EVALUATION OF PELLETING PROCESS PARAMETERS ON FEED NUTRIENTS, STARCH GELATINIZATION AND PIG GROWTH PERFORMANCE

by

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B.S., Kansas State University, 2011

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Approved by:

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Abstract

In two experiments, conditioning time and temperature of swine feed were altered to determine effects of starch, vitamin retention, and swine growth performance. A third experiment evaluated methodologies for estimating gelatinized starch in swine feed. Across all experiments, diet formulation was constant. In Exp. 1, treatments were arranged in a 2 × 3 factorial design plus a control, including 2 conditioning temperatures (77 vs. 88°C) and 3 conditioner retention times (15, 30, and 60 s). A mash diet was added for a total of 7 treatments. Total starch was affected by conditioning temperature ($P = 0.04$) but not time ($P = 0.50$). Similar results were observed for gelatinized starch ($P = 0.005$ and 0.65, respectively). Sample location also affected total starch ($P = 0.0002$) and gelatinized starch ($P = 0.0001$), with the greatest increase in gelatinization occurring between conditioned mash and hot pellets. Conditioning alone did not influence gelatinization as evidenced by similar values between cold and hot mash ($P > 0.05$). Neither conditioning temperature nor time affected vitamin concentrations ($P > 0.50$). A portion of these treatments were then fed to 180 nursery pigs (PIC 327 × 1050; initially 12.6kg) in an 18-d study. Treatments included: 1) non-processed mash (negative control); 2) pelleted diet conditioned for 30 s (positive control); 3) pelleted diet conditioned for 15 s and reground; 4) pelleted diet conditioned for 30 s and reground, and 5) pelleted diet conditioned for 60 s and reground. Observed growth performance differences appear to be due to feed form, not conditioning time. Average daily gain and G:F did not differ ($P > 0.12$) between treatments, but ADFI was decreased ($P = 0.03$) as expected for pigs fed the positive control pelleted diet compared to all other diets. There were no differences ($P > 0.05$) in any growth performance variables amongst the three conditioning temperatures. In Exp. 3, it was determined that the method developed by Mason et al. (1982) was the best indicator of gelatinization in livestock

feed. In summary, feed form, but not conditioning time affected gelatinized starch and swine growth performance.

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Acknowledgements

I would like to thank DSM Nutritional Products for their contributions towards vitamin assays. I would also like to express my gratitude to Wenger Manufacturing for their contributions and expertise in regards to starch gelatinization methodologies, with a special thanks to Brian Plattner and Rhonni Mitchell. I would also like to recognize all staff and faculty involved for their support and collaboration.

Dedication

I would like to dedicate this to all my family and friends that supported me throughout this process and pushed me to get to where I am today. I would like to say a special thanks to my loving wife Kellie, for constantly encouraging me while working towards my Master's degree.

Chapter 1-Literature Review

Introduction

The energy component of animal growth is modulated by carbohydrates, fats, and protein. There are many energy sources available, but due to the economics of food animal production, cereal grains have become the basis for our food animal diets. Starch is the primary source of carbohydrates in animal feed. Starch granules are micro molecules that can range between 1 and 100 microns in diameter (Haralampou 1999). These granules are made up of glucose units. Glucose units make up amylose and amylopectin structures within the granules. Amylose are essentially linear structures, whereas amylopectin are branched.

Resistant starch

There are three categories of starch that include readily digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). The RDS tends to be easily susceptible to enzymatic digestion and is mostly digested proximal to the jejunum. This is important because the primary absorption site for glucose is in the jejunum. Slowly digestible starch is much slower to digest, although nearly all is still broken down proximal to the ileum. The remaining starch that is undigested distal to the ileal-cecal juncture is coined as RS (Sajilata et. al. 2006). The RS manages to pass completely through the small intestine without digestion or absorption. Starch digestibility can be increased by hydration or gelatinization. Starch particles in their dehydrated form are tightly packed and virtually impervious to enzymatic digestion, while gelatinized granules are susceptible to enzymatic digestion (Haralampou 1999). Thus, improvements in starch digestibility are often observed when ingredients are processed or heated, such as through

an extruder or steak flaker, to partly gelatinize the starch granules and make them more readily digestible when introducing enzymes (Holm and Bjorck 1988).

There are four types of RS that have been identified: type I, II, III, and IV (Haralampou 1999). Type I consists of very tightly packed structures, usually found in cracked or whole grains. In animal feed production, type I is primarily found in mash diets or preprocessing. Type II, which is similar to type I, has a high tolerance to enzymatic digestion, also due to a solid structure and lack of moisture, which are also found in cracked grains. Type III is known to be the crystalline form of starch that results from cooling of gelatinized starch, also known as retrogradation. This form is essentially indigestible by enzymatic hydrolysis and unaffected by hydrothermal treatment (Ring et. al. 1988; Haralampou 1999). Type IV is chemically modified to increase the formation of RS, often used to help control appetite (Sajilata et. al. 2006).

Regardless of type, all starch categories are comprised of two main structures: amylose and amylopectin, which are usually found in cereal grains in quantities of 15-20% and 80-85%, respectively (British Nutritional Foundation 1990). Certainly these concentrations vary based on different ingredient types and starch sources. Amylose and amylopectin are composed of oligosaccharides and polysaccharides and are held together by alpha-D-(1,4) and alpha-D-(1,6) linkages. Amylose has approximately 1000 straight alpha-D-(1,4) chains linking glucose molecules. Amylopectin, being much larger and branched, via alpha-D-(1,4) and alpha-D-(1,6), has approximately 4000 linked glucose molecules. These glucose chains include an aldehyde group (CHO), four stereocenters (a hydrogen bonded with a hydroxyl group), and an alcohol group (CH_2OH) (Zobel 1988). A large proportion of all starch is said to be amorphous, or lacking a uniform structure. The amorphous portion can be nearly 70% of the total starch depending on the ingredient. This starch structure can make up nearly all of amylose and the

majority of amylopectin (Sajilata et. al. 2006). However, these molecules can erupt during thermal processing when the glass transition temperature is reached with addition of hydration (Jacobs and Delcour, 1998). The water from hydration reactions, in combination with heat, causes the amorphous regions to absorb additional moisture, prompting the structure to convert from rigid to elastic in form. These structures swell and erupt, exposing potentially digestible amylose and amylopectin (Jacobs and Delcour, 1998). This may allow starch to become more readily digestible after consumption by an animal, resulting in better nutrient utilization, energy supplementation, and feed efficiency (Owens et. al. 1997).

Starch availability can be manipulated by a number of different factors, including the temperature, moisture content, acid hydrolysis, cooling process, and addition of agents such as alkaline earth metals, halide salts, sulfates, and phosphates. Hydrothermal processing steps are used primarily to improve starch availability and can consist of baking, extruding, pelleting, autoclaving, or any other heating and cooling steps involving moisture (Patel et. al. 2005; Singh et. al. 2010). Starch begins to gelatinize when the granules are heated from 40°-120°C. The temperature required for gelatinization is dependent on the starch source and its amylose content (Haralampou 1999). The heat, with addition of moisture over short periods of time, allows for gelatinization of starch (Sievert et. al. 1989a). The gelatinization process breaks molecular bonds, such as hydroxyl groups and hydrogen bonds, which allows for further enzymatic digestion (Zobel 1988). Although gelatinization increases available starch content, other factors must be considered.

The gelatinization process, followed by cooling, may result in starch retrogradation (Hoover 1995). Retrogradation causes reformation of crystallite through hydrogen bond linkages. These hydrogen bonds form double helices, which merge together and reconstruct the molecules

to form RS (Morris 1990). During the crystallization process, both amylose and amylopectin are reconstructed. Amylopectin, after crystallization, can be easily returned to its digestible state, whereas amylose requires much higher temperatures (100°-160°C). Furthermore, amylose retrogradation may cause damage to the nutrient composition, which is primarily due to the high density of amylose and correlated extreme temperatures (Miles et. al. 1985). There again, amylopectin being less dense in nature, is more likely to form crystallite structures if the ratios of amylopectin to amylose are in favor of the amylose. Because amylose is linearly structured, it can pack very tightly when crystallites bond, making it difficult to partition post-crystallization (Zobel 1988). However, amylopectin crystallite bonds are less dense because the branching nature does not allow for it to pack as closely as the amylose. This structure enables amylose to boil at a lower temperature and consequently its crystallization is more reversible, even as low as room temperature, depending on the molecular associations (Zobel, 1988).

Multiple reheating serves to increase resistant starch (Szczodrak et. al. 1992). Upon the first thermal processing step, gelatinization occurs and RS forms. After repeated heating to gelatinization temperatures and subsequent cooling, the overall RS increases. The RS is very heat tolerant, and accordingly, takes high levels of heat and moisture to rehydrate (Haralampou, 1999). In addition to repeated gelatinization, the rate of cooling also affects RS content (Niba, 2003). In general, research supports that the faster the cooling process, the higher the RS yield (Miles et. al. 1984). This is caused by accelerated crystallization formation.

To achieve optimum starch utilization, it becomes imperative to select the correct starch sources. High amylose starches reduce overall weight gain due to higher levels of RS post processing (Bird et. al. 2007). Therefore, if ingredients are to be thermally processed, lower amylose starches should be selected. In one experiment comparing pig diets with either high or

low amylose starch, the low amylose starch was nearly completely digested proximal to the large intestine and had only 3.66 gram of starch excretion per day, while the high amylose starch had approximately 59% more (8.85g starch/day) digesta upon excretion, indicating higher levels of RS for high amylose diets (Brown et. al. 1997).

Reducing resistant starch

Resistant starch is formed during recrystallization of gelatinized starch. Franco et. al. (1995) reduced RS by manipulation of water:starch ratios, which have been shown to have a profound effect on digestible starch. When comparing diets with moisture levels of 18 and 27% through hydrothermal processing, the higher moisture content reduced crystallization formation during retrogradation, thus reducing RS content, indicating more digestible starch. Additional methods for decreasing RS involve inclusion of simple and complex sugars, such as glucose, ribose, sucrose and maltose (Buch and Walker 1988; Eerlingen et. al. 1994). During the retrogradation process, bonding starch molecules interact with these supplemented sugars, reducing RS bonding and helix formation (Sajilata et. al. 2006). Lipid-amylose complexes have been shown to be more susceptible to enzyme digestion upon retrogradation. (Szczodrak 1992; Sajilata 2006). However, other research has shown that lipid-amylose starch was less digestible during the gelatinization phase or shortly after, until post retrogradation, in which the levels of digestible lipid-amylose complexes reverted back and became readily digestible to α-amylase (Cui et. al. 1999). However, based on what we know about retrogradation and crystallization, this theory becomes muddled due to the rapid formation of crystallite structures generally associated with retrogradation. The theories on lipid-amylose complexes are inconclusive, as to whether they are considered as a type of RS or not (Sajilata 2006).

Animal performance

Processing of starch and its effects on animal performance has been widely researched within the last 60 years. Numerous ingredients have been evaluated under a number of different processing conditions. Some of the diets used in these processing studies have also been utilized in animal feeding experiments, allowing for a relatively broad knowledge base of information on the correlation of gelatinized starch and subsequent animal performance (Hongtrakul et. al. 1998; Vicente et. al. 2008; Mudd and Perry 1969).

Unfortunately, there are many research studies that contradict each other regarding the influence of starch gelatinization on food animals (Moritz et. al. 2005; Fancher et. al. 1996; Parera et. al. 2010). When weighing the body of evidence, it appears as though there may be limited to no improvements in animal performance in relation to degree of starch gelatinization (Hongtrakul et. al. 1998). The discrepancies among experiments may be influenced by diet differences and processing variables. These differences may also be related to variations in animal subjects, and more specifically, their genetic makeup. Much of the research body was evaluated over 10 years ago with animals of vastly different genotypes, and therefore nutrient demand, than animals today. Thus, there is merit to reevaluating this processing research in modern animal genotypes. However, it is difficult to make these connections between previous and modern experiments without performing an identical older study with today's more modern pigs. This would require multiple replications of the same study to ensure variability in methods was not apparent.

Effects of processing

Starch gelatinization, and its effects on animal performance, is well known due to the immense amount of research in this area. Varying degrees of starch gelatinization occur due to

different processing methods. Processing methods of animal feeds typically include pelleting, expansion, extrusion, and, in some cases, baking (Behnke 1996). Pelleting is a processing method with a limited cooking effect. During processing, mash feed passes through a steam conditioner where heat and moisture is applied via steam. The retention time, or the time the mash feed remains in the conditioning chamber, generally ranges from 15-30 seconds. The mash feed is then pressed through a steel die drilled with hundreds of holes to create the pellets, resulting in its final form. The end product of this process yields pelleted feed that generally has a higher bulk density than that of extruded or mash feed.

Extrusion is primarily used in the pet food and aquaculture industry. Extrusion allows the production of physically stable kibble that can absorb high levels of fat without losing its structural integrity (Rokey 1994). Extrusion is a high energy input process that utilizes steam and mechanical energy to reach acceptable temperatures for expansion cooking; unfortunately, it also lacks production rates of other large scale feed processing methods such as pelleting. However, this process offers unique features such as control of mechanical energy, expansion of products, buoyancy, and shape characteristics (Rolfe et. al. 2001).

Lastly, expansion was developed to enhance degree of cook, or gelatinization, before the feed entered the pelleting system. Levels of high heat, pressure, and friction that soften feed particles. Upon reaching normal atmospheric temperatures, the starch particles break the hydrogen bonds of starch molecules apart, similar to the extrusion process (Fancher et. al. 1996). This provides the animal a better opportunity for energy utilization. This processing method is not commonly used in the U.S. food animal industry due to its higher processing costs. It requires high levels of mechanical energy to reach temperatures that provide fully gelatinizing temperatures. Because gelatinization does not provide enough animal performance to offset the

cost of running this type of equipment, most producers have chosen to sacrifice this improvement, utilizing the effects associated with pelleting instead.

While it is apparent that processing affects starch gelatinization to varying degrees, the question remains if the improvements from processing can be contributed to starch gelatinization in regards to animal performance. Perhaps other variables such as diet form or reduced trypsin inhibition are responsible for improved animal performance. Based on the literature, it has become difficult to determine if starch gelatinization provides significant effects to animal performance in the early stages of life (Lindemann et. al. 1986; Jones et. al. 1995). It is expected that as animals become older, their digestive systems become adaptable. Therefore starch gelatinization may have application in early nursery pig and chick diets.

Jones et. al. (1995) compared pelleted and extruded corn soy based diets in poultry. Treatments included pelleted, extruded, and crumbled diets in whole form. Body weights (BW) were recorded from 0 to 6 weeks of age. Birds fed crumbled treatments showed no significant ($P > 0.05$) difference in average BW. Those fed intact feed showed extruded kibble to have significantly ($P < 0.05$) greater BW than those fed pellets for the first 2 weeks, then significance was lost. However, no significance ($P > 0.05$) was observed in the case of gain:feed (G:F). When comparing pelleting and extrusion of diets with common particle size, there were no significant differences detected. Both pelleted and extruded diets were conditioned and cooked at 82°C and pressed through a 5/32” diameter hole, then diets were reground to a similar particle size. The retention times for each processing method were not listed. This would have established a good comparison between pelleting and extrusion from an equipment perspective. While the experiment was ideal to compare the equipment, it was not necessarily realistic because most extrusion is conducted between 100 to 200°C (Singh et. al. 2007; Bhatnagar and Hanna, 1994).

However, gelatinization can begin to occur at temperatures of 70-80°C, but full gelatinization is far from reachable (Singh et. al. 2007). Regardless, broilers had greater average daily gain (ADG) during week 1 when fed whole extruded feed ($P < 0.05$), compared to those fed mash or whole pelleted feed. This improvement may be attributed to increased retention time during the extrusion process, leading to longer cook time and thus better expression of starch. It is also possible that the mechanical pressure from extrusion processing was the cause for increased starch gelatinization. Interestingly, this significance failed to extend past the initial starter period. The extruded fed birds were still heavier than the mash-fed birds, but had no significant increase in performance when compared to the pelleted-fed birds. This suggests that starch gelatinization may improve animal performance in chicks or young animals with developing digestive tracts, but has limited effects beyond early diets.

Mortiz et. al. 2005 compared pelleted and extruded corn-based chick diets, and found that chicks fed diets with extruded corn had greater ADG compared to those fed diets manufactured with pelleted corn. Unfortunately, this study was only 3 weeks, which leaves speculation as to whether this significance would have held true if the birds were grown to market weight. Furthermore, the ADG was driven by ADFI in birds fed extruded corn-based diets, so the difference does not appear to be related to nutrient efficiency or availability of starch. It is important to point out that this experiment was confounded by particle size, which, as previously covered, alters nutrient availability. The pelleted corn was 256 microns (μm) in diameter compared to the 487 μm for extruded corn. Importantly, birds tend to perform better with particle size ranging from 600-900 μm (Amerah et. al. 2007).

While starch gelatinization may or may not be significant in early livestock and poultry diets, studies show that the effects of processing methods in later phases may not be as effective.

For example, van Der Poel et. al. 1990, used an in vitro starch digestion and in vivo digestibility experiments to examine the effects of processed vs. unprocessed maize. The in vivo experiment included pigs that were 38 days old. The unprocessed diet yielded approximately 20% digestible starch, whereas, in the extruded diet the starch was nearly 90% digestible. The diets consisted of extruded vs. unprocessed maize starch, which were both dry-pelleted to maintain a uniform particle size. It was not described whether or not corn was ground to a similar particle size before pelleting. There were no significant differences between the two diets, with the exception of ileal starch digestion ($P < 0.01$), which was improved in extruded diets by 1%. It is unclear and unlikely whether a 1% increase in starch digestion, although statistically significant, would truly be of biological significance. Animal performance characteristics were measured in a follow-up experiment and no significant differences were observed for ADG or G:F. The one exception ($P < 0.05$) was from the 5-8 week period when G:F ratio differed by 0.05 units. Overall, this animal performance study would indicate that extrusion processing is not effective for pigs at 3 weeks of age or older. It appears that starch gelatinization in corn-based diets has no significant impact on older animals. This is further proven by Sloan et. al. (1971), who used poultry as subjects to feed varying levels of extruded corn and sorghum up to 28 days of age. The treatments included ground corn, 50%/50% ground/extruded corn, 100% extruded corn, and reflective treatments for sorghum. Of these 6 treatments, there were no significant effects between diets ($P > 0.05$). However, both corn and sorghum diets tended to have improved F:G ratios as extruded diets replaced ground portions.

Hongtrakul et. al. (1998) compared different ingredients (sorghum, wheat flour, broken rice, and cornstarch) with and without extrusion processing on the effectiveness for each treatment. Each treatment, was ground and pelleted after processing to maintain uniformity. The

first experiment concluded that young weanling (0-7 days) pigs fed cornstarch had significantly lower ($P < 0.05$) ADG than other diets. Pigs that consumed broken rice or grain sorghum had significantly greater ($P < 0.05$) average daily feed intake (ADFI) and grain sorghum had significantly greater G:F ratio than those fed cornstarch. Unfortunately, particle size of ingredients before pelleting was not included in the paper description. This would have aided in determining whether particle size was a factor in the digestion. The results concluded no significant differences between extruded or non-extruded diets. This contradicts other research findings (Lindemann et. al. 1986, Jones et. al. 1995, and Mortiz et. al. 2005) but is comparable to others (van Der Poel et. al. 1990) who determined there were no effects in piglets fed extruded diets from 3 to 8 weeks old.

In a second experiment by Hongtrakul et. al. (1998) diets were pelleted, extruded, or expanded, then re-pelleted to maintain feed uniformity. Regrettably, it was not explained thoroughly whether the pelleted diet was pelleted twice to create consistency or if the extruded and expanded diets were processed twice. The results showed significant increases ($P < 0.05$) in ADG and ADFI when complex protein diets were extruded compared to all other complex and simple diets. The performance improvements from extrusion may not be due to gelatinized starch, but rather from the reduction of trypsin inhibition. An important factor in this study was that extruded diets reached nearly 140°C, while those expanded or pelleted were only 60°C. Most expanders would normally be run at a higher temperature and pressure. Interestingly, feed efficiencies were not altered ($P > 0.05$), suggesting limited improvement in nutrient or starch digestibility. Notably, starch gelatinization was measured to determine the degree that starch gelatinization affected growth in early weanling pig performance. The diets were balanced corn-based diets with varying degrees of gelatinized corn. The treatments consisted of corn at 14.5,

38.7, 52.7, 64.4, and 89.3% gelatinization. The results were difficult to interpret in that the minimum and maximum (14.5% and 89.3%, respectively) degrees of starch gelatinization performed better, for ADG ($P = 0.04$) and ADFI ($P = 0.01$), than any of the other three intermediate treatments. Also G:F was not significant ($P = 0.77$) across treatments, which also suggests limited improvement in digestibility. The 14.5% gelatinization was used as a control in that it did not undergo any processing measures. Due to the inconsistences from the data, the results suggest that analytical error occurred during the cook step. It is also important to mention that even though performance was increased at the lowest and highest degree of gelatinization, the lowest (14.5%) was not extruded, and still showed better ADG, ADFI, and G:F than any of the gelatinized treatments. In all other respects, this data may conclude that starch gelatinization has no effect on early weanling pigs, so these differences may be due to other variables.

Starch Analysis

Approved methods commonly used to measure total starch and starch gelatinization in food animal research include the Association of Analytical Communities (AOAC) Official Method 996.11, AOAC Official Method 979.10, the American Association of Cereal Chemists International (AACC) 76-13.01, and AACC 76-11.01.

AOAC 996.11

AOAC Official Method 996.11 utilizes amyloglucosidase and α-amylase that begins by hydrolyzing the starch and adding the α-amylase to break it down to maltodextrins. The samples are heated to 95-100°C to maximize effectiveness of the enzymes. Then amyloglucosidase is added to hydrolyze the maltosaccharides, cleaving the glucose.

Peroxidase/oxidase reagent is used to determine glucose concentration, or total starch. Samples should be run in duplicate. The procedure begins with weighing a known ground

sample weight to approximately 100 mg and placing into test tubes. Next add 0.2 mL of 80% ethanol to ensure the product is wet and stir using a test tube vortex. Then add α-1 amylase and vortex again. Tubes are then put into a boiling water bath for 6 minutes while being mixed every two minutes. Sample tubes are then placed in a 50°C water bath for 5 minutes to rest. At the end of this time, 200 mM sodium acetate buffer is added to stabilize pH, and then amyloglucosidase solution is added and tubes are vortexed. Sample tubes are then covered and placed back into 50°C water bath for 30 minutes. Then, the content of test tubes is adjusted to 10 mL volume with distilled water. The samples are centrifuged at 3,000 rpm for 10 minutes, then 1 mL aliquots are removed and further diluted to 10 mL using distilled water. From the aliquot, 0.1 mL is pipetted into separate test tubes. The glucose peroxidase-oxidase reagent is added to test tubes, which are then placed in 50°C water bath for 20 minutes. Lastly, approximately 0.3 mL is pipetted into a 96-well plate and samples are measured for absorbance at 510 nm against the reagent blank. Total starch is calculated using the following formula where A = absorbance against reagent blank, F = conversion of absorbance to micrograms of glucose (100µg glucose/absorbance for 100 µg glucose), 1,000 = is volume correction (0.1 mL taken from 1000 mL), 1/1,000 = conversion from µg to mg, 100/W = conversion to 100 mg test portion, 162/180 = conversion from free glucose, as determined, to anyhydroglucose, as occurs in starch.

$$\text{Total starch (\%)} = A \times F \times 1000 \times 1/1000 \times 100/W \times 162/180$$

$$= A \times F/W \times 90$$

AOAC 979.10

The AOAC Official method 979.10 is a glucoamylase method for determination of starch. First, moisture is determined from the ground sample. Then, 0.5 grams of sample is added to a volumetric flask. Water is added and the solution is adjusted to a pH of approximately 5 to 7.

The sample is then boiled for 3 minutes followed by autoclave cooking for 60 minutes at 135°C. Once the sample has been cooked thoroughly, it is removed and cooled to 55°C. At this point, acetate buffer is added to stabilize an acidic pH, and water is added to reach a total weight of solution. Then, the volumetric flask is placed into a shaking water bath of 55°C to optimize glucoamylase activity. Glucoamylase is added and incubated for 2 hours. The solution is then filtered into 250 mL flask, rinsed and filled to volume. Next, aliquots of 20 to 60 µg are placed into test tubes and 2 mL of enzyme-buffer-chromogen mixture is added. Tubes are shaken and incubated in the dark at 37°C for 30 minutes. During this time, the color should develop for calorimetry absorbance. After 30 minutes, 2 mL of sulfuric acid is added to halt the reaction and absorbance is measured at 540 nm. The following formula is used to determine total starch, where E = test sample (g), M = microgram D-glucose obtained using the standard curve (µm), V_0 = the initial aliquot sample from test tubes (mL), MS = is percent solid in the test tube, and V_1 = final volume from the test tube sample (mL).

$$\text{Percent starch} = 0.9 \times (M/10^6) \times (V_1/1) \times (250/V_0) \times (100/E) \times (100/MS)$$

AACC 76-13.01

In the case of high amylose maize, pretreatment involves inclusion of dimethyl sulfoxide and cooking to 100°C. Dimethyl sulfoxide is a solvent that dissolves polar and non-polar bonds of starch molecules without recrystallization. Then, the addition of α-amylase begins the initial breakdown of sugars, and splits the large molecules leaving maltodextrins and free glucose. Maltodextrin are chains of glucose, which makes them easily susceptible to glucoamylase. Once the glucoamylase is added, glucose molecules can be cleaved from the maltodextrin chains. Measurement of glucose is conducted using oxidase/peroxidase reagent, which can be viewed calorimetrically to determine glucose content. First, 100 mg of ground sample to test tubes. Next,

tubes are wetted with 80% ethanol, which aids in sample separation, followed with test tube vortex. Then, α-amylase/50 mM MOPS buffer reagent is added to the test tubes and mixed using the vortex. Samples are then placed in a boiling bath for 5 minutes to activate the enzyme solution. Test tubes are then transferred to a 50°C bath, and acetate buffer is added followed by amyloglucosidase reagent. Tubes are vortexed again and placed back into the 50°C bath for 30 minutes. Then, sample tubes are weighed and adjusted to 10.0 g plus weight of the tube (weight of tube plus 10.0 g sample). Next, the tubes are centrifuged at 3000 rpm for 10 minutes. Then 1.0 mL of supernatant (liquid) is removed and added to 9 mL of distilled water and mixed. The diluted solution (0.1 mL) is transferred to a new test tube in duplicate. Each test tube has GOPOD reagent added and is placed in a water bath at 50°C for 20 minutes. Absorbance is read at 510 nm against a blank sample. The following formula is used to determine starch content, where ΔE = absorbance against blank solution, F = 100 µg of glucose / absorbance for 100 µg of glucose, 100 = volume correction (0.1 mL from 1.0 mL), 10 = dilution, 1/1,000 = conversion from µg to mg, 100/W = starch as a percentage of flour weight, W = weight of sample in mg, and 162/180 = adjustment from free glucose to anhydro- glucose (McCleary et. al. 1997).

$$\text{Starch (\%)} = \Delta E \times F \times 100 \times 10 \times 1/1000 \times 100/W \times 162/180$$

$$= \Delta E \times F/W \times 90$$

AACC 76-11.01

Starch samples are boiled for approximately 3 minutes, then cooked in an autoclave at 135°C for 60 minutes. This provides the necessary gelatinization step of the starch in the sample. The procedure begins by adding 0.5 to 1.0 g of ground sample to Erlenmeyer flasks, then adding 25 mL of distilled water to adjust pH and separate particles. Samples are boiled for 3 minutes, and then placed in an autoclave at 135°C for 1 hour. Samples are moved to a 55°C water-bath,

where acetate buffer is added to stabilize pH and water is filled to achieve a total weight of 45 g. Flasks are then placed in an agitating water bath at 55°C for 1 hour and glucoamylase solution is added. The solution is then filtered into 250 mL flasks and filled to volume with distilled water. Then, 1 mL of solution is transferred to a test tube. Next, the enzyme buffer-chromogen solution is added and tubes are vortexed, then set in dark area at 37°C for 30 minutes and allow color to develop. The reaction is halted after 30 minutes with sulfuric acid, then absorbance is measured at 540 nm. The following formula is used to determine starch content, where E = weight of sample (g), M = weight of D-glucose (µg) from the standard curve, V_0 = volume of aliquot from the 250 mL flask (mL), MS = dry weight of sample (%).

$$\text{Starch (\%)} = 0.9 \times M/10^6 \times 250/V_0 \times 100/E \times 100/MS$$

$$= 2.25 \times M / (V_0 \times E \times MS)$$

Total starch and gelatinized starch were determined according to Mason et al. (1982). Briefly, one 0.5 g subsample was hydrolyzed in 25 mL distilled water for 20 minutes at room temperature while a second 0.5 g subsample was boiled with 25 mL distilled water for 20 minutes. The samples were then allowed to cool to ambient temperature. Next, 10 mL of acetate buffer solution was added to each flask. Then samples were hydrolyzed with 5 mL of glucoamlylase, and incubated at 40°C for 70 minutes. After incubation, 5 mL of trichloroacetic acid was added to halt hydrolysis, samples were cooled to room temperature, and then mixed with approximately 50 mL distilled water to a final volume of 100 mL. Free D-glucose was then measured using a glucose analyzer (Model 2700, YSI, Yellow Springs, OH) YSI 2700. The resulting quantity of free glucose determined in the cold water hydrolyzed sample represented the starch that was gelatinized during processing while the cooked sample represented the total

starch in the sample. The following formula was developed to measure gelatinized starch, where A = absorbance, B = absorbance of mixture after 30 minute enzymatic hydrolysis, C = absorbance of mixture after 60 minute hydrolysis, k = absorbance of 1% intact starch after 30 minute digestion (constant), and Y = gelatinized starch percentage (Thivend et. al. 1972).

$$Y = 100 (B - k) / (A – k), \text{ where } k = A (C – B) / A -2(B + C)$$

And total starch is determined by the following:

$$\text{Total starch (\%)} = ((\text{glucose} \times 0.90) / (\text{sample wt. (dry basis)})) \times 100$$

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Chapter 2 - Evaluation of conditioning time and temperature on gelatinized starch and vitamin retention in a pelleted swine diet

Abstract

Two key feed processing parameters, conditioning temperature and time, were altered to determine their effects on concentration of gelatinized starch and vitamin retention in a pelleted nursery swine diet. Diet formulation (corn-soybean meal-based with 30% distillers dried grains with solubles) was held constant. Treatments were arranged in a 2 × 3 factorial design plus a control with two conditioning temperatures (77 vs. 88°C) and three conditioner retention times (90, 60, and 30 rpm to represent 15, 30, and 60 s actual conditioning times). In addition, a mash diet not subjected to conditioning served as a control for a total of 7 treatments. Samples were collected after conditioning but prior to pelleting (hot mash), after pelleting but prior to cooling (hot pellet), and after pelleting and cooling (cold pellet) and analyzed for total starch, gelatinized starch, and riboflavin, niacin, and vitamin D_3 concentrations. Total starch was affected by conditioning temperature ($P = 0.04$) but not time ($P = 0.77$), while both substantially altered gelatinized starch. However, gelatinized starch was not further improved if diets were conditioned longer than 15 s ($P > 0.05$). The interaction between conditioning temperature and time affected both total starch and gelatinized starch ($P = 0.01$ and 0.01, respectively), with both high conditioning temperatures and longer conditioning times resulting in greater gelatinization. Sample location also affected both total starch and gelatinized starch ($P = 0.02$ and 0.0001, respectively), with the greatest increased in gelatinized starch occurring between hot mash and hot pellet samples. Interestingly, there was no increase in gelatinized starch due to conditioning as evidenced by similar values for cold mash vs. hot mash samples (17.7 vs. 20.0%, respectively;

$P > 0.05$). Finally, neither conditioning temperature nor time affected riboflavin, niacin, or vitamin D_3 concentrations ($P > 0.50$). In summary, both increasing conditioning temperature and time effect gelatinized starch, but not to the extent of physical pelleting.

Key words: conditioning, feed processing, gelatinization, pelleting, temperature

Introduction

Processing of feed ingredients by sieving, grinding, cooking, and pelleting improves nutrient availability for animal consumption. Specifically, pelleting has been shown to improve finishing pig G:F by increasing ADG while decreasing ADFI (Medel et. al., 2004). While it is widely accepted that pelleting improves G:F in modern swine genotypes by 4-8%, there is some dispute as to the source of this improvement because pelleting provides both a degree of cook from the conditioner as well as a diet form change by pressing the hot mash through a pellet die, allowing the feed to maintain a pelleted form (Behnke 1996; Miller, 2012). The process as a whole increases the gelatinized starch, or the quantity of starch molecules that have swollen during the heating process and irreversibly dissolved to allow for more hydrogen bonding sites (Jenkins and Donald, 1998). It is important to evaluate both the location and conditions that maximize gelatinized starch so that these conditions may be optimized by feed processors in an attempt to maximize animal feed efficiency.

While conditioning and pelleting ingredients can improve the availability of some nutrients, such as with starch gelatinization, the potential negative effects of these conditions should also be considered. This is particularly true regarding nutrients that may be sensitive to heat, such as vitamins. Specifically, retention of some vitamins can be as low as 50% when subjected to extreme temperatures (Beetner, 1974;Guzman-Tello & Cheftel, 1990; Killeit, 1994).

Thus, the objectives of this experiment were to determine the effects of conditioning temperature and time on both concentration of gelatinized starch and vitamin stability, as well as to determine the locations within the pelleting process that are most responsible for altering the concentration of gelatinized starch.

Materials and Methods

A single phase 3 swine nursery diet with 30% distillers dried grains with solubles (Table 1) was manufactured according to 7 different methods in an effort to elucidate differences in gelatinized starch concentrations and vitamin retention associated with pelleting and to further evaluate the individual sites in which starch gelatinization occurs. Research was conducted at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 × 28.6 mm die and a conditioner-feeder (Model C18LL4/F6, California Pellet Mill Co., Crawfordsville, IN). Treatments were arranged in a 2 × 3 factorial design plus a control with two conditioning temperatures (77 vs. 88°C) and three conditioner retention times (90, 60, and 30 rpm to represent 15, 30, and 60 s actual conditioning times). In addition, a mash diet not subjected to conditioning served as a negative control for a total of 7 treatments. There were three manufacturing runs per treatment, run order was completely randomized, and the pellet mill and conditioner were completely shut off between each run. Treatment samples were analyzed for proximate analysis, total starch, gelatinized starch, and riboflavin, niacin, and vitamin D_3 concentration. In addition, samples were collected after mixing but prior to conditioning (cold mash), after conditioning but prior to pelleting (hot mash), after pelleting but prior to cooling (hot pellet), and after pelleting and cooling (cold pellet) and these location samples were analyzed for total starch and gelatinized starch.

Estimation of proximate analysis was completed by Fourier Transform Near-Infrared Spectroscopy (FT-NIR; Bruker Optics multi-purpose Analyzer, Billerica, MA). Total starch and gelatinized starch were determined according to Mason et al. (1982). Samples were ground through a 0.5 mm screen. Briefly, one 0.5 g was hydrolyzed in 25 mL distilled water for 20 minutes at room temperature while a second 0.5 g was boiled with 25 mL distilled water for 20 minutes. The samples were then allowed to cool to ambient temperature. Next, 10 mL of acetate buffer solution was added to each flask. Then samples were hydrolyzed with 5 mL of glucoamlylase, and incubated at 40°C for 70 minutes. After incubation, 5 mL of trichloroacetic acid was added to halt hydrolysis, samples were cooled to room temperature, and then mixed with approximately 50 mL distilled water to a final volume of 100 mL. Free D-glucose was then measured using a glucose analyzer (Model 2700, YSI, Yellow Springs, OH) YSI 2700. The resulting quantity of free glucose determined in the cold water hydrolyzed sample represented the starch that was gelatinized during processing while the cooked sample represented the total starch in the sample. Samples were analyzed for vitamin concentration by DSM Nutritional Products (Parsippany, NJ) by using a combination of HPLC and tandem mass spectrometry (Schadt et al., 2012).

Data were analyzed as a completely randomized design using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with run as the experimental unit. Conditioning temperature and time served as fixed effects. The results were considered significant if $P < 0.05$, or a trend if $P < 0.10$.

Results and Discussion

Analyzed nutrient composition revealed differences between mash and thermally-processed treatments (Table 2). Specifically, moisture content rose 0.5 to 0.8% due to conditioning, which is not surprising considering that steam was added during thermal processing. Percentage crude fat increased 0.7 to 0.8% and crude protein 3.1 to 3.5% in thermally-processed treatments compared to the mash diets. Even when placed on a dry matter basis, crude fat and crude protein concentrations of thermally-processed treatments were 0.6 to 0.7% or 2.6 to 3.0% greater than the mash treatment, respectively. This was difficult to explain and limited research is available that might justify why this occurred. Theoretically, thermal processing should not affect crude nutrient concentrations. Arndt et. al. (1999) showed that soy flour exposed to high cooking temperatures decreased protein solubility due to protein denaturation; however, one would still not expect this difference to be observed in a crude protein value. Instead, these differences might suggest that FT-NIR may not accurately predict nutrient concentrations in thermally-processed livestock feeds. More research is needed to confirm this theory.

Production rate and pellet durability were recorded throughout each processing run (Table 1.3). Pellet mill production rate was relatively stable throughout all treatments and varied by no more than 3%. Pellet durability index was also consistent throughout all treatments. The high pellet quality amongst treatments should be noted as one would traditionally expect increased conditioning time to result in improved pellet quality.

Total starch was affected by the main effect conditioning temperature ($P = 0.04$), but not time ($P = 0.50$; Table 4). While significant, the total starch values due to temperature differences were numerically (35.2 and 36.1) similar, so the biological significance of this difference is

questionable. The literature denotes that total starch values should be the same throughout processing (Muir et. al., 1995; Theurer et. al., 1999).

In the case of gelatinized starch, significant differences ($P = 0.003$) were observed across all treatments (Table 1.5), demonstrating that temperature and retention time are major factors in gelatinization. Main effects altered percentage gelatinized starch (P = 0.0001) respect to temperature, but not retention time (P = 0.65), suggesting that greater temperature resulted in increased gelatinized starch. Stevens (1987) observed an opposite effect, where a temperature increase during pelleting resulted in lower levels of gelatinization. This may be explained by the method used to evaluate the gelatinized starch. Stevens (1987) utilized differential scanning calorimetry, while the current study used enzymatic methods for quantification of gelatinization. Differential scanning calorimetry is a robust method for evaluation of starch gelatinization, but is most reliable at detecting gelatinization in isolated starch compared to compound mixtures of ingredients (Sopade et al., 2004). Meanwhile, the method used in the current study was developed specifically for finished feeds with multiple ingredients. Additionally, the method used by Stevens (1987) utilized a substantially smaller sample size (0.002 vs. 0.5 g). Thus, sampling error may further affect the precision of the assay results compared to a larger batch of compound feed.

Neither temperature ($P > 0.55$) nor time ($P = 0.50$) altered riboflavin, niacin, or Vitamin D_3 concentrations. This was expected because processing up to 88°C for 60 s is not thought to affect vitamin stability. However, it has been argued that vitamin stability has not been thoroughly validated (Lešková et al., 2006). Niacin destruction occurs during the precooking process when it may leach into steep water, but processing alone is not expected to destroy the vitamin (Lešková et al., 2006). However, cooking of beef products to 57°C internal temperature

has demonstrated a loss of both riboflavin and niacin (Dawson et al., 1988). Thus, degradation of B-vitamins can occur at low temperatures; however, conditioning times relevant to animal feed pelleting do not appear to be long enough to affect vitamin concentrations.

Individual treatment effects from conditioning temperature and retention time are shown in Table 1.4. There was no treatment effect on total starch ($P = 0.15$), but differences existed in gelatinized starch ($P = 0.003$). As expected, gelatinized starch was lower ($P = 0.05$) in mash diets than any thermally-processed treatment. The treatment with numerically the greatest concentration of gelatinized starch was the feed processed with the highest temperature and longest retention time, which was greater ($P < 0.05$) than that processed at the lower temperature for either 15 or 30 s. This is in agreement with Lund (1984), who found that temperature is the single variable most responsible for starch gelatinization. Again, no effects were observed in riboflavin, niacin, or vitamin D_3 concentrations ($P = 0.98$, 0.78, and 0.92, respectively).

Finally, both total starch and gelatinized starch were altered by sample location ($P = 0.0002$ and 0.0001, respectively; Table 1.6). Mash samples collected prior to conditioning or pelleting had lower ($P = 0.0002$) total starch than conditioned mash or hot pellet samples. Meanwhile, both cold and hot mash samples had lower ($P = 0.0001$) gelatinized starch compared to hot or cold pellet samples. Notably, there was no difference in gelatinized starch between cold mash and hot mash samples. This suggests that conditioning alone did not have a significant impact on degree of gelatinization. True starch gelatinization is thought to occur at temperatures above 70°C and moisture above 25% (Lund, 1984). While the temperatures evaluated in the current experiment were above this point, the maximum moisture addition from steam in a conditioner from steam is expected to be only 6% (Leaver, 1988). This agrees with the moisture concentrations of cold mash and hot mash observed in the current experiment, which were 11.1

and 17.1%, respectively. Moisture addition during conditioning is therefore far below the 25% requirement for true starch gelatinization to occur, which confirms that conditioning alone has very little effect on starch gelatinization, regardless of temperature.

The difference in starch gelatinization between hot mash and hot pellet samples suggests that starch gelatinization does occur due to the frictional or mechanical heat of the pelleting process. This is in agreement with Wang (1993), who showed that mechanical energy was effective at increasing starch availability during extrusion processing at 50°C and Skoch et. al. (1981), who demonstrated that when cold mash was pelleted without the addition of steam, a significant amount of starch damage was observed due to frictional heat created as feed passed through the die holes through feed compression. Thus, it appears that the formation of pellets results in increased starch availability, rather than increases from temperature or conditioning.

In summary, this research found that only conditioning temperature, not time, affected available starch. There were no effects of conditioning temperature or time on vitamin retention. Finally, increasing conditioning temperature and time increased gelatinized starch, but not to the extent of physical pelleting.

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Figures and Tables

Table 1.1 Calculated diet composition (as-fed basis)

Ingredient, %	%
Corn	40.55
Soybean meal	25.25
Corn distillers dried grains with solubles	30.00
Poultry fat	0.50
Monocalcium phosphate	1.03
Limestone	1.30
Salt	0.35
L-lysine-HCL	0.45
DL-methionine	0.07
L-threonine	0.09
Vitamin premix[2]	0.25
Trace mineral premix[3]	0.15
Total	100.00
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lysine	1.26
Isoleucine:lysine	65
Leucine:lysine	156
Methionine:lysine	33
Methionine & cysteine:lysine	58
Threonine:lysine	62
Tryptophan:lysine	17.0
Valine:lysine	74
Total lysine, %	1.47
ME, kcal/kg	3,296
SID lysine:ME, g/Mcal	3.82
CP, %	24.1
Ca, %	0.76
P, %	0.69
Available P, %	0.41

[1]A single diet formulation was manufactured according to different feed processing parameters to determine effect of processing on gelatinized starch or vitamin retention.
[2]The vitamin premix provided the following per kg of feed: vitamin A, 4,400,000 IU; vitamin D3, 550,000 IU; vitamin E, 17,600 IU; vitamin B12, 15.4 mg; menadione, 1,760 mg; riboflavin, 3,300 mg; D-pantothenic acid, 11,000 mg; niacin, 19,800 mg.
[3]The trace mineral provided the following %: iron, 7.3; zinc, 7.3; manganese, 2.2; copper, 1.1; iodine, 198 ppm; selenium, 198 ppm.

Table 1.2 Analyzed nutrient concentrations

Condition temp, °C:	Mash	77			88		
Condition Retention, rpm:	Mash	15	30	60	15	30	60
Nutrient, %							
Moisture	11.1	11.6	11.8	11.6	11.6	11.7	11.7
Fat	4.3	5.1	5.0	5.0	5.0	5.1	5.0
Protein	21.9	25.0	25.0	25.2	25.4	25.2	25.4
Fiber	6.7	4.9	5.1	4.9	4.9	5.0	4.8
Ash	3.9	3.5	3.4	3.5	3.6	3.5	3.6
Starch	29.0	29.4	29.0	29.1	28.6	28.5	29.2

Table 1.3 Treatment manufacturing production specifications

Conditioning temperature, °C:		77			88		
Conditioning time, s:	Mash	15	30	60	15	30	60
Pellet mill production rate, kg/hr.	-	716	719	734	725	740	737
Pellet durability index	-	89.6	95.0	91.0	93.0	92.6	93.0

Table 1.4 Main effects of conditioning temperature and time on starch and vitamin concentrations

	Conditioning Temperature				Conditioning Time[1]				
Item;	77	88	SEM	*P* =	15	30	60	SEM	*P* =
Starch, %									
Total	35.2	36.1	0.41	0.04	35.9	35.6	35.5	0.45	0.50
Gelatinized	9.0	10.7	0.57	0.0001	9.6	9.7	10.2	0.53	0.65
Vitamin concentration, IU									
Riboflavin	3.8	3.5	0.35	0.55	3.6	3.6	3.7	0.43	0.97
Niacin	23.7	23.8	1.52	0.95	24.3	24.9	21.9	1.86	0.50
D_3	626.8	595.2	53.26	0.68	614.7	583.2	635.2	65.24	0.85

[1]Retention time was preset as specified conditioner screw rotations per minute during the manufacturing process and verified manually. The preset retention speeds of 90, 60, and 30 rotations per minute resulted in actual retention times of 15, 30, and 60 s, respectively.

Table 1.5 Treatment effects of conditioning temperature and time on starch and vitamin concentrations

Conditioning temperature, °C:		77			88				
Conditioning time, s:	Mash	15	30	60	15	30	60	SEM	*P* =
Starch, %									
Total	34.7	35.9	35.2	34.7	36.0	36.0	36.3	0.51	0.15
Gelatinized	6.1[a]	9.0[b]	8.8[b]	9.3[bc]	10.2[bc]	10.6[bc]	11.2[c]	0.72	0.003
Vitamin concentration, %									
Riboflavin	4.0	3.9	3.6	3.9	3.5	3.6	3.4	0.62	0.98
Niacin	26.8	21.3	24.8	24.8	22.5	25.1	23.8	2.50	0.78
D_3	684.0	694.7	543.7	642.0	575.7	622.7	587.3	99.99	0.92

[abc]Means within a row that lack a common superscript differ $P \leq 0.05$.

[1]Retention time was preset as specified conditioner screw rotations per minute during the manufacturing process and verified manually. The preset retention speeds of 90, 60, and 30 rotations per minute resulted in actual retention times of 15, 30, and 60 s, respectively.

Table 1.6 Effects of sample location on total starch and gelatinized starch

Form:	Cold Mash	Hot Mash	Hot Pellet	Cold Pellet	SEM	*P* =
Total starch, %	34.7[a]	36.3[b]	36.7[b]	35.2[ab]	0.39	0.0002
Gelatinized starch, %	6.1[a]	7.3[a]	11.7[b]	10.8[b]	1.02	0.0001

[ab]Means within a row that lack a common superscript differ $P < 0.05$.

[1]Cold mash samples were collected after mixing but prior to conditioning, hot mash samples were collected after conditioning but prior to pelleting, hot pellet samples were collected after pelleting but prior to cooling, cold pellet samples were collected after manufacturing was complete and pellets were completely cooled.

Chapter 3 - Effects of pelleting conditioner retention time on nursery pig growth performance

Abstract

A total of 180 nursery pigs (PIC 327×1050; initially 12.6 kg) were used in an 18-d study to determine the effects of pellet mill conditioning parameters and feed form on pig performance. All diets were similar and different feed processing parameters were used to create experimental treatments. Factors considered were conditioning time (15, 30, or 60 s conditioning time) and feed form (mash or pelleted). To remove the confounding factor of feed form, pelleted samples were reground to a similar particle size as the mash diet. Treatments included: 1) mash diet without thermal processing (negative control); 2) pelleted diet conditioned for 30 s (positive control); 3) pelleted diet conditioned for 15 s and reground; 4) pelleted diet conditioned for 30 s and reground, and 5) pelleted diet conditioned for 60 s and reground. Pigs were weaned and fed a common acclimation diet for 21 d prior to the start of the experiment. Growth and feed disappearance were then measured for 18 days. All diets had similar levels of total starch ($P >$ 0.17), but thermally processed diets had a 1.67 to 1.87 fold increase in of gelatinized starch compared to the mash diet. Average daily gain and G:F did not differ (P = 0.34 and 0.14, respectively) between treatments overall, but pigs fed the positive control pelleted diet had decreased ADFI (P = 0.03) compared to pigs fed all other diets. Pre-planned orthogonal contrasts revealed that pigs fed mash diets tended to have greater (P = 0.10) ADG compared to those fed pelleted and reground diets. This result suggests that processing may have had a negative influence on feed utilization, which is further supported by the finding that pigs fed mash diets tended to have greater (P = 0.06) ADG compared to those fed diets that were thermally

processed, regardless of regrinding. Considering these results, it was not surprising that pigs fed mash diets had greater ADG and ADFI (P = 0.05 and 0.01, respectively) than those fed pelleted diets. When directly comparing diets conditioned at 30-s, fed either as whole pellets or reground to mash consistency, pigs fed pelleted diets had improved (P = 0.01) G:F due to lower ADFI (P = 0.004) but similar ADG (P = 0.60). The expected improvement in G:F from pelleting (6.8%) was observed, but it was lost when diets were reground to near original mash particle size. This may indicate that diet form from the actual pelleting process affects G:F more than conditioner retention time.

Key words: conditioning, growth, pellet, pig, processing

Introduction

Thermal processing of diets into a pelleted form is known to improve animal feed efficiency (Miller, 2012). However, there is disagreement as to the whether the improvement is due to enhancements in nutrient utilization or to changes in diet form that minimize potential feed wastage (Medel et al., 2004). Svihus et. al. (2004) observed that pelleted wheat-based diets fed to broilers increased (P < 0.001) apparent metabolizable energy. Meanwhile, Xing et. al. (2004) found that pelleting swine diets affected (P < 0.002) dry matter, organic matter, and fat digestibility. Still others have hypothesized that conditioner retention time is irrelevant, and the alteration in diet form is most responsible for the feed efficiency improvement from pellets (Miller, 2012). Specifically, the role of conditioner retention time on efficiency of nutrient use and its impact on animal growth performance is not consistently demonstrated in previous studies. Furthermore, much of the previous research in feed processing was conducted more than 10 years ago with animals possessing less genetic potential for lean growth performance than

hose with modern genetics (Wondra et al., 1995). Factors such as gelatinized starch and other increases in nutrient availability due to increased conditioner retention time may now result in improved growth performance or feed efficiency. Therefore, the objective of this experiment was to evaluate how pellet mill conditioning retention time, regardless of diet form, affects nursery pig performance.

Materials and methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used for this experiment. This experiment was conducted at Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility is a totally enclosed, environmentally controlled, mechanically ventilated barn. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens had wire-mesh floors and allowed 0.085m^3/pig.

A total of 180 nursery pigs (PIC 327 × 1050; initially 12.6 kg) were utilized in an 18-d growth experiment. Pigs were weaned at 21-d of age and then fed common diets for 21-d until the start of the experiment. There were 6 pigs per pen and 6 pens per treatment. At 42-d of age, pigs were weighed and treatments were randomly assigned to pens in a completely randomized design. All diets were corn-soybean meal-based with 30% distillers dried grains with solubles (Table 2.1), but processed differently to form the experimental treatments. The five treatments consisted of a negative control diet that was fed in mash form, a positive control diet that was conditioned for 30 s prior to pelleting and fed in pelleted form, and 3 diets that were conditioned for 15, 30, or 60 s prior to pelleting and then reground and fed in mash form. All diets were manufactured at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 mm ×

28.6 mm die and a conditioner-feeder (Model C18LL4/F6 0.46 m x 1.22 m, California Pellet Mill Co., Crawfordsville, IN) set at an 88°C conditioning temperature. Conditioner retention times were obtained by presetting the conditioner screw to turn at 90, 60, or 30 rpm for the 15, 30, and 60 s retention times, respectively. Samples were collected after processing and analyzed for particle size, bulk density, pellet durability, total starch, and gelatinized starch. Particle size was determined according to ASABE Standard S319.3 using 100 g of sample in a 13-sieve stack placed on a Ro-Tap^{TM} sifter for 15 min with 0.5 g of flow agent. Bulk density was calculated according to the loose density method (Fairchild, 2005). Pellet durability was determined according to ASABE Standard S269.4 modified by adding five 12.7 mm hex nuts in the tumbling compartment. Total starch and gelatinized starch were determined according to Mason et al. (1982). Briefly, one 0.5 g subsample was hydrolyzed in 25 mL distilled water for 20 min at room temperature, and a second 0.5 g subsample was boiled with 25 mL distilled water for 20 min. The samples were then allowed to cool to ambient temperature. Next, 10 mL of acetate buffer solution was added to each flask, then samples were, hydrolyzed with 5 mL of glucoamlylase, and incubated at 40°C for 70 min. After the incubation period, 5 mL of trichloroacetic acid was added to halt hydrolysis, and samples were cooled to room temperature, then mixed with approximately 50 mL distilled water to a final volume of 100 mL. Free D-glucose was then measured using a glucose analyzer YSI 2700 (Yellow Springs, OH). The resulting quantity of free glucose determined in the cold water hydrolyzed sample represented the percentage of starch that was gelatinized during processing whereas the cooked sample represented the total starch with in the sample. Pigs were weighed and feed disappearance was measured on d 0 and 18 to calculate ADG, ADFI, and G:F.

Data were analyzed using the GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC) with pen as experimental unit. Treatment means were separated using pairwise comparisons of means performed using the DIFFS option from the LSMEANS statement of SAS. Preplanned orthogonal contrasts included the 3 reground diets versus mash diet; the 4 thermally-processed diets versus mash diet; pelleted diet versus mash diet; and pelleted diet conditioned for 30 s fed in pelleted form versus. fed in reground form. Results were considered significant at $P \leq 0.05$ and tendencies from $P > 0.05$ to $P \leq 0.10$.

Results and Discussion

This experiment was conducted to evaluate the effects of pellet mill conditioning parameters and feed form on pig performance. In addition to a mash form negative control and a pelleted positive control, pelleted samples that were conditioned for 15, 30, or 60 s were reground to remove the confounding factor of feed form. Thus, it is important to evaluate the physical characteristics of the final diets to confirm if this intention was achieved. The geometric mean particle size of reground samples were within 100 μm of the mash diet and within 0.3 of the mash diet geometric standard deviation (Table 2.2). Wondra et al. (1995) reported that a 200 μm difference in particle size (400 versus 600 μm) affected ADG, ($P \leq 0.05$), but only by 2.6%. No research has evaluated if a difference as small as 100 μm particle size will affect pig growth performance, but we speculate that this difference in particle size is not biologically adequate to cause a substantial growth response in nursery pigs. As expected, bulk density of pelleted diets was substantially heavier than mash or reground diets. Pelleted diets had a pellet durability index of 93.3%, confirming that the manufacturing was effective to achieve and maintain a pelleted form. Total starch was greater in thermally-processed treatments compared to the mash treatment. Total starch, theoretically, should be equal across all treatments; thus the 1.6%

observed range may be due to inherent error associated with the analyses. Interestingly, gelatinized starch was increased 1.67, 1.74, and 1.84 fold when diets were conditioned for 15, 30, or 60-s compared to unprocessed diets, regardless of diet form. Starch gelatinization is known to increase due to conditioning time and temperature, and has been theorized to improve animal growth performance by increasing starch digestibility (Behnke, 1996).

No overall treatment effect was observed on final body weight or ADG (P = 0.63 and 0.34, respectively; Table 2.3). Interestingly, treatment affected ADFI (P = 0.03) because pigs fed pelleted diets had approximately 20% less feed disappearance than those fed mash or reground diets (P = 0.03). The magnitude of this effect was similar to observations by Lundblad et al. (2011), but these authors also found a corresponding 6 to 7% improvement in G:F compared to those fed mash diets. Our experiment did not find a significant treatment effect in G:F (P = 0.14), but pigs fed the positive control pelleted diet had the numerically greatest G:F, which was 6.3% greater than those fed the negative control mash diet. Notably, no substantial differences were found between pigs fed mash diets and those fed reground diets, regardless of conditioning time. This may suggest that diet form is the primary driver of a standard pelleting response, whereas conditioning time and respective gelatinized starch has less of an effect on pig growth performance than hypothesized.

The preplanned orthogonal contrasts between the mash and reground treatments showed no differences in final body weight, ADG, ADFI, or G:F (P = 0.23, 0.10, 0.87, and 0.24, respectively; Table 2.4). This result further confirms that the 100 µm difference in particle size between the mash and reground treatments likely did not affect performance and our goal of achieving similar particle size between reground treatments and the mash diet was successful. However, the lack of effect also demonstrates that conditioning alone does not likely improve

nutrient availability enough to result in growth performance differences. This further suggests that the pellet form alone or its combination with conditioning, and not conditioning alone, is most responsible for the traditional feed efficiency improvement observed from the pelleting process.

The orthogonal contrast comparing all thermally processed treatments to mash showed no effects on final body weight, ADFI, or G:F (P = 0.16, 0.33, and 0.55, respectively). Pigs fed mash diets tended to have a greater ADG (P = 0.06) than those fed thermally processed diets, which was surprising. This may be explained if conditioning temperatures were too hot, and thermal processing caused a Maillard reaction that may have affected growth performance, (Abd El-Khalek and Janssens 2010) but if this had occurred both ADFI and G:F would be affected. In addition, feed was conditioned at 88°C, which is a high temperature for nursery diets that contain milk-based ingredients, but it is considered a normal conditioning temperature for corn-soybean meal-based diets with distillers dried grains as formulated in this experiment (Steidinger et al., 2000).

These effects are underscored when evaluating the orthogonal contrasts between pelleted and mash diets, as well as those between pelleted and reground diets conditioned for 30 s. Again, treatment did not affect final body weight or feed efficiency when comparing mash versus pelleted diets (P = 0.13 and 0.30, respectively), but both ADG and ADFI were greater (P = 0.05 and 0.01, respectively) when diets were fed in mash form. Meanwhile, treatment did not affect final body weight or ADG when comparing pelleted and reground diets conditioned for 30 s (P = 0.70 and 0.60, respectively), but ADFI was greater, and G:F was lower (P = 0.004 and 0.01, respectively) when pigs were fed reground diets compared to pelleted diets when both were conditioned for 30 seconds.

In summary, we observed the expected lower feed intake and numerically greater feed efficiency when pigs were fed pelleted diets compared to mash diets; however, these improvements appear to be due to the difference in feed form alone or in combination with conditioning because conditioning alone at 88°C for 15, 30, or 60-s appears to have little effect on nursery pig growth performance.

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Figures and Tables

Table 2.1 Calculated diet composition (as-fed basis)

Ingredient, %	%
Corn	40.55
Soybean meal	25.25
Corn distillers dried grains with solubles	30.00
Poultry fat	0.50
Monocalcium phosphate	1.03
Limestone	1.30
Salt	0.35
L-lysine-HCL	0.45
DL-methionine	0.07
L-threonine	0.09
Vitamin premix[2]	0.25
Trace mineral premix[3]	0.15
Total	100.00
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lysine	1.26
Isoleucine:lysine	65
Leucine:lysine	156
Methionine:lysine	33
Methionine & cysteine:lysine	58
Threonine:lysine	62
Tryptophan:lysine	17.0
Valine:lysine	74
Total lysine, %	1.47
ME, kcal/kg	678.1
SID lysine:ME, g/Mcal	3.82
CP, %	24.1
Ca, %	0.76
P, %	0.69
Available P, %	0.41

[1]A single diet formulation was manufactured and then processed according to different parameters to create 5 dietary treatments.
[2]The vitamin premix provided the following per kg of feed: vitamin A, 4,400,000 IU; vitamin D3, 550,000 IU; vitamin E, 17,600 IU; vitamin B12, 15.4 mg; menadione, 1,760 mg; riboflavin, 3,300 mg; D-pantothenic acid, 11,000 mg; niacin, 19,800 mg.

Table 2.2 Treatment mean particle size, bulk density, pellet durability, total starch, and gelatinized starch

Form:	Mash	Pelleted	Pelleted then reground		
Conditioning Time, s	---	30	15	30	60
Particle size					
Dgw, µm (geometric mean)	592.0	---	508.0	492.0	526.0
Sgw, µm (standard deviation)	2.4	---	2.1	2.1	2.1
Bulk density, kg/m^3	527.8	627.1	547.7	544.8	539.2
Pellet durability index, %	---	93.3	---	---	---
Total starch, %	34.7	35.5	36.0	36.0	36.3
Gelatinized starch, %	6.1	10.7	10.2	10.6	11.2

Table 2.3 Effects of feed processing parameters on nursery pig performance

Form:	Mash	Pelleted	Pelleted then reground				
Conditioning Time, s:	n/a	30	15	30	60	SEM	*P* =
BW, kg							
d 0	12.6	12.7	12.7	12.6	12.7	0.21	1.00
d 18	21.8	20.9	21.2	21.1	21.3	0.41	0.63
ADG, kg	0.51	0.46	0.48	0.47	0.48	0.02	0.34
ADFI, kg	0.81[b]	0.68[a]	0.80[b]	0.83[b]	0.78[b]	0.03	0.03
G:F	0.63	0.67	0.59	0.58	0.61	0.03	0.14

[ab]Means within a row that lack a common superscript differ $P < 0.05$.

[1]A total of 180 pigs (42 d of age; initially 12.6 kg) were utilized with 6 pigs per pen and 6 pens per treatment.

Table 2.4 Preplanned orthogonal contrasts on the effects of feed processing parameters on nursery pig performance

	Processing Treatment											
Contrast:	Reground vs. Mash			Thermally Processed vs. Mash			Pelleted vs. Mash			30 s Conditioning Time and Reground vs. 30 s Conditioning Time and Pelleted		
Form:	Reground	Mash	*P*=	Thermally Processed	Mash	*P*=	Pelleted	Mash	*P*=	Reground	Pelleted	*P*=
BW, kg												
d 0	12.7	12.6	0.94	12.7	12.6	0.94	12.6	12.6	0.98	12.7	12.6	0.99
d 18	21.2	21.8	0.23	21.1	21.8	0.16	20.9	21.8	0.13	21.1	20.9	0.70
ADG, kg	0.45	0.50	0.10	0.45	0.50	0.06	0.45	0.50	0.05	0.45	0.45	0.60
ADFI, kg	0.82	0.82	0.87	0.77	0.82	0.33	0.68	0.82	0.01	0.82	0.68	0.004
G:F	0.56	0.61	0.24	0.59	0.61	0.55	0.67	0.61	0.30	0.56	0.67	0.01

Chapter 4 - An evaluation of gelatinized starch methodologies in pelleted swine feed

Abstract

Three separate methodologies were evaluated to determine the most appropriate method for determination of total and gelatinized starch in pelleted complete swine feed samples. A modified glucoamylase method developed by Wenger Manufacturing (Sabetha, KS) was used to determine both total and gelatinized starch in samples, while the official AOAC 996.11 method was utilized to determine total starch content and differential scanning calorimetry (DSC) was utilized to determine gelatinized starch. All samples were manufactured from the same corn-soybean meal-based diet formulation with 30% distillers dried grains with solubles. The diet was processed at either 77°C or 88°C for 15, 30, or 60 seconds during pelleting. Samples were collected prior to conditioning (cold mash), after conditioning but prior to pelleting (hot mash), after pelleting but prior to cooling (hot pellet), and after pelleting and cooling (cold pellet). Diet form affected total starch according to the modified glucoamylase method and tended to affect it according to the AOAC method ($P = 0.0002$ versus 0.07, respectively). In both cases, cold mash samples were determined to have less total starch than those that were collected as hot samples ($P \leq 0.05$). The AOAC method predicted a lower total starch concentration than the modified glucoamylase method in all samples, and this difference was significant ($P \leq 0.05$) overall and in mash and hot pellet samples. With the exception of hot mash, those samples analyzed according to the DSC had nearly twice the concentration of gelatinized starch compared to those analyzed according to the modified glucoamylase method; however, this response was only significant in cold pellet samples ($P = 0.01$). Still, this finding is substantial because the majority of complete feed analyses are conducted on cold pellet samples. The elevated gelatinized starch

concentrations in cold pellet samples from DSC analyses are greater than theoretically possible. This overestimation of gelatinized starch from the DSC method may actually be protein denaturation from soybean meal within the starch gelatinization peak. Still, there were no significant differences between replications for either total or gelatinized starch for any form, regardless of method ($P > 0.05$), so intra-assay variation was not an influencing factor. In summary, analyzing pelleted swine feed according to the Official AOAC 996.11method results in lower total starch values than the modified glucoamylase method. Meanwhile, the DSC method overestimates gelatinized starch compared to the modified glucoamylase method. Thus, the DSC method is not appropriate for analyzing starch gelatinization in multi-ingredient products such as livestock feed.

Key words: conditioning, feed processing, gelatinization, livestock feed, starch

Introduction

Starch gelatinization occurs in the presence of heat and moisture during the pelleting process of livestock feed manufacturing. Pelleting improves feed efficiency in food production animals by 5 to 8% (Miller, 2012). Thomas and van der Poel (1996) have shown that starch gelatinization occurring during pellet processing may attribute to the binding properties within the pellets themselves. This binding may be the root cause of the feed efficiency improvement associated with pelleting due to increasing pellet quality and thus reduced feed wastage (Abdollahi et. al. 2011; Baird 1973). Vicente et al. (2008) demonstrated that the gelatinization of starch itself may improve nutrient digestibility in young pigs. This, however, is difficult to prove in pelleted feed because gelatinization in naturally confounded by feed form. Svihus et. al. (2004) demonstrated that gelatinization does occur during pelleting, but not to a great degree.

Stevens (1987) determined that starch gelatinization on the surface of pellets may be as great as 58%, but only 41% in whole pellets. This would suggest that the majority of gelatinization is actually occurring during the formation of the pellet compared to conditioning, but this has not been confirmed. In addition, the analytical technique used to evaluate either total starch or starch gelatinization is important. The three most common starch analysis techniques for animal feed are the AOAC Official Method 996.11 for total starch analysis, the modified glucoamylase method for total starch and gelatinized starch analysis, and differential scanning calorimetry for gelatinized starch analysis. The experiments described above were evaluated with a combination of these methods, and there is limited data describing their applicability within livestock feed and comparison to one another. Thus, the objectives of this experiment were to determine appropriate methods for starch analysis in a pelleted nursery swine diet, as well as to evaluate differences in starch analysis among different feed forms.

Materials and methods

Three methods were utilized to determine either total or gelatinized starch concentrations. A variety of samples were analyzed that were all the same diet formulation, a corn-soybean meal-based phase 3 swine nursery diet with 30% distillers dried grains with solubles (Table 1). The diet contained 40.55% yellow dent corn, which was the primary source of starch and was ground to an average geometric mean particle size of 592.0 µm prior to processing. Yellow dent corn is approximately 70% starch (Hallauer, 2004). Thus, the diet utilized in these experiments would theoretically contain approximately 28.4% starch. Total starch of the complete diet according to Fourier Transform Near-Infrared Spectroscopy (Bruker Optics Multi-Purpose Analyzer, Billerica, MA) was actually 29.0%.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit utilizing a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) fitted with a 4.4 mm × 28.6 mm die and a conditioner-feeder (Model C18LL4/F6, California Pellet Mill Co., Crawfordsville, IN). Samples were collected when manufacturing at two conditioning temperatures (77 vs. 88°C) and three conditioner retention times (90, 60, and 30 rpm to represent 15, 30, and 60 s actual conditioning times). In addition, a mash diet not subjected to conditioning served as a negative control. In addition, samples were collected after mixing but prior to conditioning (cold mash), after conditioning but prior to pelleting (hot mash), after pelleting but prior to cooling (hot pellet), and after pelleting and cooling (cold pellet) and these location samples were analyzed for total starch and gelatinized starch. These samples were then analyzed for total and/or gelatinized starch concentrations according to three methods. The AOAC Official Method 996.11 was utilized for determining total starch, the modified glucoamylase method for determining total starch and gelatinized starch, and differential scanning calorimetry for determining gelatinized starch.

The AOAC Official Method 996.11 (AOAC Int., 2007) of total starch analysis via enzymatic hydrolysis was conducted using a total starch kit (Megazyme K-TSTA, Wicklow, Ireland). Briefly, samples were ground through a 0.5 mm screen and approximately100 mg of sample was added to test tubes in duplicate. Next, 0.2 mL of ethanol (80% v/v) was added tube vortexed. Next, 3.0 mL of α-amylase/ 3-(N-morpholino)propanesulfonic acid (MOPS) reagent was added and tubes were boiled for 6 minutes. During this time, test tubes were vortexed at 2 minute intervals. Tubes were then placed in a 50°C bath to rest for 5 minutes. Sodium acetate bufferwas added (4.5 pH) followed by 0.1 mL of amyloglucosidase. Tubes were then vortexed and incubated for 30 minutes. Test tubes were then filled to a volume of 10 mL with distilled

water and centrifuged at 3,000 rpm for 10 minutes. Next, 1.0 mL aliquots were diluted with 10 mL of distilled water. Aliquots of 0.1 mL were removed from the diluted solution and placed into clean test tubes. Then, 3.0 mL of Glucose oxidase (> 12,000 U) plus peroxidase and 4-aminoantipyrine (GOPOD) reagent was added to each tube and incubated for an additional 20 minutes at 50°C. The control samples include 0.1 mL of D-glucose standard and the 3.0 mL of GOPOD reagent. The blank included 0.1 mL of water and 3.0 mL of GOPOD reagent. Using a pipette, 0.3 mL was removed and placed into a 0.5 mL well plate. Once all samples had been plated, then the plate was read for absorbance at 510 nm. Analysis was conducted in duplicate.

Both total starch and gelatinized starch were measured using the modified glucoamylase method developed by Wenger Manufactured (Sabetha, KS) according to Mason et al. (1982). Briefly, one 0.5 g subsample was hydrolyzed in 25 mL distilled water for 20 minutes at room temperature while a second 0.5 g subsample was boiled with 25 mL distilled water for 20 minutes. The samples were then allowed to cool to ambient temperature. Next, 10 mL of acetate buffer solution was added to each flask. Then samples were, hydrolyzed with 5 mL of glucoamlylase, and incubated at 40°C for 70 minutes. After the incubation period, 5 mL of trichloroacetic acid was added to halt hydrolysis, samples were cooled to room temperature, and then mixed with 50 mL distilled water to a final volume of 100 mL. Free D-glucose was then measured using a glucose analyzer (Model 2700, YSI, Yellow Springs, OH) YSI 2700. The resulting quantity of free glucose determined in the cold water hydrolyzed sample represented the of starch that was gelatinized during processing while the cooked sample represented the total starch within a sample. Analysis was conducted in duplicate.

Finally, gelatinized starch was also measured using differential scanning calorimetry. Unlike the other two enzyme hydrolysis methods, DSC is a measurement of enthalpy between

non-processed and processed samples, the difference of which represents percent gelatinization. Initially, samples were ground through a 0.5-mm screen, but subsequent analysis showed that a clear starch gelatinization peak could not be observed (Figure 1). Ensuing samples ground through a 0.2 mm screen showed more clearly discernable peaks (Figure 2) and thus it is recommended to grind all complete livestock feed samples through a 0.2 mm screen prior to DSC analysis. After grinding, approximately 10 mg of feed sample and deionized water (1:2, feed/water, w/w) were weighed into a stainless steel pan, sealed, and allowed to equilibrate overnight. Thermal scans were conducted using a DSC (Q100, TA Instrument, New Castle, NJ) using an empty pan as a reference. Enthalpy (ΔH) of feed was determined by heating the sample from 10°C and scanning at 10°C/min. to 160°C.

Data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Individual sample served as the experimental unit. Main effects evaluated were conditioning temperature (77°C versus 88°C) vs. conditioning time (15 versus 30, versus 60 s); diet form (cold mash versus hot mash versus hot pellet versus cold pellet); or analytical method (AOAC 996.11 vs. modified glucoamylase for total starch or DSC vs. modified glucoamylase for gelatinized starch. Results were considered significant if $P \leq 0.05$ and trends if $0.05 < P < 0.10$.

Results and Discussion

The main effect of conditioning temperature did not affect ($P = 0.33$) total starch concentration according to the AOAC method, but was affected by the modified glucoamylase method ($P = 0.04$; Table 2). Additionally, analysis by the modified glucoamylase method showed that gelatinized starch was affected by conditioning temperature ($P = 0.01$). Specifically, conditioning at 88°C resulted in 0.9 and 1.7% greater total and gelatinized starch, respectively, compared to conditioning at 77°C. One would not expect total starch concentration to change

based on thermal processing, so the increase in total starch according to the modified glucoamylase method is interesting. Kingman and Englyst (1994) showed that different cooking treatments can lead to variable outcomes in total starch, but the difference was likely due to analytical error. Gomez et. al. (1983) explained that degree of gelatinization is the difference between total starch and processed starch values. Total starch analytical methods require complete cooking of the starch prior to sample enzymatic hydrolysis (Mason and Rokey, 1982; Rosin et al., 2002). If complete cooking is carried out effectively, one would expect similar total starch values amongst diets regardless of prior processing. Potentially, the modified glucoamylase method for total starch did not complete full cooking and was thus not truly indicative of total starch. Still, the significant effect was actually only a 0.03 magnitude difference between the two methods, so the finding is not likely to be of much biological relevance.

On the other hand, temperature is known to alter the concentration of gelatinized starch, and true starch gelatinization is thought to occur at temperatures above 70°C (Lund, 1984). Thus, the 1.7% increase in starch gelatinization according to the modified glucoamylase method between 77°C and 88°C is not surprising. Conditioning time did not affect total starch according to either method of evaluation or gelatinized starch according to the modified glucoamylase method ($P \geq 0.50$).

Opposite of the patterns observed in the main effects, the overall treatment effect showed differences for total starch when evaluated according to the AOAC method, but not the modified glucoamylase method (P = 0.0001 and 0.15, respectively; Table 3.3). There was still an overall treatment effect for gelatinized starch evaluated according to the modified glucoamylase method (P = 0.003). In both the total starch according to AOAC and the gelatinized starch cases, mash

samples were determined to have a lower percentage value than thermally-processed samples ($P \leq 0.05$). It is notable that mash samples had 6.1% gelatinization, despite not being thermally processed. Because the modified glucoamylase method is an actual measurement of free D-glucose units, the resulting conclusion is that this 6.1% is actually free glucose, not necessarily gelatinization. Thus, true gelatinization of the thermally processed samples would be their determined values (8.8 to 11.2% starch gelatinization) minus the 6.1% baseline free glucose shown in the mash diet.

When samples were evaluated by feed form, concentrations for both total and gelatinized starch varied (Table 3.4). Total starch evaluation according to the AOAC method tended ($P = 0.07$) to be affected by diet form while those evaluated according to the modified glucoamylase method were significantly affected ($P = 0.0002$) by feed form. Total starch in cold mash samples was analyzed to be substantially lower than concentrations in thermally-processed samples ($P \leq 0.05$). Again, one would not expect thermal processing to affect total starch concentrations, so perhaps error within the analytical method existed to allow for this difference. Regardless of method, there were considerable differences in starch gelatinization between hot mash samples and hot pellet or cold pellet samples ($P < 0.0001$). The values for starch gelatinization in hot mash samples according to either the DSC or modified glucoamylase method (-2.6 or 2.3%) were not theoretically possible and suggest that neither method is appropriate for evaluating starch gelatinization in conditioned mash samples. This is not altogether discouraging, because most feed samples are analyzed as cold pellet samples. Thus, the discrediting of either method for gelatinization in hot mash samples has limited implications. The gelatinization concentrations in cold pellet samples are more meaningful. According to the method, either 39.4% (DSC) or 21.5% (modified glucoamylase) gelatinized starch existed in the cold pellet form. These values

are in line with other literature. Mortiz et. al. (2005) reported 28% gelatinized starch due to pelleting. When Cramer et. al. (2003) expanded feeds at 82°C, gelatinized starch levels reached 31%. Finally, Skoch et. al. (1981) measured gelatinized starch levels while dry pelleting and found 20 to 25% starch gelatinization.

While it is important to evaluate these methods according to different processing conditions and feed forms as outlined above, an evaluation between the methods themselves is important. When using only samples processed according to a single manufacturing process (pelleting at 88°C for 60s), it was found that the analytical method type affected total starch concentrations (Table 5). The AOAC method predicted a lower total starch concentration than the modified glucoamylase method in all samples, and this difference was significant ($P < 0.05$) overall and in mash and hot pellet samples. While both methods utilize enzyme hydrolysis, the AOAC method has a 6 minute cook time compared to the 20 minute cook time of the modified glucoamylase method. This cook time difference may be the root cause of variation amongst methods as samples must be completely cooked for enzyme hydrolysis to be effective. There were no differences ($P > 0.05$) amongst replications for total starch concentrations, so intra-assay variation was not affected overall or in different diet forms.

Finally, analytical method did not affect gelatinized starch analysis overall or in hot pellet samples ($P = 0.30$ and 0.40, respectively; Table 6). Again, the values of gelatinized starch in the hot mash samples are not realistic and thus neither method should be utilized for analyzing gelatinized starch in this feed form, even though the individual replicates were relatively similar. With the exception of hot mash, samples analyzed according to the DSC had nearly twice the concentration of gelatinized starch compared to those analyzed according to the modified glucoamylase method; however, this response was only significant in cold pellet samples ($P =$

0.01). Still, this finding is substantial because the majority of complete feed analyses are conducted on cold pellet samples. Although the gelatinized starch concentrations in cold pellet samples analyzed according to the DSC method are similar to those found in literature, we question if either method is appropriate for measuring true starch gelatinization. The DSC method showed an enthalpy difference of 3.76 to 3.90 J/g for various feed forms, which is greater than theoretically possible. The enthalpy of pure starch is suggested as 9 J/g (Zhou et al., 2010). As previously described, the analyzed samples were from diets with 40.55% corn and corn is approximately 70% starch (Hallauer, 2004). Thus, the diet utilized in these experiments contained approximately 28.4% starch and therefore the theoretical maximum enthalpy change would be 2.56 J/g. The values observed by DSC were 1.2 to 1.3J/g greater than theoretically possible based on the quantity of starch in the samples. Some of this overestimation of gelatinized starch from the DSC method may actually be protein denaturation from soybean meal within the starch gelatinization peak. Other potential causes of this overestimation may include a relative high quantity of residual sugars from the distillers dried grains with solubles or unknown causes. Regardless, it must be recognized that the DSC method was developed for individual components, such as starch. Although it has some applicability to ingredients containing multiple starch components, it does not appear that DSC is an appropriate method for the evaluation of livestock feedstuffs with multiple ingredients.

Meanwhile, the starch gelatinization values according to the modified glucoamylase method appear to be lower than those found in literature and mentioned previously. Still, there were no significant differences between replications for gelatinized starch for any form regardless of method ($P > 0.50$), so intra-assay variation was not an influencing factor in method performance.

In summary, analyzing pelleted swine feed according to the Official AOAC 996.11method results in lower total starch values than the modified glucoamylase method. Meanwhile, the DSC method overestimates gelatinized starch and is not appropriate for analyzing starch gelatinization in multi-ingredient products such as livestock feed.

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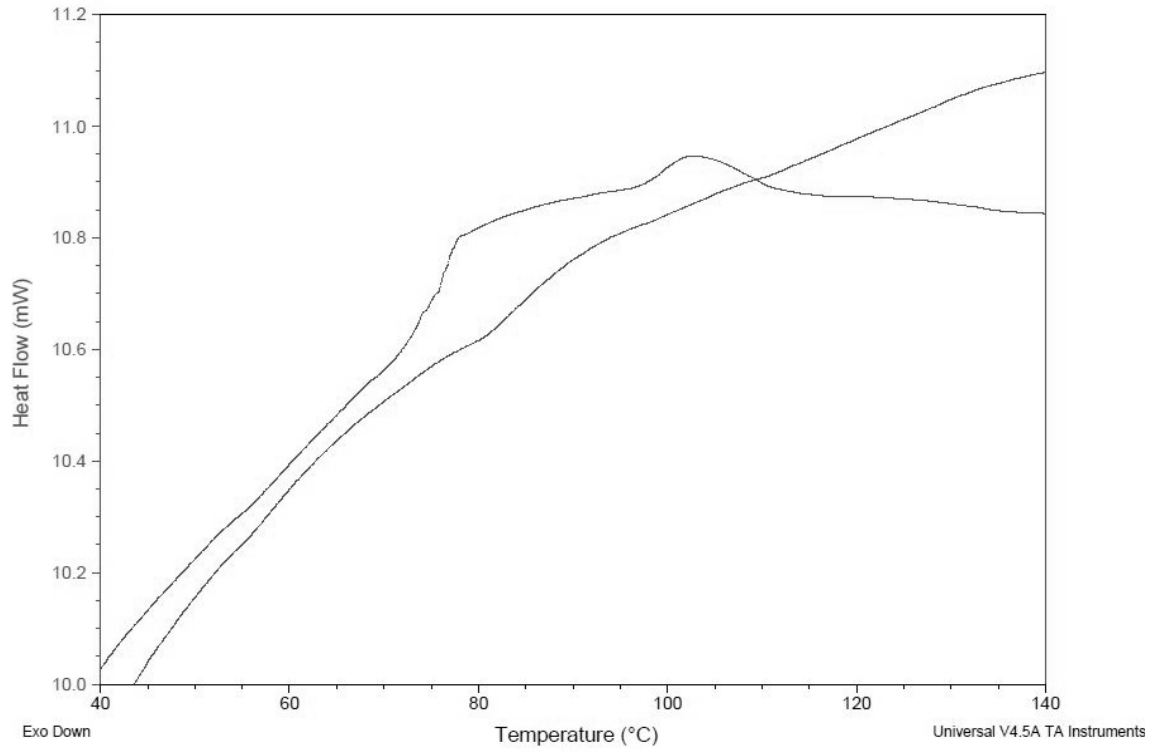
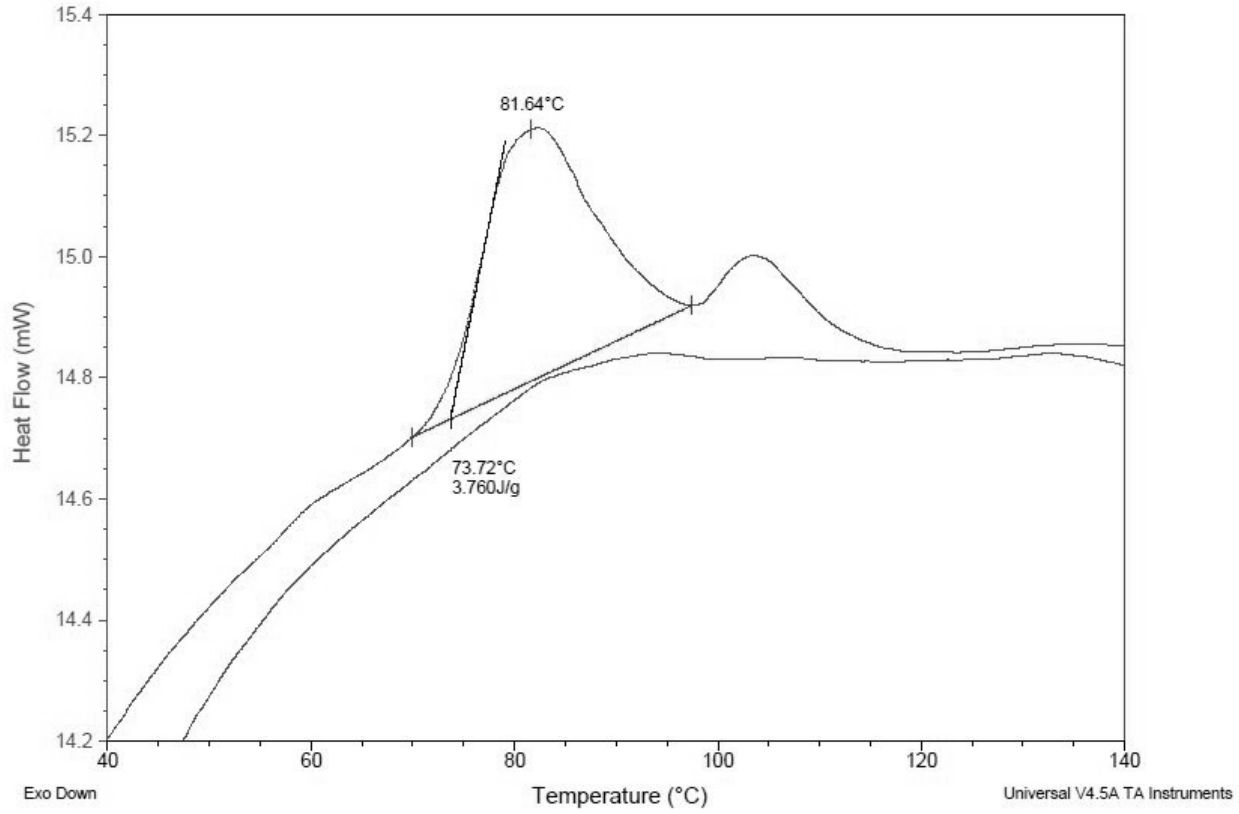
Figures and Tables

Figure 1 Starch gelatinization of complete swine feed as determined by differential scanning calorimetry



Feed sample was ground through a 0.5 mm screen, which resulted in an indiscernible starch gelatinization peak.

Figure 2 Starch gelatinization of complete swine feed as determined by differential scanning calorimetry



Feed sample was ground through a 0.2 mm screen, which resulted in a clear starch gelatinization peak.

Table 3.1 Calculated diet composition (as-fed basis)

Ingredient, %	%
Corn	40.55
Soybean meal	25.25
Corn distillers dried grains with solubles	30.00
Poultry fat	0.50
Monocalcium phosphate	1.03
Limestone	1.30
Salt	0.35
L-lysine-HCL	0.45
DL-methionine	0.07
L-threonine	0.09
Vitamin premix[2]	0.25
Trace mineral premix[3]	0.15
Total	100.00
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lysine	1.26
Isoleucine:lysine	65
Leucine:lysine	156
Methionine:lysine	33
Methionine & cysteine:lysine	58
Threonine:lysine	62
Tryptophan:lysine	17.0
Valine:lysine	74
Total lysine, %	1.47
ME, kcal/lb.	1,495
SID lysine:ME, g/Mcal	3.82
CP, %	24.1
Ca, %	0.76
P, %	0.69
Available P, %	0.41

[1]A single diet formulation was manufactured and then processed according to different parameters to create 5 dietary treatments.
[2]The vitamin premix provided the following per kg of feed: vitamin A, 4,400,000 IU; vitamin D3, 550,000 IU; vitamin E, 17,600 IU; vitamin B12, 15.4 mg; menadione, 1,760 mg; riboflavin, 3,300 mg; D-pantothenic acid, 11,000 mg; niacin, 19,800 mg.
[3]The trace mineral provided the following %: iron, 7.3; zinc, 7.3; manganese, 2.2; copper, 1.1; iodine, 198 ppm; selenium, 198 ppm.

Table 3.2 Main effects of conditioning temperature and time on starch concentrations and total of gelatinized starch concentrations according to the Official AOAC Standard 996.11 or modified glucoamylase method

	Conditioning Temperature				Conditioning Time[1]				
Item;	77°C	88°C	SEM	*P* =	15 s	30 s	60 s	SEM	*P* =
Total starch, %									
AOAC 996.11	31.7	32.1	0.35	0.33	32.1	31.7	31.9	0.44	0.82
Modified glucoamylase	35.2	36.1	0.27	0.04	35.9	35.6	35.5	0.45	0.50
Gelatinized starch, %									
Modified glucoamylase	9.0	10.7	0.57	0.01	9.6	9.7	10.2	1.71	0.65

[1]Retention time was preset as specified conditioner screw rotations per minute during the manufacturing process and verified manually. The preset retention speeds of 90, 60, and 30 rotations per minute resulted in actual retention times of 15, 30, and 60 s, respectively.

Table 3.3 Treatment effects of conditioning temperature and time on total or gelatinized starch concentrations according to the Official AOAC Standard 996.11 or modified glucoamylase method

Conditioning temperature, °C:		77			88				
Conditioning time, s:	Mash	15	30	60	15	30	60	SEM	*P* =
Total starch, %									
AOAC 996.11	27.3[a]	32.6[c]	30.8[b]	31.5[bc]	31.2[bc]	32.6[c]	32.6[c]	0.62	0.0001
Modified glucoamylase	34.7	35.9	35.2	34.7	36.0	36.0	36.3	0.51	0.15
Gelatinized starch, %									
Modified glucoamylase	6.1[a]	9.0[b]	8.8[b]	9.3[bc]	10.2[bc]	10.6[bc]	11.2[c]	0.72	0.003

[abc]Means within a row that lack a common superscript differ $P < 0.05$.

[1]Retention time was preset as specified conditioner screw rotations per minute during the manufacturing process and verified manually. The preset retention speeds of 90, 60, and 30 rotations per minute resulted in actual retention times of 15, 30, and 60 s, respectively.

Table 3.4 Effects of diet form on total gelatinized starch concentrations according to the Official AOAC Standard 996.11, modified glucoamylase method, or differential scanning calorimetry

Form:	Cold Mash	Hot Mash	Hot Pellet	Cold Pellet	SEM	*P* =
Total starch, %						
AOAC 996.11	26.5^{x}	32.7^{y}	32.5^{y}	29.4^{xy}	1.57	0.07
Modified glucoamylase	33.8^{a}	36.1^{b}	38.2^{c}	35.1^{b}	0.36	0.0002
Gelatinization, %						
Differential scanning calorimetry	-	-2.6^{a}	33.0^{b}	39.4^{b}	2.51	0.0001
Modified glucoamylase	-	2.3^{a}	18.8^{b}	21.5^{c}	0.17	0.0001

^{abc}Means within a row that lack a common superscript differ $P < 0.05$.

^{xy}Means within a row that lack a common superscript differ $P < 0.10$.

[1]Samples were conditioned at 88°C for 60s and pelleted on a CPM pellet mill. Cold mash samples were collected prior to conditioning, hot mash samples were collected after conditioning but prior to pelleting, hot pellet samples were collected after pelleting but prior to cooling, cold pellet samples were collected after manufacturing was complete and pellets were completely cooled.

Table 3.5 Effects of total starch analytical method by diet form

	Type				Replication				
Item;	AOAC 996.11	Modified Glucoamylase	SEM	*P* =	1	2	3	SEM	*P* =
Total starch,%									
Overall	30.3	35.8	0.83	0.0002	33.2	33.8	32.1	1.02	0.51
Mash	26.5	33.8	0.14	0.001	29.4	30.9	30.2	0.18	0.06
Hot Mash	32.7	36.1	0.63	0.06	34.3	34.5	34.4	0.77	0.97
Hot Pellet	32.5	38.2	0.50	0.01	35.8	35.8	34.7	0.61	0.51
Cold Pellet	32.7	35.1	0.24	0.01	33.3	34.2	34.3	0.29	0.23

[1]Samples were conditioned at 88°C for 60s and pelleted on a CPM pellet mill. Cold mash samples were collected prior to conditioning, hot mash samples were collected after conditioning but prior to pelleting, hot pellet samples were collected after pelleting but prior to cooling, cold pellet samples were collected after manufacturing was complete and pellets were completely cooled.

Table 3.6 Effects of gelatinized starch analytical method by diet form

	Type				Replication				
Item;[2]	Differential Scanning Calorimetry	Modified Glucoamylase	SEM	*P* =	1	2	3	SEM	*P* =
Gelatinization, %									
Overall	23.3	14.2	5.91	0.30	18.6	20.8	16.8	7.24	0.93
Hot Mash	-2.6	2.3	0.83	0.40	0.4	0.5	-1.3	1.01	0.51
Hot Pellet	33.0	18.8	3.21	0.09	25.4	30.3	22.1	3.93	0.47
Cold Pellet	39.4	21.5	1.20	0.01	29.9	31.7	29.8	1.47	0.65

[1]Samples were conditioned at 88°C for 60s and pelleted on a CPM pellet mill. Hot mash samples were collected after conditioning but prior to pelleting, hot pellet samples were collected after pelleting but prior to cooling, cold pellet samples were collected after manufacturing was complete and pellets were completely cooled.