

An evaluation of total starch and starch gelatinization methodologies in pelleted animal feed¹

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ABSTRACT: The quantification of total starch content (TS) or degree of starch gelatinization (DG) in animal feed is always challenging because of the potential interference from other ingredients. In this study, the differences in TS or DG measurement in pelleted swine feed due to variations in analytical methodology were quantified. Pelleted swine feed was used to create 6 different diets manufactured with various processing conditions in a 2 × 3 factorial design (2 conditioning temperatures, 77 or 88°C, and 3 conditioning retention times, 15, 30, or 60 s). Samples at each processing stage (cold mash, hot mash, hot pelletized feed, and final cooled pelletized feed) were collected for each of the 6 treatments and analyzed for TS and DG. Two different methodologies were evaluated for TS determination (the AOAC International method 996.11 vs. the modified glucoamylase method) and DG determination (the

modified glucoamylase method vs. differential scanning calorimetry [DSC]). For TS determination, the AOAC International method 996.11 measured lower TS values in cold pellets compared with the modified glucoamylase method. The AOAC International method resulted in lower TS in cold mash than cooled pelletized feed, whereas the modified glucoamylase method showed no significant differences in TS content before or after pelleting. For DG, the modified glucoamylase method demonstrated increased DG with each processing step. Furthermore, increasing the conditioning temperature and time resulted in a greater DG when evaluated by the modified glucoamylase method. However, results demonstrated that DSC is not suitable as a quantitative tool for determining DG in multicomponent animal feeds due to interferences from nonstarch transformations, such as protein denaturation.

Key words: analytical methods, animal feed, degree of gelatinization, total starch

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INTRODUCTION

Corn and soybean meal are the primary ingredients used in swine diets (Loar and Corzo, 2011). Swine diets are commonly pelleted to improve handling characteristics, lessen feed wastage, and increase feed efficiency (Miller, 2012). The pelleting process starts with feed in meal form and involves conditioning it with steam before pressing it through holes in a metal die to form cylindrical pellets, which are then cooled to retain their shape and allow moisture to evaporate for a longer shelf life. Many factors

affect pellet quality, such as diet formulation, conditioning time, conditioning temperature, particle size of the meal feed, production rate, ambient temperature, specifications of the pellet die, and cooling processes (Loar and Corzo, 2011).

Starch, as the primary component in corn, may gelatinize in the presence of heat and moisture, which results in greater energy digestibility and alters the physical characteristics of the feed products. Although pelleting typically does not introduce enough moisture and energy to cause substantial gelatinization, there is a positive impact on hydrogen bonding and, consequently, the binding properties within pellets (Thomas and van der Poel, 1996). This binding may be the root cause of the feed efficiency improvement associated with pelleting, which results in increased pellet quality and reduced feed wastage (Baird, 1973; Abdollahi et al., 2011). Therefore, it is

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Table 1. Composition of experiment diet

Ingredient	Percent
Corn	40.56
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 ¹	0.25
Trace mineral premix ²	0.15
Total	100.00

¹Provided per kilogram of diet: 11,023 IU vitamin A, 1,378 IU vitamin D₃, 44 IU vitamin E, 4 mg vitamin K, 8 mg riboflavin, 28 mg pantothenic acid, 50 mg niacin, and 0.04 mg vitamin B₁₂.

²Provided per kilogram of diet: 40 mg Mn from manganese oxide, 17 mg Fe from iron sulfate, 17 mg Zn from zinc sulfate, 2 mg Cu from copper sulfate, 0.30 mg I from calcium iodate, and 0.30 mg Se from sodium selenite.

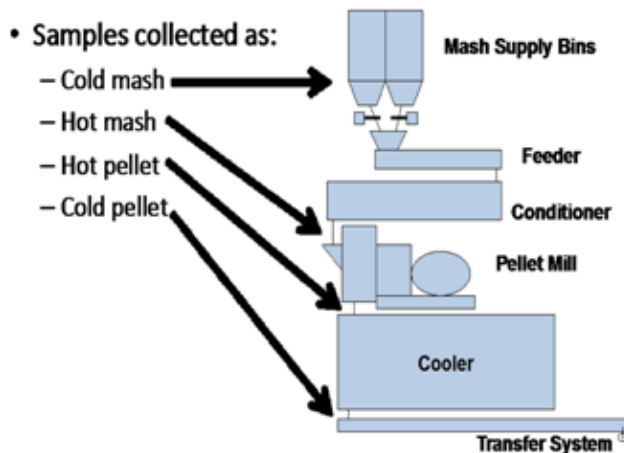
important to understand when and how gelatinization occurs at each processing stage and condition.

To understand the gelatinization changes during pelleting process, it is also important to accurately measure total starch content (TS) and degree of starch gelatinization (DG) to predict animal performance. The 3 most common starch analysis techniques are the AOAC International method 996.11 (AOAC, 2007) for TS analysis, the modified glucoamylase method for TS and gelatinized starch analysis, and differential scanning calorimetry (DSC) for gelatinized starch analysis. However, limited data is available for their applicability within multicomponent livestock feeds. Therefore, the objective of this study was to quantify the differences in TS and starch gelatinization measurement in pelleted swine feed by using different analytical methodologies and also to understand how TS and DG change at each processing stage and are affected by processing parameters. This could help optimize the conditioning temperature and retention time during processing, thereby increasing the efficiency and the quality.

MATERIAL AND METHODS

Diet and Experiment Design

All diets were manufactured from a standard swine basal formulation as shown in Table 1. In this formula, corn was the primary source of starch and was ground to an average geometric mean particle size of 592.0 μm before processing. Feed was manufactured at the North Carolina State University Feed Mill (Raleigh, NC) using a steam conditioner (model C18LL4/F6; California

**Figure 1.** Pelleting process of swine feed.

Pellet Mill Co., Crawfordsville, IN) and pellet mill (model PM1112-2; California Pellet Mill Co.) fitted with a 28.6-mm die. Six different diets were manufactured (in triplicate) with various processing conditions in a 2×3 factorial design (2 conditioning temperatures, 77 or 88°C, and 3 conditioner retention times, 15, 30, or 60 s). Samples at each processing stage (cold mash, hot mash, hot pellets, and final cold pellets) were collected for each of the 6 treatments. Cold mash samples were collected before conditioning, hot mash samples were collected after steam conditioning but before pelleting, hot pelletized feed samples were collected after conditioning and pelleting but before cooling, and final cooled pelletized samples were collected after manufacturing was complete and the pellets were completely cooled (Fig. 1).

Determination of Total Starch Content According to the AOAC International Standard Method

The TS was determined by an enzymatic colorimetric method, AOAC International method 996.11 (AOAC, 2007), with an assay kit from Megazyme International Ltd. (Wicklów, Ireland). Briefly, samples were ground through a 0.5-mm screen and 100.0 mg of sample was added to a test tube. Next, 0.2 mL of ethanol solution (80%, vol/vol) was added into the tube and mixed to wet the sample. Next, 3.0 mL of thermostable α -amylase was immediately added, and the tubes were boiled for 6 min and stirred at 2-min intervals. Tubes were then placed in a 50°C bath to rest for 5 min. Next, 0.1 mL of amylo-glucosidase was added into each tube. Tubes were then stirred and incubated for 30 min and then filled to a volume of 10 mL with distilled water followed by centrifugation at $1,800 \times g$ for 10 min at room temperature. Next, 1.0 mL of aliquots from the supernatant was diluted to 10 mL with distilled water. Then, 0.1 mL of this diluted solution was placed into a clean test tube. Glucose oxi-

dase/peroxidase reagent (3 mL) was added to each tube and incubated at 50°C for 20 min. For blanks, 0.1 mL of water was used instead of 0.1 mL of diluted solution, and the other added reagents were all the same. Samples were read for absorbance at 510 nm. Analysis was conducted in duplicate.

Determination of Total Starch Content and Degree of Starch Gelatinization According to the Modified Glucoamylase Method

Both TS and DG were measured using a modification of the glucoamylase method (developed by Wenger Manufacturing, Inc., Sabetha, KS) described by Mason et al (1982). Briefly, 0.5 g of sample was boiled with 25 mL distilled water for 20 min and then cooled to ambient temperature. Meanwhile, another 0.5 g of sample was hydrolyzed in 25 mL distilled water for 20 min at 25°C as a control. Next, 10 mL of acetate buffer solution was added into each tube followed by 5 mL of glucoamylase, and samples were incubated at 40°C for 70 min. Next, 5 mL of trichloroacetic acid was added to halt hydrolysis. After the sample was cooled to room temperature, distilled water was added to make a final volume of 100 mL. Free D-glucose was then measured using a glucose analyzer YSI 2700 (model 2700; YSI Inc., Yellow Springs, OH). The resulting quantity of free glucose determined in the control (G_{cold}) represents the percentage of starch that was gelatinized during processing. Meanwhile, the quantity of free glucose determined in the cooked sample (G_{boil}) represents the percentage of TS within a sample. The DG is then calculated as $DG (\%) = (G_{\text{cold}}/G_{\text{boil}}) \times 100$. Analyses were conducted in duplicate.

Determination of Degree of Starch Gelatinization According to the Differential Scanning Calorimetry Method

The DG was also evaluated using DSC. Unlike the other 2 enzyme hydrolysis methods, the DSC method relies on measurement of enthalpy of non-processed and processed samples, and the difference between the 2 represents the extent of gelatinization. Approximately 10 mg (dry basis) of feed sample (ground through a 0.5-mm screen) was weighed in a stainless steel pan, and deionized water (1:2, feed/water, wt/wt) was added and the sample was allowed to equilibrate overnight. Thermal scans were conducted using a differential scanning calorimeter (Q100; TA Instruments, New Castle, DE). The measurement was performed by heating the pan in the differential scanning calorimeter from 10 to 160°C at a heating rate of 10°C/min. The onset, peak, and conclusion gelatinization temperatures and the enthalpy of gelatiniza-

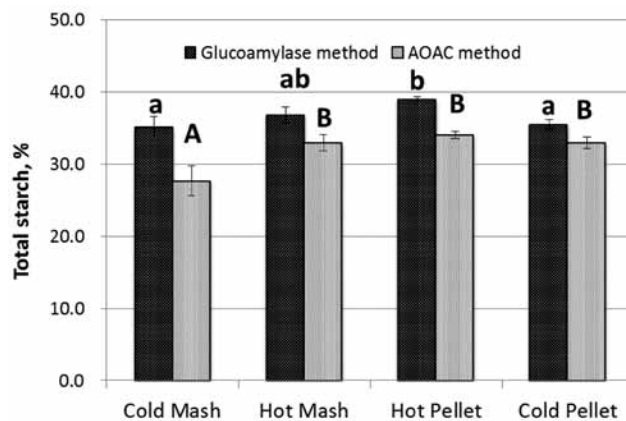


Figure 2. Total starch content in diets tested by the AOAC International method 996.11 (AOAC, 2007) and the modified glucoamylase method (conditioning temperature and retention time: 88°C and 60 s). a,b; A,B Means not sharing a common letter differ ($P \leq 0.05$).

tion (ΔH) were determined. The DG is calculated as $DG (\%) = [(\Delta H_0 - \Delta H_1)/\Delta H_0] \times 100$, in which ΔH_0 is the gelatinization enthalpy of native starch (J/g) and ΔH_1 is the gelatinization enthalpy of the cooked product (J/g). A 100% DG equates to completely cooked starch, whereas 0% equates to raw starch. All measurements were performed in triplicate.

Statistical Analysis

Analysis of variance (SPSS version 13.0; SPSS Inc., Chicago, IL) and Duncan tests were applied to determine statistical significance of measurements using different TS and starch gelatinization methods. Main effects evaluated include conditioning temperature (77°C vs. 88°C), conditioning time (15 vs. 30 vs. 60 s), diet form (cold mash vs. hot mash vs. hot pellets vs. final cold pellets), and analytical method (AOAC International method 996.11 vs. modified glucoamylase for TS and modified glucoamylase method vs. DSC for DG). Results were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Methodology Evaluation for Total Starch Content

Figure 2 shows the TS of feed tested by the AOAC International method 996.11 and the modified glucoamylase method at various processing stages (cold mash, hot mash, hot pellets, and final cold pellets) using a conditioning temperature of 88°C and retention time of 60 s. Total starch content varied with processing stages, although the trends as determined by the 2 methods were the same (hot pellet > hot mash > cold pellet > cold mash). The AOAC International method determined lower TS than the modified glucoamylase method in all samples. The TS in pelleted swine feed of common in-

Table 2. Effect of conditioning temperature and retention time on total starch content (TS) and degree of starch gelatinization (DG) in different diets according to the AOAC International method 996.11 (AOAC, 2007) and the modified glucoamylase method

Test	Cold mash	Cold pellet			Cold pellet		
		77°C			88°C		
		15 s	30 s	60 s	15 s	30 s	60 s
TS, %							
AOAC International method	27.7 ^a	34.3 ^c	31.4 ^{ab}	31.9 ^b	31.3 ^{ab}	30.6 ^{ab}	33.0 ^b
Modified glucoamylase method	35.2 ^a	36.0 ^a	35.3 ^a	34.7 ^a	36.2 ^a	36.6 ^a	35.6 ^a
Relative DG, ¹ %							
Modified glucoamylase method	0 ^a	6.2 ^b	9.1 ^c	9.4 ^c	15.1 ^d	16.6 ^e	20.8 ^f

^{a-f}Means not sharing a common letter in a row differ ($P \leq 0.05$).

¹The relative DG value shown above = tested DG value – 17.7% (tested DG value from cold mash).

redient formulation is not expected to change based on thermal processing (Kingman and Englyst, 1994). These observed variations among analytical methods may be due to incomplete cooking time which results in poorer hydrolysis, differences in glucose detection, or use of different hydrolytic enzymes.

The TS analytical methods require adequate cooking before sample enzymatic hydrolysis to achieve complete starch gelatinization (Rosin et al., 2002). If the cooking was performed effectively, one would expect similar TS values among forms, regardless of prior processing. The AOAC International method has a 6-min cooking time compared with the 20-min cooking time of the modified glucoamylase method. These differences are likely due to the substrate for which the analytical method was developed to test. The AOAC International method 996.11 was developed to assess the TS in cereal grains, which are more simplistic in structure than the multicomponent feed products intended to be analyzed by the modified glucoamylase method. This longer cooking time difference may be the root cause of variation among methods, leading to the greater TS value of pelleted feed by the modified glucoamylase method compared with the AOAC International method.

Alternatively, the variation between these 2 methods could also be caused by the difference in the detection method for glucose. For the AOAC International method, a colorimetric approach was used to evaluate the glucose concentration, in which D-glucose is oxidized to D-gluconate by glucose oxidase with the release of 1 mol of hydrogen peroxide (H_2O_2), which is quantitatively measured in a colorimetric reaction using peroxidase and the production of a quinoneimine dye. For the modified glucoamylase method, free D-glucose is measured using a glucose analyzer, YSI 2700 (model 2700; YSI Inc.), in which the D-glucose solution enters the sample chamber and then diffuses through a thin polycarbonate membrane material and encounters an extremely thin layer of the appropriate oxidase enzyme to produce H_2O_2 . The H_2O_2 then diffuses toward the platinum anode in the

probe assembly, which gives rise to the probe signal current and produces a signal in the sensor. Compared with the AOAC International detection method, the glucose analyzer detection method minimizes interference from color or turbidity of the original sample, which may lead to a more accurate result.

It should be noted that both a thermal stable α -amylase (hydrolyzing α -D-1,4 linkage only) and amyloglucosidase (hydrolyzing both α -D-1,4 linkage and α -D-1,6 linkage) are used in the AOAC International method, whereas the modified glucoamylase method uses only amyloglucosidase to hydrolyze the sample. This might contribute to the different results tested by these 2 methods. The use of α -amylase in the AOAC International method could minimize the possibility of production of maltulose (4- α -glucopyranosyl-D-fructose), which is resistant to hydrolysis by amyloglucosidase and α -amylase (Crabb et al., 2003).

It is also worth noting that both analytical methods for TS are based on assessing glucose concentration after hydrolysis. However, starch was not the only source of glucose in the multicomponent feed. Some of the analyzed glucose may be derived from nonstarch polysaccharides, oligosaccharides, and glucose from other ingredients, such as corn distillers dried grains with solubles (DDGS). Therefore, the predicted TS may be overestimated by both methods. This may explain why the analyzed TS values (Table 2) were, overall, greater than the calculated value of 28.44% in the experimental diet (40.55% corn in formula plus approximately 70% of total starch in corn = 28.4%, wt/wt) according to the composition of experiments diet as shown in Table 1.

Effect of Pelleting Stages, Preconditioning Temperature, and Retention Time on Total Starch Content

The TS in various pelleting stages for 1 processing condition (88°C and 60 s) as determined by both the AOAC International method and the modified glucoamylase method are shown in Fig. 2. The hot pellet

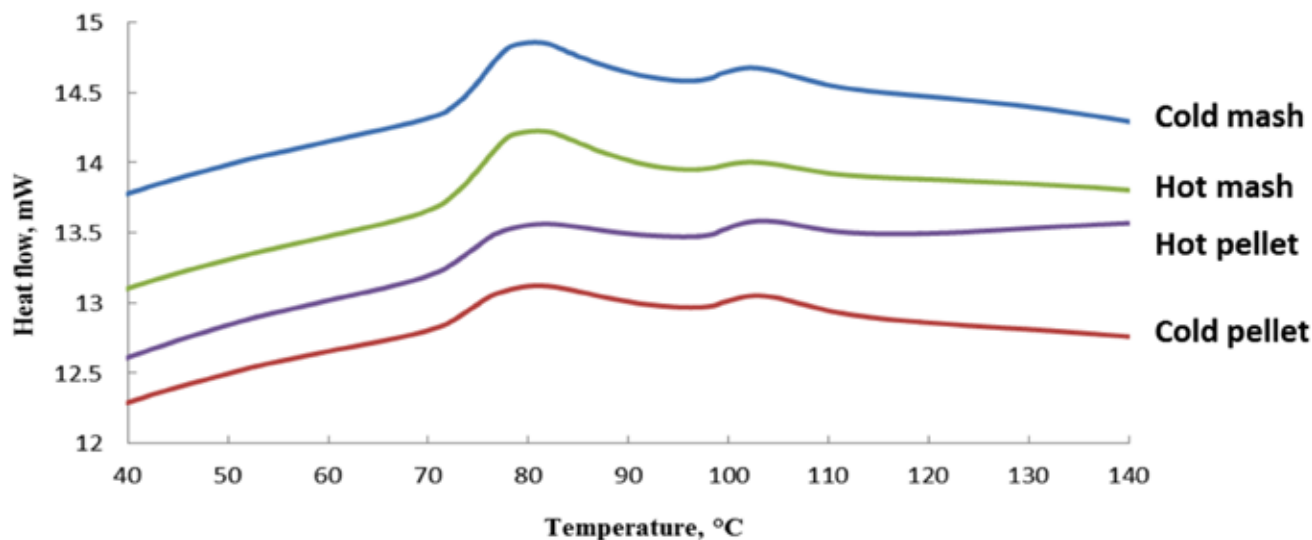


Figure 3. Differential scanning calorimetry thermograms for cold mash, hot mash, hot pellet, and cold pellet for diets with conditioning temperature and retention time of 88°C and 60 s, respectively.

demonstrated the highest TS value and followed by hot mash, cold pellet, and cold mash by both methods. This trend can be explained by the heating and mechanical pressing during the pelleting process. Native starch is packaged in granules that are semicrystalline. The crystalline areas tend to be unfavorable for enzyme attack, and in addition, the granules may contain small but variable amounts of proteins and lipids that can hinder starch–amylase interaction. The mechanical pelleting processing (heating and pressing) disrupted starch granule integrity and reduced starch degree of crystallinity and thus increased the susceptibility to amylase leading to higher TS values in hot mash and hot pellet. Englyst et al. (1992) also showed that mechanical damage to raw potato starch during analysis has been shown to increase its digestibility. Compared with hot mash and hot pellet, cold pellet showed a lower TS value, potentially due to the formation of resistant starch. It should be emphasized that both the AOAC International method and the modified glucoamylase method disregard the existence of resistant starch. Resistant starch has 4 main types: Type I is physically inaccessible or undigestible resistant starch. More extensive milling and chewing can make these starches more accessible and less resistant. Type II resistant starch is inaccessible to enzymes due to starch conformation, as in high-amylose cornstarch. Type III resistant starch is formed when starch-containing foods are cooked and cooled, due to starch retrogradation or recrystalline. Type IV resistant starch includes chemically modified starches that are used by food manufacturers to improve the functional characteristics of starch. During the pellet cooling process, gelatinized starch retrograded and recrystallized into a more ordered solid state and produced a proportion of type III resistant

starch, which was resistant to enzymes, resulting in a decreased TS value in cold pellet compare with nonretrograded samples (hot mash and hot pellet).

The TS values of final cold pellets manufactured with 2 conditioning temperatures (77 and 88°C), 3 conditioning retention times (15, 30, and 60 s), and determined by both the AOAC International method and the modified glucoamylase method are listed in Table 2. Results showed that increasing conditioning temperature and retention time have very slight impacts on the TS in cold pellet samples based on either evaluation method.

Methodology Evaluation for Degree of Starch Gelatinization

Starch will gelatinize in the presence of heat and moisture, and some starch gelatinization is likely to occur during the pelleting process. The heat flow during temperature scans of feed from different processing stages as obtained from the DSC method is presented in Fig. 3. Two peaks were observed in each scan: the large peak showing the enthalpy required to gelatinize the starch and the small peak derived from the amylase–lipid complex. It is clearly seen from Fig. 3 that the gelatinization enthalpy peak became smaller with the pelleting process order of cold mash to hot mash, hot pellet, and cold pellet, which indicated that the product was more and more gelatinized with progressive processing stages of the pelleting process.

The DG values as measured by DSC were compared with those determined by the modified glucoamylase method at different processing stages for feed produced at 88°C and 60 s conditioning temperature and retention time, respectively (Fig. 4). The DG values

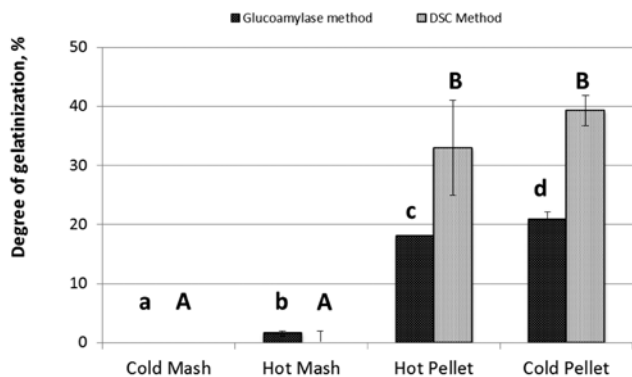


Figure 4. Degree of gelatinization for diets processed at 88°C and 60 s retention time tested by the modified glucoamylase method and the differential scanning calorimetry (DSC) method. Cold mash was used as a control; in the case of the modified glucoamylase method, the values of hot mash, hot pellet, and cold pellet were calculated by subtracting of cold mash value. ^{a-d}; ^{A,B}Means not sharing a common letter differ ($P \leq 0.05$).

obtained from either analytical method had a similar increasing trend with progression in pelleting stage. Both the DSC method and the modified glucoamylase method indicated an increased DG after pelleting. Either 20.8 (modified glucoamylase; Table 2) or 39.4% (DSC; Fig. 4) of gelatinized starch existed in the cold pellets samples compared with 0% in cold mash. These values are similar to the values reported in previous research. For example, expanded feed processed at 82°C resulted in 31% gelatinized starch, whereas pelleted feed processed at a similar temperature resulted in 28% gelatinized starch (Cramer et al., 2003). Skoch et al. (1981) measured gelatinized starch levels while dry pelleting and found 20 to 25% starch gelatinization.

Although the percentage of starch gelatinization was reasonable, the DSC method showed that the ΔH value ranged from 3.76 to 3.90 J/g for various processing stages, which is greater than theoretically possible. The enthalpy of pure starch is 9 J/g (Zhou et al., 2010). The analyzed samples were from diets containing 40.55% corn, and corn comprises approximately 70% starch (Hallauer, 2004). Therefore, the diet used in these experiments contained approximately 28.4% starch. Therefore, the theoretical maximum gelatinization enthalpy was 2.56 J/g, which is 1.2 to 1.3 J/g less than values obtained by our DSC analysis. The overestimation of gelatinization enthalpy from the DSC method may be due to the overlap of the protein denaturation peak (protein from soybean meal or corn) with the starch gelatinization peak. Another potential source of this overestimation may be the relative high quantity of DDGS (30%, wt/wt; Table 1) and a potential conformational change of the polysaccharides in DDGS during DSC measurement (Liu et al., 2014). Moreover, the sugars in DDGS may delay the temperature of starch gelatinization in samples by limiting water availability and de-

creasing water activity. It has been previously reported that sugars form bridges between starch chains and exert an antiplasticizing effect relative to water (Kim and Walker, 1992).

It is notable that cold mash samples had a DG value of 17.7% as measured by the modified glucoamylase method, despite not being thermally processed. Theoretically, there would be 0% DG in cold mash samples. There are 2 potential causes for this anomaly—1) some starch of the cold mash was gelatinized by mechanical energy during the grinding process or 2) the modified glucoamylase method actually measured some free D-glucose possibly present in DDGS—and this needs to be verified in our future research. In this experiment, the presence of free glucose in cold mash could lead to the overestimation of the DG value. Therefore, true gelatinization of the thermally processed samples would be the determined values minus the tested DG value from cold mash. For this experiment, the DG value was reported in this relative manner (Table 2; Fig. 4).

Effect of Pelleting Stages, Conditioning Temperature, and Retention Time on Degree of Starch Gelatinization

There were considerable differences in the DG of feed from the various processing stages ($P < 0.05$) when measured by both the DSC method and the modified glucoamylase method (Fig. 4). The DG value increased in each subsequent processing step. For both methods, hot pellets and final cold pellets had significantly greater DG than hot mash and cold mash. This may indicate that conditioning (in this case, at 80°C for 60 s) can gelatinize only a small percentage of starch compared with the potential gelatinization that occurs from the actual pelletizing process from pressing mash through the metal die. This result is consistent with previous reports that the frictional heat of the die is substantially more destructive to starch particles than steam conditioning (Stevens, 1987). Still, altering conditioning temperatures and time can facilitate starch gelatinization during pelleting and are important factors to consider for maximum pellet quality.

The DG of final cold pellets with various conditioning temperatures and retention times and measured according to the modified glucoamylase method are shown in Table 2. The DG of final cold pellets that was conditioned at 88°C was significantly greater than that conditioned at 77°C, and there was a trend for increased DG with increasing retention time. This was not altogether surprising because others have demonstrated that heating temperature and time alter the degree of starch cooking and that higher temperatures lead to a greater DG (Lund, 1984).

Conclusions

This was the first study that evaluated and conclusively reported the efficacy and accuracy of various methods for determination of TS and DG in pelleted animal feed. We found that increase in conditioning temperature and retention time has very slight impact on the measurement of TS during pelleting process. Increases in conditioning temperature and retention time, however, led to a higher DG in final cold pellet than hot mash, indicating that most of the gelatinization happened during the pelleting process and the conditioning, meaning that hot mash can only gelatinize small portion of starch.

As for the evaluation of different methods for analyzing starch in a pelleted animal diet, it is recommended that the modified glucoamylase method is more appropriate than the AOAC International method 996.11 for TS determination. This recommendation is based on the modified glucoamylase method having an overall greater extent of hydrolysis. In addition, less variation among various feed forms during thermal processing were detected for the modified glucoamylase method. However, both methods overestimate TS concentration in the multicomponent feed products. Future research is needed to assess correction of this overestimation. Furthermore, it is recommended that the modified glucoamylase method is appropriate for the determination of starch gelatinization in pelleted animal diets, as DG results followed a logical sequence based on changing conditioner temperatures and retention times and were similar to referenced values in the literature. However, we conclude that the DSC method is not appropriate for determining starch gelatinization in pelleted animal feed because nonstarch transformations, such as protein denaturation, makes interpretation more difficult and likely leads to an overestimation of DG.

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