Impact of Cleaning Corn on Mycotoxin Concentration, and Conditioning Temperature on Pellet Quality and Nursery Pig Performance

by

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Abstract

Three experiments were conducted to analyze the average mycotoxin concentration that may be reduced by cleaning corn, and to determine how removing broken kernels may affect nursery pig growth performance. A fourth and fifth experiment evaluated pellet processing parameters and their effects on gelatinized starch, phytase stability, pellet quality, and nursery pig growth performance. In Exp. 1 and 2, corn was divided into twenty 150 kg runs then cleaned by mechanical sieving. Run were randomly assigned to 1 of 4 experimental treatments: 1) no screen 2) 12.7 mm screen, 3) 4.8 mm screen, and 4) 12.7 + 4.8-mm screen. Across both experiments, cleaning reduced ($P < 0.05$) aflatoxin and fumonisin concentration by an average of 26% and 42.5%, respectively, compared to the original uncleaned corn level. In Exp. 3, 360 nursery pigs were evaluated to determine the impact of cleaning or pelleting on growth performance. Treatments were arranged in a 2 × 3 factorial with corn type (uncleaned vs. cleaned) and feed form (mash vs. pelleted from either mill A or B). Neither cleaning corn nor pellet mill type affected ($P > 0.19$) nursery pig growth performance. Pelleting improved ($P < 0.0001$) G:F by 7.6% compared to mash diets. This improvement in G:F is consistent when pelleting diets, however pellet processing parameters can influence this improvement percentage. For these reasons, Exp. 4 was a 3 × 4 factorial design with 3 pellet mills (model 3016-4, 1000 HD, or CL-5, California Pellet Mill Co., Crawfordsville, IN), that produced samples collected at 4 locations (initial, post-conditioner, post-die, or post-cooler). Across each pellet mill, the greatest gelatinized starch increase ($P < 0.05$) was found post-pellet die, while phytase stability decreased ($P < 0.05$) by 70% after conditioning feed to 85˚C. This decrease led to substituting phytase in the diet for other sources of phosphorus for Exp. 5, which was a 2 × 3 factorial design plus a control, with pellet diameter (4.0 or 5.2 mm), conditioning temperature (low, medium, or high), and mash, created

seven experimental treatments. Overall, neither the pellet diameter × conditioning temperature interaction, nor the main effects, affected ($P > 0.06$) nursery pig growth performance, even though pellet quality improved ($P < 0.0001$) when increasing conditioning temperature. These data suggest that cleaning is an effective method to legally reduce aflatoxin and fumonisin concentration, and that increasing conditioning temperature improves pellet quality, but neither impacts nursery pig growth performance.

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Dedication

I would like to dedicate this to my father, Andy, mother, Pam, brothers, Brandon, Kevin, and Kurt, and friends, for molding me into the person I am today, and instilling the discipline to accomplish this process. A special thank you to Lindsey and our dog Waylon for their constant love and support.

Chapter 1 - The Role of Aflatoxin in Pigs

Causes of Aflatoxin and Proliferation in Corn

Mycotoxins are fungal secondary metabolites from molds grown on cereal grains that can produce carcinogens and cause disease to both humans and animals (Eaton, 1994, Gelderblom et al., 1998, Huff et al., 1998). Among these mycotoxins, *Aspergillus flavus* and *Aspergillus parasiticus* can produce aflatoxin, specifically aflatoxin B_1, which is one of the most potent and prevalent mycotoxins (Klich, 2003). Aflatoxin concentration in grains can vary across year, stressing the importance to consistently monitor reports and have a plan to handle high aflatoxin concentration, similar to an animal disease outbreak.

What environmental factors impact prevalence of aflatoxin?

Optimal mycotoxin development conditions can vary across grain type and the relationship between moisture percentage and water activity (a_w) within grain (Table 1.1) (Magan and Olsen, 2004). Additionally, corn can become infected by mycotoxin due to plant stress such as drought, excessive heat, inadequate plant nutrition, insects, weeds, excessive plant populations, and other plant diseases (Bruns, 2003). Weather patterns can signify the probability of mycotoxin proliferation. One of these signs include drought during early crop phases followed by heavy rainfall. Aflatoxins produced and grown on corn flourish in warm and humid subtropical and tropical conditions. Optimal conditions for aflatoxin production is 33˚C at 0.99 a_w, while that for growth is 35˚C at 0.95 a_w (Magan and Olsen, 2004). Aflatoxin can continue to grow on crop residues left on the field from harvest. If using no-till practices, the following crop can absorb these molds through the roots, thus increasing aflatoxin prevalence.

What agricultural technologies can impact aflatoxin?

Agronomic technologies and practices can impact aflatoxin prevalence (Abbas et al., 2008; Bhat et al., 2010). Transgenic corn with a gene from *Bacillus thuringiensis*, or Bt corn, has been reported to have reduced risk for mycotoxins, such as fumonisin and aflatoxin. The genetically modified corn hybrid has a gene that encodes for formation of a protein that is toxic to a common pest, the European corn borer (Rankin and Grau, 2002; Wu, 2006). By reducing potential pest damage, subsequent kernel damage and mold entry points are reduced. Another practice that impacts mycotoxin prevalence is the rise of no-till practices planting. Tilling soil reduces the number of viable mold spores that survive over winter. With the rise of no-till farming practices, there is limited opportunity to reduce mold spores (Abbas et al., 2008). Crop rotation to a non-susceptible crop, such as grain sorghum, may be helpful, but is sometimes not possible or economically viable.

Is the prevalence of aflatoxin increasing over time?

Based on the annual mycotoxin surveys, aflatoxin concentration has on average increased slowly, but the prevalence of aflatoxins appears to be variable across region and crop year (Tables 1.2 and 1.3). This variability is because climate change is one factor that can increase aflatoxin prevalence by causing drought in areas that do not typically experience aflatoxin accumulation (Smith and Henderson, 1991). Furthermore, crop technology advances, laboratory method analyses improvements, and no-till management practices can have an impact on mycotoxin prevalence.

Is aflatoxin commonly found alone, or in combination with other mycotoxins?

Naturally contaminated corn can contain multiple mycotoxins. If kernels are exposed, multiple types of mold can grow and potentially produce different toxins. As a result, it is common for corn

to have multiple mycotoxins present. The combination of toxins can have additive, or even synergistic effects, to cause greater disease than just aflatoxin alone. Specifically, aflatoxin in combination with ochratoxin A has shown to increase the severity mycotoxins can have on swine growth performance (Huff et al., 1998). Technological advances such as LC-MS/MS allow for multiple mycotoxins to be detected in a single sample analysis. An experiment by Monbaliu et al. 2009 analyzed 82 feed and grain samples and found 67 out of the 82 samples (82%) were contaminated with multiple mycotoxins. This technology can lead researchers to better determine the effects that multiple mycotoxins can have on swine performance. However, the current LC-MS/MS method is expensive and time consuming, so it is currently impractical for facilities. That said, future technologies are in development to screen for multiple mycotoxins in a more rapid method.

Once harvested, are aflatoxin levels stable?

Mold, and therefore aflatoxin, can continue to grow on kernels after harvest until the water activity level is reduced to below 0.78 (Mannaa and Kim, 2017). Additionally, corn should be stored with a moisture level below 14.0%, with control for insects (Murphy et al., 1993; Jayas and White, 2003; Magan and Aldred, 2007). Therefore, drying and cooling corn is an effective method to prevent further mycotoxin growth during storage. Screening corn prior to storage to remove broken kernels should be considered because these damaged kernels contain the highest concentration of mycotoxin and are the greatest substrate for additional growth (Murphy et al., 1993; Yoder et al., 2017).

Summary:

Aflatoxin is one of the most carcinogenic natural substances known to man, and its prevalence is impacted by current agronomic practices. Plant stressors, such as drought, further impact the risk for the growth of aflatoxin and other mycotoxins. However, proper post-harvest storage and mechanically cleaning can prevent additional aflatoxin growth and even reduce contamination.

Aflatoxicosis

Aflatoxin consumption by the pig can cause serious disease, known as aflatoxicosis. The toxin dosage, dietary interactions, exposure time, and life stage of pigs all impact the severity of illness (Monbaliu et al., 2009) The liver and kidneys are the primary organs affected by aflatoxin, which in turn can impact swine health and growth performance.

What happens if pigs are fed aflatoxin?

If aflatoxins are absorbed by the pig, they will be metabolized in the liver to an epoxide. The resultant product binds to nucleic acids, such as DNA and RNA, thereby reducing protein synthesis (Kanora and Maes, 2009). In a subacute setting at low doses, the immune system is stimulated to minimize liver damage. As a result, pigs are weak, unthrifty, and have reduced growth performance or sudden death. Necropsy may reveal lesions and swollen livers. Due to a compromised immune system, pigs are more susceptible to concurrent infections, such as respiratory diseases. Over a longer exposure, there may be cirrhosis of the liver, carcinoma, and degeneration of the kidneys. (Iowa State University et al., 2018; Osweiler, 2018).

While there is much anecdotal knowledge about the feeding of aflatoxin to pigs, there is limited published data regarding its impact on growth performance. Research evaluating aflatoxin consumption by lactating sows showed that a dietary level of 500 to 700 ppb negatively impacted

suckling pig growth, but did not impact sow intake or reproductive performance over the next four gestation periods (Armbrecht, 1972; McKnight et al., 1990; Osweiler and Ensley, 2010). However, the consumption of aflatoxin by gestating sows can impact fetal growth. A study fed gestating sows 800 ppb aflatoxin from d 60 of gestation to farrowing, and led to reduced piglet birth weight (Mocchegiani et al., 1998).

In one trial with nursery pigs, feeding a diet with 140 ppb aflatoxin B_1 did not impact growth performance. Feed intake and ADG was not reduced until aflatoxin levels reached 280 ppb (Marin et al., 2002). Another researcher reported on two experiments, where each experiment used weanling pigs that were fed diets containing 551 ppb aflatoxin B_1 and 335 ppb aflatoxin B_2 per kg of feed, respectively. In trial 1, ADG did not statistically differ, yet trial 2 showed a 33% decrease in ADG. Statistical analysis was not performed for ADFI or feed efficiency (Furtado et al., 1982). As pigs grow, they become less susceptible to aflatoxin. In an experiment with growing pigs (initially 22.7 kg), three concentrations (0, 250, and 500 ppb) of aflatoxin were fed to pigs for 10 weeks. After two weeks, ADFI was reduced when feeding aflatoxin exceeding 250 ppb compared to the control, however, ADG did not statistically differ until week 6 (Rustemeyer et al., 2014). In another experiment by Southern et al., 1999, finishing pigs were fed naturally contaminated aflatoxin, at concentrations of 20, 385, 750, and 1480 ppb, for 66 days. Pigs consuming 385 ppb or above had reduced ADFI, which resulted in lower ADG compared to the control (20 ppb). F/G was statistically higher in pigs fed 1,480 ppb, with no difference across all lower concentrated treatments.

Notably, each experiment, besides feeding finishing pigs, used artificially inoculated corn to create aflatoxin concentrations. While the artificial inoculation likely created a more consistent delivery of toxin to the animal, it does not mimic natural conditions. Naturally-contaminated corn may have

other undetected mycotoxins and is unevenly distributed throughout a lot, leading to pulses of aflatoxin delivery to animals. The inconsistency of a response to aflatoxin and use of artificially-inoculated corn in most aflatoxin feeding research is important to resolve in future research.

Are all types of aflatoxin the same?

There are four types of aflatoxins that can be detected in grains: B_1, B_2, G_1, and G_2. Of these, B_1 is the most toxic and typically associated with symptoms. However, aflatoxin can be also passed through milk to suckling pigs as M_1, B_1, B_2, and G_1 (Silvotti et al., 1997). When sows ingest aflatoxin B_1, this metabolite is converted in the liver to M_1, which can be deposited in muscle tissue, urine and transmitted through milk to newborn pigs.

Can dietary consumption of aflatoxin by pigs affect pork safety?

While mycotoxins are typically readily metabolized in the liver, there have been reported deposition of aflatoxin in muscle tissue. All reported muscle tissue deposits have been extremely low, even when pigs were fed high aflatoxin concentrations for extended periods of time (Bailly and Guerre, 2009).

If there are aflatoxin residues in pork, processing it by curing or smoking has been shown to potentially increase the concentrations. However, it is more likely that if aflatoxin concentrations increase during processing, this increase is caused by the addition of potentially contaminated spices, not from residual in muscle tissue (Bailly and Guerre, 2009).

Pigs rapidly metabolize aflatoxins, so there is little concern for aflatoxin residues in pork that could affect human health (Furtado et al., 1982). For example, feeding a non-contaminated diet for one day after aflatoxin is deposited in muscle tissues has shown to significantly reduce aflatoxin

concentrations in the organs and tissues. After 4 days, there was no detectable mycotoxin in muscle tissue (Furtado et al., 1982).

What are the current regulatory aflatoxin tolerance levels?

To limit potential aflatoxicosis and maintain food safety, the United States Food & Drug Administration (FDA) has set limitations on the aflatoxin concentrations that can be fed to swine. Young and breeding animals are generally more susceptible to aflatoxins. For this reason, the maximum aflatoxin concentration allowed is much lower than that of finishing pigs (Table 1.4).

Summary:

The severity of aflatoxin affecting swine depends on dosage, dietary interactions, exposure time, and life stage of swine. For these reasons, FDA has guidelines for the maximum aflatoxin dosage that can be safely fed to swine at a certain life stage. Aflatoxins can reduce growth performance in all life stages, but research shows these concentrations are substantially higher than the FDA guidelines. Additionally, aflatoxin can be transmitted through milk and eventually consumed by newborn pigs. Lastly, aflatoxin can be deposited in pork, but reported levels are exceedingly rare and very low, so there is little concern for human health implications.

Sampling and Analysis of Aflatoxin in Corn

Mycotoxins are fungal secondary metabolites from molds grown on cereal grains that can produce carcinogens and cause disease to both humans and animals (Eaton, 1994, Gelderblom et al., 1998, Huff et al., 1998). Mycotoxins are generally concentrated in broken kernels and a small percentage of whole kernels (Smith and Henderson, 1991). This makes it challenging to obtain a

representative sample, because highly concentrated kernel fragments increase the risk of sampling error. For this reason, facilities must follow careful procedures to collect a representative sample and analyze it using scientifically-sound methods.

How is mycotoxin concentration estimated in corn?

Mycotoxin concentration in a single bulk lot of corn cannot be determined with 100% certainty because of inherent variability in both sampling and analytical procedures (van Egmond et al., 2007). The mycotoxin concentration is estimated in corn through three steps 1) collect a representative sample from the bulk lot, 2) grind the sample and collect a representative sub-sample, 3) extract and analyze the sub-sample for mycotoxin concentration (Smith and Henderson, 1991).

How should a representative sample be collected from a bulk lot for aflatoxin testing?

The greatest variability in aflatoxin analysis occurs at the bulk sampling stage. Most grain handling facilities collect a single probe of corn, which greatly limits the accuracy of estimating the aflatoxin concentration because aflatoxin is located in 'hot spots'. Due to its accumulation in broken kernels, the contamination is not evenly distributed throughout a load. Frequently, the contamination exists only in 0.1% of kernels (Rahmani et al., 2009).

To reduce variability, it is important to collect multiple samples from the lot to calculate an average mycotoxin concentration. Samples can be collected by inserting a closed probe at a 15˚ angle into the grain and withdrawn (Association of American Feed Control Officials, 2014). For a standard lot of corn, a bulk sample should be collected from combining ten 300 g samples collected from

various locations and depths of the bulk material into one composite sample (Rahmani et al., 2009; Association of American Feed Control Officials, 2014).

How should a sample be ground and sub-sampled as preparation for mycotoxin analysis?

Once a representative sample is collected from the bulk lot, it must be ground to reduce particle size. This reduction is typically required for chemical analysis, but also helps create a more uniform distribution of the toxin. Hand-held grinders, such as coffee bean grinders, or lab scale grinding equipment are efficient grinding methods for extraction analyses. Bulk samples should be ground to a size that allows at least 60% of the product to pass through a 20-mesh screen (United States Department of Agriculture, 2015). To keep the mycotoxin concentration consistent between the bulk sample and the ground sample, samples should be ground and mixed to create a more homogeneous sample as the particle size decreases (Smith and Henderson, 1991; Rahmani et al., 2009).

What are common methods for mycotoxin analysis?

There are several methods to extract and analyze mycotoxin concentration. Cost and time are both important factors that affect which method to use for mycotoxin analyses (Table 1.5). Per sample costs can range from approximately $8 to $150 USD, equipment set-up can be expensive, and chemicals may have to be purchased. Some mycotoxins can be extracted by water, while others may require solvent extraction with ethanol or methanol. Some analysis methods are rapid, and can be conducted on-site, while others require the sample to be sent to an external laboratory.

Rapid technique analyses

Non-instrumental, antibody-based tests are relatively fast and inexpensive. These mycotoxin analysis kits include lateral flow, dipstick, or flow-through tests (Köppen et al., 2010). These rapid technique methods have antibodies present on strips of cotton. When the extracted solution is placed on the end of the test strip, the antibodies on the test strip bind to the specific mycotoxin. The liquid slowly moves along the absorbent paper toward the other end of the strip, while the antibodies continue to bind. This binding reaction causes the control line and possibly test line to change in color. The control line changing color indicates the test was performed properly. If toxin was present, the test line appears. The test line color intensity can then be analyzed by a scanner to provide a more precise quantification. Due to the variability of the test line color intensity, methods for rapid detection of mycotoxins are less sensitive and precise than laboratory methods. Regardless, rapid technique methods are widely used at grain handling facilities because they are relatively accurate and can be completed in less than 20 minutes at a low cost.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay, or ELISA, is an immunologically based analysis. These immunological based analyses are made by producing artificial antibodies that are specific to the mycotoxin. When these antibodies come into contact with the specific mycotoxin that is being analyzed, they bind to the mycotoxin if found in the sample. This reaction shows that the mycotoxin is present, commonly represented by color intensity. Some facilities own a relatively inexpensive spectrophotometer to read results on-site, but others will send samples to an outside laboratory for this analysis. While more accurate than rapid method tests, ELISA tests are typically more expensive and time consuming.

Liquid chromatography-tandem mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS), has become the method of choice for trained laboratory personnel when performing mycotoxin and food analysis, due to easy handling, high sensitivity, and accuracy (Sforza et al., 2006). The required instrumentation and analytical precision is impractical for field settings, so this technology is utilized only in certified laboratories. This is a disadvantage for producers, because the results from the sample collected cannot be determined until after the corn is received and stored or utilized. Some LC-MS/MS can detect multiple mycotoxins in a sample at one time, and without required dilutions.

Which mycotoxin analysis method is best?

The best mycotoxin analysis method depends upon the situation. In field-based situations that demand fast results, rapid technique analyses are relatively precise and inexpensive. These analyses can be certified for accuracy by the Grain Inspection, Packers and Stockyard Administration of the Federal Grain Inspection Service. To maximize the accuracy of these tests, it is important to ensure proper sampling and grinding of the sub-sample, and that tested concentrations do not exceed the validated conformance range. For example, many rapid tests are validated up to 20 ppb aflatoxin, but some of the more expensive options are validated up to 300 ppb. ELISA is another field-based analytical method that has moderate costs and accuracy.

If instead, there is a desire to quantify multiple mycotoxins in a sample, or a sample with high levels of contamination, LC-MS/MS is likely the best option.

How accurate are rapid methods?

In a study conducted at Kansas State University, a rapid method estimation of the aflatoxin concentration in high aflatoxin cleaned corn was statistically similar (P = 0.23; Table 1.6) to

analysis by LC-MS/MS (732 vs. 789 ppb in rapid method vs. LC-MS/MS, respectively). However, the rapid method greatly underestimated ($P < 0.0001$) the aflatoxin concentration in uncleaned corn and corn screenings. The variability in the rapid technique analyses, especially in screenings, is likely due to the multiple dilutions necessary to estimate high aflatoxin levels. The maximum validated conformance in the rapid technique method used was 300 ppb. Thus, a 50:1 dilution was needed during rapid technique analyses to estimate aflatoxin concentration in the high aflatoxin corn screenings. Thus, the rapid method tested was relatively accurate at predicting aflatoxin concentration outside of its validated range up to a point, but each dilution introduces additional error potential.

How precise are rapid methods?

The precision of same rapid method was evaluated by using paired sub-samples, created by riffle division, and analyzed by LC-MS/MS. Fifteen rapid technique and LC-MS/MS samples were used to determine a correlation between analytical method. There was a moderate positive correlation ($r^2 = 0.49$) and a strong positive correlation ($r^2 = 0.71$) for uncleaned corn and cleaned corn, respectively (Figure 1.1 & 1.2). These results indicate cleaning aflatoxin contaminated corn improves the strength of the correlation between rapid technique and LC-MS/MS analyses. A strong positive correlation in cleaned corn compared to a moderate positive correlation in uncleaned corn is likely due to the removal of screenings. Screenings contained the highest concentration of aflatoxin, thus the presence of this fraction can increase the variability when performing analyses. These results also demonstrate the importance of analyzing multiple samples to determine a more accurate average aflatoxin concentration.

Summary:

To evaluate the aflatoxin concentration in corn, a sample must be collected from a lot, ground, sub-sampled, and extracted. Each of these steps introduces potential error, with the greatest error being ununiform sampling from the original lot. These chances of error demonstrate the importance for obtaining and analyzing multiple samples to accurately determine the average concentration. Rapid technique, ELISA, and LC-MS/MS are common methods to determine mycotoxin concentration. Rapid technique and ELISA have lower cost, and shorter analysis time, but have a limited detection range, and can only detect a single mycotoxin per sample compared to multiple mycotoxin analysis by LC-MS/MS. Rapid techniques can detect high concentrations of aflatoxin by diluting the sample. However, these dilutions can reduce the analysis accuracy.

Solutions to Aflatoxin in Corn for Pigs

In crop years when environmental conditions favor aflatoxin accumulation, farmers and producers may need to use post-harvest methods to reduce aflatoxin exposure to animals. Drying grain to less than 14% moisture is an effective method to control any further aflatoxin growth (Smith and Henderson, 1991; Jayas and White, 2003; Magan and Aldred, 2007). However, when aflatoxin concentrations exceed regulatory approval, controlling further growth during storage is not sufficient. Therefore, methods need to be developed to reduce the aflatoxin concentration or their effect on pigs.

Can I blend aflatoxin-contaminated ingredients with non-contaminated ones to improve its safety?

The FDA has determined that the blending of aflatoxin-contaminated product above action limits with other products below the action limit is not allowed. Because aflatoxin is considered an adulterant, the blending process is considered adulteration of the uncontaminated product. In certain circumstances, FDA has relaxed their 'no-blending' policies when aflatoxin contamination is highly prevalent, such as in the Midwest in 2005 and 2012 (National Grain and Feed Association, 2011).

Can I use binders to reduce the toxicity of aflatoxin to pigs?

There are no approved aflatoxin binders for use in swine in the United States. Thus, the use of binders for the purpose to bind to aflatoxins is considered a violation by the FDA. That said, there are some ingredients used in swine diets to improve pellet quality or other physical traits that can reduce aflatoxin symptoms in pigs. Aflatoxin-binding agents, such as aluminosilcates, clays and zeolites, are the most commonly researched and included in swine diets (Jacela et al., 2010). For example, sodium bentonite clay can bind and remove aflatoxin B_1 and improve ADG, ADFI and feed efficiency when fed with aflatoxin contaminated corn. While they may be effective, aflatoxin binders remain non-approved for use.

What are other methods that can reduce aflatoxin after harvest?

Several methods can reduce post-harvest aflatoxin concentrations, including chemical, radiation, ozone, and roasting (Bhat et al., 2010). However, many are not cost effective or practical in a field environment. Further research is needed to examine any potential side effects, practicality, and efficiency of these methods. For these reasons, alternative post-harvest methods need to be

implemented. Cleaning, or screening corn, is one method that may prove to be a practical method that reduces the aflatoxin concentration in corn.

How can cleaning reduce the aflatoxin level in corn?

Once present in grain, it is difficult to remove mycotoxins. That said, the removal of broken kernels or dust may reduce mycotoxin concentrations because those are the locations where mold is most likely to be located (Magan and Aldred, 2007). Prior to storage, some grain elevators remove these broken kernels and dust through a cleaning process, which involves running the bulk corn over a set of screens to remove particles smaller than a whole corn kernel. Anecdotal reports have stated this process reduces mycotoxin concentration, but there is limited research demonstrating the quantity of mycotoxin removed by physical cleaning.

An experiment conducted at Kansas State University analyzed natural mycotoxin contaminated corn that contained a high concentration of aflatoxin (1,074 ppb). This corn was screened across a commercial corn cleaner (Gentle Roll, EBM Manufacturing, Norfolk, NE) to remove broken kernels. Samples of uncleaned and cleaned corn were collected to analyze total aflatoxin. Total aflatoxin concentration was determined by performing rapid technique analyses and multiclass liquid chromatography tandem mass spectrometry (LC-MS/MS). By cleaning corn, aflatoxin concentration decreased by 26%. Cleaning corn by passing grain across a 4.8 mm screen can be a practical method to reduce average aflatoxin concentration.

How does physical screening impact storage and nutritional characteristics?

Cleaning corn can remove broken kernels and dust that are less dense than whole kernels, resulting in corn samples with a higher bulk density (Hall, 1972; Brusewitz, 1975). In our study, cleaning corn by removing 2.2% screenings increased ($P < 0.0001$) bulk density by 3.4 kg/m^3, while removing 6.0% screenings increased ($P < 0.0001$) bulk density by 8.1 kg/m^3 (Table 1.7).

The gross energy concentration of cleaned corn did not differ ($P > 0.05$) from uncleaned corn (Table 1.8). The gross energy concentration of the broken kernels was similar ($P < 0.05$) to uncleaned and cleaned corn regardless of corn source. However, in corn source 2, overs (large pieces of cob or stalk) had the lowest ($P = 0.02$) energy concentration.

Summary:

Although proven to reduce the impact of aflatoxin, blending or binding aflatoxin are not FDA approved methods to prevent its impact in pigs. A potential practical method to reduce aflatoxin concentration in post-harvest grain is mechanical screening. This screening removes broken kernels, which have an exceptionally higher mycotoxin concentration and are less dense than whole corn. This research demonstrates that removal of screenings substantially reduces mycotoxin concentration and increases bulk density with no change to gross energy concentration.

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Figures and Tables

Figure 1.1 Total aflatoxin concentration of unclean corn

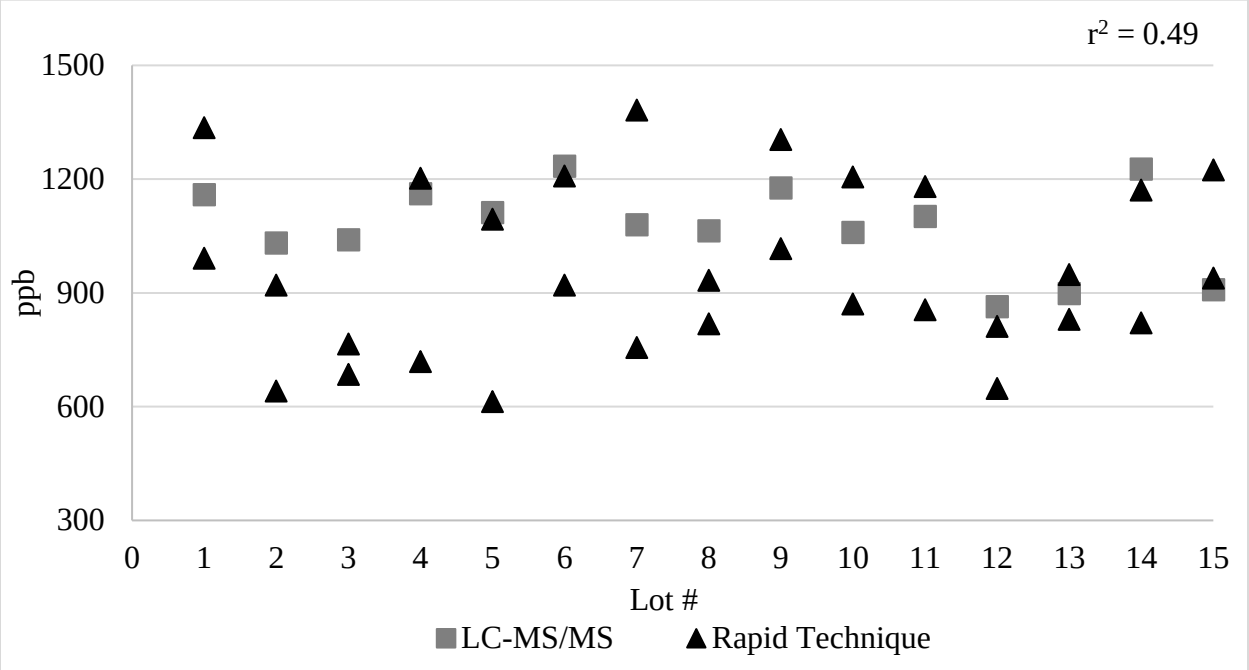

Figure 1.2 Total aflatoxin concentration of cleaned corn

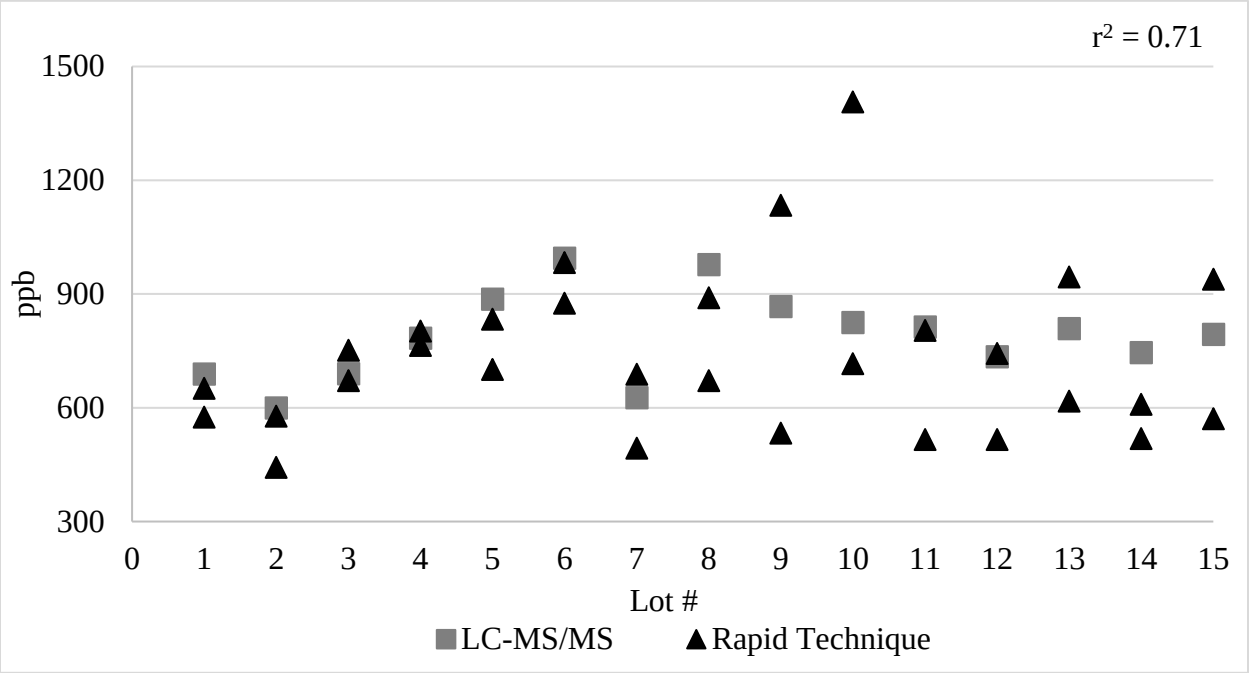

Table 1.1 Relationship between moisture content (wet weight basis, %) and water activity (aw) for some key cereals at 25˚C

	Moisture Content, %				
a_w	Corn	Wheat	Sorghum	Rice	Groundnuts
0.98	30-32	30-34	31-32	26-28	16-17
0.95	26-27	26-28	26-27	23-24	14.5-15
0.90	23-24	21-22	22.5-23	20-21	12.5-13.5
0.80	16-17	16-17	18-19	17-18	9-10
0.70	15-16	14-14.5	16-17	14-14.5	7-8

Referenced from Magan, N., Olsen, M. Mycotoxins in food. Cambridge: Woodhead Publishing Limited. 2004;69, 175-178.

Table 1.2 Average global aflatoxin prevalence (2013-2016)

	Prevalence, %				
Year	Africa	Asia	Europe	North America	South America
2013	67	28	40	26	12
2014	62	28	22	16	10
2015	33	25	11	2	18
2016	42	27	15	3	30

Referenced from Biomin Mycotoxin Report.

Table 1.3 Average global aflatoxin concentration (2013-2016)

	Concentration, ppb				
Year	Africa	Asia	Europe	North America	South America
2013	16	53	7	20	4
2014	8	104	8	29	40
2015	49	59	6	16	6
2016	52	41	10	6	6

Referenced from Biomin Mycotoxin Report.

Table 1.4 Summary of maximum concentrations of aflatoxin in swine feed and ingredients

Life Stage	Product	Level
Immature animals	Corn, peanut products, and other animal feeds and feed ingredients (excluding cottonseed meal)	20 ppb
Breeding swine	Corn and peanut products	100 ppb
Finishing swine > 100 pounds	Corn and peanut products for	200 ppb
Regardless of age or breeding status	Cottonseed meal	300 ppb

Referenced from Food and Drug Administration Center for Veterinary Medicine.

Table 1.5 Mycotoxin Analysis Methodology

Analysis Method	Location	Mycotoxin Analysis	Cost per Sample	Time
Rapid Technique	Field	Single	Low	Short
ELISA	Field/Laboratory	Single	Medium	Short-Medium
LC-MS/MS	Laboratory	Multiple	High	Long

Table 1.6 Aflatoxin Analysis Methodology Comparison

	Aflatoxin Concentration (ppb)		
Analysis Method	Sample 1	Sample 2	Sample 3
n =	20	10	10
Rapid Technique[1]	960[b]	732	2,870[b]
n =	10	10	10
LC-MS/MS[2]	1,074[a]	789	4,224[a]

[1] Neogen Corporation AccuScan Gold scanner with Reveal Q+ Max aflatoxin test strips.
[2] Multiclass liquid chromatography tandem mass spectrometry.
[ab]Means within a column with different superscripts differ $P < 0.05$

Table 1.7 Bulk density of corn fractions

	Initial Bulk Density (kg/m^3)		
Screenings removed, %	Unclean Corn	Clean Corn	Screenings
2.2	863[b]	866[a]	649[c]
6.0	861[b]	869[a]	587[c]

[abc]Means within a row with different superscripts differ $P < 0.05$

Table 1.8 Gross energy concentration of corn fractions

	Energy Concentration (kcal/kg)			
Corn source, #	Unclean Corn	Cleaned Corn	Broken Kernels	Overs
1[1]	3,725	3,719	3,712	3,632
2[2]	3,722[a]	3,715[a]	3,663[a]	3,318[b]

[1] 2.2% screenings were removed by cleaning.
[2] 6.0% screenings were removed by cleaning.
[ab]Means within a row with different superscripts differ $P < 0.05$.

Chapter 2 - Effect of Cleaning Corn on Mycotoxin Concentration and Nursery Pig Growth Performance

Abstract

Mycotoxins are naturally-produced hazards that result from molds grown on cereal grains and other commodities. These molds may produce carcinogenic mycotoxins, which can be harmful to humans and animals. Removing broken kernels has been demonstrated to reduce mycotoxin concentration, but with high variability. Therefore, two experiments were conducted to quantify the magnitude of natural mycotoxin concentration that may be reduced by cleaning corn. Two loads of corn that were naturally contaminated with mycotoxins were procured. Corn for Exp. 1 was contaminated with aflatoxin (1,074 ppb), fumonisin (8.3 ppm), and ochratoxin A (206 ppb), while corn for Exp. 2 was contaminated with only fumonisin (5.5 ppm). Corn was cleaned by mechanical sieving. For each experiment, corn was divided into twenty 150 kg runs. Run were randomly assigned to 1 of 4 experimental treatments: 1) no screen 2) 12.7 mm screen, 3) 4.8 mm screen, and 4) 12.7 + 4.8-mm screen. The corn cleaner was sanitized via aspiration between run. Three 5 kg corn samples were collected from each run, and analyzed for mycotoxin concentration. In Exp. 1, cleaning reduced ($P < 0.05$) aflatoxin and fumonisin concentration by an average of 26% and 45%, respectively, compared to the original uncleaned corn level, but did not impact ($P > 0.10$) ochratoxin A. The resultant screenings had nearly 4 times the aflatoxin (4,224 ppb) and 7.5 times the fumonisin concentration (60.4 ppm) as the uncleaned corn. In Exp. 2, cleaning reduced ($P < 0.05$) fumonisin concentration by 32%. The resultant screenings had 19.6 times the fumonisin

concentration (65.4 ppm) as the uncleaned corn. To determine the effect that cleaning corn or pelleting may have on nursery pig growth performance, 360 nursery pigs were used in Exp. 3. Treatments were arranged in a 2 × 3 factorial with corn type (uncleaned vs. cleaned) and feed form (mash vs. pelleted from either mill A or B). Neither cleaning corn nor pellet mill type affected ($P > 0.19$) nursery pig growth performance. Pelleting improved ($P < 0.0001$) G:F by 7.6% compared to mash diets. These data suggest that cleaning is an effective method to legally reduce aflatoxin and fumonisin concentration, but does not impact animal growth performance. Screenings contain the highest concentration of mycotoxin and should be distributed cautiously when feeding to animals.

Key words: corn, feed, mycotoxin, screenings

Introduction

Mycotoxins are fungal secondary metabolites from molds grown on cereal grains and other commodities, such as peanuts, cottonseed, and soybeans. These harmful metabolites can be produced by *Aspergillus, Fusarium and Penicillium* fungi. (Rodríguez et al., 2013). These genus can produce aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, T-2 toxin, and zearalenone. Aflatoxins and fumonisins are of high concern because they produce carcinogens and induce disease that can be harmful to both humans and animals (Huff et al., 1988; Eaton and Gallagher, 1994; Gelderblom et al., 1988).

Mycotoxins are often concentrated on the outer seed coat of grains or in dust and broken kernels, which are more susceptible to fungal infection and toxin contamination (Smith &

Henderson, 1991). The cracked or broken kernels may result from drought stress or insect damage that exposes nutrients for fungal growth (Magan & Olsen, 2004).

The amount of mycotoxin contamination in the corn may be reduced by mechanical processing. Screening is a mechanical process in which the grain passes over a screen that contains uniformly measured openings to separate the desired particle size from the original sample. Screenings may include light and broken grains and agricultural seeds, weed seeds, hulls, chaff, joints, straw, elevator or mill dust, sand, and dirt (AAFCO, 2018). Screening can remove broken corn and dust to lower the mean mycotoxin concentration, but with variable success (Smith & Henderson, 1991). There is limited data demonstrating how screening affects the mycotoxin concentration or how removing the screenings may affect swine growth performance. Therefore, the objective of this experiment was to evaluate how physical cleaning of corn naturally contaminated with mycotoxins impacted its resultant level of contamination, and to evaluate how cleaning impacts growth performance of nursery pigs fed mash or pelleted diets.

Materials and Methods

General

The Kansas State University Institutional Biosafety Committee and Institutional Animal Care and Use Committee approved the procedures used in this experiment (#1178 and 3529, respectively). Aflatoxin is a biosafety level-2 pathogen, and cleaning was conducted under containment conditions.

Experiment 1

A total of 3,000 kg corn that was naturally contaminated with mycotoxins was procured from a single field in central Oklahoma, and transported with Food and Drug Administration (FDA) approval to the Kansas State University Cargill Feed Safety Research Center. The dryland corn was a drought tolerant hybrid (6355 Dekalb) with 30-inch row spacing, in no-till sandy loam soil, that contained adequate nutrition by analysis. Glyphosate and atrazine were applied to reduce herbicide and pesticide occurrence. The crop experienced extremely low rainfall in May, slightly higher temperature and rainfall in July, and substantially more humidity in July, August, and September compared to the 5-year average, and was harvested after 158 days (Table 2.1).

3,000 kg of uncleaned corn was divided into twenty 150 kg runs, with run as the experimental unit. A single run consisted of three 50 kg barrels. A total of sixty 50 kg barrels were filled from the original 3,000 kg. To account for the segregation of broken kernels and foreign material while unloading product from the bin, three sets of 20 barrels were filled (1-20, 21-40, and 41-60). Each barrel within each set of 20 were randomized to run number 1 through 20, for a total of three barrels per run. Screens were cleaned by aspiration between each run to prevent cross-contamination. Runs were cleaned using 1 of 4 experimental treatments: 1) no screening (control), 2) 12.7 mm screen, 3) 4.8 mm screen, and 4) 12.7 + 4.8 mm screen. Treatments 1, 2, 3, and 4 had 20, 10, 10, and 10 replications, respectively. Corn was cleaned using a commercial corn cleaner (EBM Gentle Roll, model# 24S-1-HB-F-FF, Norfolk, NE), with resultant products being corn + thrus and overs, corn + overs and thrus, and cleaned corn (Figure 2.1). 3 samples of each resultant product of each run were collected by probing according to the Association of American Feed Controls Officials (AAFCO) Feed Inspector's Manual (2014).

Experiment 2

A total of 3,000 kg corn that was naturally contaminated with fumonisin was procured from a single field in south-east Kansas and transported to the Kansas State University Cargill Feed Safety Research Center. The irrigated corn with 30-inch row spacing, was an herbicide tolerant kernel with Glyphosate traits planted in soil that contained medium to high amounts of phosphorus pentoxide and potassium oxide by analysis. Corn kernels also contained insect resistant technology and crop rotation practices were used to reduce pest occurrence. Conventional tillage practices and storage bin aeration were performed. The crop experienced slightly less rainfall in May and June, and substantially more rainfall and humidity in July and August compared to the 5-year average (Table 2.2).

3,000 kg of uncleaned corn was again divided into twenty 150 kg runs, with run as the experimental unit. Treatments and procedures for cleaning and sampling are as described in Exp. 1.

Sample Preparation

Resultant samples were ground to below 400 μm (≈ 291μm) using a laboratory-scale hammermill (Bliss Industries, LLC, Model Eliminator rotor and screen width, Ponca City, OK) attached with a 56-liter commercial vacuum to mimic air-assist and collect the ground sample into a vacuum bag. To reduce cross-contamination, the hammermill and vacuum hose were cleaned by aspiration between samples, and a new vacuum bag was used for each sample.

Mycotoxin analyses

All samples were analyzed by a single technician. Total aflatoxin and fumonisin concentration were determined by lateral flow rapid technique analysis using an AccuScan Gold scanner with Reveal Q+ Max (Neogen Corporation, Lansing, MI) aflatoxin test strips and Reveal Q+ fumonisin test strips for Exp. 1 and 2, respectively. When sample concentration exceeded the maximum test strip threshold, samples were diluted with distilled water or a 65% ethanol solution per manufacturer recommendations. After rapid technique analysis, sub-samples were riffle divided into composite samples and analyzed for multiple mycotoxins by multiclass liquid chromatography tandem mass spectrometry (LC-MS/MS) at the North Dakota State University Veterinary Diagnostic Laboratory in Fargo, ND.

Experiment 3

The trial was conducted at the Kansas State University Segregated Early Weaning Facility. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center.

A total of 360 nursery pigs (initially 8.8 kg BW) were utilized in a 28-d-experiment. There were 5 pigs per pen and 12 pens per treatment. Upon arrival to the barn, pigs were allotted in a completely randomized design. A common starter diet was fed for 10 d post-weaning. On d 10, pens were allotted to 1 of 6 dietary treatments. Treatments were arranged in 2 × 3 factorial design with corn type (uncleaned vs. cleaned corn that was naturally contaminated with fumonisin from Exp. 2) and feed form (mash vs. pelleted from either mill A or B) The same diet formulation was used in all treatments (Table 2.3). Diets were pelleted on either pellet mill A (master model 1000 HD, California Pellet Mill Co., Crawfordsville, IN) or pellet mill B (model 3016-4, California

Pellet Mill Co., Crawfordsville, IN). While pellet mill types differed, the conditioning temperature (85°C ± 1°C), conditioner retention time (30 s), pellet die size (4.0 × 22-mm), and production rate percentage (60%) based on pellet mill capacity were held constant. Uncleaned and cleaned corn samples were analyzed by bomb calorimetry to measure gross energy concentration. Complete diets were analyzed for proximate analysis at Ward Laboratories Inc. using AOAC Methods 935.29, 990.03, 978.10, 920.39, and 942.05 for dry matter, crude protein, crude fiber, ether extract, and ash, respectively (Table 2.4). Furthermore, pelleted diets were analyzed for pellet durability index (PDI) using the Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for a 60 s test time, and pellet fines percentage by sifting pellets across a U.S. #6 sieve to determine the quantity of fines. Pigs and feeders were weighed on d 0, 7, 14, 21, and 28 of the trial to determine ADG, ADFI, and G:F.

Statistical analyses

Data were analyzed using the GLIMMIX and CORR procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) with the Kenward-Roger adjustment. In Exp. 1 and 2, run was the experimental unit, while pen was the experimental unit for Exp. 3. In Exp. 1 and 2, the average range between values for LC-MS/MS were compared using Tukey's studentized range for pairwise analysis. In Exp. 3, pre-planned contrast statements of uncleaned vs. cleaned corn, pellet mill type A vs. pellet mill type B, and mash vs. pelleted diets were conducted using the CONTRAST statement of SAS. Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant if $0.05 < P \leq 0.10$.

Results

Experiment 1

Screening impacted ($P < 0.05$) the concentration of all detectable mycotoxins in corn except Ochratoxin A ($P = 0.45$; Table 2.5). The reduction in mycotoxin concentration was due to the 4.8 mm screen. A single pass across the 4.8 mm screen reduced ($P < 0.05$) contamination in all measured mycotoxins except Ochratoxin A. Notably, cleaning corn with only a 4.8 mm screen reduced a substantial magnitude of contamination: a 36% reduction on aflatoxin and a 45% reduction in fumonisin compared to the uncleaned corn. Screening across the 12.7 mm screen alone did not impact ($P > 0.05$) corn mycotoxin concentration, and did not further reduce ($P > 0.05$) contamination when used in combination with the 4.8 mm screen. After cleaning corn across both the 4.8 and 12.7 mm screen, aflatoxin and fumonisin were reduced by 26% and 45% respectively. The difference between the 4.8 mm screen and a combination of cleaning with a 4.8 and 12.7 mm screen in aflatoxin concentration reduction is likely due to analytical variability. The rapid technique method only analyzed total aflatoxin concentration, with similar reduction ($P < 0.05$) when screened with 4.8 mm screen, but no impact ($P > 0.05$) of the 12.7 mm screen.

To determine the mycotoxin concentration of each fraction in the uncleaned corn, fractions were separated and analyzed (Table 2.6). These individual fractions included: 1) overs, or material that did not pass through the 12.7 mm screen, 2) cleaned corn, or material that passed through the 12.7 mm screen, but not the 4.8 mm screen, and 3) thrus, or material that passed through both the 12.7 and 4.8 mm screens. Overs, typically including pieces of corn cob or husk, accounted for only 0.06% of the weight of end product. Cleaned corn was 94.1% of the initial weight, with thrus, typically including broken kernels and dust, representing 5.86% of the initial weight of the uncleaned corn.

Physical cleaning impacted ($P < 0.05$) mycotoxin concentration in all fractions. Material caught by the 12.7 mm screen, or overs, had lower ($P < 0.05$) concentrations of total aflatoxin, but greater ($P < 0.05$) total fumonisin, compared to cleaned corn. There was no difference ($P > 0.05$) in ochratoxin A concentration between overs and cleaned corn. Interestingly, there were detectable levels for trichothecene (T-2) and sterigmatocystin (34.0 vs. 30.0, respectively) in the overs, despite being undetected in the cleaned corn.

As expected, material passing through both the 12.7 and 4.8 mm screens had greater ($P < 0.05$) aflatoxin mycotoxin concentrations compared to cleaned corn. The thrus had 5.3 times greater aflatoxin, 13.4 times greater fumonisin, and 2.8 times greater ochratoxin A than cleaned corn. The rapid technique analysis method only analyzed total aflatoxin concentration, with similar pattern for aflatoxin reduction ($P < 0.05$), but the means for the cleaned corn samples were more consistent with those measured by LC-MS/MS than means for overs or thrus.

Experiment 2

Cleaning corn reduced ($P < 0.05$) the concentration of fumonisin, as fumonisin was the only detectable mycotoxin in this specific source of corn (Table 2.7). Similar to Exp. 1, the reduction in fumonisin concentration was caused by removing material with a 4.8 mm screen. Notably, this led to a 32% reduction in fumonisin compared to the uncleaned corn. Again, cleaning corn across the 12.7 mm screen alone did not impact ($P > 0.05$) fumonisin concentration, and did not further reduce ($P > 0.05$) contamination when used in combination with the 4.8 mm screen. After cleaning corn across both the 4.8 and 12.7 mm screen, fumonisin were reduced by 40%. The rapid technique method only analyzed total fumonisin concentration, with similar reduction ($P < 0.05$) when screened with 4.8 mm screen, but no impact ($P > 0.05$) of the 12.7 mm screen.

Again, to determine the fumonisin concentration of each fraction in the uncleaned corn, fractions were separated and analyzed (Table 2.8). These fractions included: 1) overs, or material that did not pass through the 12.7 mm screen (0.02% by weight); 2) cleaned corn, or material that passed through the 12.7 mm screen, but not the 4.8 mm screen (97.76% by weight) and 3) thrus, or material that passed through both the 12.7 and 4.8 mm screens (2.22% by weight).

Physical cleaning impacted ($P < 0.05$) mycotoxin concentration in all fractions. Material caught by the 12.7 mm screen, or overs, had 6 times greater ($P < 0.05$) fumonisin levels compared to cleaned corn. Material passing through both the 12.7 and 4.8 mm screens had nearly 20 times greater ($P < 0.05$) fumonisin than cleaned corn. There was not sufficient sample to analyze the overs using the rapid technique method, but the thrus had over 27 times greater ($P < 0.05$) the fumonisin level of cleaned corn, with means that were numerically similar to those measured by LC-MS/MS.

Experiment 3

To understand the potential impact of corn cleaning on nursery pig performance, corn from Exp. 2 was used in a swine growth experiment. The uncleaned corn had 5.48 ppb fumonisin, while the cleaned corn (material that passed through the 12.7 mm screen, but not the 4.8 mm screen) had 3.33 ppb fumonisin. Diets were then fed as mash, or pelleted in one of two pellet mills, to see if the inclusion of overs and thrus potentially impacted dietary energy or pellet quality that could be discerned through different pellet mills. As described in Table 2.4, there was limited impact of cleaning on primary nutrient concentrations. Specifically, bomb calorimetry showed the gross energy of uncleaned corn to be 3,725 kcal/kg, compared to 3,719 kcal/kg in cleaned corn. Neither

PDI (42.9% and 43.1% for uncleaned and cleaned, respectively) nor pellet fines percentage (7.35% and 7.6% for uncleaned and cleaned, respectively) differed among corn type.

The interaction between corn type × diet form impacted ($P < 0.05$) ADG from d 14 to 28 of the experiment, but no other measured parameter ($P > 0.05$; Table 2.9). Pigs fed uncleaned corn had similar ($P > 0.05$) ADG when fed mash or pelleted diets. However, those fed cleaned corn had greater ($P < 0.05$) ADG when feed was pelleted.

Corn type did not impact ($P > 0.05$) nursery pig growth performance. Overall, pellet mill type B had a marginally significant improvement in ADG compared to the smaller sized pellet mill Type A, however, there were no differences in any other time period or response criteria. When comparing mash diets to all pelleted diets, pigs fed mash diets had greater ($P < 0.05$) ADG from d 0 to 14 than those fed pelleted diets, which was driven by greater ($P < 0.05$) feed intake, but not ($P > 0.05$) G:F. The effect was reversed in the second period, where pigs fed pelleted diets had greater ($P < 0.05$) ADG, lower feed intake, and improved G:F compared to pigs fed mash diets. Overall, pigs fed pelleted diets utilized less ($P < 0.05$) feed, but grew more efficiently ($P < 0.05$), and therefore had similar ($P > 0.05$) ADG as those fed mash diets.

Discussion

Overall cleaning implications

In our study, 26% aflatoxin and 42% fumonisin concentration, on average, were removed from cleaning corn across perforated screens. These results are similar to Scudamore and Patel, (2000), who found a 40% and 32% reduction in aflatoxin and fumonisin, respectively, when 140 samples of corn received at ports or mills were surveyed before and after cleaning. The aflatoxin and fumonisin that remains in the corn after cleaning is likely present in the whole kernel. This is

supported by research reported by Brekke et al. (1975), Broggi et al. (2002), and Saunders et al. (2001). In these studies, the bran and germ contained 2.1 times greater aflatoxin and 2.7 times greater fumonisin than the whole kernel, while the flour contained the least amount of mycotoxin. The bran and germ that are removed from the flour milling process are often used in livestock diets. Including these corn milling by-products in livestock diets holds risk to increase the overall mycotoxin concentration within the diet. Additionally, some toxins in livestock feed fractions may have the potential to become residues in animal products (i.e. aflatoxins, ochratoxin A) and still enter the human food chain (Bullerman et al., 2007). Our study differed from these previously published as the bran and germ were still intact to the kernel, which is common for grain elevator storage because removing these fractions reduces nutritive and economic value.

Alternative cleaning methods are needed to reduce the maximum amount of mycotoxin without reducing nutritive value. Shetty and Bhat et al., (1999), showed when natural fumonisin contaminated corn was immersed in water for 5 minutes, fumonisin B_1 concentration were reduced by 74%. While the same source of corn was immersed in a 30% NaCl solution, fumonisin B_1 concentration were reduced by 86%. Additionally, studies by Bullerman et al., (2007), and Pearson et al., (2004) demonstrated the effects of high-speed dual-wavelength sorting on high mycotoxin concentrated kernels. Aflatoxin and fumonisin were reduced by 81% and 85%, respectively. One negative aspect is the high amount (5-10%) of kernels removed during sorting. Despite 5-10% corn removed by sorting, the economic loss may be overcome by developing a safe feed source, especially when handling corn of high mycotoxin concentration.

In addition to aflatoxin and fumonisin, the uncleaned corn also had detectable levels of ochratoxin A. Ochratoxin A concentration was not impacted by cleaning, despite having 2.7 times greater ochratoxin A concentration in the thrus. These findings are contrary to Duarte et al., (2010),

and Scudamore et al., (2003), who reported dust, broken grain, and bran removal lowers the ochratoxin A concentration. In wheat, the ochratoxin A concentration were reduced by 44% after removing screenings and the bran. Our study did not find a difference in ochratoxin A concentration, perhaps because our cleaning methods kept the bran intact when analyzing corn mycotoxin reduction.

In Exp. 1, even after cleaning, aflatoxin concentration was substantially greater than the maximum tolerance (> 300 ppb) allowed for corn to be fed to animals (United States Food & Drug Administration, 1994). With an initial aflatoxin concentration greater than 1,000 ppb, mycotoxin concentration can be difficult to reduce to safe concentrations when using traditional grain handling facility cleaning methods.

Cleaning corn across a 4.8 mm perforated screen effectively reduced fumonisin concentration, even after initially cleaning with a 12.7 mm screen. Using a 12.7 mm screen was ineffective at reducing mycotoxin concentration. The 12.7 mm screen removed overs while the 4.8 mm screen removed thrus. Since the thrus fraction contained the highest concentration, removing this fraction reduced the overall mean of the fumonisin concentration. Even though the overs contained more fumonisin than cleaned corn, the percentage of overs in the entire corn source were less than 0.1%. Pieces of cob in between 4.8 and 12.7 mm in size were found within the cleaned corn sample. Improvements in technology to clean corn not only by size, but by shape and density could prove to further reduce the overall fumonisin concentration since the overs are more concentrated than the cleaned corn.

Screenings

The mycotoxin concentration of the overs fraction was variable across mycotoxin type. Compared to uncleaned corn, overs contained 3.6 times less total aflatoxin, 1.9 times greater total fumonisin, and identical concentrations of Ochratoxin A. Although overs contained 1.9 times greater total fumonisin than uncleaned corn, this was not a large enough impact to reduce the average total fumonisin concentration. In this experiment, based on the concentration of fumonisin within overs, it may be possible to see a reduction in total fumonisin concentration from sources of corn that contain a higher percentage of overs. While trichothecene and sterigmatocystin were undetected in the uncleaned corn, there were detectable levels within overs. Trichothecene and fumonisin are generally produced in growing cereal crops, while sterigmatocystin and ochratoxin A are produced during storage. When grains are received, screening may inhibit accumulation of sterigmatocystin and ochratoxin A during storage.

In Exp. 1, our study found that thrus had 7.5 times greater fumonisin concentration than uncleaned corn, while in experiment 2 the thrus contained 19.6 times more fumonisin, compared to uncleaned corn. These findings are supported by Murphy et al. (1993), who analyzed 160 total samples of corn from multiple states. They found that thrus contained approximately 10 times higher fumonisin B_1 concentration than uncleaned corn. The determination that thrus contain the highest mycotoxin concentration appears to be consistent, although the thrus mycotoxin concentration can be variable.

The mycotoxin concentration in the screenings are notable and can have both positive and negative implications. If feeding whole corn contaminated with aflatoxins or fumonisins, these data suggests that cleaning is an effective method to legally reduce mycotoxin contamination and render the product safer for animal consumption. If storing whole corn contaminated with

aflatoxins or fumonisins, these data suggests that cleaning prior to storage may be an important step to reduce the overall mycotoxin contamination and potential for proliferation during the storage period. When cleaning grains, shrink can be affected because small and large particles are removed. For this reason, not all manufacturers separate screenings. It is common practice to feed screenings, mostly utilized by ruminants, either as a distinct commodity or by addition to the ground corn bin, which can lead to high risk pulses of mycotoxicosis. For these reasons, caution needs to be taken with the screenings removed during cleaning.

Swine Growth Performance

While physically cleaning corn is shown in this project to reduce mycotoxin concentration, the results demonstrated in this research suggest that removing screenings does not impact corn nutrient density. Notably, a small fraction of fines was removed from this sample compared to the nearly 6% of screenings removed in Exp. 1. Manufacturers should consider not only the reduced volume of corn and mycotoxin concentration, but also the potential change in nutrient level when employing physical screening. The results from experiments 1 and 2 show the highest concentration of fumonisin is within the screenings. Screenings that are more concentrated in parts of the diet can increase the average fumonisin concentration that the pig is consuming, thus worsening performance (Osweiler et al., 1992). However, our results show that removing screenings does not affect average swine growth performance. This is likely because initial fumonisin concentrations were far below values that are shown to affect growth performance.

Pelleting improved overall feed efficiency. This improvement in feed efficiency was driven by lower ADFI in pigs fed pelleted diets, while there was no difference in ADG across feed form. These results support Jensen et al., 1965, who found pigs fed a pelleted corn-soybean meal-based

diet had lower ADFI, but no differences in ADG, which was driven by higher G:F, as compared to pigs fed a mash form feed. Interestingly in our study from d 0-14, pigs fed pelleted diets conditioned at 85˚C had lower ADG compared to mash diets, but from d 14-28 pelleted diets had higher ADG. Overall pigs fed pelleted diets had lower ADFI compared to pigs fed mash diets. This is expected because G:F increased. This improvement in G:F could be partially due to improved dry matter, nitrogen, gross energy digestibility, and apparent ileal digestibility (AID) of starch and most AA (Wondra, 1995; Rojas, et al., 2015).

Pellet mill type did not influence overall feed efficiency. This is supported by DeJong et al. 2014, who determined no differences in growth characteristics for nursery pigs fed the same diet that was produced from two different pellet mills. However, another study by DeJong et al. 2014, showed there were differences in growth characteristics for nursery pigs fed feed produced from two different pellet mills. These results are inconsistent, and the operator, conditioning temperature, conditioner retention time, and die specification may influence nursery pig growth performance if these parameters are not held constant when comparing pellet mill types. In our experiment, operator, pellet process parameters facility was kept the same for all pelleted diets.

Conclusion

In conclusion, screening corn is one method to reduce aflatoxin and fumonisin concentration. The average aflatoxin and fumonisin concentration of corn containing 5.86% of the thrus fraction can be reduced by approximately 36% and 45%, respectively, when performing a single pass across a single 4.8 mm screen. Additionally, in corn that contains 2.2% of the thrus fraction, the same cleaning method can effectively reduce fumonisin concentration by 32%. In Exp. 1 and 2, the screenings contained the highest mycotoxin concentration. Yet, neither cleaning low fumonisin

concentrated corn nor pellet mill type affected nursery pig growth performance. In years of high mycotoxin grain, screenings should be discarded or utilized for an alternative processing method that does not create livestock feed. While it is common for the feed industry to feed screenings to livestock, these results suggest that feeding screenings have heightened risk for mycotoxicosis.

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Figures and Tables

Figure 2.1 Exp. 1 & 2 treatment flow diagram

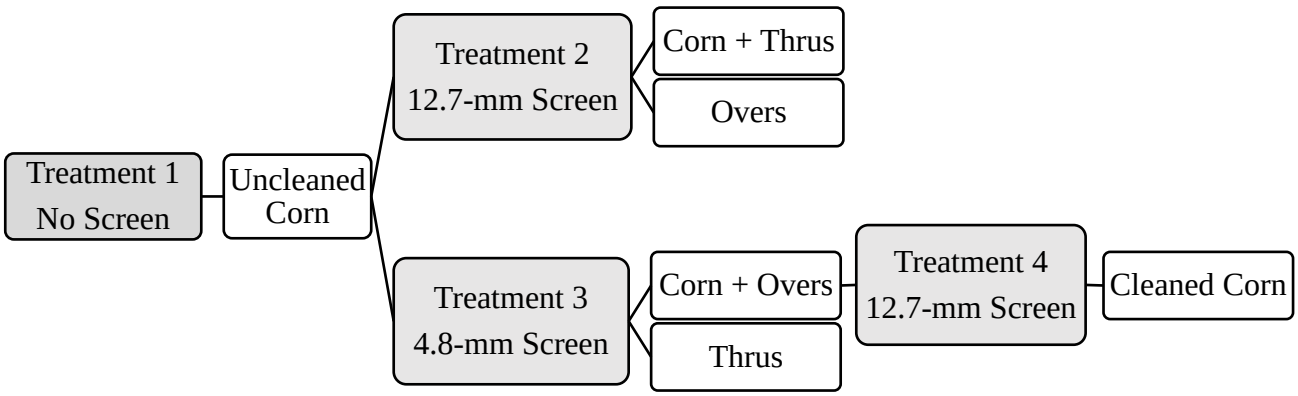

Table 2.1 Oklahoma agronomic climate conditions

	Month[1]					
Item:	April	May	June	July	August	September
2016						
Temperature (˚C)	17.2	19.6	26.1	28.2	27.4	25.3
Rainfall[2] (cm)	0.66	0.28	0.25	0.53	0.04	0.11
Humidity (%)	65.6	74.6	68.0	68.7	67.8	69.6
5-year average (2012-2016)						
Temperature (˚C)	16.2	20.3	25.9	27.8	27.3	24.4
Rainfall[2] (cm)	0.41	0.62	0.31	0.45	0.16	0.16
Humidity (%)	66.7	70.8	67.3	63.7	61.5	63.2

[1] Data collected from Mesonet database; Norman, OK site.
[2] Average daily rainfall.

Table 2.2 Kansas agronomic climate conditions

	Month[1]						
Item:	March	April	May	June	July	August	Sept.
2016							
Temperature (˚C)	11.5	15.6	18.0	26.1	27.1	26.2	22.8
Rainfall[2] (cm)	0.23	0.50	0.25	0.17	0.44	0.46	0.27
Humidity (%)	55.9	58.9	70.1	67.4	72.3	70.5	69.0
5-year average (2012-2016)							
Temperature (˚C)	10.0	14.9	19.4	25.0	26.8	25.9	22.6
Rainfall[2] (cm)	0.18	0.35	0.34	0.23	0.24	0.23	0.20
Humidity (%)	59.2	60.4	68.1	67.8	65.2	64.6	66.2

[1] Data collected from Mesonet database; Sedan site.
[2] Average daily rainfall.

Table 2.3 Composition of diets, Exp. 3

Ingredient, %	
Ground corn	64.2
Soybean meal, 46.5% CP	29.9
Monocalcium phosphate, 21% P	1.35
Limestone	0.85
Salt	0.75
L-lysine HCl	0.50
DL- Methionine	0.21
L-Threonine	0.21
L-Tryptophan	0.03
L-Valine	0.13
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Phytase[3]	0.015
Choice White Grease	1.50
Total	100.00
Calculated Analysis	
Standardized ileal digestibility (SID) amino acids, %	
Lysine	1.30
Isoleucine:Lysine	55
Leucine:Lysine	114
Methionine:Lysine	37
Met & Cystine:Lysine	58
Threonine:Lysine	63
Tryptophan:Lysine	18.5
Valine:Lysine	70
ME, kcal/kg	687
CP, %	20.4
SID Lysine:metabolizable energy, g/Mcal	3.90
Total Lysine, %	1.44
Calcium, %	0.69
Phosphurus, %	0.67
Available Phosphurus, %	0.46
Fat, %	4.1

[1] Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[2] Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

[3] HiPhos 2700 (DSM, Het Overloon, Netherlands) provided 476 phytase units (FTU)/kg of diet, for an estimated release of 0.10% available P.

Table 2.4 Chemical analysis of diets (as-fed basis), Exp. 3

Corn type	Uncleaned[1]			Cleaned[2]		
Feed form	Mash	Type A[3]	Type B[4]	Mash	Type A[3]	Type B[4]
Item, %						
DM	90.3	91.0	91.0	90.0	90.8	91.2
CP	21.1	20.7	21.0	20.2	21.0	20.8
Crude fiber	2.9	3.3	3.5	3.3	3.5	3.7
Ether extract	3.1	3.8	3.3	3.9	3.7	4.0
Ash	5.7	5.2	5.2	5.5	5.3	5.4

[1] Uncleaned corn contained broken kernels and foreign material.
[2] Cleaned corn had broken kernels < 4.8 mm and foreign material > 12.7 mm removed.
[3] Type A (master model 1000 HD, California Pellet Mill Co., Crawfordsville, IN).
[4] Type B (model 3016-4, California Pellet Mill Co., Crawfordsville, IN).
Gross energy of uncleaned corn measured 3,725 kcal/kg, compared to 3,719 kcal/kg in cleaned corn.

Table 2.5 Effect of physically cleaning corn and cleaner screen size on corn mycotoxin concentration

Item:	Uncleaned Corn	Screen size (mm) used to clean corn[1]			Pooled SEM	*P*=
		12.7[2]	4.8[3]	12.7 + 4.8[4]		
LC-MS/MS analysis						
n	15	3	3	10	-	-
Aflatoxin (total), ppb	1,074[a]	968[ab]	690[c]	789[bc]	62.3	<0.0001
B_1, ppb	1,005[a]	902[ab]	641[c]	733[bc]	59.4	<0.0001
B_2, ppb	69.3[a]	65.7[ab]	49.0[c]	56.4[bc]	4.07	<0.0001
G_1, ppb	< 20	< 20	< 20	< 20	-	-
G_2, ppb	< 20	< 20	< 20	< 20	-	-
Deoxynivalenol (DON), ppb	< 200	< 200	< 200	< 200	-	-
Fumonisin (total), ppm	8.26[a]	8.14[a]	4.60[b]	4.46[b]	0.41	<0.0001
B_1, ppm	6.88[a]	6.94[a]	3.89[b]	3.72[b]	0.36	<0.0001
B_2, ppm	1.38[a]	1.20[a]	0.71[b]	0.74[b]	0.06	<0.0001
Trichothecene (HT-2), ppb	< 200	< 200	< 200	< 200	-	-
Trichothecene (T-2), ppb	< 20	< 20	< 20	< 20	-	-
Ochratoxin A, ppb	206	227	186	198	19.6	0.453
Sterigmatocystin, ppb	< 20	< 20	< 20	< 20	-	-
Zearalenone, ppb	< 50	< 50	< 50	< 50	-	-
Rapid technique analysis						
n	20	10	10	10	-	-
Aflatoxin (total), ppb	976[a]	872[ab]	693[c]	732[bc]	46.8	<0.0001

[1] A single load of naturally-contaminated corn was cleaned by mechanical sieving. Corn was divided into twenty 150 kg runs. Run were randomly assigned experimental treatments and the corn cleaner was sanitized between run. Three 5 kg corn samples were collected from each run, ground, split, and analyzed for mycotoxin concentration by either LC-MS/MS or rapid technique analysis.

[2] Values represent material passing through the 12.7 mm screen.

[3] Values represent material not passing through the 4.8 mm screen.

[4] Values represent material not passing through the 4.8 mm screen, but passing through the 12.7 mm screen.

[abc]Means within a row with different superscripts differ $P<0.05$.

Table 2.6 Effect of fraction type on mycotoxin concentration

	Fraction Type[1]				
Item:	Overs[2]	Cleaned corn[3]	Thrus[4]	SEM	*P*=
Percentage of weight, %	0.06	94.1	5.86		
LC-MS/MS analysis					
n	3	10	3		
Aflatoxin (total), ppb	298^{c}	789^{b}	4,224^{a}	79.7	<0.0001
B_1, ppb	258^{c}	733^{b}	3,976^{a}	74.8	<0.0001
B_2, ppb	40.0^{b}	56.4^{b}	248^{a}	7.41	<0.0001
G_1, ppb	< 20	< 20	< 20	-	-
G_2, ppb	< 20	< 20	< 20	-	-
Deoxynivalenol (DON), ppb	< 200	< 200	< 200	-	-
Fumonisin (total), ppm	15.6^{b}	4.50^{c}	60.4^{a}	0.70	<0.0001
B_1, ppm	13.2^{b}	3.72^{c}	51.1^{a}	0.62	<0.0001
B_2, ppm	2.43^{b}	0.74^{c}	9.32^{a}	0.94	<0.0001
Trichothecene (HT-2), ppb	< 200	< 200	< 200	-	-
Trichothecene (T-2), ppb	34.0	< 20	< 20	-	-
Ochratoxin A, ppb	236^{b}	198^{b}	562^{a}	42.9	<0.0001
Sterigmatocystin, ppb	30.0	< 20	< 20	-	-
Zearalenone, ppb	< 50	< 50	< 50	-	-
Rapid technique analysis					
n	10	10	10	-	-
Aflatoxin (total), ppb	121^{c}	732^{b}	2,865^{a}	43.5	<0.0001

[1] A 5 kg sample were collected from each run, ground and analyzed for mycotoxin concentration.
[2] Product that did not pass through a 12.7 mm screen.
[3] Product that passed through a 12.7 mm screen but not a 4.8 mm screen.
[4] Product that passed through a 4.8 mm screen.
[abc]Means within a row with different superscripts differ $P<0.05$.

Table 2.7 Effect of physically cleaning corn and cleaner screen size on corn fumonisin concentration

Item:	Screen size (mm)[1]				SEM	*P*=
	Uncleaned Corn	12.7[2]	4.8[3]	12.7 + 4.8[4]		
LC-MS/MS analysis						
n	10	3	3	10	-	-
Aflatoxin (total), ppb	< 20	< 20	< 20	< 20	-	-
B_1, ppb	< 20	< 20	< 20	< 20	-	-
B_2, ppb	< 20	< 20	< 20	< 20	-	-
G_1, ppb	< 20	< 20	< 20	< 20	-	-
G_2, ppb	< 20	< 20	< 20	< 20	-	-
Deoxynivalenol (DON), ppb	< 200	< 200	< 200	< 200	-	-
Fumonisin (total), ppm	5.48[a]	5.17[a]	3.74[b]	3.33[b]	0.32	< 0.0001
B_1, ppm	4.51[a]	4.27[a]	3.08[b]	2.72[b]	0.38	< 0.0001
B_2, ppm	0.97[a]	0.91[a]	0.66[b]	0.60[b]	0.05	< 0.0001
Trichothecene (HT-2), ppb	< 200	< 200	< 200	< 200	-	-
Trichothecene (T-2), ppb	< 20	< 20	< 20	< 20	-	-
Ochratoxin A, ppb	< 20	< 20	< 20	< 20	-	-
Sterigmatocystin, ppb	< 20	< 20	< 20	< 20	-	-
Zearalenone, ppb	< 50	< 50	< 50	< 50	-	-
Rapid technique analysis						
n	20	10	10	10	-	-
Fumonisin (total), ppm	3.73[a]	3.76[a]	2.52[b]	2.58[b]	0.20	< 0.0001

[1] A single load of naturally-contaminated corn was cleaned by mechanical sieving. Corn was divided into twenty 150 kg runs. Run were randomly assigned experimental treatments and the corn cleaner was sanitized between run. Three 5 kg corn samples were collected from each run, ground, split, and analyzed for mycotoxin concentration by either LC-MS/MS or rapid technique analysis.

[2] Values represent material passing through the 12.7 mm screen.

[3] Values represent material not passing through the 4.8 mm screen.

[4] Values represent material not passing through the 4.8 mm screen, but passing through the 12.7 mm screen.

[abc]Means within a row with different superscripts differ $P<0.05$.

Table 2.8 Effect of fraction type on fumonisin concentration

	Fraction Type[1]				
Item:	Overs[2]	Cleaned corn[3]	Thrus[4]	SEM	*P*=
Percentage of weight, %	0.02	97.76	2.22	-	-
LC-MS/MS analysis					
n	1	10	3	-	-
Aflatoxin (total), ppb	< 20	< 20	< 20	-	-
B_1, ppb	< 20	< 20	< 20	-	-
B_2, ppb	< 20	< 20	< 20	-	-
G_1, ppb	< 20	< 20	< 20	-	-
G_2, ppb	< 20	< 20	< 20	-	-
Deoxynivalenol (DON), ppb	< 200	< 200	< 200	-	-
Fumonisin (total), ppm	21.0[b]	3.33[c]	65.4[a]	1.89	< 0.0001
B_1, ppm	18.4[b]	2.72[c]	52.4[a]	1.54	< 0.0001
B_2, ppm	2.59[b]	0.60[c]	13.0[a]	0.42	< 0.0001
Trichothecene (HT-2), ppb	< 200	< 200	< 200	-	-
Trichothecene (T-2), ppb	< 20	< 20	< 20	-	-
Ochratoxin A, ppb	< 20	< 20	< 20	-	-
Sterigmatocystin, ppb	< 20	< 20	< 20	-	-
Zearalenone, ppb	< 50	< 50	< 50	-	-
Rapid technique analysis					
n	0	10	10	-	-
Fumonisin (total), ppm	-	2.58[b]	70.8[a]	0.90	< 0.0001

[1] A 5 kg sample were collected from each run, ground and analyzed for mycotoxin concentration.
[2] Product that did not pass through a 12.7 mm screen.
[3] Product that passed through a 12.7 mm screen but not a 4.8 mm screen.
[4] Product that passed through a 4.8 mm screen.
[abc]Means within a row with different superscripts differ $P<0.05$.

Table 2.9 Effects of corn type (uncleaned or cleaned) and feed form (mash, type A, or type B pellet mill) on nursery pig growth performance

Corn type	Uncleaned			Cleaned				*P* =			
Feed form	Mash	Type A[2]	Type B[3]	Mash	Type A[2]	Type B[3]	SEM	Treatment	Uncleaned vs. Cleaned[4]	Type A vs. Type B[4]	Pellet vs. Mash[4]
BW, kg											
d 0	8.8	8.8	8.8	8.8	8.8	8.8	0.130	0.999	0.989	0.951	0.903
d 14	14.6	14.0	14.2	14.7	14.1	14.1	0.272	0.434	0.896	0.811	0.036
d 28	24.1	23.7	24.2	23.9	24.0	24.3	0.366	0.912	0.811	0.337	0.824
d 0 to 14											
ADG, g	408	371	388	420	367	378	16.9	0.181	0.944	0.392	0.012
ADFI, g	578^{ab}	506^{c}	533^{bc}	584^{a}	518^{c}	526^{c}	17.2	0.007	0.798	0.308	0.0001
G:F	0.71	0.73	0.73	0.72	0.70	0.72	0.02	0.763	0.452	0.772	0.513
d 14 to 28											
ADG, g	684^{bc}	691^{bc}	711^{ab}	657^{c}	711^{ab}	726^{a}	12.3	0.003	0.777	0.150	0.001
ADFI, g	1,074^{a}	984^{c}	1,008^{bc}	1,040^{ab}	1,009^{bc}	1,014^{bc}	18.4	0.020	0.947	0.452	0.001
G:F	0.64^{b}	0.70^{a}	0.71^{a}	0.63^{b}	0.71^{a}	0.72^{a}	0.007	<0.0001	0.654	0.324	< 0.0001
d 0 to 28[1]											
ADG, g	546	529	550	538	532	552	11.6	0.637	0.939	0.081	0.905
ADFI, g	826^{a}	743^{c}	770^{bc}	812^{ab}	758^{c}	770^{bc}	15.9	0.003	0.998	0.218	< 0.0001
G:F	0.66^{b}	0.71^{a}	0.71^{a}	0.66^{b}	0.70^{a}	0.72^{a}	0.007	<0.0001	0.681	0.169	< 0.0001

[1] A total of 360 pigs (average initial BW = 8.8 kg) were used in a nursery trial with 5 pigs per pen and 12 replicates per treatment.
[2] Master model 1000 HD (California Pellet Mill Co., Crawfordsville, IN)
[3] Model 3016-4 (California Pellet Mill Co., Crawfordsville, IN).
[4] Each contrast compared the following treatments: 1) 'Uncleaned vs. Cleaned compared the 3 unclean treatments to the 3 clean treatments; 2) 'Type A vs. Type B compared the 2 type A treatments to the 2 type B treatments; 3) 'Pellet vs. Mash' compared the 2 mash treatments to the 4 pelleted treatments.
[abcd]Means within a row with different superscripts differ $P < 0.05$.

Chapter 3 - Effects of Pellet Processing Parameters on Pellet Quality and Nursery Pig Growth Performance

Abstract

During the pelleting process, conditioning temperature can influence nutrient availability and pellet durability index (PDI). However, the impact of conditioning temperature on nursery pig growth performance is variable. Therefore, the objective of two experiments were to: 1) compare moisture percentage across 3 pellet mill series, and 2) quantify growth performance differences in nursery pigs due to diet form (mash vs. pelleted), conditioning temperature (low, medium, vs. high), and pellet diameter (4.0 vs. 5.2 mm). Exp. 1 was a 3 × 4 factorial design with 3 pellet mill series of decreasing capacity (3016-4, 1000 HD, vs. CL-5; California Pellet Mill Co., Crawfordsville, IN, for pellet mills A, B, and C, respectively) that produced samples collected at 4 locations (initial, post-conditioner, post-die, vs. post-cooler). There were 3 runs completed on each pellet mill, with the shutdown of the pellet mill indicating the end of a run. Three samples were collected from each location per run, for a total of 36 samples. Exp. 2 utilized 350 nursery pigs (DNA 200 × 400; initially 7.6 kg) in a 28-d experiment fed 2 phases, with a phase change at d 14. Seven treatments were arranged in a 2 × 3 factorial design plus an unpelleted mash control, with the factors of pellet diameter (4.0 vs. 5.2 mm), and conditioning temperature (low, medium, vs. high). Data were analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). In Exp. 1, there was a significant pellet mill × location interaction ($P = 0.012$) for moisture percentage. Because moisture is added via steam at the conditioner, only the conditioning location results are described. Regardless of pellet mill type, moisture was highest in conditioned samples. However, conditioned samples on pellet mill C had over 1% greater ($P < 0.05$) moisture than pellet mill A or B, with no difference between the two larger pellet mills. This increase in

moisture explains one reason why a higher ($P < 0.05$) PDI were found in pellets produced on pellet mill C compared to the larger size pellet mills. In Exp. 2, increasing conditioning temperature improved ($P < 0.0001$) PDI. The interaction of pellet diameter × conditioning temperature did not impact ($P > 0.10$) overall nursery pig feed efficiency. However, for overall G:F, both main effects were marginally significant ($P < 0.10$), which was caused by pigs have greater ($P < 0.05$) G:F when fed larger diameter pellets conditioned at a low temperature, compared to pigs fed smaller diameter pellets conditioned at a high temperature. Regardless of parameter, pigs fed pelleted diets had 4.5% greater ($P < 0.05$) G:F than those fed mash diets. In summary, conditioning with a CL-5 pellet mill can increase moisture percentage higher than larger scale pellet mills. Increasing conditioning temperature is one method to improve PDI, but there was marginal significance to worsen nursery pig G:F when feeding two phases of diets in a 28-d study. In conclusion, conditioning feed at lower temperatures may improve nursery pig G:F when starting pigs on a new diet.

Keywords: moisture, pelleting, starch

Introduction

Starch gelatinization is recognized to improve starch digestibility in monogastric animals because the gelatinized starch has a large capacity for water absorption and subsequent nutrient hydrolysis in the body (Smith et al., 1957, Jensen and Becker, 1965, Kotara and Fuchs et al., 2001). This leads to gelatinized starch having the ability to increase the rate of amylose hydrolysis and absorption (Rokey et al., 2005). True starch gelatinization is thought to occur at temperatures above 70°C and moisture above 25%, parameters that are met during the extrusion process (Lund, 1984). Generally, the pelleting process creates pellets from a mash moisture of 17 to 18% post-

conditioner, thus true gelatinization cannot occur across all starch molecules. That said, pelleting has been shown to gelatinize a small percentage of starch granules, while more are merely damaged due to high thermal energy generated from flowing across the pellet die holes. These damaged granules may also increase starch digestibility (Sivhus et al., 2005, Lewis et al., 2014). However, the variability in starch damage among pellet mill types or pellet dies has not been established.

Grow-finish pigs fed pelleted diets have greater ADG, partially because the damaged or gelatinized starch is more easily digested (Hanke et al., 1972, Skoch et al., 1983, Wondra, et al., 1995, Traylor et al., 1996). However, this result is inconsistent in nursery pigs (Xing et al., 2004, Zhu et al., 2007, Frobose et al., 2012, Bokelman et al., 2015). Feed efficiency (G:F), is improved in both nursery and growing-finishing pigs by an average of 4 to 7% when diets are pelleted (Hancock and Behnke, 2001). This improvement in efficiency could be partially due to less feed wastage, improved dry matter, nitrogen, gross energy digestibility, and apparent ileal digestibility (AID) of starch and most AA (Wondra, 1995; Rojas, et al., 2015). However, when conditioning at higher temperatures, stability of phytase and other enzymes must be considered (Jacela et al., 2010). Furthermore, AA, specifically lysine, may be irreversibly bound to free sugars by the maillard reaction. Because higher temperatures can have negative effects on these ingredients, such as scorching or burned pellets occuring within the pellet die, nursery pig diets that frequently have greater lactose and sugar levels are commonly pelleted at lower temperatures. Reducing conditioning temperature frequently increases pelleting time and reduces mill efficiency. As feed flows through the pellet die, temperatures can continue to increase to a higher temperature than the conditioning temperature. A strategy to reduce this frictional heat is by changing the die-hole diameter or thickness of the pellet die. However, limited data exists to understand how pelleting the same diet on different types of pellet mills or using different pelleting parameters impacts both

the physical form of starch and its utilization in the animals. Therefore, the objective of these experiments were to: 1) evaluate how different types of pellet mills impact the physical and chemical properties of a diet at various locations of the pelleting process, and 2) quantify growth performance differences in nursery pigs due to diet form, conditioning temperature, and pellet diameter, and pellet die thickness.

Materials and Methods

Experiment 1

All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan. Treatments were arranged in a 3 × 4 factorial design by producing pellets from 3 different pellet mill models (medium scale: 3016-4, small scale: master model 1000 HD, and benchtop: CL-5; California Pellet Mill Co., Crawfordsville, IN) to collect samples at 4 locations during the pelleting process (initial mash, post-conditioner, post-pellet die, and post-cooler). Run was the experimental unit, with run designated as being complete after all samples were collected, followed by the shutdown of the pellet mill. A swine finishing diet (Table 3.1) was pelleted through 3 different runs on each pellet mill. Diet type, ground corn particle size (500 µm ± 50 µm), hot pellet temperature (88°C ± 1.1°C), steam pressure (152 kPa), conditioner retention time (30 s), pellet die size (4.0 × 22 mm), length/diameter ratio (5.6), and production rate (60% of rated capacity) were held constant. Average conditioning temperature across pellet mill was 89°C ± 1.5°C. Three composite samples were collected from each run on each pellet mill at each location. Samples were collected prior to conditioning (cold mash), after conditioning but prior to the pellet die (hot mash), after the pellet die but prior to cooling (hot pellet), and after cooling (cold pellet). Each sample was analyzed for chemical and physical properties. Hot mash and hot pellet

samples were immediately placed in a freezer following sample collection. Chemical analysis included moisture percentage (AOAC method 934.01), total starch, gelatinized starch, cooked starch (as described by Lewis et al., 2014), phytase stability (AOAC Method 2000.12), proximate analysis (AOAC Methods 935.29, 990.03, 978.10, 920.39, and 942.05 for dry matter, crude protein, crude fiber, ether extract, and ash, respectively), and total and available lysine percentage (AOAC method 975.44). Physical analysis included pellet durability index (PDI) using the Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for 60 s (H100-60) and 120 s (H100-120), and the tumble box method (ASABE S269.4, 2007) modified to include either three (modified-3) or five (modified-5) 19 mm hex nuts in each chamber.

Experiment 2

All practices and procedures used in this experiment were approved by the Kansas State University Institutional Animal Care and use Committee (#3529). The trial was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center. A total of 350 nursery pigs (DNA 200 × 400; initially 7.6 kg) were utilized in a 28-d-experiment. There were 10 replicate pens per treatment and 5 pigs per pen. Pigs were allotted to pens according to BW and gender. A common starter diet was fed for 7 d post-weaning. On d 7, pens were allotted to 1 of 7 dietary treatments in a completely randomized design. A 2 × 3 factorial design plus a control, with pellet diameter (4.0 vs. 5.2 mm), conditioning temperature (low, medium, vs. high), and mash, created seven experimental treatments: 1) mash, 2) 5.2-low 3) 5.2-medium, 4) 5.2-high, 5) 4.0-low, 6) 4.0-medium, and 7) 4.0-high. The 4.0 mm and 5.2 mm pellets were produced using a 4.0 × 22.2 mm and 5.2 × 25.4 mm pellet die, respectively, attached to a 1000 HD series pellet

mill, (California Pellet Mill Co., Crawfordsville, IN). Phase 1 diets, fed from d 0-14, contained 10% spray dried whey which were conditioned to 54˚C, 66˚C, and 78˚C for low, medium, and high, respectively, and phase 2 diets, fed from d 14-28, were corn-soybean meal-based diets conditioned to 54˚C, 71˚C, and 88˚C for low, medium, and high, respectively (Tables 3.2 and 3.3). All diets within phase were compositionally the same across all treatments. Pigs and feeders were weighed on d 0, 7, 14, 21, and 28 of the trial to determine ADG, ADFI, and G:F.

Statistical analysis

Data were analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) with the Kenward-Roger adjustment. Treatment means were separated using pairwise comparisons of means performed using the DIFFS option from the LSMEANS statement of SAS. In Exp. 1, run was the experimental unit, while pen was the experimental unit in Exp. 2. In Exp. 2, pre-planned contrast statements of mash vs. pelleted diets, smaller diameter (4.0 mm) vs. larger diameter (5.2 mm) pellets, and linear and quadratic effects of conditioning temperature were conducted using the CONTRAST statement of SAS. Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant if $0.05 < P \leq 0.10$.

Results

Experiment 1

Pellet durability index (PDI) was impacted ($P < 0.001$) by pellet mill type across all methods (Table 4.4). In each PDI method, pellets produced from the CL-5 had a higher ($P < 0.05$) PDI compared to those produced from the 1000 HD and 3016-4 pellet mills. The 1000 HD produced pellets with a higher ($P < 0.05$) PDI compared to the 3016-4 when using the H100-60 and

Modified-3 PDI methods, however, there was no difference ($P > 0.05$) between the same two pellet mills when using the H100-120 and Modified-5 PDI methods, which are more abrasive PDI methods.

The interaction between pellet mill type × sample location affected ($P < 0.012$) moisture percentage (Table 5.5). Initial moisture levels across pellet mill types were similar ($P > 0.05$). Moisture is added in the conditioner, so it was predicted that moisture levels across pellet mill were highest in samples collected immediately post-conditioner. The moisture percentage of conditioned samples were highest ($P < 0.05$) at the CL-5, with no difference ($P > 0.05$) between the 1000 HD and 3016-4 models. Moisture percentage post-die decreased ($P < 0.05$) as the size of pellet mill increased. During the cooling process, pellets produced on the CL-5 had greater ($P < 0.05$) moisture content than pellets produced on the other pellet mills.

While there was no pellet mill type × location interaction for total starch ($P > 0.05$), gelatinized and cooked starch was impacted ($P < 0.05$). In diets manufactured by the CL-5, both gelatinized and cooked starch increased ($P < 0.05$) at each sampling point, with the largest increase due to feed flowing through the pellet die. In the 1000 HD, conditioning did not increase ($P > 0.05$) gelatinized starch, but did increase ($P < 0.05$) cooked starch. Extruding pellets through the pellet die, and cooling both increased ($P < 0.05$) gelatinized and cooked starch in the 1000 HD and 3016-4, but to a lower magnitude than in the CL-5. Meanwhile, cooked starch was increased ($P < 0.05$) in the 3016-4 at each processing step, with a similar ($P > 0.05$) final cooked starch level in cooled pellets level compared to the 1000 HD, but less ($P < 0.05$) compared to the CL-5.

There was an interaction for crude fat percentage. Across all factors, crude fat was highest ($P < 0.05$) at the initial location of the CL-5, yet there was no difference ($P > 0.05$) across all other CL-5 locations. For the 1000 HD pellet mill, the initial location had the greatest ($P < 0.05$) crude fat

percentage, while the conditioner and pellet die had the lowest ($P < 0.05$) with cooler being intermediate. Within the 3016-4 pellet mill, there was no difference ($P > 0.05$) in crude fat percentage across location.

There was no pellet mill type × location interaction for phytase stability, but the main effects were each significant. Detectable phytase units were reduced ($P < 0.05$) by pelleting, with the lowest phytase level measured immediately after the pellet die and in diets manufactured by the CL-5.

While there was no ($P > 0.05$) interaction for total Lys, the initial location contained greater ($P < 0.05$) total Lys with no difference ($P > 0.05$) across all other locations. A higher percentage ($P < 0.05$) of total Lys was also found when pelleting with the 1000 HD pellet mill, with no difference ($P > 0.05$) between the other pellet mills. Available Lys was marginally impacted ($P < 0.10$) by pellet mill type × location. Pelleting increased ($P < 0.05$) total detectable Lys, with the highest levels occurring in samples collected after conditioning. While available Lys followed this pattern, the magnitude of available Lys changed at sampling locations. The greatest ($P < 0.05$) available Lys after the conditioner were in diets manufactured by the CL-5, while the greatest ($P < 0.05$) available Lys in cooled pellets were in diets manufactured by the 3016-4.

Experiment 2

PDI was impacted by conditioning temperature ($P < 0.05$; Table 6.6). In Phase 1, PDI was highest ($P < 0.05$) when conditioning with high temperatures and medium temperature with a 5.2 × 25.4 mm pellet die. Inversely, PDI was lowest when conditioning at medium temperature on the 4.0 × 22.2 mm pellet die. In phase 2, Increasing conditioning temperature increased ($P < 0.05$) PDI, when pelleting across both pellet dies. When using the 4.0 × 22.2 mm pellet die, conditioning at low and medium temperatures had a lower ($P < 0.05$) PDI, but higher ($P < 0.05$) PDI when conditioning at high temperatures. Notably, PDI in Phase 2 was poor across all treatments, with those manufactured with less than 9.42% of pellets manufactured at low conditioning temperatures withstanding the H100-60 PDI method. Even though PDI % was low, pellets were still intact at the feeder (Figure 3.1).

The interaction between pellet diameter × conditioning temperature was not significant ($P > 0.05$) for any measurable growth parameter, and therefore removed from the model. Pigs fed pellet-based diets had marginally significant greater ($P < 0.10$) ADG than those fed mash-based diets from d 0 to 7, which was driven by an increase ($P < 0.05$) in G:F (Table 3.7). This effect was not observed ($P > 0.10$) from d 7 to 14.

From d 14 to 21, pellet diameter ($P < 0.05$), impacted ADG and G:F, but not ADFI. Pigs fed diets pelleted with a smaller die had greater ($P < 0.05$) ADG and G:F than those fed diets pelleted with a larger die. Low conditioning temperatures further increased ($P < 0.05$) ADG and G:F. The contrast of pigs fed mash based-diets vs. those fed pelleted diets demonstrated that regardless of die type or conditioning temperature, mash diets had greater ($P < 0.05$) ADG from d 14 to 21 than pelleted diets, which was driven by an increase in ADFI ($P < 0.05$). There was no impact ($P > 0.10$) on G:F.

There was no impact of pellet diameter or conditioning temperature on ADG or ADFI from d 21 to 28. Pigs fed diets manufactured at lower conditioning temperatures had improved ($P < 0.05$) G:F compared to those fed diets pelleted at higher temperatures. The consumption of mash-based diets caused pigs to have greater ($P < 0.05$) ADFI and poorer ($P < 0.05$) G:F than those fed pelleted diets, with no impact ($P > 0.10$) on ADG.

Overall in the 28-day experiment, both main effects moderately impacted ($P < 0.10$) G:F. This was led by greater ($P < 0.05$) G:F in pigs fed diets manufactured with a small die and low conditioning temperature compared to those fed diets manufactured with a high conditioning temperature, regardless of die size. Pigs fed mash diets had greater ($P < 0.05$) ADFI, but lower ($P < 0.05$) G:F than those fed pelleted diets, with no impact ($P > 0.10$) on ADG.

Discussion

Pellet Quality

Surprisingly, PDI differences were observed, despite consistent processing parameters across pellet mill. The CL-5 produced pellets with the highest PDI percentage across each PDI method, followed by the 1000 HD pellet mill in which PDI's were higher in the less abrasive (H100-60 and Modified-3) and unchanged in the more abrasive (H100-120 and Modified-5) PDI methods. One reason PDI values were highest in the CL-5 pellet mill is because the CL-5 had the highest amount of moisture content in the feed, prior to flowing through the pellet die. In Exp. 2, PDI values were below 50% across all conditioning temperatures in corn-soy based diets. Values were likely low due to diet formulation, pellet die thickness, and PDI method. The H100-60 is a PDI method that removes fines as the test is in progress, which can lead to lower PDI values compared to the tumble box methods, especially when pellets are of poor quality. In both experiments, pellets had poor

quality in the corn-soy based diet because the pellet die used was relatively thin (5.6 L/D ratio), causing a low degree of resistive force on the pellets. These data underscore that clear differences exist in PDI among different pellet mill series, even those from the same manufacturer when pelleting parameters are held constant. PDI method and the amount of abrasiveness within that method can greatly afffect the numerical value of PDI results. The results of this study indicate caution should be used when comparing PDI among pellet mill type and PDI method.

Nutrient Values

Conditioned mash on the CL-5 had the greatest moisture content, while there was no difference between the 1000 HD and 3016-4 pellet mills. The difference in moisture is likely due to size of conditioner, or steam quality, defined as percentage of steam-water mixture in a steam system that is in vapor phase. Moisture increases as steam quality decreases because there is more liquid present within the steam. Yet steam quality was not measured in this experiment. Additionally, the conditioner attached to the CL-5 pellet mill has a smaller diameter than larger scale pellet mills, which could lead to greater amounts of condensation accumulated on the walls of the conditioner. The higher amount of moisture added in the CL-5 led to the highest gelatinized starch percentage across pellet mill. These results are supported by Lewis, et al., 2014, who determined gelatinized starch is greater at higher conditioning temperatures, due to the extra steam added to reach higher temperatures and moisture percentages. A higher moisture percentage leading to a greater amount of gelatinized starch percentage is likely because neither the moisture of the conditioned mash nor gelatinized starch after the pellet die differed between the 1000 HD and 3016-4 pellet mill. In Exp. 1, gelatinized starch percentage, reported on a dry-matter basis, increased the greatest after flowing through the pellet die. The physical abrasion of the ingredients against the pellet die walls likely

caused the starch granules to gelatinize or damage. Interestingly, while cooling, gelatinized starch percentage continued to increase. For nutritionist, gelatinized starch may be a more practical measurement compared to cooked starch, because the actual value of gelatinized starch, damaged starch, or the combination, is known, while cooked starch is the quotient of gelatinized starch over total starch.

Phytase stability decreased on average by 70% across pellet mill. These findings are supported by Slominski et al., 2007, who used a commercially available granulated phytase product and determined a 63.6% and 55.9% loss in phytase activity from samples collected from two different pellet mills, with conditioning temperatures of 67˚C and 70˚C, respectively. In our experiment, the average hot pellet temperature across pellet mill measured 89˚C and pellet die size was 4.0 × 22.2 mm. This higher hot pellet temperature could be the reason why a greater loss in phytase activity was observed in our experiment. Unfortunately, the manufacturer of the phytase, pellet die size, steam pressure, or increase in temperature caused by steam was not described by Slominski et al., 2007. Thus, the effect of pellet die size or steam pressure on phytase stability remains unknown. Further research is needed to confirm phytase stability at different process parameters performed during the pelleting process.

Across all pellet mills, total and available lysine increased from producing pellets at the process parameters used (88˚C hot pellet temperature, 30 s retention time, 22 psi steam pressure, 4.0 × 22.2 mm pellet die size) in our experiment. This result is contrary to Mavromichalis and Baker, 1999, who determined lysine availability was not affected when conditioning at 60˚C for a 45 s retention time, with a 5.0 × 38.0 mm pellet die attached, yet steam pressure, and hot pellet temperature was not defined in their study. Based on these studies, pelleting may or may not affect lysine availability; however, compared to our study, there are clear differences in the pellet process

parameters. Regardless, total and available lysine could change when pelleting under more aggressive processing parameters.

Nursery Pig Growth Performance

Diets fed from d 0-14 contained 10% spray dried whey, while corn-soybean meal-based diets were fed from d 14-28. ADG did not differ between mash and pelleted diets, but a lower ADFI, and higher G:F was found in pigs fed pelleted diets compared to pigs fed mash. From d 0-7, ADG of pigs fed pelleted diets increased by 19.6%, yet overall there was no difference in ADG between diet form. Studies conducted by Frobose et al., 2012 and Zhu et al., 2010, found from d 0-21, and across each weighing period, nursery pigs improved in feed efficiency by having higher ADG, while ADFI remained unchanged. More research is needed to confirm ADG increases when pelleting diets.

Pellet diameter did not influence overall growth performance. This is supported by Traylor et al., 1996 and Edge et al., 2005, who found little to no difference on nursery pig growth performance when pigs were fed different pellet sizes. Conditioning temperature did not affect growth performance from d 0-14, yet when pigs were fed a corn-soybean meal-based diet from d 14-28, feed conditioned at low temperatures resulted in improved performance compared with use of medium and high conditioning temperatures. This is contrary to Lundbland et al., 2011, and Steidinger, et al., 2000, who found no differences in nursery growth performance when changing conditioning temperature of a wheat-based diet, and a diet that contained 10% spray dried whey, respectively. From d 14-21 of our experiment, pigs started consuming a corn-soybean meal-based diet. Surprisingly, the pellets conditioned at a high temperature with a smaller pellet diameter, which had a pellet die with a higher L/D ratio, had lower ADG than the pigs fed mash diets.

Furthermore, mash diets utilized feed less efficiently than pelleted diets conditioned at a low temperature and larger pellet diameter, which had a pellet die with a lower L/D ratio. In another experiment performed by Yoder et al. 2017, feeding pellets at 85˚C with a 4.0 × 22.2 mm pellet die, led to lower ADG from d 0-14, however, from d 14-28, pigs fed pelleted diets had lower ADFI, higher G:F, and higher ADG compared to pigs fed mash diets. These findings demonstrate that conditioning at higher temperatures may worsen performance during the first 14 days when fed a corn-soybean meal-based diet, but can improve ADG after d 14. Further research is needed to confirm this effect and possibly observe the effects of progressively increasing conditioning temperature when feeding a new diet and as the pig matures. In our experiment, the difference in growth performance by changing conditioning temperature was only found in the corn-soybean meal-based diets that contained no wheat or spray dried whey. Interestingly, these results were inverse of PDI values, with the highest and lowest growth performance values produced from low and high conditioning temperatures, respectively. For our experiment, feed was handled with minimal abrasive procedures, that may have otherwise caused breaks in the lower quality pellets before reaching the swine feeder. Post-cooler, pellets were transferred via drag conveyor and dropped approximately 3 meters into a surge conveyor for bagging. Bags were placed on a pallet and transferred to the farm to be hand-dumped into swine feeders. Commercial handling methods (bucket elevators, storage bins, truck delivering, automatic feeders) may decrease the amount of intact pellets once they reach the farm, thus observing a different outcome in nursery pig's growth performance due to a higher percentage of fines developed. An increase in fines can greatly reduce nursery pig growth performance (Stark, et al., 1994). In Exp. 2, percentage fines may have not exceeded the concentration to reduce growth performance, however percentage fines were not specifically measured.

Conclusion

Gelatinized starch increases at the highest degree after passing through the pellet die, while conditioning has minor improvements on gelatinized starch percentage. Conditioning temperature can substantially influence the PDI value of a corn-soybean meal-based diet. Pelleting diets improved overall feed efficiency. From d 0-7, there was no difference in ADFI, while pigs fed pelleted utilized feed more efficiently, which improved ADG by 19.6% compared to pigs fed mash diets. Overall, feed efficiency was higher for pigs fed pelleted diets, but reduced ADFI, which resulted in no difference in ADG between pigs fed pelleted or mash diets.

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Figures and Tables

Figure 3.1 Phase 2 pelleted treatments. PDI % averaged 4.23, 16.3, 43.2, 9.42, 31.2, and 37.5% for 5.2-low, 5.2-medium, 5.2-high, 4.0-low, 4.0-medium, and 4.0-high, respectively.

5.2 - Low

5.2 - Medium

5.2 - High

4.0 - Low

4.0 - Medium

4.0 - High

Table 3.1 Composition of diets, Exp. 1

Ingredient, %	
Ground Corn	61.3
Soybean meal, 46.5% CP	33.8
Monocalcium phosphate, 21% P	1.15
Limestone	0.95
Sodium chloride	0.35
L-Lysine	0.30
DL-Methionine	0.12
L-Threonine	0.115
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Phytase[3]	0.015
Choice white grease	1.50
Total	100.00

[1] Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[2] Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

[3] Commercially available phytase.

Table 3.2 Composition of diets, Exp. 2

	Phase 1[1]	Phase 2[2]
Ingredient, %		
Ground corn	52.7	65.3
Soybean meal, 46.5% CP	26.5	29.0
Spray dried whey	10.0	-
Monocalcium phosphate, 21% P	1.43	1.43
Limestone	0.80	0.83
Sodium chloride	0.55	0.60
L-Lysine	0.40	0.45
DL-Methionine	0.20	0.18
L-Threonine	0.19	0.21
L-Tryptophan	0.01	0.03
L-Valine	0.05	0.06
Trace mineral premix[3]	0.15	0.15
Vitamin premix[4]	0.25	0.25
Zinc oxide	0.25	-
Hamlet Protein 300[5]	5.0	-
Choice white grease	1.50	1.50
Total	100.00	100.00
Calculated analysis		
Standardized ileal digestibility (SID) amino acids, %		
Lys	1.35	1.24
Ile:Lys	59	57
Lue:Lys	117	119
Met:Lys	36	36
Met & Cys:Lys	58	58
Thr:Lys	65	65
Trp:Lys	18.6	18.6
Val:Lys	67	67
ME, kcal/kg	1,520	1,514
CP, %	21.6	20.0
SID Lys:ME, g/Mcal	4.03	3.72
Total Lys, %	1.48	1.38
Ca, %	0.75	0.69
P, %	0.74	0.68
Available P, %	0.45	0.38
Fat, %	3.9	4.2

[1] 350 nursery pigs (DNA 200 × 400; initially 7.6 kg)

[2] 350 nursery pigs (DNA 200 × 400; 11.8 kg)

[3] Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 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] Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

[5] Hamlet Protein, Findley, OH.

Table 3.3 Chemical analysis of diets (as-fed basis), Exp. 2

Pellet Diameter[1], mm	5.2			4.0		
Conditioning Temp.[2]	Low	Medium	High	Low	Medium	High
Phase 1						
DM	90.7	89.7	88.9	89.0	89.32	89.3
CP	22.4	21.1	21.3	21.6	21.05	20.4
Crude fiber	2.74	2.49	2.28	2.10	2.17	2.64
Ether extract	2.89	2.61	2.46	2.35	2.36	2.30
Ash	6.20	6.49	6.41	6.31	6.23	6.23
Phase 2						
DM	89.2	88.5	88.4	87.7	88.9	88.3
CP	19.6	20.4	19.5	20.3	20.0	19.9
Crude fiber	2.36	1.81	2.04	1.92	2.00	1.99
Ether extract	2.31	2.71	2.18	3.11	2.50	2.44
Ash	4.94	5.23	5.00	5.27	4.88	5.03

[1] Pellet die size was 4.0 × 22.2 mm and 5.2 × 25.4 mm for 4.0 and 5.2, respectively.
[2] Phase 1 conditioning temperature was 54˚C, 66˚C, and 78˚C for low, medium, and high, respectively; Phase 2 conditioning temperature was 54˚C, 71˚C, and 88˚C for low, medium, and high, respectively.

Table 3.4 Main effect of pellet mill model (CL-5, 1000 HD, vs. 3016-4) on PDI, Exp. 2

Pellet Mill[1]	CL-5	1000 HD	3016-4	SEM	*P* =
PDI, %					
H100-60[2]	92.9[a]	57.6[b]	37.7[c]	3.19	<0.0001
H100-120[3]	86.3[a]	22.6[b]	10.5[b]	3.71	<0.0001
Modified-3[4]	91.2[a]	67.7[b]	59.2[c]	1.76	<0.0001
Modified-5[5]	83.9[a]	49.4[b]	41.2[b]	2.57	<0.0001

[1] California Pellet Mill Co., Crawfordsville, IN.
[2] Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for 60 s.
[3] Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for 120 s.
[4] Tumble box method (ASABE S269.4, 2007) modified with three 19 mm hex nuts.
[5] Tumble box method (ASABE S269.4, 2007) modified with five 19 mm hex nuts.
[a-c]Means within a row with different superscripts differ $P < 0.05$.

Table 3.5 Interactive effects of pellet mill model (CL-5, 1000 HD, vs. 3016-4) and sample location (initial, post-conditioner, post-pellet die, vs. dryer) on chemical analysis, Exp. 1

Pellet Mill	CL-5				1000 HD				3016-4					*P* =		
Location	Initial[1]	Cond.[2]	Die[3]	Cooler[4]	Initial[1]	Cond.[2]	Die[3]	Cooler[4]	Initial[1]	Cond.[2]	Die[3]	Cooler[4]	SEM	Pellet Mill	Location	Pellet Mill × Location
Moisture, %	11.7^{f}	17.4^{a}	16.8^{ab}	12.8^{e}	11.8^{f}	16.3^{bc}	16.0^{c}	11.3^{f}	11.9^{f}	15.9^{c}	15.1^{d}	11.4^{f}	0.26	<0.0001	<0.0001	0.012
Total Starch, %	45.7	43.3	43.7	43.2	48.6	45.4	45.6	44.7	46.3	44.6	43.4	43.7	0.80	0.003	0.001	0.941
Gelat. Starch, %	8.54^{g}	10.1^{f}	15.3^{b}	17.9^{a}	8.04^{g}	8.71^{g}	11.2^{e}	13.1^{c}	8.09^{g}	10.0^{f}	11.3^{e}	12.2^{d}	0.28	<0.0001	<0.0001	<0.0001
Cooked Starch, %	18.7^{fg}	23.3^{e}	35.0^{b}	41.6^{a}	16.5^{g}	19.2^{f}	24.5^{de}	29.2^{c}	15.5^{gh}	22.5^{e}	25.7^{d}	28.0^{c}	0.75	<0.0001	<0.0001	<0.0001
Phytase, FYT/kg	841	140	72.4	189	837	301	212	301	849	318	266	280	50.7	0.006	<0.0001	0.519
Lysine, Total, %	1.51	1.65	1.59	1.53	1.42	1.61	1.56	1.52	1.49	1.62	1.58	1.55	0.02	0.039	<0.0001	0.504
Lysine, Available, %	1.48	1.61	1.52	1.45	1.45	1.56	1.52	1.47	1.38	1.58	1.55	1.51	0.02	0.022	<0.0001	0.093
Crude Fat, %	7.28^{a}	4.68^{de}	4.71^{cde}	5.07^{cd}	6.29^{b}	5.01^{de}	5.13^{cd}	5.60^{bc}	4.63^{de}	4.07^{e}	4.45^{de}	4.84^{cde}	0.31	0.001	<0.0001	0.012
Crude Fiber, %	2.70	2.84	2.42	2.43	2.40	2.54	2.35	2.15	2.36	2.37	2.22	2.20	0.09	0.002	0.001	0.634
Crude Protein, %	23.7	24.5	24.6	24.5	23.9	23.9	24.3	24.1	23.6	24.1	24.9	24.3	0.64	0.786	0.384	0.985
Ash, %	5.55	5.37	5.48	5.62	5.72	5.54	5.44	5.54	5.75	5.64	5.71	5.63	0.08	0.012	0.131	0.494

[1] Sample collected before processing.
[2] Sample collected after the conditioner, but before extruder die.
[3] Sample collected after the pellet die, but before cooler.
[4] Sample collected after the cooling process.
[a-g]Means within a row with different superscripts differ $P < 0.05$.

Table 3.6 Interactive effects of pellet diameter (4.0 vs. 5.2 mm) and conditioning temperature (low, medium, high) on pellet durability index

Pellet Diameter[1], mm	5.2			4.0				*P* =		
Conditioning Temp.[2]	Low	Medium	High	Low	Medium	High	SEM	Diameter	Temp	Diameter × Temp
PDI, %[3]										
Phase 1[4]	92.1^{b}	87.0^{d}	92.8^{ab}	88.7^{c}	93.1^{a}	92.7^{ab}	0.34	<0.0001	0.0008	<0.0001
Phase 2[5]	4.23^{f}	16.3^{d}	43.2^{a}	9.42^{e}	31.2^{c}	37.5^{b}	1.12	< 0.0001	< 0.0001	< 0.0001

[1] Pellet die size was 4.0 × 22.2 mm and 5.2 × 25.4 mm for 4.0 and 5.2, respectively.
[2] Phase 1 conditioning temperature was 54˚C, 66˚C, and 78˚C for low, medium, and high, respectively; Phase 2 conditioning temperature was 54˚C, 71˚C, and 88˚C for low, medium, and high, respectively.
[3] Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for 60 s.
[4] Corn-soybean meal-based with 10% spray dried whey.
[5] Corn-soybean meal-based diet.
[a-d]Means within a row with different superscripts differ $P < 0.05$.

Table 3.7 Effects of pellet diameter (4.0 vs. 5.2 mm) and conditioning temperature (low, medium, high) + mash on nursery pig growth performance

Pellet Diameter[2], mm		5.2			4.0				*P* =			Conditioning Temperature	
Conditioning Temp.[3]	Mash	Low	Medium	High	Low	Medium	High	SEM	Treatment	Pellet vs. Mash[4]	4.0 mm vs. 5.2 mm[4]	Linear[4]	Quadratic[4]
BW, kg													
d 0	7.61	7.63	7.63	7.61	7.59	7.63	7.64	0.09	0.999	0.868	0.997	0.879	0.851
d 7	8.69	8.99	8.79	8.81	8.81	8.91	8.94	0.16	0.848	0.264	0.835	0.868	0.791
d 14	11.7	11.8	12.0	11.6	11.4	11.9	11.9	0.25	0.625	0.790	0.654	0.506	0.283
d 21	14.9	15.1	14.9	14.6	14.2	14.6	14.6	0.27	0.407	0.429	0.101	0.812	0.598
d 28	18.9	19.3	18.7	18.4	18.2	18.5	18.6	0.34	0.430	0.522	0.212	0.439	0.942
d 0 to 7													
ADG, g	148	193	165	172	174	182	173	15.5	0.560	0.093	0.985	0.465	0.747
ADFI, g	253	256	230	239	246	259	256	14.4	0.770	0.735	0.323	0.799	0.685
G:F	0.59	0.77	0.72	0.71	0.70	0.72	0.67	0.05	0.198	0.016	0.270	0.323	0.901
d 7 to 14													
ADG, g	430	407	460	399	367	419	429	21.2	0.094	0.461	0.326	0.196	0.038
ADFI, g	534	513	527	496	483	503	518	18.1	0.469	0.174	0.472	0.621	0.430
G:F	0.81	0.79	0.87	0.80	0.75	0.83	0.83	0.03	0.130	0.996	0.495	0.108	0.027
d 14 to 21													
ADG, g	456^{a}	462^{a}	409^{bc}	428^{ab}	408^{bc}	404^{bc}	377^{c}	14.6	0.001	0.011	0.004	0.027	0.327
ADFI, g	735	675	687	674	657	669	671	19.6	0.140	0.004	0.435	0.737	0.602
G:F	0.62^{a}	0.69^{a}	0.59^{bc}	0.64^{ab}	0.63^{b}	0.60^{bc}	0.56^{c}	0.02	0.002	0.829	0.011	0.003	0.084
d 21 to 28													
ADG, g	567	599	544	548	571	560	567	21.1	0.620	0.946	0.871	0.193	0.298
ADFI, g	926^{a}	883^{ab}	840^{bc}	841^{bc}	814^{c}	834^{bc}	863^{bc}	21.5	0.012	0.001	0.335	0.875	0.470
G:F	0.61^{c}	0.68^{ab}	0.65^{bc}	0.65^{bc}	0.71^{a}	0.67^{ab}	0.65^{bc}	0.02	0.027	0.006	0.234	0.038	0.386
d 0 to 28[1]													
ADG, g	399	416	394	387	380	390	387	10.4	0.279	0.526	0.116	0.266	0.954
ADFI, g	610^{a}	582^{ab}	571^{b}	562^{b}	550^{b}	566^{b}	577^{ab}	12.4	0.045	0.003	0.479	0.770	0.939
G:F	0.65^{c}	0.72^{a}	0.69^{ab}	0.69^{b}	0.69^{ab}	0.69^{ab}	0.67^{bc}	0.01	0.003	0.001	0.073	0.015	0.858

[1] A total of 350 pigs (DNA 200 × 400; initially 7.6 kg) were used in a nursery trial with 5 pigs per pen and 10 replicates per treatment.

[2] Pellet die size was 4.0 × 22.2 mm and 5.2 × 25.4 mm for 4.0 and 5.2, respectively.

[3] d 0-14 conditioning temperature was 54˚C, 66˚C, and 78˚C for low, medium, and high, respectively; d 14-28 conditioning temperature was 54˚C, 71˚C, and 88˚C for low, medium, and high, respectively.

[4] Each contrast compared the following treatments: 1) 'Pellet vs. Mash' compared the 1 mash treatment to the 6 pelleted treatments; 2) '4.0 mm vs. 5.2 mm' compared the 3 4.0 mm pelleted treatments to the 3 5.2 mm pelleted treatments; 3) 'Conditioning Temperature' evaluated the linear and quadratic effects of the low and high conditioning temperature treatments.

[a-c]Means within a row with different superscripts have different treatment $P < 0.05$.