The increase in fluidity of the stomach contents of pigs fed a finely ground pelleted diet led to increased incidences of ulceration as well. Mösseler et al. (2014) also showed increased Cl concentrations in stomachs of pigs fed a finely ground pelleted diet compared to pigs fed a coarsely ground meal diet, which they suggested was a result of increased HCL secretion. They also noted that the pH of the stomach was more consistent in all 4 regions when compared to pigs fed a coarse meal diet. This also points to increased mixing in the stomach when a pelleted diet is fed most likely as a result of the increased fluidity of the stomach contents.

It was also noted in the current study that when pigs were fed a meal diet for the entirety, the second half of the trial, or for 2 wk before harvest, pigs had marginally significant ($P < 0.10$) or had decreased ($P < 0.05$) stomach ulcer index scores as compared to pigs fed pellets for the entirety, the second half of the trial, or for 2 wk before harvest. Ayles et al. (1996) were able to demonstrate that feeding a coarse ground meal diet for as little as a 3-wk period can improve stomach morphology when a finely ground diet was previously fed. Though both the meal and pelleted diet were from the same corn source and had identical particle sizes, it is possible that

the pelleting process may have caused a decrease in the particle size of the pelleted feed. Previous reports have shown reductions in diet particle size of 50 to 290 µm during pelleting (Svihus et al., 2004). Further grinding of the diet during pelleting could have resulted in smaller particle size of the pelleted diet, which may have contributed to the ulceration from pelleting. The possibility of a finer grind in the pelleted diet of the current study provides some reasoning to why stomach morphology scores worsened when pigs were fed pellets.

There were no differences observed in carcass characteristics. This is in agreement with Nemechek et al. (2013) who also observed no significant differences in any carcass measurements when pigs were fed a pelleted diet compared to a meal diet. Potter et al. (2010); however, conducted an experiment feeding meal and pelleted diets to finishing pigs and found that pigs fed a pelleted diet had improved carcass yield, and a tendency for increased percentage lean and decreased loin depth.

In conclusion, our data suggest that if a meal diet is rotated with pelleted diets during the finishing period, ulceration of the stomach lining may be lessened and improvements from pelleting can still be realized. Feeding a pelleted diet improved G:F but also increased the number of pigs removed during the study as a possible result of stomach ulceration.

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Table 2-1 Diet composition of experimental diets, Exp. 1 (as-fed basis)[1]

	Dietary phase				
Item	1	2	3	4	5
Ingredient, %					
Corn	61.11	67.78	72.36	73.82	84.33
Soybean meal (46.5% CP)	20.11	13.50	9.00	7.80	12.49
Choice white grease	1.00	1.00	1.00	1.00	1.00
Dried distillers grains with solubles	15.00	15.00	15.00	15.00	---
Monocalcium phosphate (21% P)	0.25	0.15	0.10	0.00	0.25
Limestone	1.15	1.20	1.20	1.20	1.00
Salt	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.43	0.44	0.44	0.38	0.21
DL-Met	0.07	0.04	0.02	---	---
L-Thr	0.10	0.10	0.09	0.07	0.06
L-Trp	0.03	0.04	0.04	0.03	0.01
VTM premix[2,3]	0.25	0.25	0.25	0.20	0.15
Total	100	100	100	100	100
Calculated analysis					
Standard ileal digestible (SID) AA, %					
Lys	1.05	0.90	0.79	0.71	0.65
Ile:Lys	61	59	58	61	66
Met:Lys	33	32	31	32	31
Met + Cys:Lys	58	58	58	61	62
Thr:Lys	62	62	62	64	67
Trp:Lys	19.0	19.0	19.0	19.0	19.0
Val:Lys	70	70	70	75	78
Total Lys, %	1.20	1.03	0.91	0.83	0.75
ME,[4] kcal/kg	3,332	3,337	3,342	3,347	3,376
NE,[4] kcal/kg	2,502	2,541	2,570	2,575	2,594
SID Lys:ME, g/Mcal	3.15	2.70	2.36	2.12	1.92
CP, %	19.0	16.5	14.8	14.2	13.0
Crude fiber, %	3.3	3.2	3.1	3.1	2.2
Ca, %	0.58	0.56	0.53	0.51	0.49
P, %	0.44	0.39	0.36	0.34	0.36
Available P, %	0.18	0.15	0.13	0.11	0.10

[1] Phase 1 diets were fed from d 0 to 28, Phase 2 from 28 to 56, Phase 3 from 56 to 84, Phase 4 from d 84 to 98, and Phase 5 from d 98 to 118.

[2] Provided per kg of premix: 4,537,205 IU vitamin A; 1,088,929 IU vitamin D_3; 19,963 IU vitamin E; 2,117 mg vitamin K; 2,722 mg riboflavin; 12,704 mg pantothenic acid; 16,334 mg niacin; and 18.1 mg vitamin B_{12}. Provided per kg of premix: 53.3 g Mn from manganese oxide; 134 g Fe from iron sulfate; 160 g Zn from zinc sulfate; 13 g Cu from copper sulfate; and 137 mg I from calcium iodate.

[3] Quantum Blue 5 G (AB Vista, Marlborough, UK.) provided 455 FTU/kg diet, with a release of 0.12% available P.

[4] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.

Table 2-2 Chemical analysis of ingredients (as-fed basis)[1]

Item	Corn[2]	Soybean meal	Dried distillers grains with solubles[3]
DM, %	88.87	91.05	90.68
CP, %	9.1 (8.2)	45.1 (46.5)	29.8 (27.7)
ADF, %	3.0	6.3	10.1
NDF, %	6.1	7.4	24.8
Ca, %	0.05 (0.02)	0.41 (0.33)	0.15 (0.20)
P, %	0.29 (0.26)	0.74 (0.71)	0.81 (0.77)
Ether extract, %	3.1 (3.5)	1.8 (1.5)	8.7 (7.3)
Starch, %	60.7	4.0	3.7

[1]A composite sample of 3 subsamples taken throughout the experiment at the feed mill were used for analysis.
[2] Values in parenthesis for corn and soybean meal were taken from NRC (2012).
[3] Values in parenthesis for DDGS are taken from Stein (2007).

Table 2-3 Chemical analysis of diets (as-fed basis)[1,2]

	Dietary phase				
Item	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
DM, %	90.90	90.43	89.92	90.19	89.39
CP, %	19.1	16.5	15.3	14.8	12.6
ADF, %	4.3	3.5	4.7	3.8	1.9
NDF, %	10.1	10.6	11.6	11.0	8.6
Ca, %	0.64	0.56	0.53	0.50	0.57
P, %	0.45	0.42	0.40	0.36	0.35
Ether extract, %	4.8	4.9	5.2	4.9	4.2
Starch, %	39.6	42.7	43.6	45.9	51.2
Particle size, µm	683	692	705	714	641
Pellet fines, %	26.7	34.6	20.3	33.1	3.7
PDI, %[3]	84.5	85.8	86.9	90.2	94.5

[1] A composite sample consisting of 6 subsamples was used for analysis.
[2] Meal and pelleted diet samples within phase were individually analyzed and results were averaged. Particle size represents the complete meal diet for each phase. Percentage fines and PDI represent the pelleted diet for each phase.
[3] PDI = pellet durability index.

Table 2-4 The effect of pellet feeding regimen on finishing pig growth performance, carcass characteristics, and stomach morphology[1]

	Diet form and period, d 0 to 70/d 70 to 118						
Item,	Meal Meal	Pellet Pellet	Meal Pellet	Pellet Meal	Rotated[2] Rotated	Rotated[3] Rotated	SEM
d 0 to 111							
ADG, kg	0.96	0.97	0.96	0.96	0.96	0.97	0.012
ADFI, kg	2.36^{a}	2.26^{c,y}	2.30^{b,c,x}	2.28^{b,c}	2.30^{b}	2.29^{b,c}	0.024
G:F	0.407^{c}	0.430^{a}	0.421^{b}	0.422^{b}	0.420^{b}	0.423^{b}	0.002
BW, kg							
d 0	31.5	31.6	31.4	31.4	31.6	31.5	0.60
d 118	135.6	136.6	136.0	134.0	135.3	136.2	1.95
Keratinization score	2.3	2.4	2.2	2.1	2.8	2.1	0.22
Ulceration score	1.5	2.0	2.2	1.6	1.8	1.6	0.20
Ulcer index[4]	5.25^{ab,z}	6.72^{a,x}	6.72^{a,x}	4.61^{b,y}	6.15^{ab,xz}	5.32^{ab,z}	0.61
Pigs removed/pen	0.50^{b}	1.92^{a}	1.06^{b}	0.93^{b}	0.85^{b}	0.92^{b}	0.265
Carcass characterisitcs[5]							
HCW, kg	97.9	99.2	98.6	97.9	98.4	98.9	1.06
Yield, %	74.8	75.2	74.7	74.8	75.3	75.6	0.50
Backfat, mm	16.7	17.1	16.8	16.5	16.8	16.8	0.26
Loin depth, mm	72.8	73.8	73.9	73.4	73.7	73.9	0.36
Lean, %[6]	56.3	56.2	56.2	56.5	56.5	56.3	0.19

^{a,b,c} Superscripts within a row are different ($P < 0.05$).

$^{x, y, z}$ Superscripts within a row tended to be different ($P < 0.10$).

[1] A total of 2,100 pigs (PIC 327×1050; initially 31.5 ± 0.13 kg BW) were used in a 118-d trial there were 25 pigs per pen and 14 pens per treatment (7 barrow and 7 gilt).

[2] Meal and pellet were rotated every 2 weeks starting with meal and ending with pellet. Pigs were fed a meal diet for 10-d prior to collecting stomach morphology scores.

[3] Meal and pellet were rotated every 2 weeks starting with pellet and ending with meal. Pigs were fed a pelleted diet for 10-d prior to collecting stomach morphology scores.

[4] An index of stomach morphology was developed by adding a pig's ulcer and keratinization score. An additional score of 4 was added to each pig that had an ulceration score greater than 1.

[5] On d 110, 4 pigs were removed from each pen and a combined keratinization and ulceration score was assigned to each stomach. On d 112 the pens remaining with barrows were marketed. On d 118 the pens remaining with gilts were marketed.
[6] Calculated using the equation: Lean % = 48.3575 - (6.38916 * backfat) + (4.424677 * loin depth).

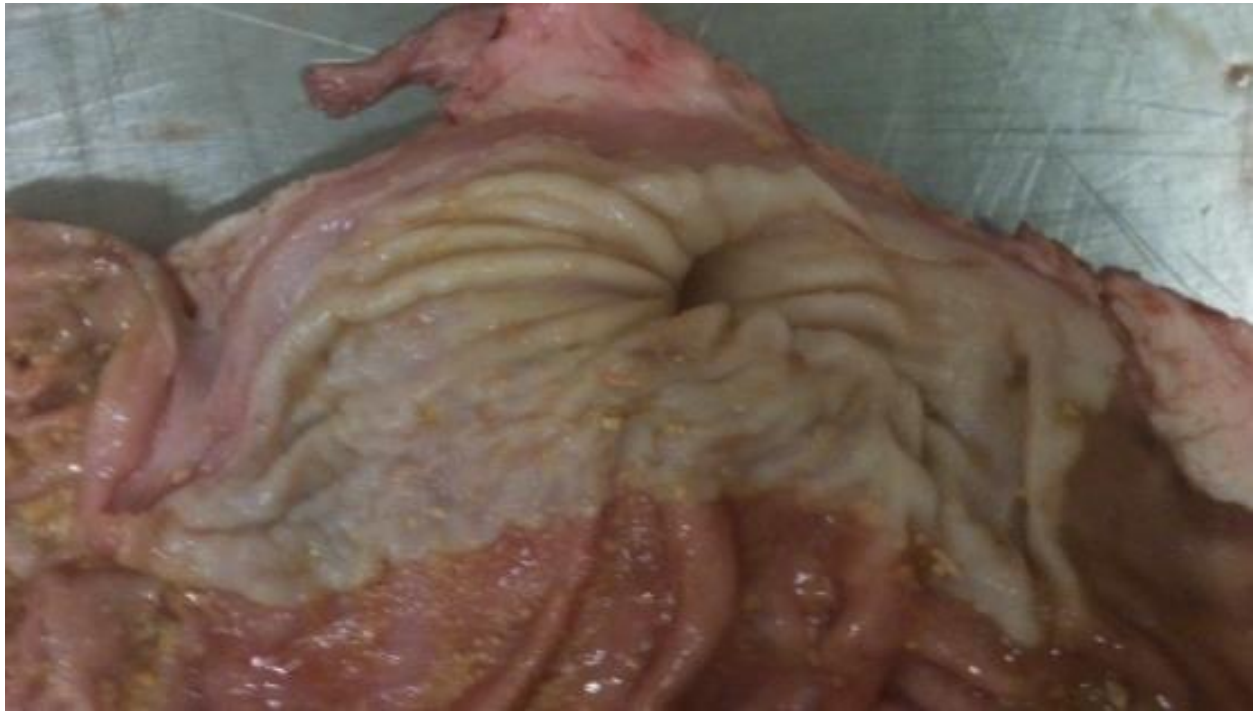

Figure 1 Esophageal opening of stomach with no keratinization or ulceration.

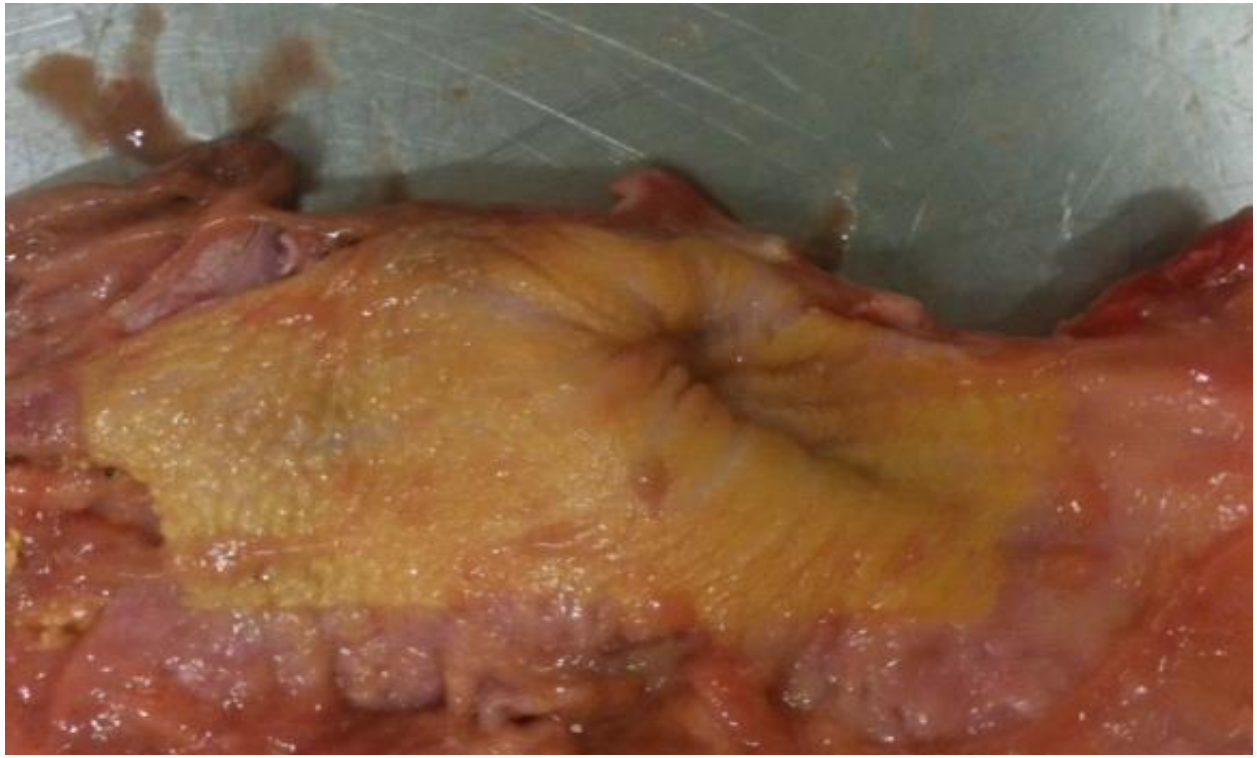

Figure 2 Esophageal opening of stomach with 100% keratinization and no ulceration.

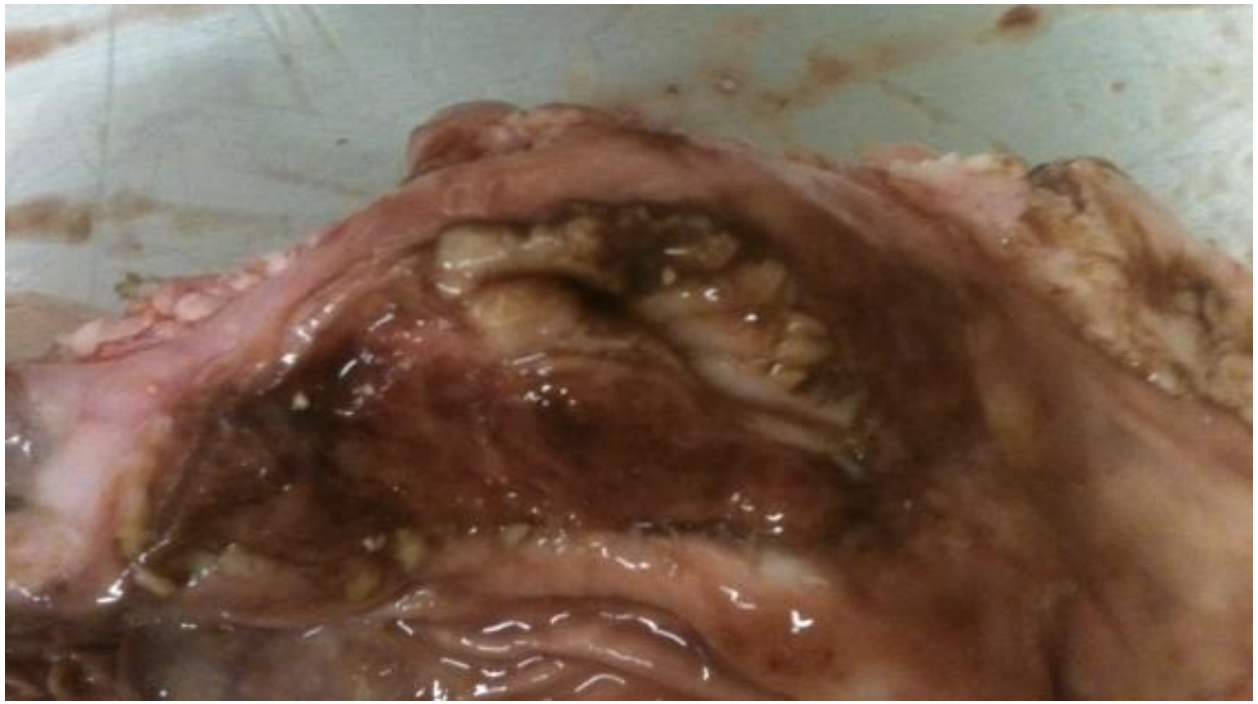

Figure 3 Esophageal opening of stomach with 100% ulceration.

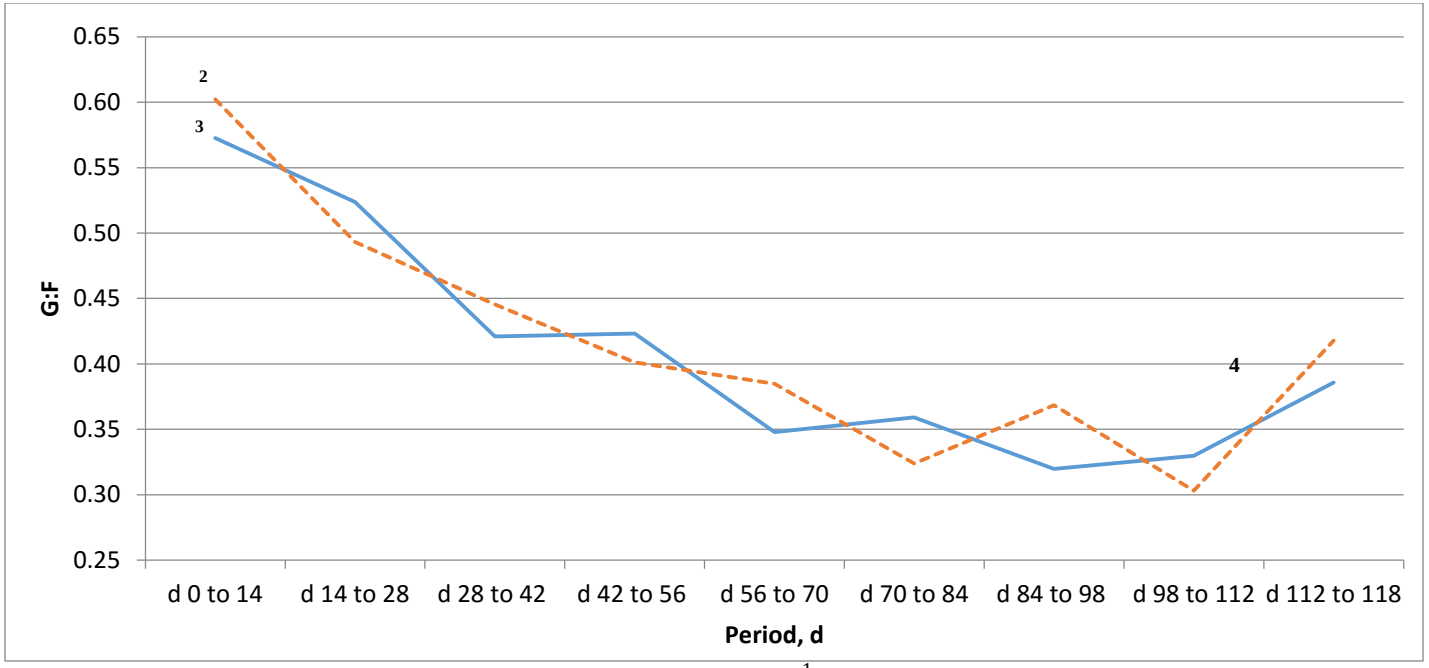

Figure 4 Effect of rotating meal and pellet every 2-wk on G:F[1]

[1] Treatments were significantly different ($P < 0.05$) at every weigh period.
[2] Treatment started on pellets and was rotated between pellet and meal every 2-wk.
[3] Treatment started on meal and was rotated between meal and pellet every 2-wk.
[4] From d 112 to 118 only gilts remained in the experiment explaining the improvement in G:F for the last period.

Chapter 3 - Formation of Pellet Fines During the Feed Manufacturing Process, Transportation and Feed Line Delivery, and Nutrient Composition of Pellets and Fines

ABSTRACT

Three studies were conducted to investigate the formation of fines during the pelleted feed manufacturing process, transportation, and feed line delivery at the farm. A second objective was to determine the chemical composition of the pellets and fines. In Exp. 1, complete feed samples in a commercial feed mill were collected at the pellet mill, pellet cooler, fat coater, and at load-out. Overall, pellet durability index (PDI) was different ($P < 0.05$) between mill locations, and increased from the pellet mill to the fat coater, but decreased between the fat coater and load-out. The largest increase in PDI was between the cooler and fat coater (6.3%). Correspondingly, percentage fines was similar from the pellet mill to the cooler, but then increased ($P < 0.05$) at the fat coater and at load-out. Dry matter and ether extract were greater ($P < 0.05$) and ADF tended to be greater ($P = 0.087$) in fines compared to pellets, whereas CP was lower ($P = 0.021$) in fines. In Exp. 2, pelleted feed was unloaded using 3 truck unloading speeds (900, 1,150, and 1,400 RPM) from each of 8 compartments of a feed truck (Walinga Inc., Guelph, Ontario). Six samples per compartment were collected creating 16 replications/unloading speed. There was an unloading speed × trailer compartment interaction ($P = 0.031$) for unloading time. The percentage of fines formed during unloading was not influenced by unloading speed but tended to increase (quadratic; $P = 0.081$) from the front to the rear compartment. In Exp. 3, pelleted feed samples were collected during unloading into a commercial feed bin at 6 barn sites with 2 adjacent feed lines, resulting in 12 replications per

feed line location. Samples were collected from the feed line at 6, 35, and 76 m from the bin. There were no interactions between feed line location and nutrient profile of the fines and pellets. There was no effect of feed line location on pellet PDI, percentage fines, percentage fines formed, or the nutrient profile of pellets or fines. Across locations, fines had decreased ($P < 0.05$) CP and P, but greater ($P < 0.05$) ADF, crude fiber, Ca, ether extract, and starch compared to the composition of pellets. Understanding the location of fines creation during the feed manufacturing and delivery process may allow for alternative methods to reduce the formation of fines.

Key words: feed line, feed mill, feed truck, fines, pellets, PDI

INTRODUCTION

Pellet quality and its subsequent effects on pig performance have been studied in recent years. Nemechek et al. (2015) found that pellet fines should be minimized to achieve the maximum benefit from pelleting for finishing pig diets. It is expected that each 10% increase in pellet fines worsens G:F by 1%. Thus, providing pigs with high quality pelleted diets with a low percentage of fines is important to ensure the positive effects of pelleting on pig performance are realized. Reducing the percentage of fines in pellets can be accomplished in a number of ways, such as by manipulating diet formulation, conditioning time and temperature, or post-pelleting handling techniques (Muramatsu et al., 2013).

When pellets exit a pellet mill, they are not immediately loaded onto a truck for delivery, but are instead moved though the feed mill for cooling and fat application and then placed into bins prior to loading onto a feed truck. Then, feed is typically transported and unloaded through a high output auger and dropped vertically into an on-farm bin. Feed truck drivers are sometimes incentivized to be efficient in the delivery process, leading them to load bins as quickly as

possible. The process that pellets must undergo during delivery and unloading is suspected to physically damage them and increase the percentage of fines; however, few studies have evaluated manufacturing and delivery factors that may influence pellet damage. If the formation of fines can be better understood, feed mills may be able to implement strategies to reduce pellet damage.

Moritz et al. (2013) observed that when pelleted turkey diets were moved along a feed line, a greater percentage of fines were present in feeders closest to the feed bin. This resulted in differences in feed nutrient content between the front and back of the barn and between the pellets and pellet fines. This difference in nutrient profile, along with the preferences of pigs to consume pellets compared to fines (Skoch et al., 1983), is compounded when separation occurs in the feed line when transferring feed from bins to feeders. This variation in nutrient content may lead to increased growth variability within a single barn. Currently, little data exists to predict the formation of fines after the pelleted feed manufacturing process or the possible differences that may exist in nutrient composition between fines and pellets in swine diets.

Thus, the objective of these studies were to determine 1) where the formation of fines occurs during feed manufacturing and the nutrient concentration of whole pellets and their fines; 2) the effect of feed truck unloading RPM on pellet quality and unloading time, and 3) the effect of feed line sampling location on pellet quality and nutrient concentration of whole pellets and their fines.

MATERIALS AND METHODS

In Exp. 1, 2, and 3, feed was manufactured at the same commercial feed mill in northwest Iowa. Diets were pelleted using a 500 horsepower pellet mill (Pioneer Pellet Mill; Bliss Industries, Ponca City, OK), counter-flow cooler (OPFLO; Bliss Industries, Ponca City, OK),

and a twin screw liquid fat coater (TMX Mistcoater; APEC, Lake Odessa, MI). All samples were analyzed for pellet durability index (PDI) via a Holmen NHP200 (Tekpro Limited, Norfolk, United Kingdom) and percentage fines (ASAE, 1987). Fines were characterized as material that would pass through a #6 Tyler Sieve (3,360-μm opening) during 15 s of manual shaking. During fines determination, a subsample of both the fines and pelleted fraction was reserved and analyzed for DM (AOAC 934.01, 2006), CP (AOAC 990.03, 2006), ether extract (AOAC 920.39 A, 2006), Ca (AOAC 965.14/985.01, 2006), P (AOAC 965.17/985.01, 2006), and ADF (Van Soest, 1963; Ward Laboratories, Inc. Kearney, NE USA). In addition, crude fiber (AOAC 978.10, 2006) and starch (AOAC 996.11, 2006) were analyzed for samples from Exp. 3.

Experiment 1

A 3-wk study was conducted where samples were collected from 4 swine and 2 turkey diets (Table 1). Samples were collected during discharge from the pellet mill, cooler, fat coater, and at loadout to determine progression of fines formation during the manufacturing process. One 1-kg sample of pelleted feed was collected every 15 to 20 min from 7 to 10 different manufacturing runs for each diet throughout the 3-wk period. Feed manufacturing runs varied from 54 to 134 tonnes of feed.

Samples collected at the pellet mill discharge were immediately placed in a bench-top pellet cooler to reduce the temperature of the pellets to ambient temperature (22± 2°C). The second sampling port was located under the screw auger immediately after the pellets were discharged from the cooler. The third sampling port was below the fat coater where post-pelleting liquid fat was added to 5 of the 6 diets. The diet that did not have fat added post-pelleting was directed through the fat coater for the duration of the experiment to replicate the

transportation process through the fat coater the other diets experienced. The last sampling occurred during load-out as feed was exiting the discharge into the feed truck.

Experiment 2

A single 8-compartment 21.7-tonne high output auger unit feed truck (Walinga Inc., Guelph, Ontario) was used in this experiment. In order to achieve different unloading speeds, the truck motor was set to 1 of 3 pre-selected speeds: 1) lowest attainable speed, 900 RPM; 2) intermediate speed, 1,150 RPM; and 3) highest attainable speed, 1400 RPM. The feed truck was equipped with a 30.4 cm diameter floor auger, 40.6 cm diameter vertical auger, and a 30.4 cm diameter boom auger measuring 9.7 m long at the posterior of the truck. The truck speed of 900 RPM resulted in the 3 augers within the trailer having speeds of 84, 207, and 280 RPM for the floor, vertical, and boom auger, respectively. The truck speed of 1,150 RPM resulted in the 3 augers within the trailer having speeds of 122, 263, and 316 RPM for the floor, vertical, and boom auger, respectively. Finally, the truck speed of 1,400 RPM resulted in the 3 augers within the trailer having speeds of 159, 318, and 354 RPM for the floor, vertical, and boom auger, respectively. Unloading speeds were randomly assigned to each compartment within the truck. Six truckloads of pelleted feed were used for this experiment, which resulted in 16 replications per unloading speed and 6 replications per compartment. The compartment located closest to the truck cab was denoted as Compartment 1 and the compartment located closest to the rear of the truck was denoted as Compartment 8.

As the truck was loaded with pelleted feed in the mill, samples were taken directly under the load-out spout for each compartment. Thus, a baseline value for percentage fines and PDI was determined for each compartment. Once loading was complete, the truck was driven by the same route to the same central location, and feed was unloaded into a different feed truck to

collect samples and auger motor load data. The boom auger was equipped with a 2.4 m × 30.4 cm reinforced cardboard sleeve to prevent spilling during the unloading process, and the boom was raised until it was approximately 6.1 m off of the ground to simulate a typical feed bin height.

Unloading time for each compartment began when the slide gate under the compartment was opened, and stopped when feed was no longer exiting the boom. Three separate samples were taken while each compartment was unloaded. Samples were taken from below the boom auger sleeve.

Experiment 3

Six identical wean-to-finish swine barns were used to determine the effects of feed line location on pellet quality and nutrient segregation. Feed samples were taken directly below the boom auger at the top of the feed bin to establish a baseline for initial PDI for each feed bin. Feed was allowed to flow into the barn for 24 to 36 h to ensure that feed initially sampled would be present at the feeders.

Each barn was approximately 79 m long × 12 m wide, and was equipped with two 6-m tall tandem bins located at the front of each barn. Two screw conveyor feed lines were present in each barn, and spanned approximately 76 m from the bin to the final feeder on each line. At 24 to 36 h after placement, 1-kg feed samples were collected from each feed line directly below the spout connection at the feeder closest to the bin (6 m), an intermediate distance to the bin (35 m), and the farthest from the bin (76 m) for each feed line. Samples were analyzed for percentage fines and PDI.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). In Exp. 1, location or feed form (pellet vs. fines) was used as the experimental unit for the physical and chemical analysis, respectively. Location, run, and location within run were considered a random effect for physical analysis. Pairwise comparisons were used to determine differences. Compartment or feed line was used as the experimental unit for Exp. 2 and 3 respectively. Preplanned contrasts were used to determine the interaction and linear and quadratic effect of unloading speed and compartment on pellet quality and unloading time for Exp. 2. The interaction of feed form and feed line location, linear and quadratic effect of feed line location, and the main effects of feed line location and feed form were determined for Exp. 3. Results were considered significant at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

RESULTS

In Exp. 1, PDI was different ($P < 0.05$) between locations in the mill; increasing from the pellet mill to the fat coater, but then decreasing between the fat coater and load-out (Table 2). The largest increase in PDI was between the cooler and fat coater (63 g/kg). Percentage fines was similar from the pellet mill to the cooler, but then increased ($P < 0.05$) as pellets exited the fat coater, and again from the fat coater to load-out. The largest increase in fines was between the cooler and fat coater and between the fat coater and load out (56 and 65 g/kg, respectively). Dry matter and ether extract were greater ($P < 0.05$) and ADF tended to be greater ($P < 0.08$) in fines compared to pellets, whereas CP was lower ($P < 0.05$) in fines compared to pellets (Table 3).

In Exp. 2, there was an unloading speed × trailer compartment interaction ($P = 0.031$) for unloading time (Table 4). The difference in unloading time from the first to last compartment was greatest at the slowest unloading speed and similar at the two highest unloading speeds (70

vs. 35 vs. 37 sec for 900, 1,150, and 1,400 RPM, respectively). The percentage of fines formed was not influenced by unloading speeds (Table 5). The percentage of fines formed during unloading tended to increase (quadratic; $P = 0.081$) from the 1st to the 8th compartment, with the maximum percentage of fines formed occurring in the 5th compartment (Table 6).

In Exp. 3, there were no interactions between feed line location and nutrient profile of the fines and pellets. There was no effect of feed line location on PDI, percentage fines, or percentage fines formed (Table 7). Fines had decreased ($P < 0.05$) CP and P, but greater ($P < 0.05$) ADF, crude fiber, Ca, ether extract, and starch when compared to the composition of pellets (Table 8).

DISCUSSION

Feeding pelleted compared to mash diets has consistently improved ADG and G:F in growing pigs (Stark et al., 1994; Potter et al., 2010; and De Jong et al., 2013) and poultry (Abdollahi et al. 2013). The improvements from pelleting swine diets is most likely a result of decreased feed disappearance from reduced feed wastage as noted by Hanrahan et al. (1984); or as a result of the improved digestibility of pelleted diets (Skoch et al., 1983). Notably, the improvements from pelleting may be limited or nullified if pellet quality suffers. Amornthewaphat et al. (2000) investigated the effects of increasing percentage fines (0, 25, and 50%) of a pelleted diet on finishing pig performance. As fines increased, ADG and G:F both decreased when a conventional dry feeder was used. Schell et al. (1998) also found pigs had poorer G:F when fines were increased from 2.5 to 40%, and decreased ADG when fines were increased from 3 to 37% fines. Stark et al. (1994) observed through 2 experiments with nursery and finishing pigs that when pigs were fed screened pellets with 0, 15, 25, or 30% fines, they had either a tendency or numerical decrease for reduced G:F with increasing percentage of fines in

the diet More recently, Nemechek et al. (2015) demonstrated that feeding a high quality pellet (< 10% fines) compared with a low quality pellet (> 33% fines) improved G:F in both the nursery and finishing phase; in fact, nursery pigs fed a poor quality pellet had G:F similar to pigs fed a mash diet. In addition, the reduction in performance from increasing pellet fines has also been widely shown in broilers (McKinney et al., 2001 and Lilly et al., 2011). The improvements in G:F from pelleting in broilers may also be a result of the decreased energy required to consume a pelleted diet compared to a meal diet (McKinney et al. 2004).

Understanding that pellet quality is an important factor to maximize the improvements in performance of growing swine and poultry has led many to attempt to improve pellet quality during feed manufacturing. In a review, Loar et al. (2011) speculated that feed formulation constitutes a large proportion of pellet quality problems. They reported that the addition of starch or protein based products were shown to improve pellet quality. The gelatinization that occurs when starch is heated during pelleting (Stevens 1987) is the most likely cause of improved pellet quality from ingredients high in starch. Buchanan et al. (2010) also found that increasing the CP of the diet improved pellet quality. Protein also unfolds during the heating process and then re-forms during cooling to provide stability within the pellet (Wood 1987). The addition of moisture during the pelleting process has also been shown to improve PDI, while the addition of fat was detrimental (Buchanan et al., 2010; Loar et al., 2011; Muramatsu et al., 2013). The worsening of pellet quality from fat applied in the mixer has led many producers to include fat after pelleting using post pellet liquid application. In the current experiment; however, PDI was reduced after fat was applied, suggesting that post pellet liquid application of fat may actually begin to soften the pellet. Other methods to improve pellet quality include using a thick pellet die and reduced production rate (Buchanan et al., 2010) or using finely ground grains (Muramatsu et

al., 2013). Slowing the production rate at most commercial mills is not a viable option to improve pellet quality due to the demands for large quantities of feed in a timely manner. Fine grinding will also slow production rate of the mill which will lead to increases in the cost of production.

Factors other than diet formulation and manipulation of the pellet mill may affect pellet quality after it passes through the die. Similar to our experimental design, Cardeal et al. (2014) investigated the effects of transport on pellet quality. Samples were collected as feed was discharged from the pellet mill, from inside the truck, as the truck discharged into an on-site bin, and inside the barn towards the front, middle, and end of the feed line. The percentage of intact pellets significantly decreased post pelleting, but only during discharge into the on-site farm bin. They noted a reduction of intact pellets of 15%, which was less than in the current study, with an associated 10% increase in pellet fines within the mill, and another 10% increase during discharge from the truck. It should be mentioned that samples were only taken at the die unlike the current experiment where samples were taken at 4 points within the mill. Similar to our findings, Cardeal et al. (2014) described an increase in PDI after the pellet die, which is most likely a result of the cooling process removing moisture from the pellet. Alternatively, the increase in PDI after the pellet die may be due to the poorest quality pellets being degraded early in the manufacturing process, leaving only the hardest and most durable pellets as they move though the feed mill. In a notable difference from our experimental design, Cardeal et al. (2014) observed that using a larger discharge opening on the feed truck auger resulted in an improved percentage of intact pellets, potentially because there was less friction within the auger, allowing pellets to maintain their structural integrity compared to pellets discharged using the smaller auger.

In the current experiment, percentage fines were reduced from discharge into the on-farm bin to the last feeder on the feed line. This was unexpected, but may be explained by Winowiski (1998), who sampled pelleted feed as it was discharged from a storage bin. Feed was sacked off as it was discharged, and every tenth bag was sampled for percentage fines. For the first 60% of the feed from the bin, percentage fines remained relatively constant (15% fines). However, as the last 40% of the bin was discharged, the percentage of fines increased from 15 to 40%. This data suggests that fines and pellets were segregating inside the bin, and that a larger of proportion of pellets were discharged at the beginning, followed by an increasing quantity of fines towards the end of discharging the bin. In the current experiment, samples were taken 24 to 36 h after feed was placed in the bin and had begun to discharge into the barn. The bins sampled were within the first 20-40% of the bins capacity, which most likely led to a larger than expected quantity of pellets being present in our samples. Thus, the quantity of fines was actually reduced in the samples collected during a single time point from entry into the bin until it was sampled at the feeders. More research is needed to fully understand the level of fines discharged as the bin empties relative to initial quantity at unloading.

In addition to measuring PDI, differences in the nutrient profiles of the pellets and their associated fines were determined in our studies. Mortiz et al. (2013) observed that when pelleted diets were moved along a feed line in a turkey barn, a greater percentage of fines were present in feeders closest to the feed bin. In contrast, Sellers et al. (2014) observed that when augering feed to feeders at the farm, the amount of pellets present at the feeder decreased along the feed line from 0 to 55 m. In the current experiment, there were no differences in pellet fines along the feed line. This may be due in part to when samples were taken as the bin unloaded or to potential differences in initial percentage of fines present in the diet. Moritz et al. (2013) also noted that

phytase, which was added using post pellet liquid application, was more concentrated in the fines compared to the pellets. The quantity of total phytase present in the feed was subsequently reduced from the first to the last feeder. In the present studies, phytase concentration was not measured; however, differences were found in fat composition between pellets and fines, which was applied through a post pellet liquid application system. This may suggest that post pellet liquid application of phytase or fat may lead to increased concentrations in the fines of the diet. This is also in agreement with Engelen and van der Poel (1999), who reported that when vitamin E and phytase were added to pelleted feed using spray application, fines had significantly greater concentrations of both vitamin E and phytase. They also reported that when xylanase was sprayed on a pelleted feed with only 9% fines, the fines had a significantly higher concentration of xylanase compared to the pellets. This resulted in fines providing 23% of the total xylanase activity, even though they physically represented 9% of the total feed amount. More recently, Sellers et al. (2014) investigated the effects of mixer or post pellet liquid addition of phytase and fat in diets with 25 or 45% fines in the diet. They reported that when fat and phytase were added at the mixer, the diet had worse pellet quality as noted by the increased amount of pellet fines.

Myers et al. (2013) suggested that poor PDI leads to increased sorting of the pellets and fines, and possible increases in feed wastage. The preference of pigs to consume pellets compared to pellet fines may lead to nutrient imbalances or possible deficiencies. The differences in CP, fiber components, Ca, and P of the pellets and associated fines as seen in the current experiment may also be cause for concern when formulating to meet the metabolic needs of the pigs when fed a pelleted diet, especially in cases where pellet fines are very high.

In conclusion, pellet quality is worsened as pellets are transported through the feed mill. As feed was then transported to the barn, it appears that the front compartments of the feed truck

closest to the cab resulted in fewer fines formed during the unloading process which was unexpected. Decreasing unloading speed significantly increased the quantity of time it took to unload a single compartment, but did not change fines formation. Thus, high unloading speeds may be used without jeopardizing pellet quality, and may improve delivery efficiency. Once pellets were delivered to the farm, pellet quality of our tested samples was similar between feed line locations within a commercial wean-to-finish barn. There were significant differences between the fines and pellet nutrient profiles, as noted by the increased concentration of ADF, crude fiber, Ca, ether extract, and starch in the fines, and decreased CP and P when compared to pellets. Understanding where the formation of fines occurs during the feed manufacturing and delivery process may allow for the development of strategies to reduce pellet deterioration.

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Table 3-1 Composition of experimental diets (as-fed basis)

	Experiment and diet formulation							
	1						2	3
Item	Swine			Turkey			Swine	Swine
Ingredient, %								
Corn	77.56	76.08	61.09	65.20	64.34	68.97	85.54	74.95
Soybean meal (46.5% CP)	17.35	11.55	8.44	20.22	18.43	16.08	9.50	19.97
DDGS	0.00	5.00	20.40	---	---	---	---	---
Wheat middlings	---	---	7.50	6.39	---	---	---	---
Meat scraps	---	---	---	--	7.54	5.22	---	---
Animal/vegetable blend fat	2.00	4.37	0.35	5.51	7.39	7.51	2.75	2.57
Limestone	0.72	0.90	1.20	0.71	0.55	0.64	0.80	0.82
Monocalcium phosphate (21% P)	---	---	---	---	---	---	0.25	0.25
Dicalcium phosphate (18% P)	1.07	0.84	---	0.60	0.10	0.00	---	---
Salt	0.42	0.46	0.41	0.41	0.28	0.32	0.35	0.35
Copper[1]	0.02	0.02	0.05	0.05	0.05	0.03	0.02	0.02
L-lysine	0.52	0.50	0.40	0.48	0.41	0.40	0.52	0.61
DL-methionine	0.05	0.01	---	0.09	0.22	0.20	---	0.09
L-threonine	0.11	0.09	---	0.11	0.01	0.01	0.09	0.16
L-tryptophan	0.01	0.01	---	---	---	---	0.03	0.03
Betaine	---	---	---	---	0.27	0.27	---	---
Vitamin trace mineral premix	0.15	0.16	0.17	0.24	0.41	0.36	0.15	0.15
Ractopamine HCL, 19.8g/lb	---	---	---	---	---	---	---	0.03
Total	100	100	100	100	100	100	100	100

[1] Copper source varied between copper sulfate and tribasic copper chloride (Micronutrients Inc., Indianapolis, IN).

Table 3-2 Effect of mill sample location on pellet durability and percentage fines (Exp. 1)[1]

Item, %	Pellet mill	Cooler	Fat coater	Load-out	SEM	Probability, $P <$
Pellet durability index	77.0[d]	78.3[c]	84.6[a]	81.9[b]	0.82	0.001
Pellet fines	9.44[c]	8.54[c]	14.20[b]	20.46[a]	0.77	0.001

[a,b,c,d] Superscripts within a row are different ($P < 0.05$).

[1] Eight to 10 samples were taken from each location in the mill within a run for 8 runs over 3 weeks.

Table 3-3 Nutrient composition of fines and pellets (Exp. 1)[1,2]

				Probability, $P <$
Item, %	Fines	Pellets	SEM	Fines vs. pellets
DM	88.83	88.32	0.16	0.031
CP	13.58	15.24	0.48	0.021
ADF	4.09	3.59	0.20	0.087
Ca	0.74	0.74	0.07	0.975
P	0.50	0.53	0.02	0.354
Ether extract	9.00	7.71	0.42	0.039

[1] Samples from the fat coater and load out were combined within run and form (pellets or fines) for analysis.
[2] One turkey and 3 swine diets were sent to a commercial lab with 5 replications within diet for a total of 20 samples of both fines and pellets.

Table 3-4 Interactive effects of trailer compartment and unloading speed on unloading time (Exp. 2)[1]

	Truck speed, RPM				Probability, $P <$
Compartment, s[2]	900	1,150	1,400	Pooled SEM	Compartment × truck speed
1	222	153	127	17.4	0.031
2	228	145	107		
3	203	134	113		
4	173	132	98		
5	173	126	123		
6	174	119	90		
7	162	114	93		
8	152	118	90		

[1] Values represent the amount of time (s) to unload 3 tons of feed. Six truck loads were used for the trial which resulted in 16 replications/unloading speed and 6 replications/compartment.
[2] The compartments were numbered 1 to 8 starting with the compartment nearest the cab and ending with the compartment nearest the rear of the trailer.

Table 3-5 Main effects of truck unloading speed on pellet quality (Exp. 2)[1]

	Truck speed, RPM				Probability, *P* <	
Item, %	900	1,150	1,400	SEM	Linear	Quadratic
Pellet durability index	83.9	80.8	84.5	2.06	0.826	0.192
Fines formed[2]	10.4	10.6	12.4	1.37	0.291	0.631

[1] Six truck loads were used for the trial which resulted in 16 replications/unloading speed.

[2] Fines formed were calculated by subtracting the amount of fines present during unloading from the amount of fines present during loading per compartment.

Table 3-6 Main effects of trailer compartment on pellet quality (Exp. 2)[1]

	Compartment[2]									Probability *P* <	
Item, %	1	2	3	4	5	6	7	8	SEM	Linear	Quadratic
Pellet durability index	85.6	76.7	85.9	81.8	77.9	85.3	85.7	85.5	3.54	0.388	0.279
Fines formed[3]	8.2	7.3	11.9	9.9	16.3	11.9	12.5	10.7	2.27	0.083	0.081

[1] Six truck loads were used for the trial which resulted in 6 replications/compartment

[2] The compartments were numbered 1-8 starting with the compartment nearest the cab and ending with the compartment nearest the rear boom auger.

[3] Fines formed were calculated by subtracting the amount of fines present during unloading from the amount of fines present during loading per compartment

Table 3-7 Effects of feed line location on pellet quality (Exp. 3)[1]

	Feeder location				Probability, $P <$	
Item, %	Front[2]	Middle	Back	SEM	Linear	Quadratic
Pellet durability index	87.9	87.5	87.3	0.40	0.838	0.270
Pellet fines	18.3	20.0	16.6	2.23	0.290	0.993
Fines formed[3]	-5.0	-3.4	-6.7	3.11	0.449	0.995

[1] Two feed lines from 6 commercial wean to finishing barns were sampled which resulted in 12 replications/feeder location sampled.
[2] The front, middle, and back feed line location were 6, 35, and 76 m from the bin respectively.
[3] The percentage of fines formed from the feed bin to the feed line location were negative due in part to the manner in which feed bins unload starting with a larger proportion of pellets and concluding with a larger proportion of fines. Samples from the study were taken in the early stages of each bin unloading resulting in larger proportions of pellets present at the feeder as compared to the samples taken while the bin was loaded.

Table 3-8 Effects of feed line location and feed form on nutrient composition (Exp. 2)[1]

	Feed form			Probability, $P <$
Item, %	Fines	Pellets	SEM[2]	Feed form main effect
CP				
Front[3]	12.3	15.2	0.14	0.001
Middle	12.4	15.5		
Back	12.5	15.2		
ADF				
Front	3.5	3.1	0.24	0.011
Middle	3.7	3.2		
Back	3.8	3.2		
Crude fiber				
Front	2.8	2.1	0.09	0.001
Middle	2.7	2.3		
Back	2.8	2.3		
Ca				
Front	0.48	0.46	0.012	0.003
Middle	0.48	0.43		
Back	0.45	0.44		
P				
Front	0.37	0.41	0.006	0.001
Middle	0.37	0.40		
Back	0.37	0.40		
Ether extract				
Front	6.2	5.2	0.11	0.001
Middle	6.3	5.3		
Back	6.1	5.2		
Starch				
Front	47.6	44.6	0.42	0.001
Middle	47.2	44.6		
Back	47.3	45.0		

[1] Two feed lines from 6 commercial wean to finishing barns were sampled which resulted in 12 replications/feed line location sampled.
[2] There were no interactions of feed line location and feed form and no main effect of feed line location.
[3] The front, middle, and back feed line location were 6, 35, and 76 m from the bin respectively.

Chapter 4 - Stability of four commercial phytase products under increasing thermal conditioning temperatures

ABSTRACT

The objective of this experiment was to determine the stability of 4 commercial phytase products exposed to increasing thermal conditioning temperatures in the pelleting process. The four commercial products used were: Quantum Blue G (AB Vista, Plantation, FL); Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (Dupont, Wilmington, DE), and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). The phytase products were mixed as part of a corn-soybean meal-based swine diet at a concentration recommended by the manufacturer to provide a 0.12% aP release. Diets were exposed to each of 4 thermal conditioning temperatures (65, 75, 85, and 95°C) and the entire process repeated on 4 consecutive days to create four replicates. Samples were taken while feed exited the conditioner and before entering the pellet die. Samples were cooled to room temperature before being stored until analysis. Phytase activity was determined from complete feed samples before conditioning to establish a baseline diet phytase activity level for each product. Phytase stability was measured as the residual phytase activity (% of initial) at each conditioning temperature. There were no product × temperature interactions for conditioning temperature, throughput, or residual phytase activity. As expected, as the target temperature was increased, conditioning temperature increased (linear; $P < 0.001$), and conditioner throughput decreased (linear; $P < 0.001$). There was no evidence for effects of phytase product on conditioning temperature or conditioner throughput. As target temperature increased, phytase activity decreased (linear; $P < 0.001$) for each product. Residual phytase activity decreased as conditioning temperature increased from 65

to 95˚C at a rate of -1.9% for every 1˚C increase in conditioning temperature. There was a significant phytase product ($P < 0.001$) main effect which was mainly driven by Microtech 5000 Plus having decreased ($P < 0.05$) phytase activity when compared to all other products. There was no evidence for residual phytase differences between the Quantum Blue G, Ronozyme Hi Phos GT, or Axtra Phy TPT products. In summary, increasing target conditioning temperatures decreased phytase stability regardless of product. In addition, Microtech 5000 Plus had decreased residual phytase activity (% of initial) when compared to all other products.

Key words: conditioning temperature, pelleting, phytase stability

BACKGROUND

Phytase is an enzyme that breaks down phytate phosphorus and when included in swine diets will increase the amount of phosphorus available to the pig (Simons et al., 1990; Harper et al., 1997; Lei et al., 1993). However, phytase, like any catalytic protein, is subject to damage when exposed to numerous feed processing parameters, including the pelleting process (Jongbloed and Kemme, 1990). Thermal processing affects stability of phytase and each manufacturer has different recommendations for the amount of residual phytase after pelleting.

Several commercial phytase products are available for use in livestock diets. While previous research (Wyss et al., 1998) has shown the heat stability of some phytase products, the heat stability of next generation products that are currently available to the market have not been compared. Thus, the objective of the current study was to evaluate 4 current commercial phytase products when exposed to increasing conditioning temperatures.

MATERIALS AND METHODS

Experimental Design and Diets

The four commercial phytase products used were: Quantum Blue G (declared potency of 5,000,000 FTU/kg; AB Vista, Plantation, FL); Ronozyme Hi Phos GT (declared potency of 2,699,282 FYT/kg; DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (declared potency of 2,500,000 FTU/kg; Dupont, Wilmington, DE), and Microtech 5000 Plus (declared potency of 5,000,000 FTU/kg; Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). One phytase unit (FTU or FYT) was defined as the amount of enzyme that catalyzes the release of 1 µmol of iP per minute from 5.1 mM sodium phytate in pH 5.5 buffer at 37˚C (AOAC 30024, 2009). Phytases were included as part of a corn-soybean meal-based complete swine diet. Concentrations used in formulation were determined by manufacturer recommendations from each product in order to release 0.12% aP. Phytase products were all commercially obtained from a third party distributor.

Each product was initially mixed with 91 kg of soybean meal using a Wenger (Wenger, Sabetha, KS) 91 kg double ribbon mixer. This was done in order to ensure proper mixing of the phytase throughout the subsequent 454 kg batches of complete feed (Table 1) used for the experiment. The phytase-soybean meal mix was then bagged in 22.7 kg bags and hand added during the batching of complete diets used in the experiment. Complete feed was sacked into 22.7 kg bags after mixing and samples were taken from 10 separate bags to form a composite sample. This composite sample was used to provide initial levels of phytase in the diet. The phytase activity from the initial samples was used as a baseline for comparison of all subsequent samples taken during the experiment

Sample Collection and Chemical Analysis

Diets were conditioned at 4 temperatures (65, 75, 85 and 95˚C) and the entire process repeated on 4 consecutive days to create four replicates. Diets were processed through a CL5 Laboratory Mill (California Pellet Mill; Crawfordsville, IN). Diets were steam conditioned in a 13 × 91 cm single screw conditioner. The mill was equipped with a 15 kg hopper located above the conditioner with a vibratory auger feeding the conditioner. A 45 s retention time was targeted for each diet. At the beginning of each day, flush feed containing no phytase was used to warm the pellet mill to the initial conditioning temperature (65˚C) at which point 15 kg of feed from 1 of the 4 products was placed in the hopper above the conditioner.

Feed was processed through the conditioner and samples were taken between the conditioner and pellet die. Temperature of the hot mash exiting the conditioner was used to determine conditioning temperature. Samples were taken at 4 time points during each run for each phytase product. Immediately after sampling, feed was transferred to a pilot scale cooler where pellet temperatures were reduced to ambient levels (21˚C) within 5 min. After cooling, the 4 sub-samples were combined for analysis.

After feed from the first phytase product exited the conditioner, 15 kg of flush diet was again added to the hopper and used to flush the system. Flush feed contained titanium dioxide as a tracer to verify when flush feed had passed through the conditioner. While the pellet mill was still at the initial conditioning temperature, feed from the second phytase product was added to the hopper. This process continued until all four products were conditioned at the initial temperature. When all products had been processed at the initial temperature, flush feed was again added to the hopper and the temperature was increased to the second conditioning temperature (75˚C) and stabilized before adding the first phytase treatment. All samples were

again processed through the conditioner using procedures similar to those used for the initial temperature samples. This process was replicated for all phytase products at each conditioning temperature. On d 2 of the trial (the next replication), similar procedures were used. However, if a phytase product had previously been conditioned 1st for all temperatures, it was rotated and conditioned 2nd on d 2, 3rd on d 3, and 4th on d 4. This was completed for all phytase products to minimize potential effects from conditioning order during each replication. All samples were sent to New Jersey Feed Lab (New Jersey Feed Lab Inc., Trenton, NJ) for analysis of phytase activity (AOAC 30024, 2009).

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC), with conditioning run as the experimental unit. Treatments were analyzed as a 4 × 4 factorial with the main effect of phytase product (Quantum Blue G, Ronozyme Hi Phos GT, Axtra Phy TPT, and Microtech 5000 Plus) and temperature (65, 75, 85, and 95˚C). Preplanned contrasts were used to evaluate the interaction between phytase product and temperature, linear and quadratic temperature effect, and the product main effect. Pairwise comparisons were also used to determine differences between products for residual phytase activity. Treatment differences were considered significant at $P < 0.05$ and were considered tendencies between $P > 0.05$ and $P < 0.10$.

RESULTS

The initial calculated and analyzed phytase activities and the ratio of analyzed to calculated of the complete feed prior to conditioning are reported (Table 2). Calculated values were determined from the minimum declared phytase concentrations as provided by each products manufacturer. The analyzed to calculated ratio was 1.17, 1.52, 1.00, and 1.66 for

Quantum Blue G, Ronozyme Hi Phos GT, Axtra Phy TPT, and Microtech 5000 Plus, respectively. The variation present in the analyzed to calculated ratios may be a result of some manufacturers providing a greater concentration of phytase then the minimum declared concentration listed for the product (Sulabo et al., 2011). The minimum declared concentrations by each manufacturer are determined using internal company assays which in general are variations from the official AOAC method used in the current experiment (AOAC 30024, 2009). Thus, differences in phytase analysis may have been present when comparing the calculated to analyzed values for d 0..

There were no product × temperature interactions observed for actual conditioning temperature, conditioner throughput, or residual phytase activity (Table 3). As the target temperature was increased, conditioning temperature increased (linear; $P < 0.001$) and conditioner throughput decreased (linear; $P < 0.001$). There was no evidence for effects of phytase product on conditioning temperature or conditioner throughput.

As target temperature increased, phytase activity decreased (linear; $P < 0.001$) for all products. Residual phytase activity decreased at a rate of -1.9% for every increase in conditioning temperature of 1˚C between 65 and 95˚C. There was a significant phytase product ($P < 0.001$) main effect which was mainly driven by Microtech 5000 Plus having decreased ($P < 0.05$) phytase activity when compared to all other products. There was no evidence for a difference in residual phytase between the Quantum Blue G, Ronozyme Hi Phos GT, or Axtra Phy TPT products.

DISCUSSION

Phytate is the primary storage form for phosphorous in most cereal grains and is unavailable to the growing pig. Of the total P in most plants, 60 to 90% is stored as phytate

(Reddy et al., 1982). Phytate P is low in digestibility and, thus, increased amount of P is present in the feces which can lead to increased amounts of P when manure is applied to soil (Greiner and Konietzny 2006). Phytates have also been shown to interfere with the availability of other nutrients in the diet including, Ca, Zn, Fe, and proteins (Cheryan and Rackis 2009). In order to supply the pig with an adequate amount of aP in the diet, inorganic products of P are used in formulation. The phytase enzyme is another option to use in formulation and is capable of breaking down phytate and releasing P to the animal. Phytase can be categorized by the site of hydrolysis of the phytate molecule as a 3-phytase or a 6-phytase, which breaks the inositol phytate ring at the 3 or 6 carbon, respectively (Selle and Ravindran 2007). Not only does phytase work to improve P availability in the diet, it also reduces the amount of P in the feces and subsequent phosphate present in the soil.

Due to the proteolytic structure of phytase, it is susceptible to protein denaturation when exposed to heat (Yao et al., 2012). In the swine industry, pelleting of diets is a common practice to improve both ADG and G:F in growing pigs (Stark et al., 1994; Potter et al., 2010; and De Jong et al., 2013). Pelleting conditions can vary, but normally consist of conditioning temperatures ranging from 65 to 95˚C, with temperatures varying depending on the feed mill equipment and diet type being pelleted. The poultry industry utilizes higher pelleting temperatures as a kill-step for bacterial and viral containment (Furuta et al.. 1980).

In order to understand the influence of pelleting temperature on phytase stability, Wyss et al. (1998) investigated three acid-based phytases under 2 conditioning temperatures (75 and 85˚C). Increasing the conditioning temperature from 75 to 85˚C reduced the percentage recovery of all three phytase products by 20 to 40 %. It was also noted that at 75˚C, the recovery of the phytase products ranged from 60 to 70% when compared to the baseline levels suggesting that

the lower conditioning temperature was already degrading the phytases. This is similar to the current experiment where all phytase products showed decreased residual phytase activity at 75˚C (21 to 78% residual activity) with phytase activities being further decreased as conditioning temperatures were increased. In addition to phytase, other in-feed enzymes may be degraded at increased conditioning temperatures. Silversides and Bedford (1999) observed decreased amounts of a xylanase enzyme in feed when conditioning temperature was increased from 70 to 95˚C.

In the current experiment, diets were formulated to allow phytase to release 0.12% aP. The decrease in phytase activity found in the current trial as conditioning temperature increased, especially at the highest conditioning temperature (95ºC), would have resulted in a reduction of approximately 0.06, 0.07, 0.08, and 0.10% aP for Axtra Phy TPT, Ronozyme Hi Phos GT, Quantum Blue G, and Microtech 5000 Plus, respectively, if analyzed values truly correlate to changes in pig performance. These decreases in aP would have resulted in diets deficient in aP if no safety margin was considered in diet formulation. Feeding growing swine diets deficient in P not only reduces growth performance (Coalson et al., 1972; Mahan 1982; Ruan et al., 2007) but it can also decrease plasma P concentrations and bone mineralization (Nicodemo et al. 1998). Understanding and accounting for the phytase activity of a diet post-pelleting is crucial to maximize performance of growing pigs and to ensure P deficiencies do not occur.

It should be noted that reductions in the phytase activity from conditioning in the current experiment were beyond what was expected based on manufacturers recommendations. Commercial feed mills currently utilize a wide variety of conditioning temperatures and retention times when manufacturing pelleted feeds (McCracken 2002). In addition, different conditioner and pellet mill models exist across feed mills. The variation in equipment as well as mill ambient

temperature can create a large amount of variance in the processing methods used to pellet swine feed. In the current experiment, a lab scale conditioner was utilized. Slominksi et al. (2007) evaluated two phytase products at 2 separate mills in Canada. Hot pellet temperatures for the two mills were 67 and 70˚C, respectively. There were no differences in the change in phytase activity of the pelleted feed between the two mills or between the 2 products. However, pelleting at 67 and 70˚C reduced phytase activity from 50 to 63%, respectively, as compared to the baseline sample. In addition to the commercial study, they also conducted an in vitro study evaluating the same 2 phytase products at 60 and 70˚C. Both phytase products had significantly reduced activity at the 70˚C. Eeckhout (2002) evaluated the effects of die opening diameter, die channel length, and diet composition on phytase stability during pelleting. They observed that a small die opening reduced phytase activity when compared to a larger die opening which was most likely a result of the increased friction and heat produced from the smaller die. In addition at moderate conditioning temperatures, a longer channel length within the die resulted in decreased phytase activity, which again, was most likely a result of the increase in friction and heat created by a long channel. Lastly, a diet with a greater percentage of fat (22%) and low crude fiber (6%) was also capable of retaining phytase activity during the pelleting process as compared to a diet with low fat (10%) and high crude fiber (16%) content. Again, the added fat would have reduced the heat and friction at the die which could have been the reason for improved phytase activity. Data from Slominski et al. (2007) and Eeckhout (2002) both suggest that phytase products may respond differently in a commercially operated mill when compared to a lab scale pellet mill and may also respond differently across mills depending on pellet mill style, operation parameters, and diet composition. As previously stated, this should be taken into account when utilizing data

from the trial herein where a single conditioner and diet composition was used in a lab scale pellet mill.

Increasing conditioning temperature linearly reduced residual activity regardless of product. Also, Microtech 5000 Plus had significantly less activity after conditioning compared with other products with no differences among the other three products.

Conditioning temperatures may vary from 65 to 95˚C under normal commercial mill conditions depending on the type of diet. The current data suggests that conditioning temperatures at and above 65˚C negatively affects the phytase activity of each product used in this experiment. While the present data was measured in a lab scale pellet mill, additional research in different commercially operated pellet mills to further understand thermal feed processing on phytase stability is warranted.

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Table 4-1 Diet composition (as-fed basis)

Item	
Ingredient, %	
Corn	61.37
Soybean meal (46.5% CP)	33.79
Choice white grease	1.50
Monocalcium phosphate (21% P)	1.05
Limestone	1.00
Salt	0.35
L-lysine HCl	0.30
DL-met	0.12
L-thr	0.12
Vitamin premix[1]	0.15
Trace mineral premix[2]	0.25
Phytase[3]	---[4,5,6,7]
Total	100
Calculated analysis	
Standard ileal digestible (SID) amino acids, %	
Lysine	1.24
Iso:lys	63
Leu:lys	128
Met:lys	33
Met & Cys:lys	57
Thr:lys	63
Trp:lys	18.7
Val:lys	68
Total lys, %	1.39
ME, kcal/kg[8]	3,341
NE, kcal/kg[8]	2,471
SID lys:ME, g/Mcal	3.71
CP, %	21.6
Crude fiber, %	2.5
Ca, %	0.70
P, %	0.63
Available P, w/o phytase, %	0.30
Available P, %	0.42
Phytase, FTU/kg	---[9]

[1] Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B12.

[2] Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[3] Phytase products were added at levels expected to release 0.12% available P based on manufacturer recommendations.

[4] Quantum Blue G (AB Vista, Plantation, FL) included at 0.015%.

[5] Ronozyme Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ) included at 0.022%.
[6] Axtra Phy TPT (Dupont, Wilmington, DE) included at 0.007%.
[7] Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) included at 0.017%.
[8] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC.
[9] Quantum Blue G (AB Vista, Plantation, FL), Ronozyme Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ), Axtra Phy TPT (Dupont, Wilmington, DE) and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) provided 350, 550, 375, and 850 FTU/kg respectively.

Table 4-2 Calculated and analyzed phytase values of initial feed samples[1]

Item	Calculated, PU[2]/kg	Analyzed, PU/kg	Ratio[3]
Quantum Blue G[4]	350	409	1.17
Ronozyme Hi Phos GT[5]	550	835	1.52
Axtra Phy TPT[6]	375	376	1.00
Microtech 5000 Plus[7]	850	1,410	1.66

[1] Values represent means of 2 replicate samples each analyzed in duplicate (AOAC method 30024:2009; New Jersey Feed Labs, Trenton, NJ).
[2] PU = phytase units
[3] Analyzed to calculated ratio.
[4] AB Vista, Plantation, FL.
[5] DSM Nutritional Products, Parsippany, NJ.
[6] Dupont, Wilmington, DE.
[7] Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China.

Table 4-3 Effect of target conditioning temperature and phytase product on actual conditioning temperature, throughput, and residual phytase activity[1]

	Conditioning temperature, ˚C					Probability, $P <$		
Item	65	75	85	95	SEM	Product × temperature	Linear temperature	Product main effect
Conditioning temperature, ˚C								
Quantum Blue G[2]	66.8	75.2	85.5	93.8	1.11	0.992	0.001	0.761
Ronozyme Hi Phos GT[3]	66.1	75.4	85.2	93.4				
Axtra Phy TPT[4]	66.4	74.7	85.5	93.2				
Microtech 5000 Plus[5]	66.6	75.8	85.3	93.7				
Throughput, kg/hr								
Quantum Blue G	65	61	55	57	3.9	0.621	0.001	0.916
Ronozyme Hi Phos GT	63	65	58	52				
Axtra Phy TPT	66	57	59	50				
Microtech 5000 Plus	64	57	62	57				
Residual phytase activitiy,[6] %								
Quantum Blue G	99.0	78.2	37.9	21.1	8.80	0.385	0.001	0.001
Ronozyme Hi Phos GT	87.5	59.7	43.3	22.9				
Axtra Phy TPT	80.6	62.0	36.2	33.1				
Microtech 5000 Plus	37.6	21.4	3.5	3.5				

[1] Four replicate conditioning runs were completed for each product at each temperature. Within conditioning run, a composite sample consisting of 4 sub-samples was used for analysis. Samples were taken as feed exited the conditioner.
[2] Quantum Blue G (AB Vista, Plantation, FL).
[3] Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ).
[4] Axtra Phy TPT (Dupont, Wilmington, DE).
[5] Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China).
[6] Stability was measured as the analyzed phytase concentration divided by phytase concentration prior to conditioning.

Chapter 5 - Stability of Commercial Phytase Sources Under Different Environmental Conditions

ABSTRACT

A 300-d study was performed to evaluate the storage stability of 4 phytase products under varied environmental conditions. The 4 commercially available phytase products used were: 1) Quantum Blue G (AB Vista, Plantation, FL); 2) Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); 3) Axtra Phy TPT (Dupont, Wilmington, DE); and 4) Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). Products were stored as pure forms, in a vitamin premix, or in a vitamin trace mineral (VTM) premix. Pure products were stored at either -20, 4, 22, or 35˚C (75% humidity). Vitamin and VTM premixes were stored at 22 and 35˚C (75% humidity). Samples were stored in open topped paper bags and were sampled on d 30, 60, 90, 120, 210, and 300. Stability was determined as the amount of residual phytase activity (% of initial) at each sampling point. For the pure forms, all interactive and main effects of phytase product, time, and storage temperature were significant ($P < 0.05$). From d 30 to 300, products had similar reductions in phytase activity at the 3 highest temperatures; however, Quantum Blue G, Ronozyme HiPhos GT, and Axtra Phy TPT had reduced ($P < 0.05$) phytase activity as compared to Microtech 5000 at -20˚. In general, as storage time increased, residual phytase activity decreased ($P < 0.05$) from d 30 to 300 regardless of product and storage temperature. Also, when product was stored at 4 and 22˚C, phytase activity was greater than that of product stored at -20 and 35˚C, and Microtech 5000 Plus had greater ($P < 0.05$) stability regardless of time and temperature as compared to the other 3 products. For vitamin and VTM premixes, a time × temperature × product interaction ($P < 0.05$) was observed. When stored at 22˚C, Axtra Phy TPT and Microtech 5000 Plus had reduced residual phytase activity ($P < 0.05$)

when compared to the other 2 products; however, when stored at 35˚C Axtra Phy had even further reduced ($P < 0.05$) activity than the other 3 products regardless of which form the products were stored in. From d 30 to 300 Axtra Phy TPT and Microtech 5000 Plus had the lowest ($P < 0.05$) residual phytase activity when compared to the other 2 products regardless of storage form and temperature. The VTM premixed had decreased ($P < 0.05$) residual phytase activity when compared to the pure product and vitamin premixes. In conclusion, phytase stored for longer than 90 to 120 d, at both high (35˚C) and low (-20˚C) temperatures when in pure form, or when stored as a VTM premix had reduced residual phytase activity.

Key words: phytase, storage, stability, vitamin premix, vitamin trace mineral premix

INTRODUCTION

Currently, phytase sales represent over one-third of the entire feed enzyme market (Yang et al. 2007). Global expansion in phytase use has led to a large variety of phytases in the marketplace. In addition, phytase has repeatedly been shown to improve the digestibility of P in both swine and poultry (Simons et al., 1990; Lei et al., 1993; and Kornegay et al., 2001). The improved digestibility leads to reduced P excretion from animals and a subsequent improved environmental impact of livestock production (Greiner and Konietzny 2006; Selle and Ravindran, 2007).

Phytase is susceptible to damage from heat applied to feed during the feed manufacturing process (Jongbloed and Kemme, 1990; Spring et al., 1996). Many phytase manufacturers currently provide products that are claimed to provide protection during pelleting, and thus, most research has focused on the ability of phytase to withstand the increased temperature and moisture during the pelleting process (De Jong et al., 2015). However, little research has focused on the shelf life and stability of phytase when stored, especially for longer than 3 to 6 mo. The

potential for degradation of phytase from storage conditions or the interaction of phytase with other ingredients in a vitamin or vitamin trace mineral (VTM) premix was reported by (Sulabo et al., 2011). Since the publishing of their findings, several manufacturers have released a new generation of phytase products that have not been tested for shelf life stability. Thus, the objective of our study was to determine the storage stability of 4 commercially available phytase products under varying environmental conditions over 300 d.

MATERIALS AND METHODS

General

This study was conducted at the O.H. Kruse Feed Science and Research Center and at Shellenberger Hall at Kansas State University (Manhattan, KS).

Phytase Sources

This experiment utilized 4 commercially available phytase products: 1) Quantum Blue G (minimum declared concentration of 5,000,000 FTU/kg; AB Vista, Plantation, FL); 2) Ronozyme Hi Phos GT (minimum declared concentration of 2,699,282 FYT/kg; DSM Nutritional Products, Parsippany, NJ); 3) Axtra Phy TPT (minimum declared concentration of 2,500,000 FTU/kg; Dupont, Wilmington, DE); and 4) Microtech 5000 Plus (minimum declared concentration of 5,000,000 FTU/kg; Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). One phytase unit (FTU or FYT) was defined as the amount of enzyme that catalyzes the release of 1 µmol of iP per minute from 5.1 mM sodium phytate in pH 5.5 buffer at 37˚C (AOAC 30024:2009). Each phytase product was obtained through a third party distributor.

Pure Products

On d 0, 1.8 kg of each of the pure phytase products were placed into 8 open, single-lined paper bags. Two bags (observations) of each product were stored in a freezer (-20˚C), in a

refrigerator (4˚C), at room temperature (22˚C), and in a controlled environment chamber set at 35˚C and 75% humidity. At sampling, approximately 50 g was removed from each bag on d 0, 30, 60, 90, 120, 210, and 300. Samples were immediately sent to New Jersey Feed Labs (Trenton, NJ) for phytase analysis (AOAC 30024:2009).

Premixes

Each phytase product was added and mixed with either a vitamin premix or a vitamin and trace mineral **(VTM)** premix (Table 1). The amount added for each phytase product was determined such that including 0.25% vitamin premix and 0.4% VTM would provide 0.12 aP in the diet as noted by the manufacturers recommendations (350 FTU/kg, Quantum Blue G; 550 FYT/kg, Ronozyme Hi Phos GT; 375 FTU/kg, Axtra Phy TPT; and 850 FTU/kg, Microtech 5000 Plus). Vitamin or VTM premixes were mixed at the aforementioned levels with each phytase source using a 10 kg ribbon mixer. Each 2 kg batch was then placed in open, single-lined paper bags. Two bags of vitamin and VTM premix with each phytase product were stored at room temperature (22˚C) or in the environmentally controlled chamber set at 35˚C and 75% humidity. Sampling occurred on d 0, 30, 60, 90, 120, 210 and 300 at which point samples were immediately sent to the same laboratory used for phytase analysis of the pure samples. Each sample weighed approximately 200 g.

Statistical Analysis

Data were analyzed using a mixed model (MIXED procedure; SAS Inst. Inc., Cary, NC) to determine the interactive and main effects of storage temperature and time on the activity of 4 commercially available phytase products. Because the vitamin and VTM premixes were stored only at room temperature and in the environmentally controlled heat chamber, 2 analyses were performed. The first analysis was with the pure forms only. The second analysis was for the pure

forms, vitamin premixes, and VTM premixes stored at 22 and 35°C. When treatment was a significant source of variation, differences were determined by using the preplanned pairwise comparisons (PDIFF option of SAS). Results were considered significant at $P \leq 0.05$ and considered marginally significant between $P > 0.05$ and a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

The initial (d 0) calculated and analyzed phytase activities and the ratio of analyzed to calculated of the pure products, vitamin premix or VTM premixes are reported in Table 2. Calculated values were determined from the minimum declared phytase concentrations as provided by each products manufacturer. For the pure products, phytase activity ranged from 1.00 for Axtra Phy TPT to 1.64 times greater than the calculated values for Ronozyme Hi Phos GT. For the vitamin premixes, phytase activity ranged from 0.83 for Quantum Blue G to 1.84 times greater than the calculated values for Microtech 5000 Plus. In regards to the VTM premixes, phytase values ranged from 0.37 for Axtra Phy TPT to 1.61 times greater than the calculated values for Microtech 5000 Plus. The variation in the analyzed to calculated ratios between storage forms and products is a result of a number of areas. It has been shown that the actual concentration of the product may be higher than the minimum guarantee (Sulabo et al., 2011). Second, it has been shown that there is large analytical variation associated with the laboratory assays utilized to measure phytase activity (Jones et al., 2010). In addition the differences in the coating methods among the products may have inherently led to differences in assay results. Ronozyme HiPhos GT, Axtra Phy TPT, and Microtech 5000 Plus all utilize a fat based coating which may interfere with the AOAC lab assay. However, more research is necessary to determine if product coating can affect lab assay. Lastly, the minimum declared concentrations are determined using internal assays from each company which in general are

variations on the official AOAC method used in the current experiment (AOAC 30024, 2009). These internal assays were used to provide concentrations for the calculated values. Thus, differences may have been present when comparing the calculated to analyzed values for d 0 due to variations in the analysis method.

De Jong et al. (2015) showed that a 75˚C conditioning temperature resulted in residual phytase activity of 21 to 78% while a 95˚C conditioning temperature resulted in residual phytase activity ranging from 3 to 33%. This suggests that at normal conditioning temperatures phytase products may be affected by added heat and moisture. They also noted that Microtech 5000 Plus had less activity regardless of pelleting temperature when compared to Quantum Blue G, Ronozyme HiPhos GT, and Axtra Phy TPT. In the current experiment, Microtech 5000 Plus and Ronozyme HiPhos GT had greater ($P < 0.05$) stability (96 and 94%) regardless of time and temperature as compared to Quantum Blue G and Axtra Phy TPT (91 and 90% respectively; Table 3; Figure 1). It appears that stability during pellet conditioning does not correspond to stability during storage, especially in regards to Microtech 5000 Plus.

Phytase is exposed to varied temperatures and humidity levels during storage. Depending on location and season, phytase may be exposed to temperatures up to 35˚C and relative humidity of 75 to 90% during summer. Sulabo et al. (2011) previously reported that when stored at 23˚C or less, pure phytase products retained the most phytase activity when stored for 120 d. This would also agree with research evaluating other enzymes by El-Shirbiny and El-Chaghaby (2012) who found that a liquid enzyme mix (xylanase, amylase, and cellulose) had greater stability when stored at 4˚C as compared to 30˚C. In the current experiment, products had similar reductions in phytase activity from d 30 to 300 at the 3 highest temperatures; however, for Quantum Blue G, Ronozyme HiPhos GT, and Axtra Phy TPT (58, 53, 66%) phytase activity

decreased ($P < 0.05$) as compared to Microtech 5000 (83%) on d 300 when stored at -20˚C. Also, when products were stored at 4 and 22˚C, phytase activity improved (99 and 97%) compared to product stored at -20 and 35˚C (87 and 89%). It is interesting to note that pure product stored at the lowest temperature (-20˚C) had worse stability than pure product stored at the highest temperature. This finding may have been an artifact of the freezing and thawing that occurred during sampling throughout the duration of the trial. Cao et al. (2003) noted that proteins were more susceptible to denaturation and destruction when exposed to fast freezing and slow thawing in an aqueous solution. The authors noted that fast freezing proteins are more exposed to ice crystals which can lead to protein degradation. This could have occurred during sampling in the current experiment as samples were removed from the freezer to weigh and sample product which inevitably led to slight amounts of thawing and subsequent re-freezing. Sulabo et al. (2011) also stored products at similar low temperatures but didn't report negative results of freeze storing as seen in the current experiment. The difference may be due in part to the storage protocols used in their study that allowed product to remain in the freezer until sampling occurred unlike the current experiment where samples were removed every time sampling occurred. In addition, Grieff and Kelley (1966) and Whittam and Rosano (1973), showed that continued freezing and thawing reduced enzyme activity of lactic dehydrogenase and α amylase respectively. Thus, this may have occurred with the phytase products used in the present study.

In addition, Sulabo et al. (2011) also suggested that the reduced phytase activity may have been attributed to the 75% humidity employed at the highest temperature. Yang et al. (2007) showed that as humidity was increased from 53 to 89%, phytase activity. Iyer and Ananthanarayan (2008) also noted that moisture can lower the temperature required to un-fold an

enzyme, suggesting that added moisture at the highest temperature herein may have added to the denaturation of the phytase enzyme. In general, it appears that storing phytase products at temperatures below 35˚C and above -20˚C and at low humidity levels should result in the greatest phytase retention.

Under commercial conditions phytase is stored at variable temperatures and for varying lengths of time. Though most feed mills ensure turnover of their micro-ingredients within 3 to 6 months after receiving them, it is possible that product may remain in storage for long periods of time in low volume mills. It is also possible that sudden dietary changes may require less of an ingredient, such as phytase, resulting in increased storage time prior to use. In the present study, as storage time increased, residual phytase activity decreased ($P < 0.05$) from d 30 to 300 (98 to 80%) regardless of product and storage temperature. Sulabo et al. (2011) also reported that phytase stored as pure product had significantly reduced phytase activity when stored for as little as 60 d. Lu et al. (2013) and Naves et al. (2012) both found that when pure product was stored for 180 d, phytase activity was reduced by 25 and 67%, respectively. They speculated that decreases in phytase activity over time may be attributed to the natural proteolytic degradation of the peptide ring during storage as a result of the increased temperatures or exposure to oxygen radicals. More recently, the stability of Ronozyme HiPhos GT was reported (European Food Safety Authority, 2012) to retain >90% activity when stored for 18 mo at 5 temperatures (-18, 10, 25, 35, and 40˚C). This disagrees with the current experiment where Ronozyme HiPhos GT had >90% retention only when stored at 4 and 22˚C. Phytase retention was similar from d 0 to 120 for pure product stored at -20 and 35˚C but was reduced to 52 and 59% at d 300. The differences in the current data and data from European Food Safety Authority (2012) are currently unknown but may have been caused by the thawing and re-freezing at the lowest

temperatures and the high humidity utilized at the highest temperature. It is important to note that both experiments found reductions over time in phytase activity regardless of temperature. In general, it appears that time, in combination with moisture and temperature may lead to protein degradation minimizing pure product's efficacy during extended storage.

In the study herein, when stored at 22˚C, Axtra Phy TPT and Microtech 5000 Plus' residual phytase decreased ($P < 0.05$) when compared to the other 2 products (Table 4) regardless of storage form. However, when stored at 35˚C, Axtra Phy TPT phytase activity decreased more ($P < 0.05$) than the other 3 products (Figure 2). It appears that Axtra Phy TPT was less stable at high temperatures and long storage periods than the other 3 products. In addition, from d 30 to 300, Axtra Phy TPT and Microtech 5000 Plus had the lowest ($P < 0.05$) residual phytase activity when compared to the other 2 products regardless of storage form and temperature (Figure 3). This disagrees with the current data for pure products where Microtech 5000 Plus was the most stable. The presence of vitamins or vitamins and trace minerals in the premixes appear to have had a larger degrading effect on Axtra Phy TPT and Microtech 5000 Plus. This is especially true for Microtech 5000 Plus as it was the most stable product when stored in pure form and one of the least stable when stored as a vitamin or VTM premix.

In general, phytase activity decreased ($P < 0.05$) when product was stored as a VTM premix when compared to the pure product and vitamin premixes (Figure 4). This could have resulted from phytase and micro-ingredients present in the VTM premix interacting negatively with each other. Greater destruction of phytase when stored as part of a VTM premix as compared to the pure product or a vitamin premix has been previously shown by both Sulabo et al. (2011) and Naves et al. (2012). Both reported lower phytase activities throughout their storage studies when phytase was stored as part of a VTM premix as compared to either pure

phytase or product stored as a vitamin premix. Lu et al. (2013) speculated that the reduced phytase activity in VTM premixes may have been caused by the Cu product used in the premix. Thus, they stored phytase with either tribasic copper chloride (TBCC) or $CuSO_4$ for 0 to 40 d and at 3 temperatures (23, 32, or 38˚C). Phytase stored with TBCC had improved retention as compared to phytase stored with $CuSO_4$, which the authors noted may have been a result of the decreased water solubility and disassociation to form cations of TBCC. This is similar to Liu et al. (2005) who also found that TBCC in a complete poultry feed resulted in higher phytase retention after 21 d when compared to $CuSO_4$. In the current study, $CuSO_4$ was used in the VTM premixes which may have led to the decreased phytase retention when compared to the pure products or vitamin premixes. In addition, Chantasartrasamee et al. (2005) also reported that when stored with either Fe^{2+}, Ca^{2+}, Mn^{2+}, or Mg^{2+} phytase had similar or even improved activity; however, when stored with Zn^{2+} phytase activity was reduced to levels that were un-detectable. The authors speculated Zn may have been binding with the phytase enzyme, inhibiting its activity. Shurson et al. (2011) also showed that inorganic trace mineral sources were more destructive to vitamins present in the premix as compared to metal specific amino acid complexes. Though phytase was not present in their study inorganic mineral sources may also have a more negative effect on phytase storage. It appears that Cu, Zn, or other inorganic mineral sources may have negative effects on phytase activity when stored in a VTM premix which supports the current findings of the VTM phytase retention.

When stored as a vitamin premix, Quantum Blue and Axtra Phy had greater ($P < 0.05$) residual phytase activity than Ronozyme HiPhos GT and Microtech 5000 Plus (Figure 5). When stored as a VTM premix Ronozyme HiPhos GT had the greatest ($P < 0.05$) residual phytase activity as compared to the other 3 products. Currently, the reason for differences between

phytase products stored in the different forms is unknown. However, Sulabo et al. (2011) reported that coated products were more stable during storage compared to similar, un-coated products. This disagrees with the current experiment where Quantum Blue G had improved stability as a vitamin premix even though it was the only uncoated product. It is also possible that phytase coating may be interfering with the assay utilized in the current experiment; however, further research is needed to determine the effect of coating on assay efficacy.

In conclusion, when storing phytase in pure form, it appears residual phytase activity is maximized when stored between 4 and 22˚C. When phytase was stored as part of a VTM premix residual phytase activity was reduced by the greatest magnitude compared to the pure form and vitamin premix. In general phytase stored for longer than 90 to 120 d, at high or low temperatures in pure form, or as a VTM premix, had reduced residual phytase activity. Procedures should be in place to ensure that phytase storage time prior to feed manufacturing is minimized and products are stored at temperatures between 4 and 22˚C in order to minimize degradation and ensure phytase levels in complete feed are similar to those used in formulation.

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Table 5-1 Composition of the vitamin and vitamin trace mineral (VTM) premixes[1]

Item, amount/kg	Vitamin premix	Vitamin-trace mineral premix
Vitamin		
Vitamin A, IU	4,408,000	2,644,800
Vitamin D, IU	551,000	330,600
Vitamin E, IU	17,632	10,579
Menadione, mg	1,763	1,058
Vitamin B12, mg	15	9
Niacin, mg	19,836	11,902
Pantothenic acid, mg	11,020	6,612
Riboflavin, mg	3,306	1,984
Trace Mineral		
Copper ($CuSO_4$), mg	---	3,960
Iodine [$Ca(IO_3)_2$], mg	---	71
Iron ($FeSO_4$), mg	---	26,280
Manganese (MnO), mg	---	7,920
Selenium ($NaSeO_2$), mg	---	71
Zinc ($ZnSO_4$), mg	---	26,280

[1] The amount added for each phytase source was determined such that including 0.25% vitamin premix and 0.4% VTM would provide 0.12 aP in the diet as noted by the manufacturers recommendations (136 and 85 FTU/kg, Quantum Blue G (AB Vista, Plantation, FL); 218 and 140 FYT/kg, Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); 141 and 90 FTU/kg, Axtra Phy TPT (Dupont, Wilmington, DE); and 318 and 203 FTU/kg, Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China)) for the vitamin and VTM premixes, respectively.

Table 5-2 Calculated and analyzed phytase value on d 0[1]

Item	Calculated, PU[2]/kg[3]	Analyzed, PU/kg	Ratio[4]
Pure product			
Quantum Blue G[5]	5,000,000	5,195,000	1.04
Ronozyme Hi Phos GT[6]	2,700,000	4,420,000	1.64
Axtra Phy TPT[7]	2,500,000	2,510,000	1.00
Microtech 5000 plus[8]	5,000,000	8,055,000	1.61
Vitamin premix			
Quantum Blue G	136,000	113,000	0.83
Ronozyme Hi Phos GT	218,000	422,000	1.94
Axtra Phy TPT	141,000	145,500	1.03
Microtech 5000 plus	318,000	584,000	1.84
Vitamin and trace mineral premix			
Quantum Blue G	85,000	62,500	0.74
Ronozyme Hi Phos GT	140,000	155,500	1.11
Axtra Phy TPT	90,000	33,500	0.37
Microtech 5000 plus	203,000	327,500	1.61

[1] Values represent means of 2 replicate samples each analyzed in duplicate (AOAC method 30024:2009; New Jersey Feed Labs, Trenton, NJ).
[2] PU = phytase units.
[3] Calculated values were determined from manufacturers guaranteed minimum.
[4] Analyzed to calculated ratio.
[5] AB Vista, Plantation, FL.
[6] DSM Nutritional Products, Parsippany, NJ.
[7] Dupont, Wilmington, DE.
[8] Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China.

Table 5-3 Probabilities of interactive and main effects of storage time, temperature, and phytase product on stability (as defined by percentage of initial phytase activity) of phytase products in pure forms

Item	Probability, $P <$
Interactive effect	
Time × temperature × product	0.001
Time × temperature	0.001
Time × product	0.001
Temperature × product	0.001
Main effect	
Time	0.001
Temperature	0.001
Product	0.001

Table 5-4 Probabilities of interactive and main effects of storage time, temperature, and phytase product on stability (as defined by percentage of initial phytase activity) of phytase products

Item	Probability, $P <$
Interactive effect	
Time × temperature × product × form	0.123
Time × temperature × product	0.018
Time × temperature × form	0.932
Temperature × product × form	0.138
Time × temperature	0.750
Time × product	0.001
Time × form	0.001
Temperature × product	0.001
Temperature × form	0.049
Form × product	0.001
Main effect	
Time	0.001
Temperature	0.001
Form	0.001
Product	0.001

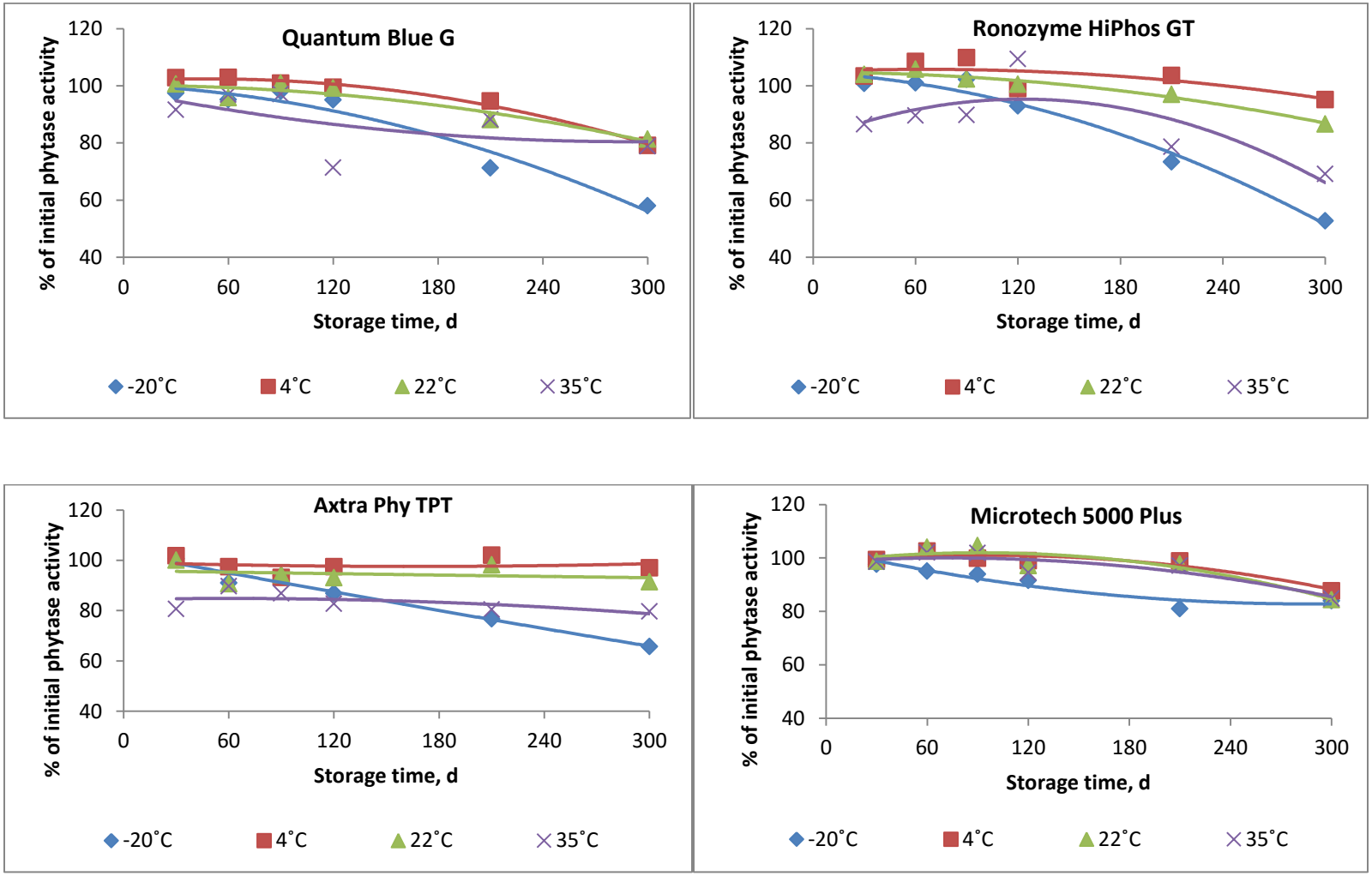
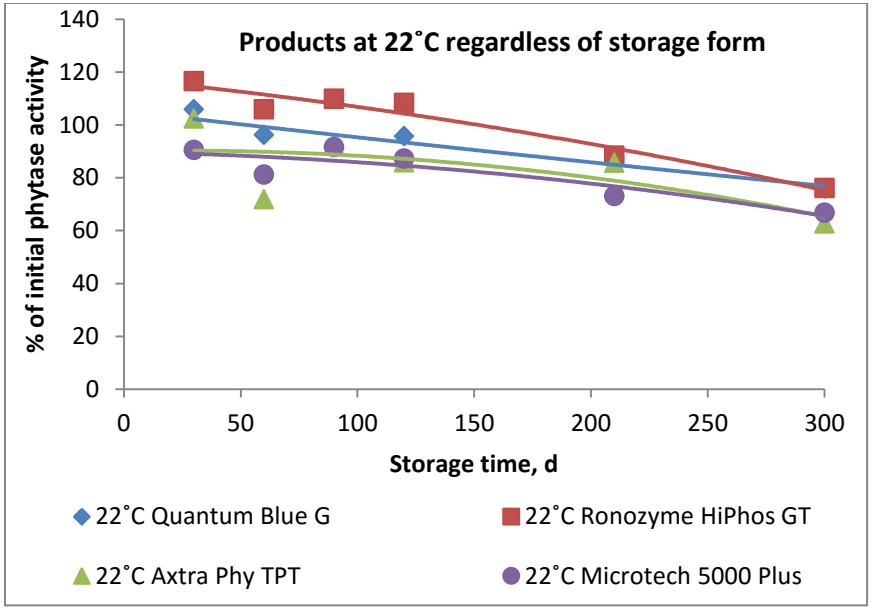


Figure 5 Residual phytase activity (% of initial) for Quantum Blue G (AB Vista, Plantation, FL), Ronozyme HiPhos GT (DSM Nutritional Products, Parsippany, NJ), Axtra Phy TPT (Dupont, Wilmington, DE), and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) as affected by storage temperature [freezer (-20˚C), refrigerator (4˚C), room temperature (22˚C), and controlled environment chamber (35˚C and 75% humidity)] and time (30 to 300 d). Each data point is the mean of 2 observations.



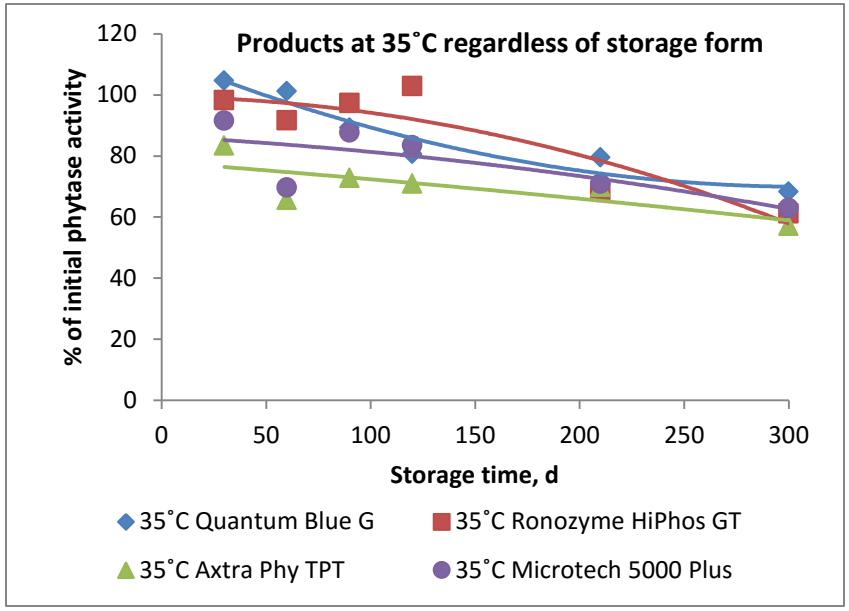


Figure 6 Residual phytase activity (% of initial) for Quantum Blue G (AB Vista, Plantation, FL); Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (Dupont, Wilmington, DE) and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) as affected by storage temperature (22˚C and 35˚C) and time (30 to 300 d) and regardless of storage form. Each data point is the mean of 6 observations.

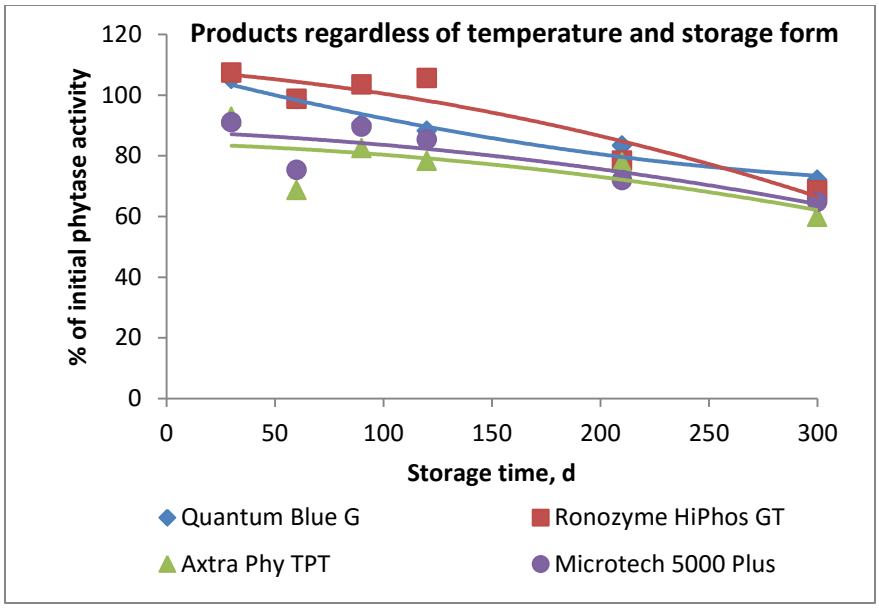


Figure 7 Residual phytase activity (% of initial) for Quantum Blue G (AB Vista, Plantation, FL); Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (Dupont, Wilmington, DE) and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) as affected by time (30 to 300 d) and regardless of storage form and temperature. Each data point is the mean of 12 observations.

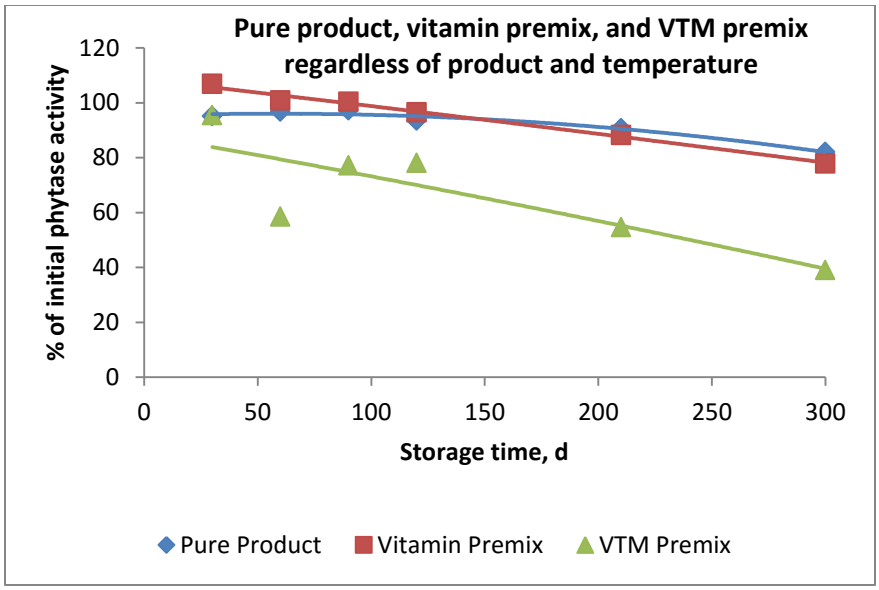


Figure 8 Residual phytase activity (% of initial) for phytase stored as pure phytase, vitamin premix, and VTM premix as affected by time (30 to 300 d), regardless of product and temperature. Each data point is the mean of 16 observations.

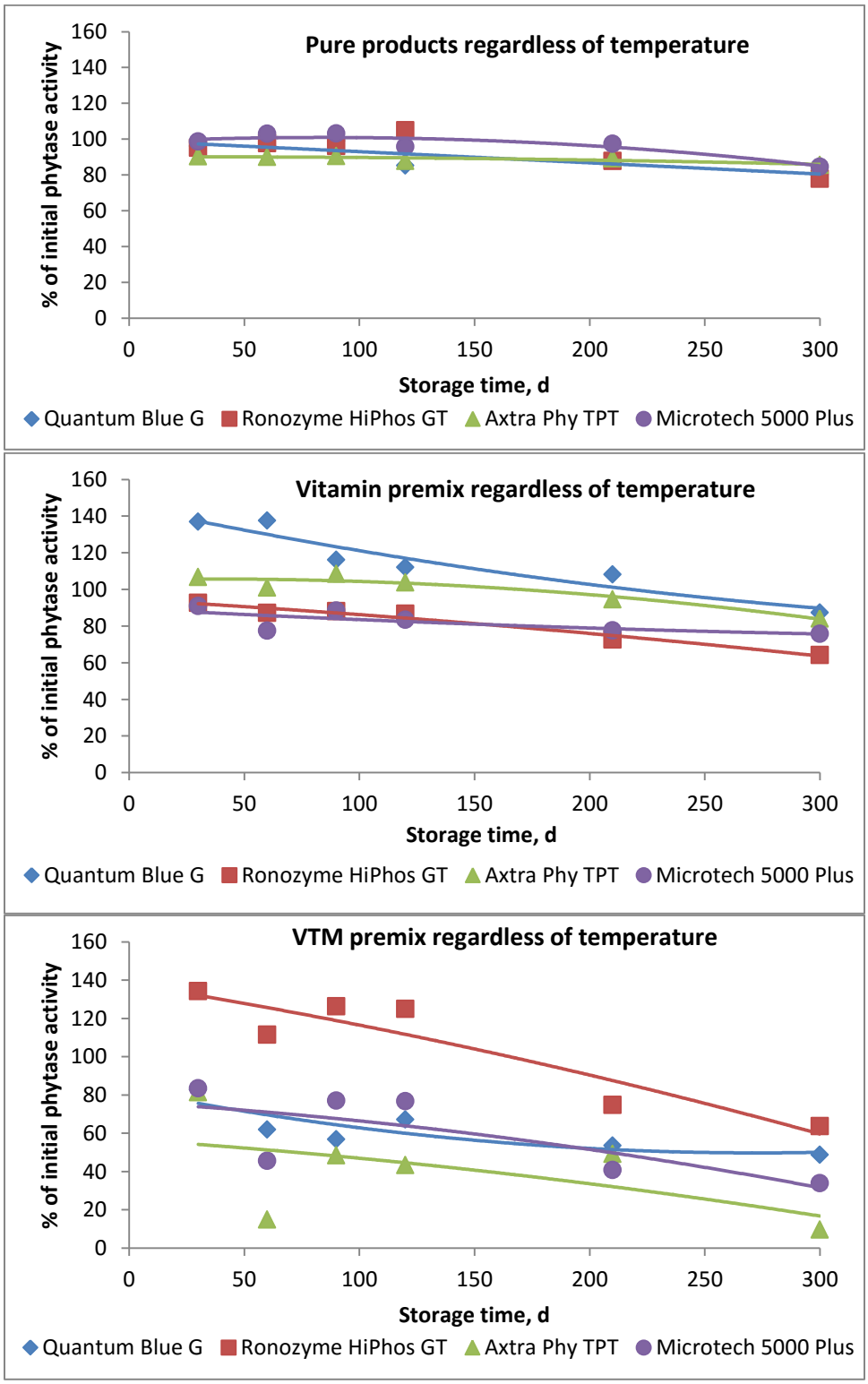


Figure 9 Residual phytase activity (% of initial) for Quantum Blue G (AB Vista, Plantation, FL); Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (Dupont, Wilmington, DE) and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong,

China) stored in pure form, vitamin premix, or VTM premix regardless of storage temperature. Each data point is the mean of 4 observations.