Influence of ingredient quality and diet formulation on amino acid digestibility and growth performance of poultry and swine

by

Kara Michelle Dunmire

B.S., Penn State University, 2016
M.S., Texas A&M University, 2017

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

Ingredient varieties and heat processing applied to ingredients or complete diets can influence nutrient utilization for monogastric species and introduce variability in the final product sold for feed use or consumption. Thus, seven experiments were used in accomplishing the objective of this dissertation: to determine the influence of ingredient quality and diet formulation on amino acid (AA) digestibility and subsequent effects of growth performance for poultry and swine. First, the effects of using excessive thermal treatment of soy white flakes as a model for soybean meal (SBM) quality determined by official analytical methods and near infrared reflectance spectroscopy (NIRS) prediction equations was evaluated. Crushed soy white flakes (SWF) only exposed to mechanical pressing were ground and autoclaved at 128ºC for 0, 5, 10, 15, 30, 45, and 60 min at 200 kPa. Official analytical methods and NIRS were highly correlated for total Lys, available Lys, and Lys/CP. However, bias correction is needed to use the NIRS calibration set for SBM to predict SBM values from SWF. The next set of 3 experiments evaluated different soybean varieties with varying levels of crude protein (CP) when fed to broilers. Dietary treatments for these experiments consisted of 1 of 4 soybean sources varying in quality determined by CP content and processed into SBM. Two sources consisted of soybeans from a similar region and processed either commercially solvent extracted or experimentally solvent extracted. It was concluded that, broilers fed commercially processed SBM had improved AA digestibility compared to those fed experimentally processed soybeans from a similar region. Increasing CP content increased AA digestibility in both studies with no evidence for differences in high CP SBM and conventionally processed SBM. However, when broiler growth performance was evaluated, broiler performance was improved in broilers fed conventionally processed SBM compared to experimentally processed SBM. Within experimentally processed

SBM treatments, when formulating diets using previously determined AA digestibility's there was no evidence of difference in growth performance. The fifth study determined the influence of dietary fat and crystalline AA inclusion on broiler diet formulation and pellet quality. Dietary treatments consisted of a corn and SBM-based control, the control with crystalline valine (Val), and the control with crystalline Val and isoleucine (Ile). As crystalline AA increased in the diets, corn concentrations increased as SBM and the fat source were removed to balance for nitrogen-corrected metabolizable energy (MEn). Diets with increasing crystalline AA, Val, and Val + Ile, led to improved pellet quality which can be explained by the 0.4% or 0.6% reduction in added fat with increasing crystalline AA and balancing for MEn in the diet. The final two studies determined the effect of the pelleting process on diet formulations with varying levels of crystalline AAs and reducing sugars (RS) on digestibility and growth performance in growing pigs. Diets were formulated with low or high crystalline AA and low or high RS provided by co-product ingredients, DDGS and bakery meal. Digestibility and growth performance results concluded that pelleting diets with increased crystalline AA or RS did not affect from the pelleting response due to the Maillard reaction.

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Approved by:

Major Professor
Dr. Chad B. Paulk

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Dedication

This dissertation is dedicated to my parents, Jeff and Ann Dunmire.

Chapter 1 - Overprocessing soy white flakes to demonstrate differences in soybean meal quality determined by official analytical methods and NIRS prediction equations

ABSTRACT

This study aimed to evaluate the correlations between chemical assay and near-inferred reflectance spectroscopy (NIRS) when measuring protein quality. Crushed soy white flakes (SWF) exposed to mechanical pressing but not heat processing were autoclaved to simulate heat processing. Samples of 500 g were ground using a blender and autoclaved at 128ºC for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa. A total of 2 samples per treatment were autoclaved at 3 time points to provide 3 replications per treatment. The duplicate samples were divided and analyzed using NIR or official analytical procedures. Available Lys, Lys:CP, available Lys: total Lys, KOH solubility, and Lys decreased (quadratic, $P < 0.05$; DM basis) and DM, where Leu, Met, Thr, and Val increased (linear, $P < 0.05$; DM basis) using official analytical procedures. Increasing SWF exposure time to the autoclave decreased (linear, $P < 0.05$; DM basis) DM, Arg, His and decreased (quadratic, $P < 0.05$; DM basis) Lys, Lys:CP and reactive Lys. However, there was an increase (quadratic, $P < 0.05$; DM basis) in Leu, Met, Phe, Thr, and Val using NIRS. There was a positive correlation ($r = 0.82$, $r = 0.98$, $r = 0.99$) between NIR Lys and analytical Lys; NIRS reactive and analytical available Lys; and NIRS Lys:CP and analytical Lys:CP, respectively. A linear model was best fit for NIRS CP, total Lys, reactive Lys and Lys:CP compared to official analytical methods. Thus, in this study bias correction is needed in order to use the NIRS calibration set for SBM to predict SBM values from SWF. In conclusion, increasing soybean autoclave exposure decreased soybean meal quality as indicated by official

analytical methods for available Lys, Lys:CP, available Lys:total Lys, KOH solubility, and Lys. Official analytical methods and NIR were highly correlated for total Lys, available Lys, and Lys:CP.

INTRODUCTION

Soybeans are the most abundant oilseed worldwide which provide byproducts including soybean meal and oil via additional processing. Multi-step processing provides opportunity to introduce variability in the final product sold for feed use. The quality of soybean meal must be evaluated when formulating poultry diets. After dehulling and solvent extraction, soybean meal is further processed by toasting to destroy antinutritional factors. However, there can be negative effects of both under-heating and over-heating. The effects of under heating will increase trypsin inhibitors and urease levels in soybean meal. Trypsin inhibitor can prevent the pancreatic enzyme secreted in the duodenum from hydrolyzing peptide bonds. Urease occurs naturally in soybean meal and converts urea to ammonia, potentially increasing ammonia excretion in ruminants. The effects of overheating include the Maillard browning reaction and loss of amino acid (AA) availability to the animal. In the Maillard reaction, a free amine group of an amino acid, most commonly Lys, will bind to a carbonyl group of a sugar, forming an unstable Schiffs base. To achieve stability, the Schiffs base will rearrange to form an irreversible Amadori compound, denaturing the Lys residue and decreasing detection in analytical assay and availability for growth. Only Lys with a free amino group is defined as reactive Lys and considered as biologically available Lys for body deposition (Rutherford and Moughan, 1997). In a study evaluating corn and distillers dried grains with solubles, the concentration of standardized ileal digestible Lys in corn was correlated with reactive Lys concentration (Pahm et

al., 2009). In a study evaluating canola meal quality, over processing provided a reduction of reactive Lys of 10% (Spragg and Mailer 2007).

Variation in soybean meal protein quality can vary by location, time of year and country of origin. While soybean meal sourced from Brazil and India has higher concentrations of CP and AA; China and United States SBM have greater apparent ileal digestibility (AID) and standard ileal digestibility (SID; Lagos et al., 2017). Soybeans sourced from the United States and Brazil also have less variability among sources compared to Argentina, China, or India (Lagos et al., 2017). Therefore, the ability to quickly identify under- and over processed soybean meal is important for suppliers, nutritionists, and feed manufacturers. Traditional analytical methods are expensive, skill intensive and time consuming. Near-infrared reflectance spectroscopy (NIRS) is a tool that requires minimal sample preparation and user training, providing rapid and economical results when compared to official analytical analysis. Therefore, the objective of this study was to create a range of soy white flake (SWF) quality to evaluate the correlations between official analytical methods and established NIRS equations (AB Vista, Plantation, FL) used to measure the protein quality of soybean meal.

MATERIALS AND METHODS

A total of 900 kg of SWF were collected from a soybean crush plant (Mark Hershey Farms, Lebanon, PA). The SWF were mechanically pressed for oil extraction at the soybean crush plant and defined as soybean meal prior to the heating step of soybean meal processing. Soy white flakes were ground using a coffee grinder (Hamilton Beach Fresh Grind, Hamilton Beach Brands, Inc), and then two 500 g samples were autoclaved (Yamato SE510, Yamato Scientific America, Santa Clara, CA) at 128°C for 0, 5, 10, 15, 30, 45, or 60 min. A total of 2

samples per treatment were autoclaved in 3 blocks to provide 3 replications per treatment and 6 observational units per treatment. Samples were split to be evaluated by official analytical methods and NIRS analysis. Treatment run times were randomized within block to ensure no effects of time or autoclave order. All treatments within block were run in the same week as the first sample of the block. Samples were collected from each treatment and analyzed for total AA, available Lys, urease, trypsin inhibitor unit, and KOH protein solubility, as described below.

The autoclave process consisted of a 15 min warm-up to bring the chamber temperature and pressure to 128°C and 200 kPa, respectively. The chamber temperature and pressure remained as required (either 0, 15, 30, 45 or 60, and 200 kPa). The samples were cooled for 5 min at 110°C and 14 kPa before discharge from the chamber.

Sample analysis

Official analytical methods were performed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri. Complete AA profiles were analyzed according to AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006. Available Lys was analyzed using the fluorodinitrobenzene (FDNB) method, according to AOAC official method 975.44. Protein solubility using the KOH method was determined according to Parsons et al. (1991) Trypsin inhibitor activities were measured according to AACC official method 22-40, 2006. Urease activity was measured according to AACC International Method 22-90.01. Once treatments were analyzed via official analytical methods, data were used to compare to established NIRS equations (AB Vista, Plantation, FL) using a NIRS DS2500 (FOSS, Eden Prairie, MN). The NIR equations estimated CP and reactive (or available) Lys based on a global soybean meal database. Additionally, analytical methods used to measure available Lys

compared the florodinitrobenzene method (FDNB) and guanidination method for the official analytical methods and NIRS established equations, respectively.

Statistical analysis

Data were analyzed as a randomized complete block design using the GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC) with soybean meal sample as the experimental unit, autoclave time as a fixed effect and period as a blocking factor. Linear and quadratic orthogonal contrasts were used, with coefficients for the unequally spaced contrasts being derived using the IML procedure in SAS. Results are expressed as least square means for each independent variable. Correlation analysis was performed to determine the degree of association between official analytical and NIRS results. Linear and/or quadratic regression was used to develop models for predicting official analytical total and available Lys, and Lys:CP using NIRS estimates. Results were considered significant if $P \leq 0.05$.

RESULTS

Soybean meal quality

Increasing SWF autoclave exposure time darkened the color of the SWF and created visual evidence of the Maillard browning reaction, varying in quality (Figure 1). Therefore, increasing SWF exposure time to the autoclave decreased (linear, $P < 0.05$; DM basis) DM, Arg, His, and Cys when measured using NIR (Table 1). There was an increase (linear, $P < 0.05$; DM basis) in Ala, Pro, Tyr when increasing autoclave exposure time. Reactive Lys, Lys:CP and Lys decreased (quadratic, $P < 0.05$; DM basis) when measured using NIR with increasing soybean meal exposure time to the autoclave. There was an increase (quadratic, $P < 0.05$; DM basis)

when measuring Ile, Leu, Met, Phe, Thr, Val, Asp, Glu, Gly, and Ser with increasing autoclave exposure time.

Dry matter and CP decreased (linear, $P < 0.05$; DM basis) when measured with official analytical results with increasing autoclave exposure time (Table 2). There was an increase (linear, $P < 0.05$; DM basis) measuring Ala, Glu, Gly, Leu, and Val when soy flakes increased with autoclave exposure. Increasing autoclave exposure time decreased (quadratic, $P < 0.05$; DM basis) Lys:CP, available Lys, available Lys:total Lys, KOH solubility, trypsin inhibitor, urease, Lys and Cys when measured using official analytical results. Additionally, there was an increase (quadratic, $P < 0.05$; DM basis) in Ile when SWF were exposed to the autoclave.

Predictability of official analytical results using NIRS

Crude protein, Lys:CP, and AA were analyzed to determine the correlation coefficient and coefficient of determination for official analytical method results and NIRS results (Table 3). There was a positive correlation ($P < 0.05$) between official analytical method and NIRS results for CP ($r = 0.546$; $R^2 = 0.298$), Lys:CP ($r = 0.977$; $R^2 = 0.954$), reactive Lys: total Lys ($r = 0.953$; $R^2 = 0.981$), total AA ($r = 0.819$; $R^2 = 0.538$), Ala ($r = 0.491$; $R^2 = 0.241$), Cys ($r = 0.924$; $R^2 = 0.853$), Lys ($r = 0.975$; $R^2 = 0.950$), and a negative correlation for Thr ($r = -0.666$; $R^2 = 0.444$). The NIR reactive Lys was positively correlated ($r = 0.935$; $r^2 = 0.981$) to official analytical available Lys.

For soybean meal quality measurements, official analytical method results for available Lys were positively ($P < 0.05$) correlated with official analytical method results for KOH solubility ($r = 0.879$; $R^2 = 0.772$), trypsin inhibitor ($r = 0.798$; $R^2 = 0.637$), urease ($r = 0.758$; $R^2 = 0.574$), Lys/CP ($r = 0.987$; $R^2 = 0.424$), and total Lys ($r = 0.995$, $R^2 = 0.991$; Table 4).

However, the model fit improved when adding a quadratic function to official analytical method results for available Lys. Therefore, official analytical method available Lys (quadratic) was a good predictor ($P < 0.05$) of quality measurements: KOH solubility ($R^2 = 0.829$), trypsin inhibitor ($R^2 = 0.951$), urease ($R^2 = 821$), Lys:CP ($R^2 = 0.991$) and total Lys ($R^2 = 0.992$).

In addition, NIR results for reactive Lys were positively ($P < 0.05$) correlated with official analytical method results for available Lys ($r = 0.976$; $R^2 = 0.954$), KOH solubility ($r = 0.817$; $R^2 = 0.667$), trypsin inhibitor ($r = 0.686$; $R^2 = 0.471$), urease ($r = 0.684$; $R^2 = 0.467$), Lys:CP ($r = 0.986$; $R^2 = 0.470$), and total Lys ($r = 0.974$, $R^2 = 0.949$). However, the model fit improved when adding a quadratic function to NIRS results for reactive Lys. Therefore, NIRS reactive Lys (quadratic) was a good predictor ($P < 0.05$) of soybean meal quality measurements: available Lys ($R^2 = 0.988$), KOH solubility ($R^2 = 0.825$), trypsin inhibitor ($R^2 = 0.875$), urease ($R^2 = 0.799$), Lys/CP ($R^2 = 0.975$), and total Lys ($R^2 = 0.968$).

The NIRS results for total Lys were positively ($P < 0.05$) correlated with official analytical method results for KOH solubility ($r = 0.867$; $R^2 = 0.753$), trypsin inhibitor ($r = 0.789$; $R^2 = 0.623$), urease ($r = 0.734$; $R^2 = 0.539$), and Lys:CP ($r = 0.990$; $R^2 = 0.980$). The model was better fit when adding a quadratic function to NIR results for reactive Lys. Therefore, NIR reactive Lys (quadratic) was a good predictor ($P < 0.05$) of soybean meal quality measurements: KOH solubility ($R^2 = 0.817$), trypsin inhibitor ($R^2 = 0.958$), urease ($R^2 = 0.780$), and Lys/CP ($R^2 = 0.989$). Statistically, quadratic responses are of higher order and are typically discussed in the case that there is both a linear and quadratic response. However, to achieve the objective of this study a linear relationship of official analytical methods to NIRS was best used to describe the data to determine predictability of NIRS values to true analytical values.

A linear model was best for NIRS CP (Figure 2), NIRS total Lys (Figure 3), reactive Lys (Figure 4), and and Lys: CP (Figure 5) predicting official analytical results of CP (R^2 = 0.489), total Lys (R^2 = 0.950), available Lys (R^2 = 0.954) and Lys: CP (R^2 = 0.954).

DISCUSSION

Soy white flakes were heated to create an experimental model for soybean meal and establish correlations between official analytical methods and NIRS results using equations established for soybean meal. These soybeans contained 39.3% crude protein (official analytical method) with a minimum of 18.8% crude fat (guaranteed analysis). Soybeans used in this study were sourced prior to heat processing, mechanically flaked, and ground before exposure to the autoclave. Therefore, to apply thermal processing an autoclave was used to establish a model to simulate the heating step of soybean meal processing. Autoclaves are traditionally used for sterilization which is accomplished with high temperature, pressure, and steam. The use of steam in the autoclaving process led to an increase in moisture content of the sample in this study. A range of overprocessed soybean meal was established when samples were exposed to the autoclave conditions. Common analytical measures used to determine soybean meal quality include trypsin inhibitor unit, urease activity, and potassium hydroxide (KOH) solubility and were also used in this study to define soybean meal quality after the autoclave processing. Traditionally, trypsin and urease analyses are used to determine under processed soybean meal where protein solubility in KOH, total Lys and reactive or available Lys are used to determine overprocessed soybean meals. Considering KOH solubility, TIU, and urease, there was a drastic decrease when the heat from the autoclave was applied from 0 to 5 min, with a gradual decrease observed from 10 to 60 min of autoclave exposure. Therefore, suggesting that targeting a time

interval in the 0 to 5 min range for heat exposure in this study could lead to a more gradual decrease of soybean meal quality. As for measures of under processing of soybean meal, from time 0 to 5 min, there was a decrease from 3.2 to 0.5 TIU/mg and 0.7 to 0.2 ΔpH for urease, respectively, with little change to samples autoclaved beyond 5 min. To measure overprocessed soybean meal with KOH solubility values, there was a gradual decrease from 0 to 60 min, ranging from 89.9 to 16.8% solubility. Target values for quality soybean meal include between 78 to 84%, less than 2.5 TIU/mg, and Δ 0.05 – 0.2 pH rise for KOH solubility, trypsin inhibitor units, and urease activity, respectively. The use of an autoclave to model soybean meal processing was also validated by Batal et al., (2000) who reported a reduction in trypsin inhibitor units from 44.2 to 2.6 TIU/mg, decrease in urease index from 2.4 to 1.0 ΔpH and decrease in KOH solubility from 97 to 78% with increasing autoclave time up to 36 min at 121°C for SWF. The American Feed Manufacturers Association (AFMA) recommended urease activity index values between 0.05 and 0.20 ΔpH (AFMA, 1979). Whereas urease levels between 0.05 and 0.20 have been considered optimum, levels below 0.05 do not necessarily mean that SBM has been overheated (Waldoup et al., 1985; Araba and Dale, 1990; Pacheco, 2014). Some issues with urease activity as an indicator of soybean meal quality have been identified, as no change in pH does not always indicate overheating and low activity levels are not correlated to chick performance (Araba and Dale, 1990).

Dozier and Hess (2011) observed that KOH solubility values below 74% have been considered an indication of excessive heat treatment and values above 85% have been associated with insufficient heat treatment. Therefore, the recommended range of KOH solubility is 78 to 84% (Parsons et al., 1991). Combining soybean meal quality measurements may be more effective in establishing quality than using each measurement alone. For example, trypsin

inhibitor or urease activity combined with KOH solubility can provide insight into SBM under processing and overprocessing, respectively. Ruiz and de Belalcàzar (2005) concluded that optimum residual concentration of trypsin inhibitor is < 2.0 mg/g with at least 78% KOH solubility. Therefore, in this study, soybean meal was considered over processed once autoclave heat was applied.

Soy white flakes not exposed to heat processing (autoclave) contained 39.3% CP analyzed using official analytical methods which was lower than that of conventional soybean meal (46 to 48% CP). However, the United Soybean Board reports values of SWF of 35% CP. The difference in CP values between conventional soybean meal and SWF can be explained by difference in processing from SWF to soybean meal. Conventional soybean meal is processed by solvent extraction and heat processing, whereas SWF are only mechanically crushed. When SWF with no heat processing were analyzed via NIRS, CP values were higher than that of official analytical methods which could be explained by the official analytical value for CP being outside the detectable range of the NIRS calibration equation. This is because NIRS calibration equations were created using SBM which would have more CP than SWF. Thus, the NIRS predicted higher than expected CP values and can also explain underestimated reactive lysine values. Nevertheless, total and reactive Lys content in this experiment agrees with soybean meal analyzed by Kim et al., (2012) where total Lys content ranged from 27.5 to 19.2 g/kg and reactive Lys ranged from 23.4 to 11.7 g/kg with increasing heating time. Therefore, this model was successful at establishing overprocessed soybean meal. To determine under processed soybean meal, a look into the timeframe from 0 to 5 min in this study would be necessary to use the calibrations for under processed soybean meal.

Soy white flakes sourced for this study had a guaranteed analysis of 18% crude fat while solvent extracted SBM typically used in diet formulation contains 1.5% (NRC. 2012). Because the SWF that were used did not undergo the solvent extraction or a heating step needed to create soybean meal, this may have increased the number of volatile compounds present compared to typical soybean meal at processing. These volatile compounds include alcohols, aldehydes, esters and lactones, ketones, and terpenoids which are used to determine quality of soybean varieties indicating developmental maturity and as biochemical markers (Ravi et al., 2019). Since only mechanical pressing was applied to these soybean seeds, these compounds were likely present and may have potentially interfered with analytical procedure.

Because soybean meal was considered over processed once autoclave heat was applied, the NIRS method was able to provide correlations between NIRS compared to official analytical methods for overprocessed soybean meal. Use of animal digestibility assays may be the best way to determine a feedstuff's amino acid availability to the animal; however, this method comes with added time, cost and inherent variability. The use of NIRS to measure the nutritive value of a feedstuff has grown in popularity as a rapid and economical tool that requires little training and minimal sample preparation compared to traditional analytical methods. The NIRS utilizes wavelengths from different characteristics of chemical bonds to provide absorbance values that correspond to different nutritive components of a feedstuff or complete feed (Samadi et al., 2020). Calibration of these wavelengths is important in determining coefficients for specific nutrients to establish matrix values for precision diet formulation. In this study the NIRS was able to provide crude protein, total Lys, reactive Lys, as well as additional amino acids. These values provided by NIRS were precise, however the accuracy of prediction was skewed providing a greater gap in results (NIRS vs. wet chemistry) due to equations established for SBM

rather than SWF. Since Lys is a key limiting amino acid and most likely to be bound in the in the Maillard reaction, this provides valuable information. Established regression correlations between official analytical methods and NIRS for total Lys, available Lys: reactive Lys, and Lys: CP had no less than a 0.95 correlation coefficient. Therefore, the correlation between official analytical methods and NIRS was highly successful in confirming NIRS calibration for overprocessed soybean meal. Additionally, there is potential for the range of CP for NIRS equations to be narrow and the range for Lys to be greater. As samples were heated, correlations (Figure 3 and 4) became more accurate; however, when samples were heated less, separation in the accuracy of the values where NIRS results should equal official analytical results became greater. This would also explain why there is a quadratic response but should only be explained as a linear relationship. The likelihood of more volatile compounds influencing results has a greater chance in samples with less exposure to the autoclave where increasing temperature would ultimately destroy volatile compounds.

There is extensive terminology around Lys and its accessibility determined by analytical method and even differences among researchers, causing considerable confusion. Terminology clarified by Rutherford and Moughan (2007) provides the following definitions for total Lys, available Lys, and reactive Lys. Total Lys refers to reactive Lys and reverted Lys, where reverted Lys is Lys from early Maillard products that can be reversed prior to Amadori rearrangement during the Maillard reaction. Available Lys refers to undamaged Lys residues digested and absorbed by an animal, therefore potentially available for protein synthesis. Reactive Lys refers to the undamaged Lys residues or Lys that have not been subject to the Maillard reaction, leaving the side chain amino group free to react. This refers to any analytical method that targets the ε -amino group of Lys including the florodinitroebenzene (FDNB) and guanidination method. In

this study, reactive Lys was determined by both the FDNB and guanidination methods via the wet chemistry and NIRS, respectively. The FDNB method measures the colorimetric change converting Lys to dinitrophenyl-Lys to be measured by HPLC. However, converted dintrophenyl- Lys can be destroyed during acid hydrolysis leading to underestimation of reactive Lys. The guanidination method is a reaction of the ε – amino group and o – methylisourea to produce stable homoarginine. The guanidination reaction happens prior to acid hydrolysis therefore Lys cannot be reverted from homoarginine. However, it is important that the reaction to homoarginine is complete. Additional challenges with the FDNB method includes reacting with both the α – and ε – amino groups where guanidination method will only react with ε – amino groups. In this case, the FDNB method can result in overestimation of reactive Lys. If analyzing synthetic amino acids, popular in complete feeds in monogastric animals, the use of HPLC in the FDNB method can be particularly challenging because of the separation of quantification of free Lys, however this does not occur using the guanidination method. Rutherford and Moughan (2007) reactive Lys content determined using the guanidination or FDNB method was highly correlated for a range of animal feedstuffs.

The thermal stress of the autoclave led to the formation of Maillard reaction products. In this model, there was decrease in AA content and further decrease in Lys and Cys with increasing autoclave exposure, which is supported by Fontaine et al., (2007) and Gonzalez-Vega et al., (2011). This can be explained due to advanced Maillard reactions in which Lys is the most susceptible and Cys is more likely affected in advanced reactions due to the formation of crosslinked compounds between Lys and other AA. These advanced reactions produce deoxykeytosol and melanoidin compounds. These melanoidins products may decrease

proteolytic enzyme efficacy by blocking absorption sites and reducing AA digestibility. (Hurrel, 1990; Moughan and Rutherfurd, 1996; Martinez-Amezcua et al., 2007).

Implications of underheated and overheated soybean meal is observed in digestibility of feedstuffs. Fernanadez and Parsons (1996) fed autoclaved soybean meal to cecectomized roosters and confirmed that Lys from heat damaged soybean meal was not bioavailable for protein synthesis. Multiple studies have shown true ileal digestibility (TID), apparent ileal digestibility (AID), and standard ileal digestibility (SID) of amino acids decreases with increased soybean exposure to heat (Karr-Lilienthal et al., 2004; Gonzalez-Vega et al., 2011; Kim et al., 2012). In a validation study, Kim et al., (2012) determined linear regression equations to predict amounts of AID, SID, and TID of total and reactive Lys from total and reactive Lys contents in SBM based *in vivo* ileal digestible Lys contents. The consequence of underestimating Lys availability in poultry diets is detrimental to growth.

While the predictability of results was correlated, the NIRS did not accurately predict the official analytical procedures due to the need to bias the equations. This can be explained by the values used to calibrate the equations for soybean meal. It should be noted that calibrations for NIRS CP measurements were built around soybean meal samples containing 46% CP, however these samples of SWF contained 38% CP. Therefore, the analyzed samples were outside of calibration range used to develop the NIRS soybean meal equations. In addition, the NIRS estimates of total Lys and reactive Lys were highly correlated with the analytical measurements for total Lys and available Lys, however, they underestimated the analyzed values. These concentrations are lower in SWF compared to SBM which was used to develop the calibrations and demonstrate the need to bias equations when using NIRS to analyze these ingredients.

In conclusion, increasing SWF autoclave exposure time decreased quality and indicated overprocessing as measured by KOH solubility, Lys, available Lys, Lys:CP, and available Lys:total Lys. These results created a model to estimate the relationship between NIRS and official analytical results. Regression models were successful at using NIRS Lys, available Lys, their ratio, and Lys:CP. This demonstrated the ability of NIRS to be used as a tool to determine soybean meal quality. However, bias correction equations may need to be used to improve the accuracy of measurements using SWF to predict SBM using NIRS.

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Figure 1.1 Soy white flakes with no prior to heat processing were ground and exposed to an autoclave at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa, simulating the heating step in soybean meal processing to create a range of soybean meal quality.

Table 1.1 Effect of autoclave heating time on crude protein, AA, Reactive Lys, and Lys/CP using near-infrared spectroscopy (NIRS; DM basis)[1]

	Autoclave time (min)								Probability, *P*<	
Item, %	0	5	10	15	30	45	60	SEM	Linear	Quadratic
DM	93.15	88.48	88.61	88.80	88.74	87.18	86.26	0.634	0.001	0.084
CP	42.50	41.59	41.67	42.14	41.87	41.60	40.99	0.452	0.061	0.619
Lys/CP	4.74	4.21	3.96	3.72	3.15	2.62	2.10	0.062	0.001	0.001
Reactive Lys	1.63	1.31	1.15	1.01	0.65	0.30	-0.04	0.046	0.001	0.005
Total AA	45.79	46.89	46.43	45.82	44.15	44.18	43.05	0.640	0.001	0.901
Indispensable AA										
Arg	3.32	3.49	3.39	3.31	3.06	2.96	2.79	0.074	0.001	0.646
His	1.49	1.47	1.45	1.43	1.36	1.38	1.33	0.025	0.001	0.228
Ile	2.03	2.25	2.25	2.25	2.23	2.27	2.27	0.027	0.001	0.001
Leu	3.30	3.68	3.65	3.63	3.57	3.63	3.55	0.037	0.124	0.001
Lys	2.17	1.98	1.86	1.76	1.49	1.25	1.00	0.036	0.001	0.029
Met	0.69	0.77	0.76	0.76	0.76	0.78	0.76	0.008	0.001	0.001
Phe	2.76	3.04	3.04	3.03	2.95	3.03	3.00	0.035	0.031	0.013
Thr	1.53	1.77	1.76	1.73	1.70	1.75	1.73	0.026	0.020	0.005
Val	1.90	2.23	2.23	2.22	2.22	2.28	2.29	0.031	0.001	0.001
Dispensable AA										
Ala	1.92	1.97	1.97	1.96	1.96	2.02	2.01	0.018	0.003	0.888
Asp	5.43	5.81	5.80	5.79	5.74	5.86	5.85	0.054	0.002	0.042
Cys	0.42	0.40	0.38	0.36	0.30	0.26	0.22	0.012	0.001	0.105
Glu	6.65	7.65	7.57	7.45	7.34	7.45	7.30	0.092	0.170	0.001
Gly	1.82	1.94	1.95	1.95	1.97	2.04	2.04	0.024	0.001	0.033
Pro	2.54	2.54	2.54	2.52	2.54	2.70	2.80	0.054	0.001	0.085
Ser	1.97	2.31	2.28	2.23	2.16	2.15	2.07	0.030	0.041	0.000
Tyr	1.74	1.73	1.73	1.73	1.73	1.78	1.79	0.025	0.008	0.191

[1] Soy white flakes after mechanical pressing but prior to heat processing, were ground using a blender and 500 g samples were put into calico cotton bags to be autoclaved. Samples were autoclaved at 128ºC for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa. A total of 2 samples per treatment were autoclaved in 3 blocks to provide 3 replications per treatment.

Table 1.2 Effect of autoclave heating time on soybean meal quality when measured using official analytical procedures (DM basis)[1]

	Autoclave time (min)								Probability, *P*<	
Item, %	0	5	10	15	30	45	60	SEM	Linear	Quadratic
DM	94.18	89.65	89.54	89.37	89.52	87.76	86.63	0.643	0.001	0.0866
CP	39.27	38.74	38.43	38.46	38.31	38.23	38.16	0.358	0.060	0.268
Available Lys	2.59	2.20	2.01	1.91	1.57	1.39	1.22	0.028	0.001	0.001
Lys:CP	6.40	5.66	5.53	5.37	4.95	4.55	4.25	0.064	0.001	0.001
Available Lys:total Lys	0.97	0.90	0.85	0.83	0.74	0.70	0.65	0.009	0.001	0.001
KOH solubility[2]	95.43	66.22	42.49	35.56	27.00	23.99	19.34	7.312	0.001	0.001
Trypsin inhibitor index, TIU/mg	3.40	0.54	0.47	0.43	0.04[3]	0.15	0.22	0.082	0.001	0.001
Urease, ΔpH	0.79	0.24	0.18	0.18	0.16	0.14	0.12	0.060	0.001	0.001
Total AA[2]	30.64	30.63	30.65	30.52	30.49	30.43	30.32	0.184	0.031	0.976
Indispensable AA										
Ile	1.96	1.97	1.99	2.00	2.00	2.01	2.00	0.008	0.002	0.036
Leu	3.20	3.23	3.24	3.26	3.27	3.29	3.31	0.020	0.001	0.100
Lys	2.67	2.45	2.38	2.31	2.12	1.98	1.87	0.023	0.001	0.001
Met	0.61	0.62	0.63	0.62	0.64	0.64	0.66	0.010	0.001	0.913
Thr	1.59	1.61	1.62	1.63	1.64	1.65	1.68	0.015	0.001	0.647
Val	2.14	2.18	2.18	2.19	2.19	2.22	2.21	0.019	0.002	0.187
Dispensable AA										
Ala	1.79	1.83	1.83	1.84	1.85	1.85	1.87	0.008	0.001	0.067
Asp	4.77	4.82	4.83	4.82	4.82	4.80	4.81	0.024	0.882	0.293
Cys	0.68	0.62	0.61	0.59	0.56	0.54	0.50	0.004	0.001	0.001
Glu	7.33	7.40	7.43	7.43	7.46	7.52	7.56	0.046	0.001	0.562
Gly	1.81	1.85	1.86	1.86	1.88	1.89	1.90	0.010	0.001	0.006
Pro	2.10	2.08	2.08	2.00	2.08	2.06	1.95	0.122	0.392	0.742

[1] Soy white flakes after mechanical pressing but prior to heat processing, were ground using a blender, and 500 g samples were put into cotton bags to be autoclaved. Samples were autoclaved at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa. A total of 2 samples per treatment were autoclaved in 3 blocks to provide 3 replications per treatment.

[2] CP = crude protein. AA = amino acids. KOH = protein solubility in potassium hydroxide.

[3] Samples from 2 of 3 replications were undetected therefore a value of zero was used.

Table 1.3 Near-infrared reflectance spectroscopy (NIRS) Pearson correlation coefficient (*r*) and coefficient of determination (R^2) to analytical official analytical method values[1]

Item	Pearson *r*	R^2	Probability, *P*<
CP[2]	0.546	0.298	0.011
Lys:CP	0.977	0.954	0.001
Reactive Lys:total Lys	0.935	0.981	0.001
Total AA[2]	0.819	0.538	0.001
Indispensable AA			
Ile	-0.214	0.046	0.352
Leu	-0.138	0.019	0.551
Lys	0.975	0.950	0.001
Met	0.021	0.000	0.928
Thr	-0.666	0.444	0.001
Val	-0.413	0.170	0.063
Dispensable AA			
Ala	0.491	0.241	0.024
Asp	0.119	0.014	0.607
Cys	0.924	0.853	0.001
Glu	-0.270	0.073	0.237
Gly	0.108	0.012	0.641
Pro	-0.021	0.000	0.929

[1]Predictability of the variance between NIRS and official procedures for individual AA analysis for under-processed, adequately processed, and over-processed soybeans.
[2]CP = crude protein. AA = amino acids.

Table 1.4 Using official analytical method values for available Lys and total Lys and near-infrared reflectance spectroscopy (NIRS) reactive Lys to determine Pearson correlation coefficient (*r*) and coefficient of determination (R^2) to official analytical method[1]

Item	Pearson *r*	R^2	Probability, *P* <
Available Lys Linear[2]			
KOH	0.879	0.772	0.001
Trypsin Inhibitor	0.798	0.637	0.001
Urease	0.758	0.574	0.001
Lys/Crude protein	0.987	0.424	0.001
Total Lys	0.995	0.991	0.001
Available Lys Quadratic[2]			
KOH	-	0.829	0.001
Trypsin Inhibitor	-	0.951	0.001
Urease	-	0.821	0.001
Lys/Crude protein	-	0.991	0.001
Total Lys	-	0.992	0.001
Reactive Lys Linear[3]			
Available Lys	0.976	0.954	0.001
KOH	0.817	0.667	0.001
Trypsin Inhibitor	0.686	0.471	0.001
Urease	0.684	0.467	0.001
Lys/Crude protein	0.986	0.470	0.001
Total Lys	0.974	0.949	0.001
Reactive Lys Quadratic[3]			
Available Lys	-	0.988	0.001
KOH	-	0.825	0.001
Trypsin Inhibitor	-	0.875	0.001
Urease	-	0.799	0.001
Lys/Crude protein	-	0.975	0.001
Total Lys	-	0.968	0.001
Total Lys Linear[2]			
KOH	0.867	0.753	0.001
Trypsin Inhibitor	0.789	0.623	0.001
Urease	0.734	0.539	0.001
Lys/Crude protein	0.990	0.980	0.001
Total Lys Quadratic[2]			
KOH	-	0.817	0.001
Trypsin Inhibitor	-	0.958	0.001
Urease	-	0.780	0.001
Lys/Crude protein	-	0.989	0.001

[1] Predictability of the variance between NIR and official procedures for individual AA analysis for under processed, adequately processed and over processed soybeans.

[2] Official analytical methods
[3] Near inferred reflectance spectroscopy (NIRS) method

Figure 1.2 Linear regression analysis near-infrared reflectance spectroscopy (NIRS) crude protein (CP) compared with official analytical results. Ground soy white flakes after oil extraction but prior to heat processing were autoclaved at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa in 3 blocks and 3 replicates per treatment.

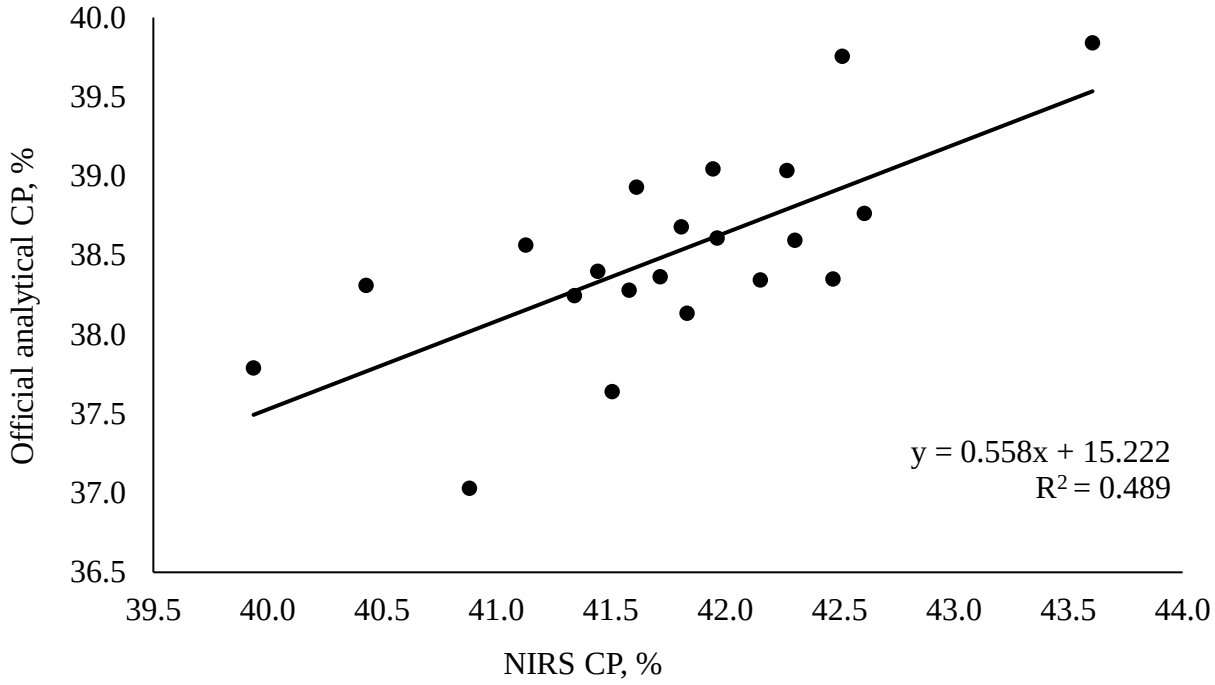


Figure 1.3 Linear regression analysis of near-infrared reflectance spectroscopy (NIRS) total Lys compared with official analytical results. Ground soy white flakes after oil extraction but prior to heat processing were autoclaved at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa in 3 blocks and 3 replicates per treatment.

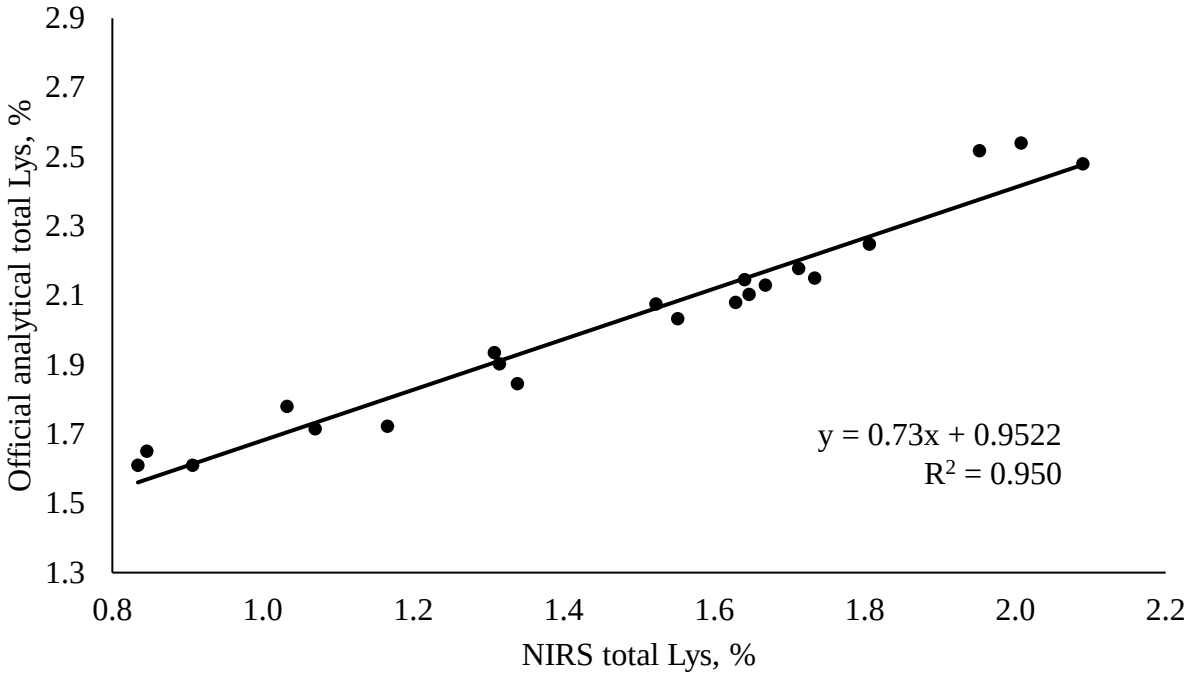


Figure 1.4 Linear regression analysis of near-infrared reflectance spectroscopy (NIRS) reactive Lys compared with official analytical results. Ground soy white flakes after oil extraction but prior to heat processing were autoclaved at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa in 3 blocks and 3 replicates per treatment.

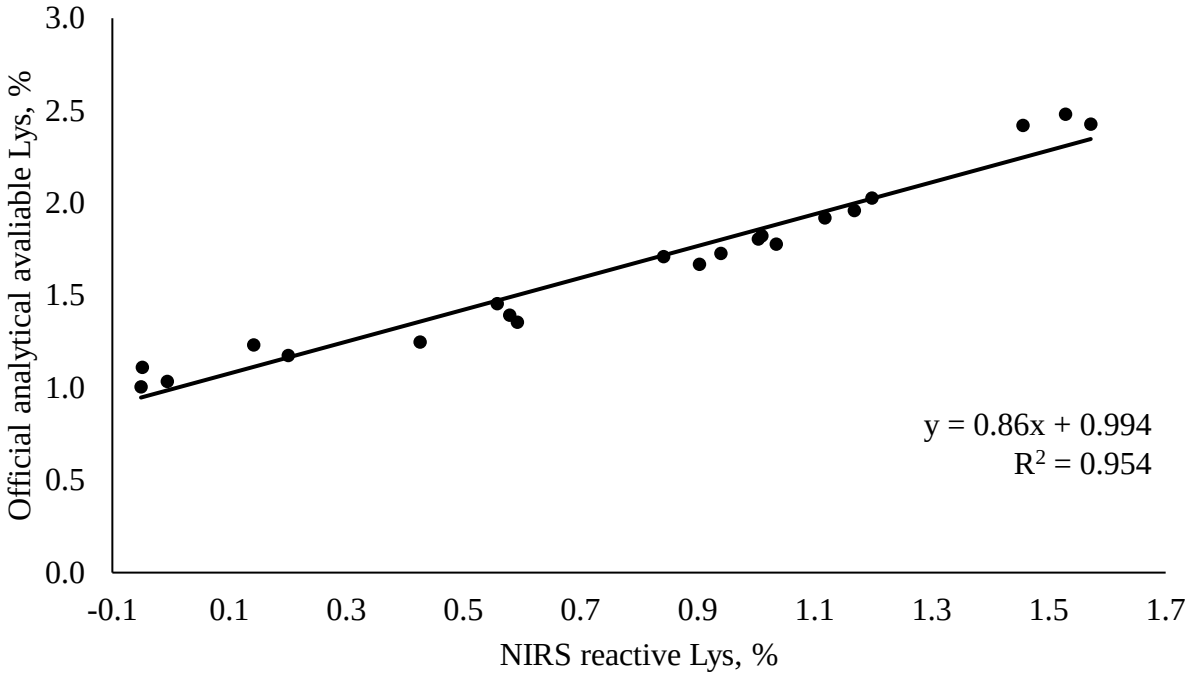
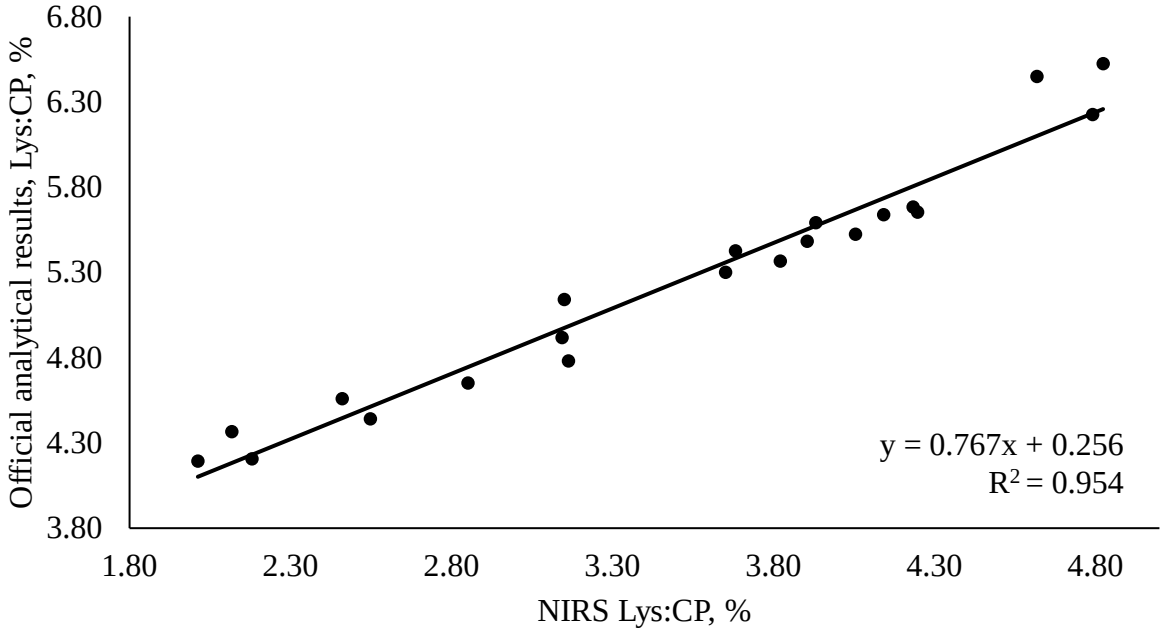


Figure 1.5 Linear regression analysis of near-infrared reflectance spectroscopy (NIRS) Lys:CP compared with official analytical results. Ground soy white flakes after oil extraction but prior to heat processing were autoclaved at 128°C for 0, 5, 10, 15, 30, 45, or 60 min at 200 kPa in 3 blocks and 3 replicates per treatment.



Chapter 2 - Determining the apparent ileal digestibility of amino acids for soybean meal from select soybean varieties with varying levels of crude protein

ABSTRACT

A total of 240 Ross 308 one-day old male broilers were placed in batteries for a 15-d study to evaluate apparent ileal digestibility (AID) of amino acids (AA) for soybean meal (SBM) from specialty variety soybeans grown in South Carolina. There were 10 replicates per treatment with 6 broilers per cage. On d 9, cages were allotted to 1 of 4 dietary treatments within location block. Treatments consisted of 1 of 4 soybean sources varying in crude protein (CP) content. Two sources consisted of soybeans from a similar region (Midwest) and solvent extracted either commercially or experimentally at Texas A&M University. Thus, dietary treatments contained either commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% CP (52SBM), or 56% CP (56SBM). Assay diets were dextrose-SBM based and formulated to supply 20% dietary CP. On d 15, ileal samples were collected for determination of AID of AA. Data were analyzed using the GLIMMIX procedure in SAS 9.4. Broilers fed CON and 56SBM had increased ($P < 0.003$) AID of total AA, Arg, His, Lys, Thr, and Trp compared to those fed PCON and 52SBM. The AID of Ile and Phe increased ($P < 0.001$) in broilers fed 56SBM compared to CON and 52SBM, with PCON intermediate to CON and 52SBM. Broilers fed 56SBM had increased ($P < 0.001$) AID of Leu compared to all other sources. The AID of Met increased ($P = 0.007$) in broilers fed CON and 56SBM compared to 52SBM with no evidence for differences between those fed PCON and all other sources. Broilers fed CON and 56SBM had increased ($P < 0.001$) AID of Val compared to PCON and 52SBM,

with no evidence for differences between those fed CON and PCON. Therefore, broilers fed commercially processed SBM had improved AA digestibility compared to those fed experimentally processed soybeans from a similar region. The high CP SBM variety had increased AA digestibility compared to PCON and 52SBM.

INTRODUCTION

Soybean meal (SBM) is the primary protein source used in broiler diets for its complimentary nutrient profile to cereal grains including high protein and amino acid (AA) profile (Kim et al., 2012). Quality of SBM can be influenced by many factors determined by processing parameters and soybean variety. Conventional processing of soybeans into SBM involves several steps including dehulling, solvent extraction, and heat processing to destroy antinutritional factors. While heat processing can destroy antinutritional factors such as trypsin inhibitors this also introduces potential AA digestibility loss and the Maillard reaction. The Maillard reaction can be accelerated by increasing temperature, binding free AAs and reducing sugars (Pahm et al., 2008). Lysine, a limiting AA in corn-SBM based broiler diets, is often the free AA bound having two free binding sites for reducing sugars. Thus, the multi-step processing of getting soybeans to an acceptable feeding quality introduces opportunity for variation in SBM.

Selection of soybean varieties have led to increasing protein content of soybeans; therefore, leading to increased CP and AA concentrations of SBM. It has been reported that a CP increased in soybeans selected for high crude protein compared to conventional SBM from 47.47 to 54.86%; lysine also increased from 3.14 to 3.56%, respectively (Baker et al., 2011). Therefore, this suggests a greater concentration of AA in soybeans selected for high crude protein compared to conventional. Determining SBM value based on digestible AA can provide opportunity for

cost savings through precision diet formulation. While traditionally SBM has 45-47% CP, the opportunity for higher CP and AA content can increase SBM value ultimately decreasing diet inclusion and lowering diet costs. It is essential to focus on optimizing SBM quality to improve the economics of broiler production. Selecting for soybean varieties that lead to increased digestible AA concentrations in SBM will provide an improvement in the feeding value of SBM. Therefore, the objective of this study was to determine the apparent ileal digestibility (AID) of AA for select varieties of SBM varying in CP content when fed to broilers.

MATERIALS AND METHODS

Experimental processing of soybean varieties

For the three experimentally processed soybeans at TAMU (PCON, 52SBM and 56SBM), whole soybeans were cracked using cracking rolls (Ferrel-Ross, Hereford, TX) and aspirated to remove any larger contaminants and hulls. Cracked material was then conveyed into a French stack cooker and conditioned for 20 min at 71°C ± 7°C. The conditioned cracked material was then flaked to approximately 0.36-mm using flaking rolls (Bauermeister, Memphis, TN). Flaked material was extracted using a countercurrent extractor (Crown Model 2, 0.21 m3 capacity). Commercial hexane was used at a rate of 1:1 (wt:wt) at a temperature of 43-60°C for 45 min. To remove residual hexane and yield toasted SBM, the flaked material was fed directly into a desolventizer – toaster (Crown Iron Works, Blaine, MN) with a product discharge temperature of 107°C ± 7°C. The degree of cooking (toasting) was monitored with a reference sample. Final toasted meal was ground with a hammermill equipped with a 6.35-mm screen and mixed.

Husbandry

The Institutional Animal Care and Use Committee at Kansas State University (Manhattan, KS) reviewed and approved the protocols used in this study.

A total of 240 one-day-old male broilers (Ross 308, Aviagen, Sallisaw, OK) were obtained and transported to the Kansas State University Poultry Facility (Manhattan, KS) to be used in a 6-d digestibility study. Broilers were placed in 2 Petersime batteries (Petersime Brood Unit, Gettysburg, OH) with 6 broilers per cage (dimensions, 96.5 × 33 cm), balanced by BW and provided a common corn SBM-based diet. On d 9, cages were randomly assigned to 1 of 4 dietary treatments within location block and balanced by BW with 10 replicates per treatment. Broilers were maintained on a 24-hr lighting schedule in a thermostatically controlled room. Illumination was provided by fluorescent bulbs for the duration of the experiment. A HOBOware data logger was used to record temperature, relative humidity, and light intensity of the battery room. Feed was provided ad libitum in one pan feeder (approximately 2 kg capacity) per cage. Water was provided ad libitum through water troughs. Mortalities were recorded daily and used for adjustments in growth performance.

Dietary treatments

Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by CP content and processed into SBM (Table 2). Two sources consisted of soybeans from a similar region (Midwest) and processed either commercially solvent extracted or experimentally solvent extracted at Texas A&M University (TAMU; College Station, TX). Therefore, dietary treatments consisted of a commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% (52SBM), or 56% CP (56SBM). Soybean meals from similar location but processed either conventionally or experimentally were CON and PCON,

respectively. Sources included varieties from the Midwest (CON and PCON) along with high protein varieties from South Carolina (52SBM and 56SBM). Processing conditions consisted of conventional (CON) and experimental (PCON, 52SBM and 56SBM) solvent extraction. Assay diets were dextrose and SBM-based and formulated to supply 20% dietary CP with titanium dioxide as an indigestible marker for determination of AID of AA.

Ileal collection

On d 15, all broilers (307.0 g) were euthanized by CO2 inhalation and ileal samples were collected for AA analysis. Ileal contents were collected beginning 1-cm. posterior to the Meckel's diverticulum and ending 1 cm. prior to the ileocecal junction. Total ileal samples were collected in conical tubes by pen. Composite samples were stored at -20□C prior to lyophilization (University of Illinois, Champaign, IL). Samples were finely ground to pass through a 0.02-in. screen and sent to the University of Missouri Agricultural Experiment Station (Columbia, MO) for analysis.

Chemical analysis

Individual SBM (Table 1) and diet samples (Table 2 and 4) were analyzed for proximate analysis and complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006), available Lys (AOAC Official Method 975.44, chp. 45.4.03, 2006), protein solubility in potassium hydroxide (KOH method, Parsons et al., 1991) and trypsin inhibitor activity (AACC Official Method 22-40, 2006). Additionally, diets and ileal samples were analyzed for titanium dioxide (Myers et al., 2004) as an indigestible marker. Ileal contents were analyzed for dry matter and complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006) for determination of AID of AA.

To determine evidence of the Maillard reaction, melanoidin was measured via methods define by Del Castillo et al., (2002) and Shen et al., (2018). Briefly, a 50 mg sample was dispersed in 3 ml ethanol solution (47.5%, v/v) in a 5 mL tube that was vortexed for 30 s and further shook in a water bath for 30 min at 50 °C and 120 rpm oscillation. Then, the sample was vortexed again for 1 min and then mixed vigorously for another 30 min. The sample was centrifuged at 10,000 × g for 10 min, and the supernatant collected for analysis. Each sample was extracted and analyzed in duplicate. The relative amount of melanoidin in the extract was analyzed using a double beam spectrophotometer (VWR UV-6300PC) at 420 nm. Thus, increasing absorbance values indicate increasing presence of Maillard reaction end products.

Calculations and statistical analysis

Calculations for AID of AA were calculated using the following equation (Kong and Adeola, 2014):

Equation 2.1 Apparent ileal digestibility of amino acids (AA)

$$\mathrm{AID_{AA}}\ (\%) = \left[1 - \left(\frac{\mathrm{AA_{diet}}}{\mathrm{AA_{digesta}}}\right) \times \left(\frac{\mathrm{TiO_{2,digesta}}}{\mathrm{TiO_{2,diet}}}\right)\right] \times 100,$$

where $AA_{digesta}$ and AA_{diet} represent the AA concentrations (g/kg) in digesta and diet DM, respectively, and $TiO_{2,\ diet}$ and $TiO_{2,\ digesta}$ represent the titanium dioxide digestible marker concentrations (g/kg) in diet and digesta DM, respectively.

All data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC), with pen as the experimental unit, pen location as the blocking factor and adjusted using Tukey-Kramer multiple comparisons. Results were considered significant if $P \leq 0.05$.

RESULTS

Analysis of individual soybean varieties

Individual SBM samples contained 44.8, 50.1, 52.4, and 56.2% CP, and 2.90, 3.05, 3.11, and 3.47% total Lys in the CON, PCON, 52SBM, and 56SBM, respectively (Table 1). The PCON was sourced from the same geographical region as the CON, therefore it was expected these would be similar in CP content. The PCON, 52SBM, and 56SBM were processed into SBM on a pilot scale crush facility at TAMU. These samples had increased DM compared to the CON. Therefore, the CON and PCON had similar CP and Lys when expressed on a DM basis. Individual SBM samples contained 2.79, 2.80, 2.90, and 3.24% available Lys and 0.6, 0.5, 2.1, and 1.1 crude fat in the CON, PCON, 52SBM, and 56SBM, respectively. Starch was greatest in CON (3.09%) followed by PCON (1.17%), 56SBM (0.19%), and 52SBM (0.12%). Trypsin inhibitor (TIU/mg) and KOH protein solubility decreased in experimentally processed SBM compared to conventionally processed. Within experimentally processed SBM, KOH protein solubility increased with increasing CP. Melanoidins numerically increased in SBM processed experimentally compared to conventionally.

Apparent ileal digestibility of amino acids

The HOBOware data logger indicated, for the first 9-d, averages were 24.8°C, 53.5%, and 122.7 lum/m^2, followed by 23.3°C, 51.2% and 122.7 lum/m^2 for the remainder of the experiment for temperature, relative humidity, and light intensity, respectively. Broilers fed CON and 56SBM had increased ($P < 0.003$) AID of total AA, Arg, His, Lys, and Thr compared to those fed PCON and 52SBM (Table 4). The AID of Trp increased ($P < 0.001$) in broilers fed 56SBM compared to PCON and 53SBm with CON intermediate. The AID of Ile and Phe increased ($P < 0.001$) in broilers fed 56SBM compared to CON and 52SBM, while PCON was

intermediate to CON and 52SBM. Broilers fed 56SBM had increased ($P < 0.001$) AID of Leu compared to all other sources. The AID of Met increased ($P = 0.007$) in broilers fed CON and 56SBM compared to 52SBM while there was no evidence for differences between those fed PCON and all other sources. Broilers fed CON and 56SBM had increased ($P < 0.001$) AID of Val compared to PCON and 52SBM with no evidence for difference between those fed CON and PCON.

DISCUSSION

The aim of this study was to determine the AID of AA for select soybean varieties with varying CP content. Soybean meal quality was defined by CP content in this study thus increasing CP content increased SBM quality.

Two commonly used measures of SBM quality are KOH protein solubility and trypsin inhibitor used to assess over processing and under processing, respectively. Parsons et al., (1991) identified target KOH protein solubility values between 78 to 84%, where the higher the number the greater the digestibility of nitrogen. In this study, KOH protein solubility was greatest in CSBM (89%) followed by 56SBM and 52SBM with PCON having the lowest. Comparing conventional to experimental processing from SBM of similar sources indicated a decrease in KOH protein solubility suggesting over processing in the experimentally processed SBM. Additionally, all experimentally processed SBM were below that of CSBM. Within SBM source experimentally processed, KOH protein solubility increased with increasing CP.

Trypsin inhibitor is an antinutritional factor that inhibits the enzymatic digestion of protein by binding trypsin and chymotrypsin (Liener, 1994) which can be seen in unheated or under processed SBM. Thus, the presence of trypsin inhibitor should be less than 2.5 TIU/mg. In

this experiment, all SBM sources were below the suggested threshold. Conventionally processed SBM indicated a greater TIU/mg compared to experimental processed SBM suggesting a higher degree of heat processing to destroy antinutritional factors for PCON, 52SBM and 56SBM.

Soybeans from similar varieties, processed under different conditions suggested that experimentally processed SBM was more harshly processed compared with conventionally processed SBM. Therefore, this suggested the potential for the Maillard reaction, influenced by processing time, moisture, pressure, and temperature under experimental processing conditions. In the Maillard reaction, free amine groups at the N-terminus of an amino acid interact with the free aldehyde group of reducing sugars, such as glucose, galactose, and fructose. The Maillard reaction can lead to decrease the availability and utilization of those free amino acids and reducing sugars (Pahm et al., 2008). The structure of lysine makes it particularly susceptible to the Maillard reaction as it has two free amino groups. Maillard reactions with lysine result in a reduction of both lysine concentration and lysine digestibility (Gonzalez-Vega et al., 2011). Increasing temperature has a positive linear effect on the Maillard reaction, therefore at temperature increases, so does the Maillard reaction (Ajandouz et al., 2008), indicating the potential for heat processing of SBM to contribute to reduced lysine digestibility and ultimately decreased performance. While visual indication of Maillard reaction can be seen in dark colored SBM, establishing an analytical test to evaluate evidence of the Maillard reaction is necessary. Evidence of the Maillard reaction can be seen in Maillard reaction end products such as melanoidin which can be measured by increasing absorbance values. In this study, melanoidins numerically increased in SBM processed experimentally compared to conventionally, indicating harsher conditions under experimental processing procedure. This could also be observed where KOH protein solubility decreased by at least 10% in experimentally processed SBM compared to

conventionally processed SBM. Additionally, analytical values for total AA, lysine, and available lysine increased with increasing CP. Available Lys:CP, Lys:CP, crude fiber, and starch concentration was greatest in conventionally processed SBM (CON). In diets used to determine digestibility, diets were balanced for CP by varying levels of SBM in the diet. Since SBM was the only protein source, dextrose replaced SBM as SBM inclusion varied. Analysis of the experimental diets confirmed that CP was held constant at 20%.

Evidence of an effect of processing was further determined in differences in AID among broilers fed CON or PCON. For SBM from the same source but processed either conventionally (CON) or experimentally (PCON), AID of AA decreased in SBM processed experimentally. Among SBM processed experimentally, AID of AA increased in broilers fed 56SBM. The effect of the heat processing has been shown to decrease analyzed AA availability (Dunmire et al., 2019) and influence growth of swine and poultry (Gonzalez-Vega et al., 2013, Parsons et al., 1991). Parsons et al., (1991) observed that increasing autoclave time decreased AA digestibility. In this study, it was determined that processing SBM experimentally provided harsher conditions of heat processing compared to SBM processed conventionally. Gonzalez -Vega et al., (2011) found similar results in autoclaved SBM fed to pigs and attributed the reduced AA digestibility to the Maillard reaction. Experimentally processed SBMs in this study were analyzed for melanoidins, a product of the Maillard reaction. The absorbance values for melanoidin indicated harsher processing conditions for experimentally processed SBM which could lead to free AA and reducing sugars to bind together.

As indicated by Ravidran et al., (2014), it is generally assumed that the digestible AA content of SBM per unit of CP is constant. The AID of AA for experimentally processed SBM showed the relationship described by Ravidran et al., (2014) for CP and AA. However, for

PCON and 52SBM, no differences were observed likely because of marginal differences in CP, 50 and 52% CP, respectively. While analytical values suggested the observed results for AID of AA when fed to broilers, AID of AA was similar in broilers fed conventionally processed SBM and high CP (56SBM). It can be suggested that CON (45% CP) would have less available AA compared to 56SBM (56% CP), however AID among these sources showed no evidence for differences. No differences observed for CSBM and 56SBM are likely due to differences in SBM processing conditions. Therefore, the potential for greater AID of AA based on CP content for 56SBM was diminished by thermal processing indicating no evidence for differences in AID of AA between CON and 56SBM.

Soybean meal from different locations either the Midwest (CON or PCON) or South Carolina (USA; 52SBM or 56SBM) indicated no evidence for differences based on source, rather differences were based on processing parameter and CP content. Additionally, in a study evaluating SBM source in the United States, Sotak-Peper et al., (2017) analyzed 22 sources of SBM finding only minor differences in CP and AA concentrations and digestible AA content remained constant regardless of geographical location (Northern Midwest, central Midwest, and Eastern United States). Therefore, the protein value of SBM in the United States was not influenced by location, however greater differences were observed in SBM source outside the United States (Lagos et al., 2017).

In conclusion, SBM from a similar source processed either conventionally or experimentally, AA digestibility was greatest in SBM processed conventionally. For SBM experimentally processed from 2 high quality soybean varieties from South Carolina resulted in increased AA digestibility compared to PCON. The 56SBM had increased digestibility of indispensable AA compared to SBM from PCON and 52SBM when fed to 15-day-old broilers.

The increased AID of AA and increased AA content of high CP SBM resulted in an increase in digestible amino acid content provided by SBM.

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Table 2.1 Chemical analysis of individual soybean meal varieties (as-is basis)[1,2]

Item, %	CON	PCON	52SBM	56SBM
Dry matter	89.82	95.51	95.44	91.40
Crude protein	44.81	50.12	52.35	56.17
Crude fat	0.55	0.48	2.08	1.14
Crude fiber	5.17	4.85	4.67	4.27
Starch	3.09	1.17	0.12	0.19
Ash	6.44	7.09	6.67	6.53
Available Lys	2.79	2.80	2.90	3.24
Available Lys: crude protein	6.23	5.59	5.54	5.77
Lys:CP	6.47	6.09	5.94	6.18
KOH protein solubility	89.05	69.80	76.31	78.87
Trypsin inhibitor, TIU/mg	1.71	0.42	0.39	0.71
Melanoidin, abs at 420nm	0.158	0.183	0.421	0.215
Total AA	44.46	49.64	49.90	56.16
Indispensable AA				
Arg	3.20	3.50	3.71	4.29
His	1.17	1.31	1.39	1.46
Ile	2.23	2.46	2.50	2.78
Leu	3.48	3.87	3.91	4.33
Lys	2.90	3.05	3.11	3.47
Met	0.61	0.68	0.62	0.69
Phe	2.30	2.58	2.65	2.92
Thr	1.76	1.98	1.92	2.09
Trp	0.62	0.70	0.69	0.71
Val	2.25	2.52	2.55	2.86

[1]A total of 240 one-d old male broilers (Ross 308, Aviagen, Sallisaw, OK) were placed in battery cages with 6 broilers per cage and 10 replicates per treatment.

[2]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM. Two sources consisted of soybeans from a similar region and processed either commercially or experimentally solvent extracted at Texas A&M University. Therefore, dietary treatments consisted of a commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% (52SBM), or 56% CP (56SBM).

Table 2.2 Diet composition balanced at 20% crude protein[1,2]

Ingredient, %	CON	PCON	52SBM	56SBM
Soybean meal[3]	44.1	40.2	37.7	34.8
Dextrose	49.8	53.7	56.2	59.0
Soybean oil	2.0	2.0	2.0	2.0
Dicalcium phosphate	1.9	1.9	1.9	1.9
Limestone	1.0	1.0	1.0	1.0
Sodium bicarbonate	0.2	0.2	0.2	0.2
Sodium chloride	0.2	0.2	0.2	0.2
Titanium dioxide	0.5	0.5	0.5	0.5
Vitamin trace mineral premix[2]	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
Calculated analysis				
ME, kcal/kg	3,049	3,097	3,128	3,161
Crude protein, %	20	20	20	20
Digestible amino acids, %				
Lys	1.31	1.19	1.12	1.03
Arg	1.43	1.31	1.22	1.13
His	0.53	0.48	0.45	0.42
Ile	0.98	0.90	0.84	0.78
Leu	1.56	1.43	1.34	1.24
Met	0.29	0.26	0.24	0.23
Total sulfur AA	0.60	0.55	0.51	0.47
Phe	1.04	0.95	0.89	0.82
Total aromatic AA	1.82	1.66	1.55	1.44
Thr	0.78	0.72	0.67	0.62
Trp	0.28	0.26	0.24	0.22
Val	1.02	0.93	0.87	0.80

[1] A total of 240 one-d old male broilers (Ross 308, Aviagen, Sallisaw, OK) were placed in battery cages with 6 broilers per cage and 10 replicates per treatment.Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM. Two sources consisted of soybeans from a similar region and processed either commercially or experimentally solvent extracted at Texas A&M University. Therefore, dietary treatments consisted of a commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% (52SBM), or 56% CP (56SBM).

[2]Provided per kg of premix: 4% Zn, 2% Fe, 4% Mn, 4,500 ppm Cu, 600 ppm I, 60 ppm Se, 3,083,700 IU vitamin A, 1,101,322 IU vitamin D_3, 6,608 IU vitamin E, 4.4 mg vitamin B_{12}, 330 mg menadione, 2,643 mg riboflavin, 441 mg thiamine, 2,643 mg pantothenic acid, 11,013 mg niacin, 551 mg vitamin B_6, 275 mg folic acid, 154,185 mg choline, and 13 mg biotin.

Table 2.3 Chemical analysis of experimental diets (as-is basis)[1,2]

Item, %	CON	PCON	52SBM	56SBM
Dry matter	91.01	93.30	93.15	91.83
Crude protein	21.41	19.82	18.57	20.82
Crude fat	1.79	1.39	2.10	1.79
Crude fiber	2.20	1.93	1.65	1.69
Ash	5.51	5.87	5.14	5.75
Available lysine	1.15	1.16	1.05	1.28
Lysine: crude protein	5.51	6.05	6.03	6.44
Available Lys: crude protein	5.37	5.85	5.65	6.15
Total AA	18.55	19.57	18.08	22.05
Indispensable AA				
Arginine	1.31	1.34	1.31	1.68
Histidine	0.49	0.52	0.48	0.57
Isoleucine	0.92	0.98	0.91	1.08
Leucine	1.44	1.53	1.42	1.71
Lysine	1.18	1.20	1.12	1.34
Methionine	0.25	0.25	0.22	0.25
Phenylalanine	0.95	1.02	0.96	1.17
Threonine	0.73	0.78	0.69	0.83
Tryptophan	0.23	0.28	0.25	0.25
Valine	0.95	1.01	0.93	1.11
Dispensable AA				
Alanine	0.82	0.87	0.79	0.93
Aspartic acid	2.14	2.27	2.05	2.51
Cystine	0.29	0.30	0.29	0.31
Glutamic acid	3.46	3.68	3.38	4.29
Glycine	0.81	0.86	0.77	0.93
Proline	0.90	0.94	0.87	1.10
Serine	0.80	0.86	0.80	1.00
Tyrosine	0.62	0.64	0.60	0.74

[1] A total of 240 one-d old male broilers (Ross 308, Aviagen, Sallisaw, OK) were placed in battery cages with 6 broilers per cage and 10 replicates per treatment.

[2]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM. Two sources consisted of soybeans from a similar region and processed either commercially or experimentally solvent extracted at Texas A&M University. Therefore, dietary treatments consisted of a commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% (52SBM), or 56% CP (56SBM).

Table 2.4 Effect of soybean meal source on apparent ileal digestibility of amino acids[1,2,3]

Item, %	CON	PCON	52SBM	56SBM	SEM	Probability, *P*
Total AA	77.29[a]	71.64[b]	69.00[b]	81.49[a]	1.366	0.001
Indispensable AA						
Arg	85.23[a]	80.46[b]	79.57[b]	88.48[a]	1.213	0.001
His	80.20[a]	74.09[b]	72.15[b]	83.14[a]	1.361	0.001
Ile	76.93[b]	72.80[bc]	70.54[c]	81.61[a]	1.384	0.001
Leu	76.50[b]	72.96[b]	70.85[b]	82.10[a]	1.149	0.001
Lys	79.65[a]	71.44[b]	69.24[b]	82.21[a]	1.944	0.001
Met	80.15[a]	73.86[ac]	70.86[bc]	81.33[a]	2.321	0.007
Phe	77.73[b]	74.71[bc]	72.50[c]	83.15[a]	1.299	0.001
Thr	77.39[a]	69.82[b]	66.20[b]	80.03[a]	1.127	0.001
Trp	75.74[ab]	73.77[b]	72.60[b]	82.47[a]	1.872	0.003
Val	74.16[ab]	69.71[bc]	66.27[c]	79.09[a]	1.570	0.001

[1] A total of 240 one-d old male broilers (Ross 308, Aviagen, Sallisaw, OK) were placed in battery cages with 6 broilers per cage and 10 replicates per treatment. Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM. Two sources consisted of soybeans from a similar region and processed either commercially or experimentally solvent extracted at Texas A&M University. Therefore, dietary treatments consisted of a commercially processed SBM with 44% CP (CON) or experimentally processed SBM with 50% CP (PCON), 52% (52SBM), or 56% CP (56SBM).

[2]Means within a row followed by a different letter (a-c) are significantly different ($P \leq 0.01$).

[3]AA = amino acids.

Chapter 3 - Determining the apparent ileal digestibility of amino acids of soybean meal from soybean varieties with varying levels of crude protein and subsequent effects on broiler growth performance

ABSTRACT

Two studies were conducted to determine apparent ileal digestibility (AID) of AA and growth performance in broilers fed soybean meal (SBM) with varying CP. Two sources consisted of soybeans from a similar region (Midwest, USA) and processed either conventionally or experimentally solvent extracted. Additional sources included a low quality and high-quality soybean, experimentally solvent extracted into SBM. Therefore, dietary treatments consisted of a conventionally processed SBM with 47% CP (CSBM), or experimentally processed SBM with 42% (42SBM), 49% (49SBM), or 52% (52SBM) CP. In Exp. 1, 240 one-day old Cobb500 male broilers were placed in Petersime batteries with 6 broilers per cage and 10 replicates per treatment. Dietary treatments were dextrose and SBM-based and fed from d 10 to 18. On d 18, broilers were euthanized, and ileal contents collected. In Exp. 2, 360 one-day old Cobb500 male broilers were placed in Petersime batteries with 6 broilers per cage and 15 replicates per treatment. Broilers were balanced by BW and assigned to 1 of 4 dietary treatments within location block. Dietary treatments were corn and SBM-based, formulated to 90% digestible Lys, and balanced based on digestible AA from Exp. 1. Data were analyzed with cage as the experimental unit, cage location as the blocking factor. Broilers fed CSBM had increased ($P < 0.05$) AID of total AA, Arg, His, Lys, and Thr compared to 49SBM. Increasing the CP content of SBM from 42SBM, 49SBM, to 52SBM increased ($P < 0.05$) AID of total AA, Arg, His, Leu, Lys, Thr, and Val. Broilers fed CSBM, 49SBM, and 52SBM had increased ($P < 0.001$) Ile, Met,

Phe, and Trp compared to 42SBM. From d 0 to 18, BWG and d 18 BW increased ($P < 0.001$) in broilers fed CSBM, compared to 42SBM, 49SBM, and 52SBM. In conclusion, broilers fed CSBM had increased AID of AA, d18 BW, and BWG compared to experimentally processed SBM, suggesting harsh experimental processing conditions of SBM compared to SBM processed conventionally.

INTRODUCTION

Soybean meal (SBM) is the primary plant protein source fed to broilers for its consistent amino acid profile and containing high CP by which it is often formulated and sold (Kim et al., 2012). Geographical location, growing conditions, soybean variety, and processing method are all factors that can affect SBM quality (Dozier and Hess, 2011). Sotak-Peper et al. (2017) analyzed 22 sources of SBM finding only minor differences in CP and AA concentrations and digestible AA content remained constant regardless of geographical location within the United States (Northern Midwest, central Midwest, and East). Therefore, the protein value of SBM in the United States was not influenced by location, however greater differences have been observed in SBM source outside the United States (Lagos et al., 2017).

A recent shift in competition for SBM usage in swine and poultry diets has occurred. Ingredient cost and availability has increased utilization of more economical crystalline amino acids and by-product ingredients. Thus, it is essential to focus on optimizing SBM quality to maintain its competitive edge in livestock and poultry diets and continue to find ways to reduce diet cost for producers. Selecting for soybean varieties that result in increased AA concentrations in SBM will provide an improvement in the feeding value of SBM. However, it is important to determine if there is a difference in the digestibility of those AA to further influence the feeding

value of SBM. Therefore, the objective of these studies was to determine the apparent ileal digestibility (AID) of AA for SBM varieties varying in CP content when fed to broilers and subsequent effects of growth performance.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Kansas State University (Manhattan, KS) reviewed and approved the protocols used in these studies.

Soybean varieties and processing

A total of 4 soybean varieties were used for both experiments. Two sources consisted of soybeans from a similar region and processed either conventionally or experimentally solvent extracted at Texas A&M University. Additional sources included a low quality and high-quality soybean, experimentally solvent extracted into SBM at Texas A&M University. Therefore, SBM utilized in treatments consisted of a conventionally processed SBM with 47% CP (CSBM), or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM), with CSBM and 49SBM processed from soybeans of similar origin.

For the three experimentally processed soybeans (42SBM, 49SBM and 52SBM), whole soybeans were cracked using cracking rolls (Ferrel-Ross, Hereford, TX) and aspirated to remove large contaminants and hulls. Cracked material was then conveyed into a French stack cooker and conditioned for 20 min at 71°C ± -7°C. The conditioned, cracked material was flaked to approximately 0.36-mm using flaking rolls (Bauermeister, Memphis, TN). Flaked material was then extracted using a countercurrent extractor (Crown Model 2, 0.21 m^3 capacity). Commercial hexane was used at a rate of 1:1 (wt:wt) at a temperature of 43-60°C for 45 min. To remove residual hexane and yield toasted meal, the flaked material was fed directly into a desolventizer –

toaster (Crown Iron Works, Blaine, MN) with a product discharge temperature of 107°C ± 7°C. The degree of cooking (toasting) was monitored with a reference sample and final toasted meal was hammermilled through a 6.35-mm screen and mixed.

Experiment 1

Apparent ileal digestibility

Husbandry

A total of 240 one-day old male broilers (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were obtained and transported to the Kansas State University Poultry Facility (Manhattan, KS) to be used in an 18-d AA digestibility study. Broilers were placed in Petersime batteries (Petersime Brood Unit, Gettysburg, OH) with 6 broilers per cage (dimensions, 96.5 × 33 cm), balanced by BW and provided a common corn-SBM crumble diet (DuMOR Chick Starter/Grower Feed 20% CP). On d 10 (d 0 of the experiment), cages were randomly assigned to 1 of 4 dietary treatments within location block and balanced by BW to provide 10 replicates per treatment. Illumination was provided by fluorescent bulbs for the duration of the experiment. A HOBOware data logger was used to record temperature, relative humidity, and light intensity of the battery room. Feed was provided *ad libitum* in a single pan feeder (approximately 2 kg capacity) per cage. Water was provided *ad libitum* through water troughs.

Dietary treatments

Assay diets were dextrose and SBM-based, fed in meal form, and formulated to supply 20% CP in the diet (Table 2). All diets used titanium dioxide as an indigestible marker. Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM as previously described.

Ileal collection

On d 18 of the study, broilers (533.4 g) were euthanized by CO_2 inhalation and ileal contents were collected for AA analysis. Ileal contents were collected beginning 1-cm posterior to the Meckel's diverticulum and ending 1-cm prior to the ileocecal junction. Ileal contents were collected and pooled by cage. Composite samples were stored at -20°C prior to lyophilization. Samples were finely ground to pass through a 0.5-mm screen and sent to the University of Missouri Agricultural Experiment Station (Columbia, MO) for analysis.

Experiment 2

Broiler growth performance

Husbandry

A total of 360 one-d old male broilers (initially 41.5 g; Cobb500, Cobb-Vantress, Siloam Springs, AR) were used in an 18-d study to determine effects of SBM quality on broiler growth performance. Broilers were placed in Petersime batteries (Petersime Brood Unit, Gettysburg, OH) with 6 broilers per cage (dimensions, 38.0 × 13.0 in.), balanced by BW. Cages were randomly assigned to 1 of 4 dietary treatments within location block to provide 15 replicates per treatment. The treatments were replicated in 15 blocks, and each treatment was randomized within each block. Illumination was provided by fluorescent bulbs for the duration of the experiment. A HOBOware data logger was used to record temperature, relative humidity, and light intensity of the battery room. Feed was provided *ad libitum* in a one pan feeder (approximately 2 kg capacity) per cage. Water was provided *ad libitum* through water troughs. Broilers were weighed on day 0, 7, and 18 for calculation of BWG, FI, and FCR. Body weight

and feed consumption were measured on d 0 and 18. Mortalities were recorded daily and used to adjust for growth performance.

Dietary treatments

Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into SBM as previously described. Dietary treatments were corn-SBM based and formulated to 90% of the digestible Lys requirement suggested by Cobb 500 broiler recommendations (Table 4). Vegetable oil and L-Lys remained constant for all treatments. Standardized ileal digestibility (SID) AA values of SBM were based on amino acid concentrations previously determined in Exp. 1. Additionally, vegetable oil dietary inclusion kept constant, thus energy (MEn) was not balanced in these diets. Calcium, available phosphorus, and AA:Lys ratios were balanced to meet or exceed Cobb 500 broiler recommendations.

Chemical analysis

Experiment 1 and 2

Individual SBM (Table 1) and diet samples (Table 2 and 4) were analyzed for proximate analysis and complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006), available Lys (AOAC Official Method 975.44, chp. 45.4.03, 2006), protein solubility in potassium hydroxide (KOH method, Parsons et al., 1991) and trypsin inhibitor activity (AACC Official Method 22-40, 2006). Experiment 1 diets and ileal contents were analyzed for titanium dioxide (Myers et al., 2004) as an indigestible marker. Ileal contents were analyzed for dry matter and complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006) for determination of AID of AA.

Calculations

Experiment 1 calculations for AID of AA were calculated using the following equation (Kong and Adeola, 2014):

Equation 3.1 Apparent ileal digestibility of amino acids (AA)

$$\mathrm{AID_{AA}}\ (\%) = \left[1 - \left(\frac{\mathrm{AA_{diet}}}{\mathrm{AA_{digesta}}}\right) \times \left(\frac{\mathrm{TiO_{2,digesta}}}{\mathrm{TiO_{2,diet}}}\right)\right] \times 100,$$

where $AA_{digesta}$ and AA_{diet} represent the AA concentrations (g/kg) in digesta and diet DM, respectively, and $TiO_{2,\ diet}$ and $TiO_{2,\ digesta}$ represent the digestible marker concentrations (g/kg) in diet and digesta DM, respectively (Table 6).

Statistical analysis

Experiment 1 and 2

All data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC), with cage as the experimental unit, cage location as the blocking factor and adjusted using Tukey-Kramer multiple comparisons. Results were considered significant if $P \leq 0.05$.

RESULTS

Analysis of individual soybean varieties

Individual SBM samples contained 46.9, 41.8, 49., and 51.8% CP and 2.97, 2.60, 3.09, and 3.21% total Lys in the CSBM, 42SBM, 49SBM, and 52SBM, respectively (Table 1). The 49SBM was sourced from the same geographical region as the CSBM, therefore it was expected these would be similar in CP content. The 42SBM, 49SBM, and 52SBM were processed into SBM on a pilot scale crush facility at Texas A&M University. These samples had an increased DM compared to the CSBM. Therefore, the 49SBM and CSBM had similar CP and Lys when

expressed on a DM basis. Individual SBM samples contained 2.80, 2.43, 2.89, and 3.08% available Lys and 86.27, 74.30, 85.72, and 76.95% KOH protein solubility in the CSBM, 42SBM, 49SBM, and 52SBM, respectively. In addition, individual SBM samples contained 5.83, 1.56, 4.43, and 6.78 TIU/mg for trypsin inhibitor units, respectively. Crude fat in individual soybean samples contained 1.6, 1.9, 1.6, and 1.8% in CSBM, 42SBM, 49SBM, and 52SBM, respectively. High CP soybean samples had lower NDF, where NDF was 12.0, 14.2, 7.8, and 8.2% for CSBM (46% CP), 42SBM, 49SBM, and 52SBM, respectively. Chemical analysis results for ADF were 6.7, 7.8, 6.8, and 6.9% for CSBM, 42SBM, 49SBM, and 52 SBM respectively where low CP soybean had higher ADF.

Experiment 1

Apparent ileal digestibility of amino acids

The HOBOware data logger indicated, for the first 7 d, average temperature, relative humidity, and light intensity were 30.8°C, 61.3%, and 143.2 lum/m^2, followed by 28°C, 71.5%, and 142.1 lum/m^2 for the remainder of the experiment, respectively. There was an overall treatment effect ($P < 0001$) for AID of total AA and all indispensable AA (Table 6). For soybeans grown in a similar region, broilers fed CSBM, processed conventionally, had increased ($P < 0.05$) AID of total AA, Arg, His, Lys, and Thr compared to 49SBM, processed experimentally. There was no evidence of difference in total AA, indispensable AA, or dispensable AA digestibility in broilers fed the CSBM and 52SBM (Table 3). The AID of total AA, Arg, His, Lys, and Thr was greater ($P < 0.001$) in CSBM compared to 49SBM. Soybean meal with increasing CP content from 42SBM, 49SBM, and 52SBM had increased ($P < 0.001$)

AID of total AA, Arg, His, Leu, Lys, Thr, and Val. The AID of Ile, Met, Phe, and Trp decreased ($P < 0.01$) in broilers fed 42SBM compared to CSBM, 49SBM, and 52SBM.

Experiment 2

Broiler growth performance

The HOBOware data logger indicated, for the first 7 d, average temperature, relative humidity, and light intensity were 30.8°C, 57.2%, and 80.7 lum/m^2, followed by 27.6°C, 66.4% and 80.7 lum/m^2 for the remainder of the experiment, respectively. From d 0 to 18, BWG and d 18 BW increased ($P < 0.002$) in broilers fed the CSBM compared to 42SBM, 49SBM, and 52SBM (Table 7). Broilers fed CSBM had increased ($P < 0.001$) FI compared to 42SM and 52SBM, with 49SBM intermediate. There was no evidence for differences in FCR from d 0 to 18 for broilers fed any SBM source. There was no evidence of difference in FCR of broilers fed 42SBM, 49SBM, and 52SBM when fed SBM at 38.0%, 29.5%, and 27.5% of the diet.

DISCUSSION

The experiments herein aimed to determine the feeding value of SBM defined by the CP content when fed to broilers by determining the AA profile and subsequent effects on broiler growth performance. The CSBM was processed conventionally while the other three SBM sources were processed experimentally. The CSBM and 49SBM were from the same source but processed under different conditions while 42SBM was considered low quality and 52SBM was considered high quality based on CP content of the variety. Upon individual analysis of SBM, NDF was greatest in CSBM and 42SBM, 12.0 and 14.2%, respectively. Available Lys, total AA and total Lys increased with increasing CP regardless of processing technique. Two common

measures of SBM quality used to assess under- or overprocessing are KOH protein solubility and trypsin inhibitor unit. By determining KOH solubility, this indicates the nitrogen (%) digestible by the animal (Stein et al., 2005). In general, target values for KOH solubility are between 78 to 84% where the higher the number the greater the digestibility of nitrogen (Parsons et al., 1991), while values from 84 to 89% would indicate slightly under processed and values under 74% indicate overprocessing (Araba and Dale, 1990). The presence of trypsin inhibitor in under processed SBM will bind trypsinogen and chymotrypsin, inhibiting enzymatic protein digestion (Araba and Dale, 1990). As a measure of the presence of this primary antinutritional factor, trypsin inhibitor should be less than 2.5 TIU/mg. Trypsin inhibitor was greatest in high CP (52SBM) and lowest in low CP (42SBM) SBM, however these values were below the suggested threshold. For the SBM used in these experiments, the KOH solubility for all sources was above 74% and below 89% suggesting no indication of under- or overprocessing. There were differences in dry matter content among SBM processed conventionally versus experimentally where SBM processed under experimental conditions contained more dry matter. The increased dry matter observed in experimental SBM suggests a lower moisture content, potentially concentrating nutrients or indicating more evaporative moisture from experimental thermal processing.

When SBM was added to experimental diets to determine AID of AA, SBM was the only protein source provided in the diet. Diets were balanced for CP therefore varying levels of SBM in the diet was replaced by dextrose. Analysis of the experimental diets confirmed that CP was held constant at 20%. When fed to broilers to determine AID of AA, there was a greater AID among broilers fed CSBM (47% CP) and 52SBM compared to 42SBM with 49SBM intermediate. Therefore, broilers had greater amino acid digestibility when fed high CP SBM or

SBM processed under commercial conditions. Baker et al., (2011) determined the nutritional value of SBM tended to see greater digestibility of Lys in high protein SBM (54.9%) compared to CSBM (47%) with no differences among digestibility of other AA. The study reported herein observed a similar response to digestibility of Lys; however, there were also observed improvements in digestibility of other indispensable AA with increasing SBM CP content. The quality of SBM as described by KOH protein solubility indicated similar quality. However, it was observed that SBM from a similar source, processed under different conditions, had increased AID of Lys (CSBM). Therefore, potential differences due to processing conditions were only observed in differences in digestibility values rather than analytical values.

The effect of SBM processing has been thoroughly studied. During SBM processing the soybean must be heated to destroy antinutritional factors such as trypsin inhibitor to achieve high quality SBM (52SBM). Following dehulling and oil extraction, the heat processing step can largely affect SBM quality by destroying AAs resulting in AA digestibility loss and initiating the Maillard reaction. The Maillard reaction is exacerbated in the presence of high temperature, pressure, and moisture in combination with a free AA and reducing sugars. Lysine is most often involved due to its structure containing two free AA binding sites that can bind to reducing sugars such as glucose. Thus, making the AA less bioavailable and ultimately decreasing performance. Although there were differences in AA digestibility, there was no other indicators of over or under processing.

When SBM was added to experimental diets to determine broiler growth performance, diets were formulated to a constant digestible Lys concentration based on previously determined values, therefore leading to varying levels of SBM in the diet. As CP of SBM and digestible Lys decreased, SBM increased, and corn decreased in experimental diets. These data indicate that

processing of SBM using pilot scale solvent extraction led to a decrease in feed intake and gain of broilers. Broilers fed the experimentally processed SBM (42SBM, 49SBM, and 52SBM) had decreased FI which resulted in decreased BWG compared to those fed CSBM, while there was no difference in BWG and FI between broilers fed experimentally processed SBM. However, FCR was unchanged in broilers fed any of the different SBM treatments. Baker et al., (2011) performed a similar study and found no differences in growth performance among broiler fed high CP (54%) and conventional SBM (49%). However, a similar conclusion to Baker et al., (2011) in that high protein soybeans could be utilized to decrease amount of SBM needed in the diet. The reason for the differences observed in this study between digestibility and growth performance of experimentally processed SBM versus conventionally processed SBM is largely unknown.

A decrease in digestibility of higher fiber SBM could further explain decreased digestibility for broilers fed 42SBM containing high NDF values. Complex carbohydrates will solubilize in the intestinal tract leading to decreased rate of feed passage, reduced feed intake, and proliferation of bacteria in the gastrointestinal tract (Abdollahi et al., 2013). Analysis of experimental diets for Exp. 1 indicated higher NDF values for 42SBM. However, analysis of experimental diets for Exp. 2 indicated marginal differences ranging from 10.25 (49SBM) and 10.83% (52SBM) for NDF and 3.78 (52SBM) and 5.87% (42SBM) for ADF.

This research focused on the AA digestibility in reference to SBM quality and does not take into consideration components that have a greater influence on the energy value and potential immune benefits. Areas of soybean quality that may warrant further investigation of routine quality testing include presence of oligosaccharides, glycinin and β-conglycinin, and soy lectins. Oligosaccharides, raffinose and stachyose, are carbohydrate structures containing

monosaccharides abundant in SBM. The influence of oligosaccharides in digestion for monogastric is the inability to breakdown α-1,6 galactosyl bonds due to the absence of α-galactosidase enzyme. Therefore, reducing the presence of oligosaccharides via genetic selection or processing could increase the SBM quality (Hymowtiz et al., 1972). Glycinin and β-conglycinin are considered to have allergic and antinutritional effects. Effects of glycinin can be associated with impaired immune function, damaged intestinal morphology, and reduced nutrient digestibility in rats (Liu et al., 2008) with most of these effects seen in the presence of β-conglycinin. However, both can be destroyed via heat processing. The presence of lectin in soybeans acts as protection for the plant from insects and phytopathogenic organisms, thus potentially contributing issues in digestion by binding and damaging enterocytes and disrupting nutrient absorption (Pusztai, 1996).

In conclusion, SBM processed from low, medium, and high CP soybeans resulted in increased concentrations of crude protein and indispensable AA. Broilers fed conventionally processed SBM had improved AA digestibility compared to those fed experimentally SBM from similar sources. Broilers fed conventionally processed SBM had improved d18 BW, BWG, and FI. Soybean meal processed from low, medium, and high CP soybeans resulted in 42%, 49%, and 52% CP, and 2.60, 3.90, and 3.21% total Lys, respectively. There was no evidence for differences in growth performance among broilers fed experimentally processed SBM. A key area of future research will be identifying and quantifying unknown variation in SBM quality and its influence on broiler performance.

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Table 3.1 Chemical analysis of individual soybean varieties (as-is basis)[1,2,3]

Item, %	CSBM	42SBM	49SBM	52SBM
Dry matter	89.53	93.74	92.62	93.74
Crude protein	46.86	41.82	49.19	51.79
Crude fat	1.58	1.94	1.61	1.84
Crude fiber	3.85	5.91	3.60	3.41
ADF	6.68	7.75	6.83	6.93
NDF	12.03	14.24	7.83	8.24
Ash	6.89	6.37	6.93	6.82
Available Lys	2.80	2.43	2.89	3.08
Available Lys:crude protein	5.97	5.81	5.87	5.95
Lys:crude protein	6.34	6.22	6.28	6.20
KOH solubility	86.27	74.30	85.72	76.95
Trypsin inhibitor, TIU/mg	5.84	1.57	4.44	6.78
Total AA	45.30	40.56	47.85	50.64
Indispensable AA				
Arg	3.25	2.79	3.42	3.67
His	1.20	1.10	1.28	1.33
Ile	2.23	2.00	2.35	2.48
Leu	3.55	3.19	3.75	3.98
Lys	2.97	2.60	3.09	3.21
Met	0.65	0.59	0.70	0.73
Phe	2.36	2.10	2.49	2.64
Thr	1.78	1.65	1.90	1.97
Trp	0.64	0.64	0.72	0.74
Val	2.31	2.09	2.45	2.56
Dispensable AA				
Ala	1.98	1.84	2.11	2.20
Asp	5.14	4.56	5.44	5.77
Cys	0.71	0.64	0.75	0.78
Glu	8.25	7.12	8.69	9.32
Gly	1.96	1.81	2.07	2.14
Pro	2.28	2.04	2.37	2.57
Ser	2.00	1.83	2.12	2.25
Tyr	1.66	1.52	1.78	1.89

[1]Soybean meal (SBM) sources consisted of a control (47% CP; CSBM), a low quality SBM (42% CP; 42SBM), a medium quality SBM (49% CP; 49SBM), and a high quality SBM (52% CP; 52SBM) where 42SBM, 49SBM, and 52SBM soybeans were processed into SBM using a pilot scale facility at Texas A&M University and the CSBM was processed at a commercial soybean crush facility.
[2]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.

Table 3.2 Diet composition balanced at 20% CP, Exp. 1[1,2,3]

Ingredient, %	CSBM	42SBM	49SBM	52SBM
Soybean meal[3]	43.80	47.25	41.03	38.29
Dextrose	50.08	46.63	52.85	55.59
Vegetable oil	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.90	1.90	1.90	1.90
Limestone	1.00	1.00	1.00	1.00
Sodium bicarbonate	0.20	0.20	0.20	0.20
Sodium chloride	0.20	0.20	0.20	0.20
Titanium dioxide	0.50	0.50	0.50	0.50
Vitamin trace mineral premix	0.32	0.32	0.32	0.32
Total	100	100	100	100
Calculated analysis				
ME, kcal/kg	3,053	3,011	3,086	3,120
Crude protein, %	20	20	20	20
Amino acids, %				
Lys	1.30	1.40	1.22	1.14
Arg	1.42	1.54	1.33	1.24
His	0.53	0.57	0.49	0.46
Ile	0.98	1.05	0.91	0.85
Leu	1.55	1.68	1.46	1.36
Met	0.28	0.31	0.27	0.25
Total sulfur AA	0.60	0.64	0.56	0.52
Phe	1.03	1.12	0.97	0.90
Total aromatic AA	1.80	1.95	1.69	1.58
Thr	0.78	0.84	.073	0.68
Trp	0.28	0.30	0.26	0.25
Val	1.01	1.09	0.95	0.88

[1]A total of 240 one-d old male broilers (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were placed in battery cages with 6 broilers per cage to provide 10 replicates per treatment.

[2]Dietary treatments consisted of 1 of 4 soybean meal sources included in diets formulated to 20% crude protein (CP).

[3]Dietary treatments consisted of one of the following soybean meal (SBM) sources: control (47% CP; CSBM), a low quality SBM (42% CP; 42SBM), a medium quality SBM (49% CP; 49SBM), and a high quality SBM (52% CP; 52SBM) where 42SBM, 49SBM, and 52SBM soybeans were processed into SBM using a pilot scale facility at Texas A&M University and the CSBM was processed at a commercial soybean crush facility.

Table 3.3 Chemical analysis of experimental diets, Exp 1[1,2]

Ingredient, %	CSBM	42SBM	49SBM	52SBM
Dry matter	90.96	92.64	92.08	92.52
Crude protein	20.83	19.65	20.31	19.96
Crude fat	2.26	2.34	2.19	2.32
Crude fiber	1.93	2.72	1.74	1.30
ADF	2.18	3.02	2.33	1.58
NDF	3.34	5.89	3.40	2.84
Ash	6.32	6.21	6.02	6.00
Lysine: crude protein	6.58	6.26	6.11	6.36
Total AA	21.48	19.35	19.59	20.00
Indispensable AA				
Arginine	1.53	1.30	1.37	1.42
Histidine	0.57	0.52	0.52	0.52
Isoleucine	1.06	0.96	0.97	0.98
Leucine	1.68	1.52	1.53	1.56
Lysine	1.37	1.23	1.24	1.27
Methionine	0.30	0.27	0.28	0.27
Phenylalanine	1.12	1.01	1.02	1.03
Threonine	0.83	0.77	0.76	0.77
Tryptophan	0.28	0.31	0.29	0.28
Valine	1.11	1.01	1.00	1.01
Dispensable AA				
Alanine	0.95	0.88	0.86	0.88
Aspartic acid	2.46	2.18	2.24	2.29
Cystine	0.33	0.30	0.31	0.30
Glutamic acid	3.98	3.44	3.61	3.75
Glycine	0.93	0.87	0.85	0.86
Proline	1.05	0.99	0.96	0.98
Serine	0.91	0.85	0.85	0.88
Tyrosine	0.72	0.66	0.65	0.66

[1]A total of 240 one-d old male broilers (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were placed in battery cages with 6 broilers per cage to provide 10 replicates per treatment.

[2]Dietary treatments consisted of 1 of 4 soybean meal sources included in diets formulated to 20% CP. Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into soybean meal (SBM). Treatments consisted of a conventionally processed SBM with 47% CP (CSBM) or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM).

Table 3.4 Diet composition, Exp 2 (as-is basis)[1,2]

Ingredient, %	CSBM	42SBM	49SBM	52SBM
Corn	66.01	57.68	66.06	68.02
Soybean meal	29.50	38.00	29.50	27.50
Vegetable oil	0.70	0.70	0.70	0.70
Lys	0.15	0.15	0.15	0.15
Met	0.23	0.22	0.21	0.21
Thr	0.05	---	0.03	0.04
Monocalcium phosphate	1.25	1.20	1.25	1.25
Calcium carbonate	1.35	1.30	1.35	1.37
Sodium chloride	0.40	0.40	0.40	0.40
Choline chloride	0.10	0.10	0.10	0.10
Vitamin trace mineral premix[3]	0.25	0.25	0.25	0.25
Phytase[4]	0.01	0.01	0.01	0.01
Total	100	100	100	100
Calculated analysis				
Nitrogen-corrected metabolizable energy (MEn), kcal/kg	3,223	3,165	3,144	3,355
Crude protein, %	19.62	20.95	20.28	20.19
Ca, %	0.90	0.90	0.90	0.90
Available P, %	0.47	0.47	0.47	0.47
Digestible amino acids				
Lys	1.05	1.05	1.05	1.05
Arg	1.06	1.08	1.09	1.11
His	0.44	0.45	0.45	0.45
Ile	0.73	0.78	0.76	0.76
Leu	1.50	1.54	1.55	1.57
Met	0.49	0.49	0.48	0.48
TSAA	0.76	0.76	0.76	0.76
Phe	0.82	0.86	0.85	0.86
Thr	0.67	0.67	0.67	0.67
Trp	0.19	0.22	0.21	0.21
Val	0.82	0.86	0.84	0.84

[1]A total of 360 one-d old Cobb500 male broilers were placed in battery cages with 6 broilers per cage to provide 15 replicates per treatment.
[2]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into soybean meal (SBM). Treatments consisted of a conventionally processed SBM with 47% CP (CSBM) or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM).
[3] Provided per kg of premix: 4% Zn, 2% Fe, 4% Mn, 4,500 ppm Cu, 600 ppm I, 60 ppm Se, 3,083,700 IU vitamin A, 1,101,322 IU vitamin D3, 6,608 IU vitamin E, 4.4 mg vitamin B12, 330 mg menadione, 2,643 mg riboflavin, 441 mg thiamine, 2,643 mg pantothenic acid, 11,013 mg niacin, 551 mg vitamin B6, 275 mg folic acid, 154,185 mg choline, and 13 mg biotin.
[4]Quantum Blue 10G (AB Vista, Plantation, FL) provided 500.4 FTU/kg to release 0.15% P.

Table 3.5 Chemical analysis of experimental diets, Exp 2[1,2]

Ingredient, %	CSBM	42SBM	49SBM	52SBM
Dry matter	88.55	89.60	89.04	89.03
Crude protein	19.19	20.94	20.60	20.75
Crude fat	2.78	2.90	2.98	3.07
ADF	4.69	5.87	3.97	3.78
NDF	10.41	10.69	10.25	10.83
Ash	4.84	5.06	4.95	4.53
Lysine: crude protein	6.10	5.87	5.73	6.02
Total AA	19.45	20.44	20.02	19.89
Ile	0.88	0.95	0.91	0.89
Lys	1.17	1.23	1.18	1.25
Met	0.45	0.47	0.43	0.46
Thr	0.76	0.79	0.75	0.75
Trp	0.22	0.24	0.24	0.23
Val	0.96	1.04	1.00	0.97

[1]A total of 360 one-d old Cobb500 male broilers were placed in battery cages with 6 broilers per cage to provide 15 replicates per treatment.
[2]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into soybean meal (SBM). Treatments consisted of a conventionally processed SBM with 47% CP (CSBM) or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM).

Table 3.6 Effect of soybean meal source on apparent ileal digestibility of amino acids, Exp 1[1,2,3]

Item, %	CSBM	42SBM	49SBM	52SBM	SEM	Probability, *P*<
Total AA	87.63[a]	80.33[c]	84.94[b]	87.32[a]	0.583	0.001
Indispensable AA						
Arg	93.12[a]	88.20[c]	90.99[b]	92.73[a]	0.374	0.001
His	89.81[a]	82.66[c]	87.53[b]	89.68[a]	0.514	0.001
Ile	87.65[a]	82.50[b]	85.99[a]	88.09[a]	0.556	0.001
Leu	87.73[ab]	83.50[c]	86.44[b]	88.59[a]	0.542	0.001
Lys	89.65[a]	81.54[c]	86.25[b]	88.41[a]	0.535	0.001
Met	90.44[a]	85.61[b]	89.35[a]	90.75[a]	0.528	0.001
Phe	88.95[a]	85.19[b]	87.69[a]	89.61[a]	0.509	0.001
Thr	81.68[a]	72.97[c]	77.97[b]	81.53[a]	0.876	0.001
Trp	89.18[a]	85.41[b]	88.28[a]	89.84[a]	0.507	0.001
Val	86.05[ab]	80.24[c]	83.91[b]	86.45[a]	0.649	0.001
Dispensable AA						
Ala	87.11[ab]	81.26[c]	84.97[b]	87.37[a]	0.584	0.001
Asp	87.01[a]	76.15[c]	82.43[b]	84.87[ab]	0.645	0.001
Cys	72.87[ab]	61.07[c]	69.47[b]	75.84[a]	1.247	0.001
Glu	91.89[a]	85.00[c]	88.99[b]	90.65[ab]	0.449	0.001
Gly	84.62[a]	75.04[c]	81.21[b]	84.31[a]	0.759	0.001
Pro	86.70[a]	79.67[c]	83.76[b]	86.49[a]	0.643	0.001
Ser	85.03[a]	77.82[b]	82.41[a]	85.97[a]	0.764	0.001
Tyr	88.16[a]	82.39[c]	85.88[b]	88.48[a]	0.577	0.001

[1]A total of 240 one-d old male broilers (Cobb 500, Cobb-Vantress, Siloam Springs, AR) were placed in battery cages with 6 broilers per cage to provide 10 replicates per treatment.
[2]Dietary treatments consisted of 1 of 4 soybean meal sources included in diets formulated to 20% crude protein (CP).
[3]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into soybean meal (SBM). Treatments consisted of a conventionally processed SBM with 47% CP (CSBM) or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM).
[4]Means within a row followed by a different letter ([a-c]) are significantly different ($P \leq 0.05$).

Table 3.7 Effect of soybean varieties with varying levels of crude protein on broiler growth performance, Exp 2[1,2]

Item	CSBM	42SBM	49SBM	52SBM	SEM	Probability, *P*
BW, g						
d 0	41.5	41.4	41.5	41.5	0.099	0.904
d 18	688.2[a]	640.0[b]	635.7[b]	635.0[b]	11.10	0.002
d 0 to 18						
BWG, g	646.7[a]	598.6[b]	594.2[b]	593.5[b]	11.08	0.002
FI, g	828.3[a]	787.3[ab]	757.2[b]	763.5[b]	14.65	0.005
FCR	1.28	1.28	1.30	1.29	0.013	0.694
Mortality, %	0.8	2.5	2.5	3.3	---	---

[1]A total of 360 one-d old Cobb500 male broilers were placed in battery cages with 6 broilers per cage to provide 15 replicates per treatment.
[2]Dietary treatments consisted of 1 of 4 soybean sources varying in quality determined by crude protein (CP) content and processed into soybean meal (SBM). Treatments consisted of a conventionally processed SBM with 47% CP (CSBM) or experimentally processed SBM with 42% CP (42SBM), 49% (49SBM), or 52% CP (52SBM).
[3]Means within a row followed by a different letter ([a-c]) are significantly different ($P \leq 0.05$).
[4]BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio.

Chapter 4 - Influence of dietary fat and crystalline amino acid inclusion on broiler diet formulation and pellet quality

ABSTRACT

A total of 3 broiler diets were pelleted to determine the effects of diet formulation on pellet quality. Dietary treatments consisted of corn and soybean meal-based control, the control with crystalline valine (Val), and the control with crystalline Val and isoleucine (Ile). As crystalline amino acids (AA) increased in the diets, corn concentrations increased as soybean meal and the fat source were removed to balance for nitrogen-corrected metabolizable energy (MEn). There were 3 replicates per treatment with time of processing as a blocking factor and treatment order randomized within each block. Cooled pellet samples were collected to determine pellet fines, standard pellet durability index (PDI), modified PDI, and Holmen NHP100. Pellet mill kW increased ($P < 0.05$) in pelleted Val + Ile diets compared to the control and Val diets. Pellet fines decreased ($P < 0.01$) and PDI increased ($P < 0.01$) as crystalline AA increased and added fat decreased in the diet. In conclusion, diets with increasing crystalline AA, Val, and Val + Ile, led to improved pellet quality which can be explained by the 0.4% or 0.6% reduction in added fat with increasing crystalline AA and balancing for MEn in the diet.

INTRODUCTION

Pelleting poultry diets is commonly used to improve nutrient utilization, feed efficiency, feed handling characteristics and bulk density (Behnke 1994). Components of the pelleting process, such as steam conditioning and feed retention time in the conditioner and die, expose feed to various degrees of heat, moisture, pressure, and sheer changing the feeds' physical and

chemical characteristics. When ingredients that make up a diet formulation are exposed to the steam conditioning process and extrusion through the die, the heat and moisture plasticize the soluble fractions of the diet and increase the agglomeration of dietary components (Lundblad et al., 2009). In addition to processing parameters, ingredients included in diet formulations can also influence pelleting efficiency and pellet quality. Ingredients that are known to decrease pellet quality include fats and oils, however they provide the most lubrication and improvement in pelleting efficiency. When broiler diets are pelleted, an increase in pellet durability can improve feed conversion ratio (FCR) up to 1.5 kg of feed per kg of gain and increase dietary caloric value by 40 kcal/kg of nitrogen corrected metabolizable energy (MEn; McKinney and Teeter et al., 2004). Thus, the overall goal to improve pellet quality is to decrease percent fines and increase pellet durability.

Commercial broiler diet formulation has been influenced by the increasing use of crystalline amino acids (AA) to reduce diet cost and dietary crude protein by reducing soybean meal diet inclusion. Valine (Val) is recognized as the fourth limiting amino acid for poultry, followed closely by isoleucine (Ile). The addition of dietary crystalline AA can be used to reduce dietary crude protein by up to 1% (Lee, 2020). A reduction of dietary crude protein by 1% will reduce nitrogen excretion by 10% and ammonia emissions by 25% (Ospina-Rojas et al., 2014). As soybean meal and fat are removed due to increasing crystalline AA, corn inclusion will increase to balance for energy (MEn). Therefore, the objective of this study was to determine the effects of formulating broiler diet with crystalline Val and Ile on pellet quality via pellet fines and pellet durability index (PDI) methods.

MATERIALS AND METHODS

Diet manufacture

Feed was manufactured in accordance with current good manufacturing practices (CGMPs) at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). A total of 3 diets were pelleted to determine the effects of broiler diet formulation with crystalline Val and Ile and varying fat concentrations on pellet quality. Dietary treatments consisted of corn and soybean meal-based control, the control formulated with crystalline Val, and the control formulated to include crystalline Val and Ile. Diets were formulated to be isocaloric and balanced for lysine concentration (Table 1). As crystalline amino acids (AA) increased in the diets, corn concentrations increased, and soybean meal and choice white grease (CWG) were removed to balance for MEn.

Corn was ground with a three-high roller mill (Model 924, RMS, Harrisburg, SD) to approximately 1,000 µm. All ingredients were weighed on certified scales, ingredient lot numbers recorded, and amounts verified. Minor and micro ingredients were weighed on a certified platform scale (FG-60KAL, A&D Weighing; capacity 60 × 0.02 kg). A total of 500 kg of broiler feed per treatment was mixed in a 907 kg horizontal twin shaft counterpoise mixer (Model TRDB63-0152, Hayes and Stolz, Fort Worth, TX). All batches of feed were mixed for a total of 180 s with 60 s for dry ingredients and an additional 120 s when liquid fat was added. Each 500 kg batch per treatment was di-vided into three batches prior to pelleting. Each treatment was pelleted in 160 kg batches during 3 separate periods in order to provide 3 replicates per treatment.

Time of processing served as a blocking factor and order of pelleting each treatment was randomized within each block. Prior to pelleting dietary treatments, the pel-let mill was brought

to target conditioning temperature (85°C) with 454 kg of basal feed which included holding conditioning temperature for a minimum of 15 min.

Diets were steam conditioned (25.4 ×139.7 cm twin staff pre-conditioner, Model 150, Wenger, Sabetha, KS) to a target conditioning temperature of 85°C for approximately 30 s and pelleted on a 907 kg 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill, Crawfordsville, IN) equipped with a 4.8 × 31.8 mm pellet die (L:D 6.67). Due to the consistency of the conditioning temperature and process, it is expected the condition mash and hot pellet moisture content is consistent across treatments. The feeder was set at a constant rate to achieve approximately 907 kg per hour. Pellet samples were taken at the die and cooled in an experimental counter flow cooler for 15 min. Hot pellet temperatures and production rates were collected at three timepoints during the pelleting process. Samples of the cool pellets were collected for determination of pellet quality.

Sample collection and pellet durability

Three 4.5 kg samples were collected from the cooler during each replication for analysis of pellet fines and pellet durability index. Pellet fines was determined by sift-ing pellet samples to determine the number of intact pellets. Pellet durability index, used to predict intact pellets at the feeder, was determined using the standard and modified tumble box method as well as the Holmen NHP100 model set at 60 s as a provided option of the equipment.

To determine pellet fines, a sample of cool pellets was taken, and the fines sifted off by using the corresponding sieve stack (ASAE S269.3). Sifted material and intact pellets were weighed separately. Calculation of pellet fines was achieved by subtracting the weight of fines from initial sample weight and dividing by initial sample weight.

For the standard method of PDI, a sample of cool pellets was taken, and the fines sifted off by using the corresponding sieve stack (ASAE S269.3). Sifted pellets were split using a riffle divider and 500 g weighed for analysis. The 500 g sample was placed in the designated chamber (30.5 × 14.0 × 30.5-cm) of the tumble box and run for 10 min at 50 rpm. After tumbling, the sample was collected from the compartment and sifted using the same sieve as used previously. The remaining sifted pellets were weighed with PDI being calculated by dividing this final sample weight by the 500 g initial sample weight.

For the modified method, a sample of cool pellets was taken, and the fines sifted off by using the corresponding sieve stack (ASAE S269.3). Sifted pellets were split using a riffle divider and 500 g weighed for analysis. The 500 g sample was placed in the designated chamber (30.5 × 14.0 × 30.5-cm) of the tumble box with the addition of three 2-cm hexnuts. The sample along with the hexnuts was tumbled for 10 minutes at 50 rpm. After tumbling, the sample was collected from the compartment and sifted using the same sieve as used previously. The hexnuts was removed and the remaining sifted pellets was weighed with PDI being calculated by dividing this final sample weight by the 500 g initial sample weight.

For the Holmen method, a sample of cool pellets was taken, and the fines sifted off by using the corresponding sieve stack (ASAE S269.3). Sifted pellets were split using a riffle divider and 100 g weighed for analysis. The 100 g sample was placed into the hopper of the Holmen NHP100 and the desired run time selected (60 s). Once completed, the sample is removed from the hopper and weighed. PDI was calculated by dividing this final sample weight by the 100 g initial sample weight.

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Experimental unit was pelleting run treatment with a random effect of pelleting period. Results were considered significant if $P \leq 0.05$.

RESULTS

Diets were formulated to be isocaloric and isonitrogenous, therefore added fat was removed as crystalline amino acids increased in the diets (Table 1). Hot pellet temperature decreased ($P < 0.01$) in the control diet compared to valine and valine + isoleucine diets, which were 84.7, 85.1, 85.0°C, respectively. Pellet mill kilowatts (kW) were 9.1, 8.9, and 10.3 for control, Val, and Val + Ile diets, respectively. Pellet mill kilowatts (kW) increased ($P < 0.05$) in pelleted valine + isoleucine diets compared to the control and valine diets (Table 2). The Val + Ile diet had a higher hot pellet temperature because of the decrease in added fat from 2.5% in the control diet to 2.1 and 1.9% in the Val and Val + Ile diets, respectively. Therefore, diets pelleted with 1.9% added fat had greater die friction and increased kW compared to those pelleted with 2.5 and 2.1% added fat.

For the control, Val, and Val + Ile diets, pellet fines were 14.2, 12.1 and 10.1, respectively. Pellet fines decreased ($P < 0.01$) as crystalline amino acids increased and added fat decreased in the diet. The PDIs were 66.5, 73.6 and 76.6% for the standard, 37.1, 46.9, and 52.8% for the modified and 53.4, 67.8 and 73.7% for the Holmen NHP100 for 60 s methods for the control, Val, and Val + Ile diets respectively. Therefore, pellet durability index for the standard, modified and Holmen NHP100 (60 s) methods increased ($P < 0.01$) as crystalline amino acids increased and added fat de-creased in the diet (Table 3).

DISCUSSION

Feed pelleting occurs first, by conditioning mash feed through addition of steam into the conditioner. The steam conditioned feed then passes through a pellet die where the feed encounters the sheer force and pressure from compaction as it's pushed through the die holes. While pelleting factors, including conditioning, feed exposure to the die, and the retention time, certainly influence pellet feed quality, dietary components of a formulation will also directly influence those pelleting factors.

More recently, formulating with crystalline AA has become increasingly popular to decrease diet costs and crude protein levels through decreasing inclusion of soybean meal and increasing inclusion of crystalline AA. It has been established that crystalline AA are rapidly absorbed and highly digestible (Chung et al., 1992) and feeding crystalline AA supplemented diets to broilers can be accomplished with-out negatively impacting performance (Hilliar et al., 2020). Therefore, the goal of this study was to demonstrate diet formulation with increasing crystalline AA and the subsequent effects this would have on pellet quality. Diet formulations in this study showed that as crystalline AA increase, soybean meal decreases but corn must also increase, consequently increasing MEn as a result. To decrease the added energy from increasing corn in the diet, mixer added fat must then decrease in the formulation.

The influence of mixer added fat on PDI is largely impacted by the fat acting as a lubricant between the feed and pellet die surface (Pope et al., 2020). In this study, the lubrication effects of mixer added fat can be seen by the ΔT between the conditioning temperatures and hot pellet temperatures off the die. The ΔT numerically increased as mixer added fat decreased. Therefore, as conditioning temperature was held constant, hot pellet temperature increased with

decreasing mixer added fat. It has been found that increasing fat level from 1% to 3% will decrease pellet durability (Fahrenholz, 2012). This experiment also suggests that decreasing mixer added fat will improve pellet quality. Decreasing mixer added fat by 0.4 or 0.6% from 2.5% dietary fat inclusion improved pellet durability by 7 and 10%, respectively when compared to the control. Previous research has also determined that dietary fat and conditioning temperature have the greatest proportional effect on PDI compared to factors such as corn particle size, percent inclusion of dried distillers' grains with solubles (DDGS), production rate, conditioning retention time, and die length:diameter ratio (Fahrenholz, 2012). Opportunities to mitigate the negative effects of mixer added fat include the use of post-pellet liquid application (PPLA) of fat after the pellet die (Stark, 2016). However, while the use of PPLA systems can be helpful with pellet quality concerns, they also require an initial investment and management challenges may be a concern.

Broiler feed pelleting should be a balance between emphasis on pellet quality and pellet mill energy consumption. Greater pellet mill energy consumption leads to higher diet costs, but improved pellet durability will lead to improved broiler growth performance metrics. In this study, diets pelleted with 1.9% added fat encountered greater die friction increasing kW compared to those pelleted with 2.5 and 2.1% added fat. This agrees with previous research, where the higher fat level inclusion led to a reduction in energy consumption (Fahrenholz, 2012). Mixer added fat concentration, conditioning temperature and die length:diameter ratio has the greatest effect on energy consumption (Fahrenholz, 2012; Pope et al., 2020). Previous research also found that when mixer added fat was removed, the coefficient of friction of the feed increased pellet mill energy consumption (Fahrenholz, 2012). Ultimately mixer added fat acts as a lubricant between the feed and pellet mill die wall (Thomas, 1998; Fahnrenholz, 2012). It has

also been found that removing mixer added fat increased the coefficient of the feed increasing the change in temperature and pellet mill energy consumption (Pope et al., 2020). In general, as the die becomes thicker, feed will encounter more shear, heat, and pressure for a longer period of time creating a longer residence time promoting particle binding, improving PDI, increasing the change in temperature and increasing pellet mill energy consumption (Stark, 1994).

Pellet quality is a key indicator to predict broiler performance and can be defined by PDI, which determines the percent of intact pellets and pellet fines. Both PDI and pellet fines are a predicted measure aimed to represent what broilers consume at the feeder after the pelleted feed is exposed to potential breakage through feeding systems and transportation. In this study, PDI was measured three ways with increasing abrasiveness of standard, modified, and Holmen NHP 100 PDI methods. The standard and modified utilize a tumble box where the modified method increases the chances of pellet breakage with the addition of hex nuts. The Holmen NHP100 method utilizes forced air at a designated time (30, 60, 90, or 120) to agitate pellets and mimic the transportation process from the pellet mill to the feeder. It is widely accepted that increasing pellet quality will increase broiler performance (McKinney and Teeter, 2004). In this study, PDI increased as mixer added fat decreased and crystalline amino acids increased using all PDI methods. Therefore, a greater percentage of intact pellets would be present at the feeder for broiler consumption. Previous research suggests that a pellet quality of 40% with 60% of the diet being pellet fines is the minimum PDI to fines ratio where nutritional benefit may still be observed (Fahrenholz, 2012). In this study fines decreased with decreasing mixer added fat, decreasing die lubrication, and increasing frictional heat across the die surface. While broiler growth performance was not evaluated in this experiment, there is evidence that the improvement in pellet quality can improve performance. Previous research found that when

pellet fines were fed to broilers, BWG increased from 20 to 60% and feed intake decreased 7.9% and 5.8%, respectively (Greenwood et al., 2004). In a study evaluating birds fed mash versus pellet with treatments decreasing pellet fines (increasing intact pellets), birds were more likely to selectively consume pellets (Ospina-Rojas et al., 2014).

In conclusion, diets with increasing crystalline amino acids, Val, and Val + Ile, led to 0.4 or 0.6% reduction in added fat when balancing for MEn. This reduction in added fat led to improvements in pellet quality and pellet mill energy consumption. Therefore, decreasing added fat by 0.6% led to a 10.1, 15.7 and 20.3% increase in PDI for increasing pellet agitation method of standard, modified, and Holmen methods, respectively.

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Table 4.1 Diet composition[1,2]

Item, %	Control	Val	Val + Ile
Corn	54.20	56.44	57.48
Soybean meal (46.5% CP)	39.12	37.13	36.16
Choice white grease	2.52	2.11	1.92
Limestone	1.11	1.12	1.12
Dicalcium phosphate	1.81	1.83	1.84
Salt	0.34	0.35	0.35
Copper sulfate	0.05	0.05	0.05
L-Lysine-HCL	0.15	0.22	0.24
L-Methionine	0.31	0.32	0.33
L-Threonine	0.10	0.12	0.14
L-Valine (Val)	---	0.03	0.05
L-Isoleucine (Ile)	---	---	0.02
Choline chloride	0.05	0.05	0.05
Mineral-vitamin premix	0.25	0.25	0.25
Total	100	100	100
Calculated nutrient analysis			
MEn, kcal/kg	3,042	3,042	3,042
Crude protein, %	24.43	23.73	23.40
Crude fat, %	4.95	4.60	4.44
Ca, %	0.96	0.96	0.96
Available P, %	0.48	0.48	0.48
Total Lysine, %	1.41	1.41	1.41

[1]A starter broiler diet composed of corn (ground to 1000 µm) and soybean meal and with added crystalline amino acids was mixed in a 907 kg Hayes & Stolz horizontal twin shaft counterpoise mixer. Diets were steam pelleted (24.5 × 139.7-cm length Wenger twin staff preconditioner, Model 150) for approximately 30 s at 85˚C targeted conditioning temperature on a 907 kg 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4.8 × 139.7-cm pellet die (L:D 6.67).

[2]Diets were formulated to be isocaloric and isonitrogenous.

Table 4.2 Pellet mill production data[1, 2, 3]

Item[4]	Control	Val	Val + Ile	SEM	Probability, *P* <
Production rate, kg/min	15.4	15.5	15.5	---	---
Conditioning temperature, ˚C	84.7	85.1	85.0	---	---
Hot pellet temperature, ˚C	87.2[a]	87.7[b]	87.8[b]	0.237	0.01
kW	9.1[a]	8.9[a]	10.3[b]	0.392	0.05

[1]A starter broiler diet composed of corn (1000 µm) and soybean meal and with added crystalline amino acids was mixed in a 907 kg Hayes & Stolz horizontal twin shaft counterpoise mixer. Diets were steam pelleted (25.4 × 139.7 cm length Wenger twin staff preconditioner, Model 150) for approximately 30 s at 85˚C targeted conditioning temperature on a 907 kg 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4.8 × 31.8-mm pellet die (L:D 6.67).
[2]Pelleting order in each time period consisted of control, valine (Val), and Ile (isoleucine); Val, Ile, control; and Ile, control, Val for time 1, 2 and 3 respectively.
[3]An average initial mash temperature among the 3 treatments was 20.7˚C.
[4]Reported values represent an average of 3 collected samples for each treatment.

Table 4.3 Effects of including valine (Val) and isoleucine (Ile) in broiler diets on pellet quality.[1]

Item, %	Control	Val	Val + Ile	SEM	Probability, *P*<
Pellet fines[2]	14.2[a]	12.1[b]	10.1[c]	0.154	0.01
Standard method[3]	66.5[a]	73.6[b]	76.6[c]	0.327	0.01
Modified method[3]	37.1[a]	46.9[b]	52.8[c]	0.501	0.01
Holmen NHP100 method (60s)[3]	53.4[a]	67.8[b]	73.7[c]	0.967	0.01

[1]A starter broiler diet composed of corn (1000µm) and soybean meal and with added crystalline amino acids was mixed in a 907 kg Hayes & Stolz horizontal twin shaft counterpoise mixer. Diets were steam pelleted (25.4 × 139.7 cm length Wenger twin staff. preconditioner, Model 150) for approximately 30 s at 85˚C targeted conditioning temperature on a 907 kg 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 4.8 × 31.8 mm pellet die (L:D 6.67).
[2]Reported values represent entire collected sample sifted (#5 screen = 4 mm).
[3]PDI methods were run in duplicate on the 3 collected samples for each treatment.

Chapter 5 - Effect of the pelleting process on diet formulations with varying levels of crystalline amino acids and reducing sugars on digestibility in growing pigs

ABSTRACT

The objective of this study was to determine effects of pelleting on the standardized ileal digestibility (SID) of amino acids (AA) in diets with or without increased concentrations of free AA and reducing sugars (RS). Eight individually housed, ileal cannulated barrows (initially 31.4 kg) were allotted to a replicated 8 × 8 Latin square with 8 diets and eight 7-d periods with ileal digesta collected on d 6 and 7. Treatments were arranged in a 2 × 2 × 2 factorial with main effects of diet form (mash or pellet), crystalline AA (low or high), or reducing sugars (low or high), provided by dried distillers grains with solubles and bakery meal. Diets were pelleted to achieve a hot pellet temperature of 85 to 88°C. Data were analyzed as a Latin square design using the GLIMMIX procedure of SAS 9.4. A feed form × RS interaction ($P < 0.026$) for SID of tryptophan was observed. Feeding pelleted low RS diets improved SID of tryptophan compared with mash high and low RS diets, and pelleted high RS diets. For main effects of feed form, the SID of total AA, CP, and indispensable AA increased ($P < 0.042$) in pigs fed pelleted diets compared with mash diets. For main effects of crystalline AA, pigs fed high crystalline AA had increased ($P = 0.007$) SID of tryptophan and decreased ($P = 0.050$) SID of histidine compared with those fed low crystalline AA diets. For main effects of RS, pigs fed high RS diets had decreased ($P < 0.05$) SID of total AA, CP and indispensable AA. In conclusion, pelleting diets with increased crystalline AA or RS did not affect the improvement in AA digestibility from pelleting. Pelleting diets improved AA digestibility. Diets formulated with high crystalline AA

had increased SID of tryptophan. Formulating diets with high RS resulted in decreased AA digestibility compared with corn-soybean meal-based diets.

INTRODUCTION

Pelleting swine diets is commonly used to improve feed efficiency, feed handling characteristics, and bulk density while decreasing feed wastage (Behnke, 1994). Growing concerns for feed safety issues and the importance of pellet quality have led feed manufacturing companies to steam condition swine diets at higher temperatures during the pelleting process. Current trends in diet formulation in the swine industry have focused on increasing the use of crystalline amino acids (AA). Another common practice for swine nutritionists is to use byproduct ingredients to reduce feed costs. Common byproduct ingredients include distillers dried grains with solubles (DDGS) from the ethanol industry and bakery meal from the food and confectionary industry. Corn and soybean meal are commonly used in finishing pig diets and contain trace amounts of reducing sugars (RS). The reducing sugars glucose, sucrose, maltose, and fructose typically have concentrations of 0.66, 1.14, 0.23, and 0.4% in corn; 5.03, 4.91, 2.85, and 4.71% in bakery meal; and 1.84%, 0.19%, 2.28%, and 0.74% in DDGS (Rojas et al. 2013). Soybeans contain glucose ranging from 0.07 to 0.40% (DM-basis), however, glucose is destroyed in the crushing of soybeans to produce soybean meal (Grieshop et al 2003). The reducing sugars in feed ingredients may pose nutritional challenges when pelleting diets at increased temperatures.

Over-processing can potentially lead to a reduction in amino acid availability due to the Maillard reaction. The Maillard reaction is a non-enzymatic browning reaction between an amino group from amino acids, peptides, or proteins and a carbonyl group of reducing sugars

such as glucose, fructose, or lactose. Variables optimizing the Maillard reaction include high temperatures and moisture levels, which occur during the feed pelleting process (Ajandouz et al. 2008). Thus, pelleting diets containing the combination of crystalline amino acids and by-products with reducing sugars may increase the risk for reduced amino acid availability (Pahm et al. 2008a). Therefore, the objective of this experiment was to test the hypothesis that pelleting swine diets containing free amino acids and reducing sugars at high temperatures will reduce the digestibility of amino acids.

MATERIALS AND METHODS

The University of Illinois Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the University of Illinois, Swine Research Center (Champaign, IL).

Animals

A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) had a T-cannula installed in the distal ileum and were allotted to an 8 × 8 Latin square design with the 8 diets and eight 7-d periods. Thus, each pig was fed each diet in one period and no pig received the same diet more than once. Pigs were limited to 3 times the maintenance requirement for metabolizable energy (i.e., 198 kcal ME per kg $BW^{0.60}$; NRC 2012). Throughout the experiment, pigs had free access to water. Pigs were deprived of feed overnight at the end of each experimental period, to be fed with a new diet the following morning. Each period lasted 7 days with the initial 5 days being the adaptation period, and ileal digesta was collected for 9 hours on d 6 and 7. All collected digesta samples were lyophilized and analyzed for crude protein. Amino

acid values and standardized ileal digestibility (SID) were calculated as described in Stein et al. (2007).

Dietary treatments

Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of diet form (mash or pellet), crystalline amino acid level (AA; low or high), and reducing sugars (RS; low or high; Table 1). For crystalline amino acid treatments, diets were considered low or high based on the inclusion of crystalline AA, with high crystalline AA diets having increased concentrations of added lysine, threonine, and tryptophan compared to low crystalline AA diets. Valine and isoleucine were also included as needed in the high crystalline AA diets. Reducing sugars were naturally occurring in ingredients (corn and soybean meal-based diets; low) or increased by adding DDGS and bakery meal (20 and 15%, respectively; high). All diets contained 0.5% titanium dioxide as an indigestible marker to allow for calculation of SID of AA as described below.

Feed processing

Diets were manufactured at the Kansas State University O.H. Kruse Feed Technology and Innovation Center (Manhattan, KS). Whole grain ingredients were ground with a three-high roller mill (RMS Model 924) to an approximate particle size of 600 microns. Feed was mixed in a 1-ton Hayes and Stolz double ribbon mixer. Treatments were pelleted using a 5-ton, 100-horsepower pellet mill (Model PM3016-4, California Pellet Mill) equipped with a 4 × 35-mm die (L:D = 8.75). The conditioning temperature ranged from 79 to 85°C to achieve a hot pellet temperature of 85 to 88°C, achieved by adjusting steam addition (Table 4). The pelleted diets were cooled in an experimental counterflow cooler for 15 minutes. To minimize the effect of pellet quality differences, pellets passed through a sifter to remove fines before transport.

A sample of cool pellets was collected, and the fines sifted off by using the corresponding sieve stack (ASAE S269.4). Sifted pellets were split using a riffle divider and 100 g used for analysis. The 100 g sample was placed into the hopper of the Holmen NHP100 for 60 seconds to simulate pellet quality at the feeder. The fines were removed as the sample was run. Once completed, the sample was removed from the hopper and weighed. The pellet durability index (PDI) was calculated by dividing this final sample weight by the 100 g initial sample weight.

Chemical analysis

Representative samples of corn, bakery meal, DDGS, soybean meal and treatment diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for dry matter (DM; AOAC Official Method 934.01, 2006), crude protein (AOAC Official Method 990.03, 2006), crude fat (AOAC Official Method 920.39 (A)), crude fiber (AOAC Official Method 978.10, 2006), ash (AOAC Official Method 942.05), complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006), available lysine (AOAC Official Method 975.44, chp. 45.4.03, 2006), protein solubility in potassium hydroxide (KOH) (Parsons et al. 1991) and Maillard reaction end products (melanoidins). Melanoidins were measured using approximately 50 mg of each sample which was accurately weighed and dispersed in 3 ml ethanol solution (47.5%, v/v) in a 5 mL tube. The tube was then vortexed for 30 s and further shook in a water bath for 30 min at 50 °C and 120 rpm oscillation. After that, the sample was vortexed again for 1 min and then mixed vigorously for another 30 min. Afterward, the sample was centrifuged at 10,000 × g for 10 min, and the supernatant was collected for analysis. Each sample was extracted and analyzed in duplicate. The relative amount

of melanoidin in the extract was analyzed using a double beam spectrophotometer (VWR UV-6300PC) at 420 nm (Del Castillo et al. 2001; Shen et al. 2018; Tables 2 and 3).

Ileal digesta contents were analyzed for dry matter (AOAC Official Method 934.01, 2006), crude protein (AOAC Official Method 990.03, 2006), and complete AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006). Diet and ileal digesta samples were also analyzed for titanium dioxide (Myers et al. 2004).

Calculations and statistical analysis

Values for SID (%) were calculated as indicated below using Stein et al., (2007). First, AID of AA was calculated as:

Equation 5.1 Apparent ileal digestibility of amino acids (AA)

$$\mathbf{AID_{AA}}\ (\%) = \left[\mathbf{1} - \left(\frac{\mathbf{AA_{diet}}}{\mathbf{AA_{digesta}}}\right) \times \left(\frac{\mathbf{Ti_{digesta}}}{\mathbf{Ti_{diet}}}\right)\right] \times \mathbf{100},$$

where $AA_{digesta}$ and AA_{diet} represent the AA concentrations (g/kg) in digesta and diet DM, respectively, and Ti_{diet} and $Ti_{digesta}$ represent the indigestible marker concentrations (g/kg) in diet and digesta DM, respectively. Then, to calculate SID of AA the following equation was used.

Equation 5.2 Standardized ileal digestibility of amino acids (AA)

$$\mathrm{SID_{AA}}\ (\%) = \mathrm{AID_{AA}} + \left[\left(\frac{IAA_{end}}{\mathrm{AA_{diet}}}\right) \times 100\right],$$

where AID_{AA} was previously calculated in equation 1, IAA_{end} represents basal endogenous losses where average values were used from digestibility experiments conducted at the University of Illinois, Champaign, Il., and AA_{diet} represents the AA concentrations (g/kg) in diet DM.

Specific energy consumption (kWh/MT) was calculated as described in Stark et al. (1994);

Equation 5.3 Pellet mill specific energy consumption (SEC)

$$\text{SEC} = \frac{\text{I} \times \text{E} \times \text{PF} \times 1.73}{\text{PR} \times 1000},$$

where I is amperage, E is voltage, PF is power factor set to 0.85 and PR is production rate.

Data were analyzed as a completely randomized Latin square design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC) with pig as the experimental unit. Fixed effects included feed form, crystalline AA, RS inclusion, and all possible interactions. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Results were considered significant at $P \leq 0.05$ and a trend at $P < 0.10$.

RESULTS

Chemical analysis

Chemical analysis of major ingredients is reported in Table 1. Analyzed crude protein and lysine content of corn was numerically greater than reference values used (10.07 vs 8.24 for crude protein and 0.43 vs 0.25 for lysine). Analyzed lysine content for corn was higher than expected at 0.43% compared to NRC (2012) analyzed nutrient value of 0.25%. Analyzed nutrient values of soybean meal were similar to the reference values used. Dried distillers' grains with solubles had a numerically decreased analyzed crude fat (5.05 vs 7.50%) and increased total lysine (1.00 vs 0.79%) compared to reference values used. Bakery meal had a numerically

increased ash content (6.02 vs 3.22%) and total lysine (0.75 vs 0.54%) compared to reference values used. In addition, melanoidins were measured on individual ingredients as evidence of Maillard reaction. All ingredients that undergo processing prior to inclusion in diets (soybean meal, DDGS, and bakery meal) had increased absorbance values for melanoidins as compared to ground corn.

Chemical analysis of experimental diets indicated that analyzed CP ranged from 13.3 to 19.3% with the expected range of 14.8 to 19.1% for both mash and pelleted diets (Table 2). The high crystalline AA × low RS diets contained the lowest CP and the low crystalline AA × high RS diets contained the highest CP (Table 3). Analyzed available lysine content was 0.85% to 1.02%, where the lowest was high crystalline AA × high RS mash diets and highest for the low crystalline AA × low RS mash diet. The high RS diets (containing 20% DDGS and 15% bakery meal) had increased absorbance values for melanoidins as compared to the low RS diets (corn and soybean meal-based diets). In addition, when pelleting low RS diets there was little numerical increase in melanoidins absorbance values. However, when pelleting high RS diets there was a greater numerical increase in melanoidins absorbance values. The high RS diets (containing 20% DDGS and 15% bakery meal) had decreased KOH protein solubility compared to low RS diets. In addition, pelleting the high crystalline AA and high RS diets resulted in decreased KOH protein solubility compared to the mash diet. However, pelleting the remaining treatments resulted in increased KOH protein solubility compared to the same diet in mash form.

Feed processing

The experiment was designed to pellet diets at a conditioning temperature of 88°C. However, at the desired conditioning temperature a consistent pelleting run could not be

achieved. Therefore, diets were pelleted to a target hot pellet temperature between 85 and 88°C, which was achieved by pelleting at a conditioning temperature range of 79 to 85°C (Table 4). The PDI for diets containing low RS increased by more than 10% compared with those containing high RS. These differences in PDI could possibly be explained by increases in added fat aiding in die lubrication in diets containing high RS. Differences in PDI were alleviated by sifting the pellets post-pelleting to remove excessive fines prior to feeding.

Standardized ileal digestibility of amino acids

There was no feed form × crystalline AA × RS interaction observed for SID of AA. There were also no 2-way interactions of feed form × crystalline AA for SID of total AA, indispensable, or dispensable AA (Table 5). There were no 2-way interactions of feed form × RS observed for SID of total AA, CP, or dispensable AA (Table 6). There were no 2-way interactions of feed form × RS observed for SID of indispensable AA, except for tryptophan. Pigs fed pelleted high RS diets resulted in lower SID tryptophan than mash high RS diets, mash low RS diets, and pelleted low RS diets.

There were no 2-way interactions of crystalline AA × RS observed for SID of total AA, CP, indispensable AA, or dispensable AA (Table 7).

For the main effects of feed form, the SID of total AA, CP, indispensable AA, alanine, aspartic acid, glutamic acid, and serine increased ($P < 0.042$) in the pelleted diets compared with mash diets (Table 8).

For the main effects of crystalline AA, pigs fed low or high crystalline AA had SID of total AA and CP that were not different. Pigs fed high crystalline AA had increased ($P = 0.007$; Table 8) SID of tryptophan compared with those fed the low crystalline AA diet. The SID of

lysine tended to increase ($P = 0.076$) in pigs fed high crystalline AA diets compared with those fed low crystalline AA diets. Pigs fed high crystalline AA had decreased ($P = 0.050$) SID of histidine compared with those fed low crystalline AA diets. The SID of arginine and isoleucine tended to decrease ($P < 0.079$) in pigs fed high crystalline AA. In pigs fed high crystalline AA, the SID of serine and glycine decreased ($P < 0.042$) compared with those fed low crystalline AA.

For the main effects of RS diets, pigs fed high RS diets had decreased ($P < 0.05$) SID of total AA, CP, indispensable AA, alanine, aspartic acid, cysteine, glutamic acid, and serine (Table 8).

DISCUSSION

The present experiment was designed to determine if pelleting various swine diets influenced the digestibility of AA. Diets were formulated with low or high crystalline AA, low or high reducing sugars (from DDGS and bakery meal) and fed in either mash or pelleted form to increase the likelihood of binding lysine via the Maillard reaction. Components of the pelleting process, such as steam conditioning and feed retention time in the conditioner and die, expose feed to various degrees of heat, moisture, pressure, and sheer altering the feeds' physical and chemical characteristics. When ingredients that make up a diet formulation are exposed to the steam conditioning process and extrusion through the die, the heat and moisture plasticize the soluble fractions of the diet and increase the agglomeration of dietary components (Lundblad et al., 2009). Pelleting swine diets is commonly used to improve nutrient utilization, feed efficiency, feed handling characteristics and bulk density (Behnke, 1994). In addition, previous research observed that feeding pelleted diets increased DM, N, and GE digestibility compared to feeding meal diets when fed to pigs (Wondra et al., 1995). More recently, Rojas et al., (2017)

observed pelleting a corn-soybean meal diet increased digestibility of DM, N, and GE by 5 to 8% compared when feeding the same diet in a mash form. Observed improvements to protein and AA digestibility could be a result of pelleting due to protein denaturation under processing conditions (Lancheros et al., 2020). The results of this experiment agree with previous research in that pigs fed pelleted diets had increased SID of total AA, CP, indispensable AA, alanine, aspartic acid, glutamic acid, and serine compared with pigs fed mash diets.

The pelleting process may result in heat damage to protein (González-Vega et al., 2011) as well as the formation of antinutritional reaction products. Such antinutritional products are a result of the Maillard reaction. In the Maillard reaction, free amino acids bind to reducing sugars, decreasing the availability and utilization of those nutrients by the animal (Pahm et al., 2008b), making amino acids unavailable for protein synthesis**.** Free amine groups at the N-terminus of an amino acid interact with the free aldehyde group of reducing sugars, such as glucose, galactose, and fructose. There is potential for the pelleting process to contribute to reduced amino acid, particularly lysine, utilization ultimately decreasing digestibility.

Evidence of the Maillard reaction can be determined from a reactive cyclic intermediate compound containing amino groups that polymerize to a dark-colored, insoluble material containing nitrogen called melanoidin, which vary in color, molecular weight, nitrogen content and solubility (Fennema et al., 2008). These end products from this “browning reaction” are commonly observed in food science processes such as baking bread. Melanoidin research in feed ingredients and complete diets is limited with most research evaluating early reaction productions (Amadori compounds). However, Amadori compounds can interfere with amino acid analysis giving an inaccurate measure of lysine concentrations by overestimating lysine content in the sample (Stein et al. 2005). On the human food side, melanoidins are measured for

indication of favorable products for flavor and antioxidant potential. Shen et al., (2018) demonstrated that melanoidins increased during bread making with longer baking time, higher baking temperature, or higher sugar inclusion. In this study, ingredients that undergo processing prior to diet formulation (i.e., soybean meal, DDGS, and bakery meal) had increased melanoidin absorbance values compared to ground corn. Therefore, diets containing DDGS and bakery meal and reduced concentrations of ground corn had increased melanoidin absorbance values. Pelleting the corn and soybean meal-based diets (low RS) only resulted in minor numerical increases in melanoidin absorbance values. There tended to be a greater numerical increase in melanoidin absorbance values when high RS diets were pelleted compared to the mash high RS diets. Additionally, diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with corn-soybean meal-based diets. However, further research is needed to determine the influence of melanoidins on digestibility in swine.

Additional analytical techniques to measure degree of processing for feed ingredients or complete diets included Lysine:CP, KOH protein solubility, and available lysine. The relationship of Lysine:CP gives an indication of the extent of conversion of lysine to melanoidins, and the heat damage that has occurred, where lower than expected values can indicate heat damage of processed ingredients (Stein et al., 2005). Expected Lysine:CP values for corn, DDGS, and bakery meal are about 3.0, 3.1 and 3.2%, respectively where expected soybean meal value is about 6.2% (NRC 2012). In this experiment, the Lysine:CP ratio did not decrease in the pelleted diets compared to the mash diets. Therefore, there was no evidence of the Maillard reaction based on Lysine:CP.

The KOH solubility (%) indicates the nitrogen (%) that is solubilized in a potassium hydroxide solution. Previous research has determined that KOH solubility is well correlated to

the digestibility of protein in the animal (Araba and Dale, 1990). In individual ingredients, KOH solubility was greatest in soybean meal (75.1%) and least in DDGS (26.7%), with corn (50.0%) and bakery meal (49.7%) being intermediate. For complete diets, high RS diets (containing 20% DDGS and 15% bakery meal) had decreased KOH protein solubility compared to low RS diets. This is largely driven by DDGS and bakery meal having the lowest KOH solubility as well as high RS diets having lower inclusions of soybean meal. Although KOH solubility has previously been correlated with soybean meal lysine digestibility, Stein et al., (2005) suggest that the KOH solubility was not accurate at predicting AA digestibility of DDGS. In this experiment, pelleting low RS & low crystalline AA, low RS and high crystalline AA, and high RS and low crystalline AA diet did not reduce KOH solubility compared to the respective mash diets. However, pelleting the high crystalline AA and high RS diets numerically decreased KOH protein solubility compared to the mash diet, which could be explained by the possible initiation of the Maillard reaction. However, this did not correspond with the AA digestibility results. Thus, analyzing KOH solubility in complete diets warrants further research to understand the association of KOH solubility with digestibility. Pelleting the remaining treatments resulted in increased KOH protein solubility compared to the same diet in mash form. Higher KOH solubility would indicate a greater nitrogen digestibility.

There are several factors that influence the rate of Maillard product formation, including temperature, pH, water activity, pressure, and reactant concentration (Rutherford and Moughan, 2007). Pelleting provides the potential for ideal conditions for the Maillard reaction. Ajandouz et al., (2008) utilized an aqueous model system via spectrophotometer absorbance of intermediate and final stages of the reaction indicating that increasing temperature increases the Maillard reaction. In contrast to reducing AA digestibility, Lundblad et al., (2012) found that

hydrothermal treatment improved AID for total indispensable AA, arginine, isoleucine, lysine, and threonine when compared to pigs fed a mash control diet. Additionally, AID of lysine was improved by hydrothermal treatment compared to mash with further improvement in pigs fed an extruded diet compared to a mash diet. These improvements in AID of AA could be due to processing partly denaturing dietary proteins, increasing digestion by proteases in the small intestinal (Rojas et al., 2016; Svihus and Zimonja, 2011).

Unlike amino acid chains that bind to form whole proteins, crystalline amino acids are in the free, unbound form, and thus have more available amino groups to bind reducing sugars. The structure of lysine in particular makes it very susceptible to the Maillard reaction as it has two free amino groups. Maillard reactions with lysine result in a reduction of both lysine concentration and lysine digestibility (Gonzàlez-Vega et al., 2011). It has been established that crystalline AA are rapidly absorbed and 100% digestible (Chung and Baker, 1992). Recent trends in swine diet formulation are to increase the levels of crystalline amino acids and by-product ingredients to reduce diet cost. In this study, no differences were observed for SID of total AA, indispensable AA or CP pigs fed low or high crystalline AA. However, the SID of tryptophan increased in diets containing higher concentrations of crystalline AA. The SID of lysine tended to increase in pigs fed high crystalline AA diets compared with those fed low crystalline AA diets.

It has been demonstrated that the Maillard reaction can negatively impact AA digestibility (Pahm et al., 2008b). Gonzàlez-Vega et al., (2011) determined that SID of all AA in autoclaved SBM was reduced as the autoclaving time increased from 0 to 30 min. Therefore, as soybean meal was exposed to moisture, pressure, high temperature (125°C) and increasing time in the autoclave, the Maillard reaction occurred in the soybean meal. However, as soybeans were

exposed to an oven drying thermal process, at the same time and temperature (125°C for 30 min) there were minimal effects on the SID of AA in SBM (Gonzàlez-Vega et al., 2011). This suggests more severe processing conditions with added moisture and pressure increase the formation of Maillard reaction products and destruction of AA. Further, Gonzàlez-Vega et al., (2011) suggests that with increased pressure AAs become less stable and (Qain et al., 1993) and an increase in humidity will increase the reaction rate between the amino group of the AA and glucose (Schwartz and Lea, 1952). Rojas et al., (2016) demonstrated that CP and AA digestibility improved in pelleted diets compared to mash diets containing corn, soybean meal and DDGS. In this experiment, there was no evidence for interaction between diets, demonstrating that diet type did not accelerate the Maillard reaction to a point to reduce AA digestibility during the pelleting process. Pigs fed pelleted diets did demonstrate improved SID of AA, in agreement with Rojas et al., (2016).

In this study, both DDGS and bakery meal byproducts were utilized and accounted for 35% of total dietary inclusion. Diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with the corn-soybean meal-based diets. Amino acid digestibility of byproduct ingredients has been evaluated throughout literature which can explain the main effect differences observed for RS diets. Almeida et al., (2011) observed that bakery meal is a poor source of digestible AA when compared with corn and corn coproducts, likely due to bakery meal variability. Additionally, the SID of lysine and CP for bakery meal was not different from SID values found for DDGS. For all other indispensable AA except tryptophan, SID values in bakery meal were less than in pigs fed corn, with no differences found between corn and DDGS (Almeida et al., 2011). This did not translate into differences in AA digestibility due to diet type and the pelleting process.

Amino acid digestibility of swine diets containing free amino acids and reducing sugars pelleted at high temperatures was evaluated. There was no evidence of interactions between diet types and diet form, indicating that increasing amounts of crystalline AA and RS did not reduce AA digestibility when pelleting diets using the reported conditions. Additionally, pelleting diets resulted in improved AA digestibility compared to mash diets. Diets formulated with 20% DDGS and 15% bakery (high RS) resulted in decreased AA digestibility compared with the corn-soybean meal-based diets. Crystalline AA concentration did not influence AA digestibility of indispensable AA, except for SID of tryptophan which was increased in diets with higher concentrations of crystalline AA. Further research should focus on the pelleting temperature breakpoint (conditioning and/or hot pellet temperature) where digestibility decreases due to the Maillard reaction.

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Table 5.1 Diet composition (as-fed basis)[1,2]

Crystalline AA Reducing sugars	Low Low	Low High	High Low	High High
Ingredient, %				
Corn	75.00	44.11	79.68	52.98
Soybean meal	21.00	15.70	16.19	6.85
Dried distiller's grain with solubles	---	20.00	---	20.00
Bakery meal[3]	---	15.00	---	15.00
Soybean oil	1.23	2.70	0.90	2.10
Calcium carbonate	0.70	0.83	0.70	0.85
Monocalcium P, 21%	1.00	0.60	1.10	0.70
Sodium chloride	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.18	0.23	0.33	0.50
DL-Methionine	----	---	0.04	---
L-Threonine	0.09	0.04	0.16	0.16
L-Tryptophan	0.01	0.01	0.04	0.06
L-Valine	---	---	0.07	---
L-Isoleucine	---	---	0.01	0.01
Trace mineral premix[4]	0.15	0.15	0.15	0.15
Vitamin premix[5]	0.15	0.15	0.15	0.15
Titanium dioxide	0.50	0.50	0.50	0.50
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lysine[6,7]	0.83	0.83	0.83	0.83
Isoleucine:lysine	69	76	60	60
Leucine:lysine	156	187	142	162
Methionine:lysine	29	34	30	29
Methionine and cysteine:lysine	57	65	56	55
Threonine:lysine	70	70	70	70
Tryptophan:lysine	21.0	21.0	21.0	21.0
Valine:lysine	77	90	75	72
Histidine:lysine	47	46	42	36
Total lysine, %	0.95	0.99	0.93	0.97
ME, kcal/kg	3,355	3,393	3,344	3,371
NE, kcal/kg	2,599	2,599	2,599	2599
Crude protein, %	16.4	19.1	14.8	16.0
Ca, %	0.59	0.59	0.59	0.59
P, %	0.56	0.55	0.56	0.53
STTD P, %	0.33	0.33	0.33	0.33

[1]Dietary treatments were arranged in a 2 × 2 × 2 factorial. Main effects consisted of crystalline AA (low vs. high), reducing sugars (low vs. high), and diet form (mash vs. pellet).
[2]Experimental diet was formulated for 22.7- to 38.6-kg pigs.
[3]Quincy Farm Products, Quincy, IL.

[4]Provided per kg of premix: 73g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.
[5] Provided per kg of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.
[6]To ensure the ability to detect a difference in AA utilization, these diets were formulated to 85% of the recommended SID lysine requirement of pigs.
[7]National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.
[8]AA = amino acids. ME = metabolizable energy. NE = net energy. STTD P = standardized total tract digestible phosphorus.

Table 5.2 Chemical analysis of individual major ingredients (as-fed basis)[1,2,3]

Item, %	Corn	Soybean meal	DDGS	Bakery meal
Dry matter	88.10 (88.31)	88.94 (89.98)	90.39 (89.31)	87.28 (86.30)
Crude protein	10.07 (8.24)	45.44 (47.73)	29.77 (28.15)	17.32 (14.30)
Fat	2.25 (3.48)	0.54 (1.52)	5.05 (7.50)	4.03 (5.10)
Crude fiber	1.81 (1.98)	3.73 (3.89)	7.64 (7.28)	8.00
Ash	1.57 (1.30)	6.09 (6.27)	5.00 (4.24)	6.02 (3.22)
Melanoidin,[4] abs	0.105	0.206	0.215	0.306
KOH protein solubility[5]	49.95	76.10	26.67	49.71
Available lysine	0.42	2.87	0.92	0.68
Total AA	9.67	45.09	27.95	16.29
Lysine:CP	4.27	6.49	3.36	4.32
Indispensable AA				
Arginine	0.51 (0.37)	3.27 (3.45)	1.36 (1.20)	1.04 (0.68)
Histidine	0.29 (0.24)	1.22 (1.28)	0.81 (0.73)	0.43 (0.22)
Isoleucine	0.39 (0.28)	2.23 (2.14)	1.21 (1.05)	0.79 (0.56)
Leucine	1.03 (0.96)	3.52 (3.62)	3.40 (3.23)	1.42 (1.1)
Lysine	0.43 (0.25)	2.95 (2.96)	1.00 (0.79)	0.75 (0.54)
Methionine	0.19 (0.18)	0.63 (0.68)	0.54 (0.57)	0.25 (0.21)
Phenylalanine	0.47 (0.39)	2.36 (2.40)	1.49 (1.38)	0.94 (0.50)
Threonine	0.36 (0.28)	1.77 (1.86)	1.10 (1.02)	0.67 (0.52)
Tryptophan	0.08 (0.06)	0.66 (0.66)	0.21 (0.22)	0.15 (0.18)
Valine	0.48 (0.38)	2.27 (2.23)	1.50 (1.39)	1.04 (0.72)
Dispensable AA				
Alanine	0.64 (0.60)	1.97 (2.06)	2.04 (1.99)	0.95
Aspartic acid	0.76 (0.54)	5.08 (5.41)	1.94 (1.88)	1.33
Cysteine	0.22 (0.19)	0.71 (0.70)	0.60 (0.53)	0.28 (0.18)
Glutamic acid	1.80 (1.48)	8.28 (8.54)	4.45 (4.49)	2.50
Serine	0.44 (0.38)	2.01 (2.36)	1.29 (1.22)	0.71
Tyrosine	0.22 (0.26)	1.66 (1.59)	1.06 (1.07)	1.10 (0.55)
Glycine	0.42 (0.31)	1.91 (1.99)	1.15 (1.07)	0.84
Proline	0.73 (0.71)	2.25 (2.53)	2.35 (2.16)	0.86

[1]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[2]Values in parentheses indicate values used in diet formulation. These values were acquired from the NRC (2012) and bakery meal values were provided by supplier. The soybean meal net energy value assigned was 88% of corn.
[3]National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.
[4]Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.
[5]Protein solubility in potassium hydroxide (KOH)
AA = amino acids. DDGS = dried distillers grains with solubles.

Table 5.3 Chemical analysis of experimental diets (as-fed basis)[1,2]

	Mash				Pellets			
Crystalline AA	Low	Low	High	High	Low	Low	High	High
Reducing sugars	Low	High	Low	High	Low	High	Low	High
Item, %								
Dry matter	87.37	87.81	87.43	87.88	87.88	87.91	87.83	88.65
Crude protein	15.78	18.71	13.31	15.73	15.61	19.29	14.42	16.99
Fat	1.20	3.69	0.95	2.89	2.13	4.53	2.30	4.40
Fiber	1.60	3.26	1.51	3.04	1.87	3.68	1.78	3.08
Ash	4.19	5.28	4.09	4.84	4.47	5.54	4.31	5.11
Melanoidin, abs[3]	0.081	0.119	0.081	0.123	0.088	0.146	0.087	0.143
KOH protein solubility[4]	63.45	48.62	62.07	54.66	81.76	57.33	71.73	46.15
Available lysine	1.02	0.96	0.93	0.85	0.98	0.94	0.90	0.98
Total AA	15.04	18.16	13.90	14.88	16.38	19.36	13.95	16.29
Lysine:CP	5.90	6.59	5.83	6.15	6.40	7.04	5.83	6.78
Indispensable AA								
Arginine	0.93	1.05	0.81	0.79	1.02	1.15	0.82	0.88
Histidine	0.41	0.49	0.37	0.39	0.44	0.53	0.37	0.43
Isoleucine	0.66	0.80	0.60	0.60	0.72	0.86	0.60	0.67
Leucine	1.36	1.77	1.27	1.51	1.47	1.87	1.28	1.63
Lysine	0.93	1.04	0.92	0.97	1.01	1.11	0.92	1.07
Methionine	0.26	0.32	0.27	0.28	0.28	0.34	0.27	0.29
Phenylalanine	0.75	0.94	0.67	0.74	0.82	1.00	0.68	0.81
Threonine	0.62	0.72	0.63	0.68	0.67	0.78	0.62	0.74
Tryptophan	0.18	0.20	0.20	0.20	0.22	0.22	0.21	0.23
Valine	0.74	0.94	0.72	0.73	0.80	1.01	0.71	0.81
Dispensable AA								
Alanine	0.81	1.08	0.75	0.94	0.87	1.14	0.77	1.02
Aspartic acid	1.43	1.60	1.24	1.14	1.57	1.72	1.24	1.26
Cysteine	0.28	0.34	0.26	0.29	0.31	0.37	0.25	0.31
Glutamic acid	2.70	3.12	2.43	2.44	2.93	3.30	2.44	2.68
Serine	0.65	0.77	0.58	0.61	0.71	0.84	0.59	0.70
Tyrosine	0.53	0.71	0.49	0.61	0.57	0.75	0.48	0.66
Glycine	0.63	0.79	0.56	0.62	0.69	0.84	0.57	0.70

[1]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline amino acids (AA) (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet).
[2]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.
[3]Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.
[4]Protein solubility in potassium hydroxide (KOH).

Table 5.4 Feed processing and pellet quality of pelleted diets[1,2]

Crystalline amino acids	Low	Low	High	High
Reducing sugars	Low	High	Low	High
Item				
Production rate, kg/h	3,810.2	3,628.7	3,719.5	3,447.3
Conditioning temperature, °C	85.1	83.6	83.0	79.7
Hot pellet temperature, °C	89.3	87.4	87.6	85.0
Pellet durability index (PDI), %3	71.5	53.7	72.2	59.6
Energy consumption. kWh/MT	9.7	8.2	9.7	9.5

[1]Treatments were pelleted using a 5-ton 100-horsepower pellet mill (Model PM3016-4, California Pellet Mill) equipped with a 4 × 35-mm die (L:D = 8.75).
[2]Pellets were sifted to remove fines to ensure no effect of pellet quality on pig performance.
[3]Holmen NHP100 for 60 seconds.

Table 5.5 Standardized ileal digestibility of crude protein (CP) and amino acids (AA) as a 2-way interaction of feed form × crystalline AA[1,2]

Form	Mash		Pellet			
Crystalline AA	Low	High	Low	High	SEM	*P*-value[3]
SID, %						
Total AA	81.71	79.84	84.36	84.83	1.066	0.263
Crude protein	78.51	75.24	81.84	82.84	1.559	0.194
Indispensable AA						
Arginine	92.52	90.67	96.51	95.64	0.787	0.513
Histidine	81.83	79.64	84.36	82.95	0.924	0.663
Isoleucine	81.41	77.85	84.64	84.06	1.102	0.170
Leucine	81.35	79.93	85.19	85.44	1.068	0.425
Lysine	85.24	86.28	86.56	89.01	0.992	0.470
Methionine	86.81	86.01	90.89	90.85	1.100	0.721
Phenylalanine	81.73	79.38	85.09	85.14	1.061	0.247
Threonine	75.23	76.09	77.16	80.33	1.283	0.357
Tryptophan	86.64	88.69	87.88	92.07	1.141	0.337
Valine	77.37	74.36	80.72	80.52	1.266	0.257
Dispensable AA						
Alanine	79.58	77.91	83.85	84.82	1.246	0.281
Aspartic acid	76.80	74.52	79.09	78.62	0.998	0.354
Cysteine	69.75	66.75	71.31	70.02	1.277	0.495
Glutamic acid	82.26	82.02	84.10	85.73	0.931	0.304
Serine	82.02	77.98	85.83	84.89	1.228	0.199
Tyrosine	85.89	84.05	88.75	89.34	0.821	0.133
Glycine	66.04	58.46	66.38	64.51	2.293	0.205
Proline	104.11	98.59	106.28	105.27	5.160	0.653

[1]A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 8 Latin square design with the 8 diets and eight 7-d periods.

[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet). This table represents the 2 × 2 factorial of crystalline AA (low vs. high) and diet form (mash vs. pellet).

[3] Probability, $P <$ of a 2-way interaction of feed form and crystalline AA.

Table 5.6 Standardized ileal digestibility of crude protein (CP) and amino acids (AA) as a 2-way interaction of feed form × reducing sugar (RS) fed to pigs.[1,2]

Form	Mash		Pellet			
RS	Low	High	Low	High	SEM	*P*-value[3]
SID, %						
Total AA	82.98	78.57	87.31	81.87	1.066	0.618
Crude protein	78.86	74.89	85.10	79.29	1.559	0.545
Indispensable AA						
Arginine	93.84	89.36	98.43	93.72	0.787	0.885
Histidine	84.12	77.35	87.79	79.52	0.924	0.404
Isoleucine	82.54	76.71	87.72	80.97	1.100	0.670
Leucine	82.74	78.54	87.60	83.02	1.068	0.854
Lysine	88.18	83.34	91.46	84.11	0.992	0.198
Methionine	89.51	83.30	94.74	87.01	1.100	0.478
Phenylalanine	82.44	78.66	87.39	82.84	1.061	0.710
Threonine	78.14	73.18	82.66	74.83	1.283	0.256
Tryptophan	87.53	87.80	92.38	87.58	1.141	0.026
Valine	78.92	72.81	84.68	76.56	1.266	0.416
Dispensable AA						
Alanine	80.24	77.25	86.89	81.79	1.246	0.385
Aspartic acid	79.46	71.86	83.34	74.37	0.998	0.484
Cysteine	71.56	64.94	75.11	66.21	1.277	0.361
Glutamic acid	84.66	79.62	86.53	83.30	0.931	0.321
Serine	83.31	76.70	89.27	81.46	1.228	0.619
Tyrosine	84.82	85.12	89.30	88.79	0.821	0.611
Glycine	63.02	61.48	67.60	63.29	2.293	0.537
Proline	100.10	102.60	104.3	107.26	5.160	0.964

[1]A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 8 Latin square design with the 8 diets and eight 7-d periods.

[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet). This table represents the 2 × 2 factorial of RS (low vs. high) and diet form (mash vs. pellet).

[3]Probability, $P <$ of a 2-way interaction of feed form and reducing sugars.

Table 5.7 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) as a 2-way interaction of crystalline AA inclusion × reducing sugar (RS). [1,2]

Crystalline AA	Low		High			
RS	Low	High	Low	High	SEM	*P*-value[3]
SID, %						
Total AA	85.17	80.91	85.13	79.54	1.066	0.520
Crude protein	82.32	78.03	81.63	76.15	1.559	0.699
Indispensable AA						
Arginine	96.62	92.42	95.65	90.66	0.787	0.605
Histidine	86.44	79.75	85.47	77.12	0.924	0.358
Isoleucine	85.55	80.49	84.71	77.19	1.101	0.255
Leucine	85.15	81.39	85.19	80.18	1.068	0.555
Lysine	89.23	82.57	90.41	84.88	0.992	0.557
Methionine	91.29	86.41	92.96	83.91	1.100	0.056
Phenylalanine	85.10	81.72	84.74	79.78	1.060	0.443
Threonine	79.62	72.77	81.17	75.24	1.283	0.711
Tryptophan	88.90	85.62	91.01	89.75	1.141	0.364
Valine	81.58	76.51	82.03	72.86	1.266	0.102
Dispensable AA						
Alanine	83.281	80.15	83.84	78.89	1.246	0.456
Aspartic acid	81.35	74.55	81.45	71.68	0.998	0.131
Cysteine	74.07	66.99	72.61	64.16	1.277	0.578
Glutamic acid	84.66	81.70	86.53	81.22	0.931	0.199
Serine	87.02	80.84	85.56	77.31	1.228	0.389
Tyrosine	87.66	86.98	86.47	86.93	0.821	0.478
Glycine	67.75	64.67	62.97	60.10	2.293	0.945
Proline	104.05	106.34	100.35	103.51	5.160	0.931

[1]A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 8 Latin square design with the 8 diets and eight 7-d periods.

[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet). This table represents the 2 × 2 factorial of crystalline AA (low vs. high) and RS (low vs. high)

[3] Probability, $P <$ of a 2-way interaction of crystalline AA and reducing sugar.

Table 5.8 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) main effects of feed form, crystalline AA, and reducing sugar (RS)[1,2]

	Form		Crystalline AA		RS			*P*-value[3]		
SID, %	Mash	Pellet	Low	High	Low	High	SEM	Form	AA	RS
Total AA	80.78	84.60	83.04	82.33	85.15	80.22	0.737	0.001	0.500	0.001
Crude protein	76.88	82.20	80.18	78.89	81.98	77.09	1.078	0.001	0.340	0.002
Indispensable AA										
Arginine	91.60	96.08	94.52	93.15	96.14	91.54	0.544	0.001	0.079	0.001
Histidine	80.74	83.66	83.10	81.30	85.96	78.44	0.639	0.002	0.050	0.001
Isoleucine	79.63	84.34	83.02	80.95	85.13	78.84	0.762	0.001	0.059	0.001
Leucine	80.64	85.31	83.27	82.67	85.78	80.78	0.739	0.001	0.577	0.001
Lysine	85.76	87.79	85.90	87.65	89.82	83.72	0.686	0.041	0.076	0.001
Methionine	86.41	90.87	88.85	88.43	92.13	85.16	0.759	0.001	0.698	0.001
Phenylalanine	80.55	85.12	83.41	82.26	84.75	80.75	0.733	0.001	0.269	0.001
Threonine	75.66	78.74	76.20	78.21	80.40	74.01	0.888	0.017	0.113	0.001
Tryptophan	87.66	89.98	87.26	90.38	89.95	87.69	0.789	0.042	0.007	0.046
Valine	75.87	80.62	79.04	77.44	81.80	74.68	0.875	0.001	0.200	0.001
Dispensable AA										
Alanine	78.75	84.34	81.71	81.37	83.56	79.52	0.862	0.001	0.776	0.002
Aspartic acid	75.66	78.85	77.95	76.57	81.40	73.11	0.690	0.002	0.160	0.001
Cysteine	68.25	70.66	70.53	68.38	73.34	65.57	0.883	0.058	0.090	0.001
Glutamic acid	84.14	84.92	83.18	83.89	85.60	81.46	0.644	0.004	0.442	0.001
Serine	80.00	85.36	83.93	81.43	86.29	79.08	0.849	0.001	0.042	0.001
Tyrosine	84.97	89.05	87.32	86.70	87.06	86.96	0.568	0.001	0.436	0.897
Glycine	62.25	65.44	66.21	61.48	65.31	62.39	1.586	0.157	0.039	0.194
Proline	101.35	105.78	105.19	101.93	102.2	104.93	3.568	0.380	0.517	0.587

[1]A total of eight individually housed growing barrows (initially 31.4 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 8 Latin square design with the 8 diets and eight 7-d periods.

[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA (low vs. high), reducing sugar (low vs. high), and diet form (mash vs. pellet).

[3] Probability, $P <$ for the main effects of form, crystalline AA, or reducing sugar.

Chapter 6 - Effect of the pelleting process on diet formulations with varying levels of crystalline amino acids and reducing sugars on nursery pig growth performance

ABSTRACT

Pelleting swine feed combined with the use of crystalline amino acids (AA) and byproduct ingredients can potentially create ideal conditions that further facilitate the Maillard reaction, combining an amino group of a free AA and a carbonyl group of a reducing sugar (RS), making the AA less available. The objective of this study was to determine the effects of pelleting swine diets containing free AAs and RS at high temperatures on nursery pig growth performance. A total of 360 pigs (DNA 200 × 400; initially 11.3 kg) were used to evaluate the effect of crystalline AA, RS, and feed form on growth performance of nursery pigs. Treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA concentration (low vs. high), RS (low vs. high), and diet form (mash vs. pellet). Diets were formulated with low or high crystalline AA and low or high RS provided by co-product ingredients, DDGS and bakery meal. Diets were pelleted to a conditioning temperature of 86°C. When pigs weighed approximately 11.3-kg, they were weighed, and pens were randomly assigned treatments. There were 9 replications per treatment and 5 pigs per pen. There were no 3-way or 2-way interactions. For the main effect of form, there was no evidence of difference in ADG, and ADFI increased (P = 0.001) in pigs fed mash diets compared to pellets. Feed efficiency and caloric efficiency improved (P = 0.001) in pigs fed pelleted diets compared to mash diets. For the main effect of crystalline AA, there was no evidence of difference in ADG or G:F; however, pigs fed high crystalline AA had increased (P = 0.024) ADFI compared to those fed low crystalline AA diets.

For the main effect of RS inclusion, pigs fed low RS diets had increased ($P < 0.041$) ADG and ADFI compared to pigs fed high RS inclusion diets. There was an improvement ($P = 0.019$) in G:F and caloric efficiency for pigs fed high RS inclusion diets compared to those fed low RS diets. In conclusion, there was no evidence of interactions between diet types, indicating that increasing amounts of crystalline AA and RS did not increase the Maillard reaction or reduce growth performance when pelleting diets by using the reported conditions. Pigs fed pelleted diets had similar ADG and an 8% improvement in feed efficiency compared to those fed mash diets. Pigs fed the high RS diets had reduced feed intake, which resulted in reduced gain and improved feed and caloric efficiency. Additionally, pigs fed high AA diets had increased feed intake.

INTRODUCTION

Pelleting swine feed is commonly used to improve feed efficiency, feed handling characteristics, and bulk density while decreasing feed wastage (Behnke, 1994). Yet, to obtain the growth performance improvements from pelleting nursery pig feed, pellet quality must be upheld by minimizing pellet fines at the feeders to less than 20% (Nemechek et al., 2015). Recent issues of feed safety and the importance of pellet quality have led feed manufacturing companies to steam condition the swine diets at higher temperatures during the pelleting process. There is concern that over-processing can potentially lead to a reduction in amino acid availability due to the Maillard reaction. The Maillard reaction is a non-enzymatic browning reaction between an amino group from amino acids, peptides, or proteins and a carbonyl group of reducing sugars such as glucose, fructose, or lactose. Variables optimizing the Maillard browning reaction include high temperatures and moisture content, which may occur during feed

processing. Steidinger et al., (2000) observed negative effects on feeding value and growth performance of nursery pigs with mash feed conditioning temperatures above 77°C.

It is the combination of pelleting feed and current swine diet formulation trends including the combination of co-product ingredients, such as DDGS and bakery meal, with increasing inclusion of crystalline amino acids (AA) that could create ideal conditions for the Maillard browning reaction. The reducing sugars glucose, sucrose, maltose, and fructose typically have concentrations of 0.66, 1.14, 0.23, and 0.4% in corn; 5.03, 4.91, 2.85, and 4.71% in bakery meal; and 1.84%, 0.19%, 2.28%, and 0.74% in DDGS (Rojas et al. 2013). Soybeans contain glucose ranging from 0.07 to 0.40% (DM-basis), however, glucose is destroyed in the crushing of soybeans to produce soybean meal (Grieshop et al., 2003).

Additionally, increasing inclusion of crystalline AAs provides free amino acids which are more susceptible to binding in the Maillard reaction. Unlike amino acid chains that bind to form whole proteins, synthetic amino acids are in the free, unbound form, and thus have more available amino groups to bind reducing sugars. The structure of lysine makes it very susceptible to the Maillard reaction as it has two free amino groups. Thus, pelleting diets containing crystalline amino acids and co-products with reducing sugars may increase the risk for reduced amino acid availability for growth. The objective of this experiment was to test the hypothesis that pelleting swine diets containing free amino acids and reducing sugars at high temperatures will reduce nursery pig growth performance.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center (Manhattan, KS).

Animals

A total of 360 pigs (DNA 200 × 400; initially 11.3 kg) were used in an 18-d growth study. Pigs were weaned at approximately 21-d of age, weighed, and assigned to pens in a completely randomized design with 5-pigs per pen with 3 barrows and 2 gilts or 3 gilts and 2 barrows. Pigs were housed in 1.2 × 1.2-m pens containing a three-hole dry self-feeder and one cup waterer to provide *ad libitum* access to feed and water. When pigs weighed approximately 11.3-kg, each pen was randomly assigned to 1 of 8 dietary treatments from 4 formulations provided in mash or pelleted form (Table 1).

Dietary treatments

Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline amino acid level (AA; low vs. high); reducing sugars (RS; low vs. high); and diet form (mash vs. pellet; Table 1). For crystalline amino acid treatments, diets were considered low or high based on the inclusion of crystalline AA with high crystalline AA diets having increased concentrations of lysine, threonine, and tryptophan compared with low crystalline AA diets. Valine and isoleucine were included as needed in the high crystalline AA diets. Reducing sugars were naturally occurring in ingredients (corn and soybean meal-based diets; low) or increased by adding DDGS and bakery meal (20 and 15%, respectively; high).

Feed processing

Diet manufacture occurred at the North Carolina State University Feed Mill Education Unit (Table 2; Raleigh, NC). Whole grain ingredients were ground with a double-high roller mill (Model C128829, RMS, Harrisburg, South Dakota). Feed was mixed in a 126-ft^3 Hayes and Stolz counterpoise mixer for a standard time of 270 sec (Model TRDB126-0604, Hayes and Stolz, Fort Worth Texas). Treatments were pelleted using a 1-ton 30-horsepower pellet mill (Model PM1112-2, California Pellet Mill) equipped with a 4.4" × 34.9-mm die (L:D = 8.1) and pelleted to a hot pellet temperature of 86°C. To minimize the effect of pellet quality differences, pellets were passed through a sifter to remove fines before transport. Motor load was recorded every 5 sec using a data logger to determine energy consumption (kWh). A sample of cool pellets was collected and analyzed for pellet durability index using the Holmen NHP100.

A sample of cool pellets was collected, and the fines sifted off by using the corresponding sieve stack (ASAE S269.4). Sifted pellets were split using a riffle divider and 100 grams used for analysis. The 100-g sample was placed into the hopper of the Holmen NHP100 for 60 seconds. The fines were removed as the sample was run. Once completed, the sample was removed from the hopper and weighed. The PDI was calculated by dividing the final sifted sample weight by the 100-g initial sample weight.

Chemical analysis

Representative samples of corn, bakery meal, DDGS, soybean meal and treatment diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for dry matter (AOAC Official Method 934.01, 2006), crude protein (AOAC Official Method 990.03, 2006), crude fat (AOAC Official Method 920.39 (A)), crude fiber (AOAC Official Method 978.10, 2006), ash (AOAC Official Method 942.05), complete

AA profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006), available Lys (AOAC Official Method 975.44, chp. 45.4.03, 2006), and protein solubility in potassium hydroxide (KOH) (Parsons et al. 1991; Tables 2 and 3). Melanoidins were measured using approximately 50 mg of each sample which was accurately weighed and dispersed in 3 ml ethanol solution (47.5%, v/v) in a 5 mL tube. The tube was then vortexed for 30 s and further shook in a water bath for 30 min at 50 °C and 120 rpm oscillation. After that, the sample was vortexed again for 1 min and then mixed vigorously for another 30 min. Afterward, the sample was centrifuged at 10,000 × g for 10 min, and the supernatant was collected for analysis. Each sample was extracted and analyzed in duplicate. The relative amount of melanoidin in the extract was analyzed using a double beam spectrophotometer (VWR UV-6300PC) at 420 nm (Del Castillo et al. 2001; Shen et al. 2018; Tables 2 and 3).

Calculations and statistical analysis

Feeders and pens of pigs were weighed, and feed disappearance calculated on d 0 and 18 of the experiment to determine ADG, ADFI, and G:F. Feed additions were recorded for each individual pen.

Pellet mill energy consumption (kWh/MT) was calculated using average logged motor load divided by 100 times the rated 30 horsepower rating for the pellet mill. Final energy consumption was determined by kWh divided by production rate.

Data were analyzed as a completely randomized design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and blocked by gender. Fixed effects included feed form, crystalline AA, and RS inclusion; and the interaction between all three. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Results will be considered significant at $P \leq 0.05$.

RESULTS

Chemical analysis

Chemical analysis of major ingredients used in in this experiment are reported in Table 2. Analyzed nutrient values of corn, soybean meal, and bakery meal were similar to the reference values used. Dried distillers grains with solubles had a numerically decreased analyzed crude fat (4.09 vs 7.50%) and increased total lysine (0.93 vs 0.79%) compared to reference values used. In addition, melanoidins were measured on individual ingredients as evidence of Maillard reaction. All ingredients which had been previously processed (soybean meal, DDGS, and bakery meal) had increased absorbance values for melanoidins as compared to ground corn.

Chemical analysis of experimental diets indicated CP ranged for 17.0 to 24.7% with the formulated range being 18.1 to 24.3% for both mash and pelleted diets (Table 3). The high crystalline AA × low RS inclusion diets contained the lowest CP and the low crystalline AA × high RS inclusion diets contained the highest CP. Analyzed available lysine content ranged from 1.2% to 1.4% where the lowest was both the low crystalline AA × low RS inclusion and high crystalline AA × low RS inclusion pelleted diets and highest being the low crystalline AA × high RS pelleted diet. The high RS diets (containing 20% DDGS and 15% bakery meal) had increased absorbance values for melanoidins as compared to the low RS diets (corn and soybean meal based diets). However, melanoidins absorbance values were similar between mash and pelleted diets. The high RS diets (containing 20% DDGS and 15% bakery meal) had decreased KOH solubility compared to low RS diets. In addition, pelleting each type resulted in increased KOH solubility compared to the similar diet in mash form.

Feed processing

Mean particle size of the complete diets ranged from 900 to 1380 µm. The high RS diets (low crystalline AA = 1078 µm and high crystalline AA = 900 µm) had decreased diet particle size compared to the low RS diets (low crystalline AA = 1380 µm and the high crystalline AA = 1335 µm). This is a result of variation in ingredient particle size and the actual corn particle size being greater than the targeted particle size. The experiment was designed to pellet diets at a conditioning temperature of 88°C. However, average conditioning temperature was achieved at 86°C with average hot pellet temperature of 90°C (Table 4). The PDI ranged from 63 to 82% with lowest PDI being low crystalline AA × high reducing sugar diet and highest PDI was the high crystalline × low reducing sugar diet. These differences in PDI could possibly be explained by variations in added fat in diets. Differences in PDI were alleviated by sifting pellets post-pelleting to remove excessive fines before transport.

Growth performance

There were no 3-way interactions of feed form × crystalline AA × RS for any of the response criteria. There were no 2-way interactions of feed form × crystalline AA or crystalline AA × RS for any of the response criteria. There was a tendency ($P = 0.066$) for 2-way interaction of feed form × RS for G:F and caloric efficiency. Pigs fed high RS diets tended to have improved G:F and NE caloric efficiency when fed mash diets; however, pigs fed pelleted high RS and low RS pelleted diets had similar G:F and NE caloric efficiency (Table 5). Additionally, pigs fed either diet pelleted had improved G:F and NE caloric efficiency compared to those fed either mash diet.

For the main effect of crystalline AA, there was no evidence of differences ($P > 0.10$) between pigs fed either crystalline AA diet for BW, or ADG. Pigs fed high crystalline AA diets

had increased ($P = 0.024$) ADFI compared to those fed low crystalline AA diets. Pigs fed low crystalline AA inclusion tended to have improved ($P = 0.073$) G:F and NE caloric efficiency compared to those fed high crystalline AA inclusion.

For the main effect of RS inclusion, pigs fed low RS diets had increased ($P < 0.041$) ADG, ADFI, G:F, and NE caloric efficiency compared to pigs fed high RS inclusion diets.

DISCUSSION

Pelleting swine and poultry diets is commonly used to improve nutrient utilization, feed efficiency, feed handling characteristics and bulk density (Behnke, 1994). Components of the pelleting process, such as steam conditioning and feed retention time in the conditioner and die, expose feed to various degrees of heat, moisture, pressure, and shear forces which change the physical and chemical characteristics of the feed. When ingredients that are included in a diet are exposed to the steam conditioning process and extrusion through the die, the heat and moisture plasticize the soluble fractions of the diet and increase the agglomeration of dietary components (Lundblad et al., 2009). One potential negative chemical change due to pelleting is the occurrence of the Maillard reaction. This reaction is optimized at high temperatures and moisture levels where free amino acids bind to reducing sugars such as glucose, fructose, or lactose. This experiment was designed to determine if pelleting swine diets containing free amino acids and reducing sugars at high temperatures would reduce growth performance of late nursery pigs. Diets were formulated with low or high crystalline AA and low or high reducing sugars provided by co-product ingredients, DDGS, and bakery meal to increase the possibility of binding lysine via the Maillard reaction. Additionally, diets were formulated to contain equal amounts of SID

lysine, NE, Ca, and STTD P. Other amino acid: lysine ratios were balanced between treatments when possible or exceeded the recommended requirement.

As discussed in a complementary study, similar diets and analytical procedures for individual ingredients and complete diets were utilized to evaluate digestibility of AA for growing pigs (Dunmire et al., 2020). For this subsequent study evaluating growth performance, the discussion for the topic of ingredients and diet analysis is similar. During the Maillard reaction, a reactive cyclic intermediate compound and compounds containing amino groups polymerize to dark-colored, insoluble material containing nitrogen. These compounds are called melanoidin, which vary in color, molecular weight, nitrogen content and solubility (Pahm et al., 2008). Melanoidin values represent the absorbance of the extract measured using spectrophotometer at 420 nm, where higher values indicate the presence of the late Maillard reaction product, melanoidin. Ingredients that had been previously processed (i.e. soybean meal, DDGS, and bakery meal) had increased melanoidin absorbance values compared to ground corn. This suggests that the heating processing step that occurs in soybean meal, DDGS and bakery meal processing creates the Maillard reaction prior to formulation. Therefore, diets containing DDGS and bakery meal and reduced concentrations of ground corn had increased melanoidin absorbance values. Pelleting the corn and soybean meal-based diets (low RS) resulted in no numerical differences in melanoidin absorbance values between mash and pelleted diets. The melanoidin absorbance values increased in diets containing high reducing sugars; however, values did not increase in pelleted diets compared to mash. This can potentially be explained by the increased Maillard reaction products (melanoidin) absorbance values in previously processed by-products included in the diet.

The analyzed KOH solubility values for individual ingredients were lowest in DDGS and bakery meal followed by soybean meal then corn. In complete diets, KOH solubility ranged from 28.0% in the high crystalline AA × high RS inclusion mash diet to 80.5% in the high crystalline AA × low RS inclusion pelleted diet. While traditionally KOH solubility is used to analyzed protein solubility in potassium hydroxide for individual ingredients, the results for complete diets are also in agreement with results for individual ingredients. Thus, experimental diets with the greatest amount of corn had the highest KOH solubility. There has been some uncertainty around if KOH solubility is appropriate to determine heat damaged in DDGS to predict AA digestibility for DDGS (Stein et al., 2005). Such uncertainty stems from low correlation between *in vitro* digestibility of protein and the value for SID *in vivo* (Stein et al., 2005). Higher KOH solubility indicates greater nitrogen digestibility. Since there is low association of DDGS to KOH solubility analysis, and all previously heat-treated ingredients have lower KOH solubility, this may indicate lower N availability among these ingredients. When mash diets were pelleted, KOH protein solubility increased indicating greater nitrogen digestibility. Therefore, this suggests no indication of a reduction in protein quality due to the Maillard reaction based on KOH protein solubility.

Available lysine refers to undamaged lysine residues digested and absorbed, therefore potentially available for protein synthesis **(**Rutherford and Moughan, 2007). Available lysine is determined by the ε - amino group of Lys. Analytical procedures such as the fluorodinitrobenzene method used in this study measures the conversion of Lys to dinitrophenyl-Lys as a colorimetric change via HPLC. Available lysine was greatest in soybean meal (3.0%) followed by DDGS (0.87%), bakery meal (0.57%) and corn (0.26), which was expected with total AA and CP results. In experimental diets, available lysine showed little to no numerical

differences among diets with no differences between mash and pelleted diets. This suggests that free lysine was not bound in the presence of reducing sugars.

Pellet quality is highly influenced by diet formulation as well as processing parameters. Additionally, pellets will undergo rigorous transportation prior to consumption. Therefore, PDI is used as a measure of pellet quality to predict the percentage of intact pellets at the feeder. In this study, the PDI was lowest in the low crystalline AA × high RS diet and best in the high crystalline AA × low RS diet. However, since differences in PDI were expected, pellets were sifted post-pelleting to remove excessive fines before transport. Diets with the highest PDI also had the highest hot pellet temperature at the die suggesting greater frictional heat from diets being pushed through the die resulting in an increased agglomeration of dietary components. Differences in PDI and hot pellet temperature could be explained by the amount of mixer added fat in the diet to balance dietary NE. It has been well established that added fat can decrease pellet quality however, it can result in decreased pellet mill energy consumption (Dunmire et al., 2019). The high crystalline AA × low RS diet also had the least amount of mixer added fat (< 1%) where the diet with the lowest PDI had the greatest inclusion of mixer added fat (> 3%).

Numerous studies have been conducted to determine the effect of mash verses pelleted diets on pig performance. In this study, pigs fed pelleted diets had similar ADG and an 8% improvement in feed efficiency compared to those fed mash diets. A summary of 16 studies determined there is an average improvement of 6% ADG and 6 to 7% G:F improvement when pelleting diets for growing and finishing pigs (Paulk, 2011). Another primary reason that swine diets are pelleted is to reduce feed wastage (Behnke, 1994). In this study, a decrease in feed wastage was potentially demonstrated from a decrease in feed intake or feed disappearance when pigs were fed pelleted diets. Additionally, previous research observed that feeding pelleted diets

increased DM, N, and GE digestibility compared to mash diets when fed to pigs (Wondra et al., 1995). More recently, Rojas and Stein, (2017) observed pelleting a corn-soybean meal diet increased digestibility of DM, N, and GE by 5 to 8% compared with feeding the same diet in a mash form. Although, these reports demonstrated improvements in digestibility, there is still discussion around how pelleting affects different nutritional components and which nutrients are leading to the benefits in performance.

There was no evidence of interactions between diet types and diet form, indicating that increasing amounts of crystalline AA and RS did not reduce growth performance when pelleting diets by using the reported conditions. It was hypothesized that the conditioning temperature or even the frictional heat at the die would create conditions for the Maillard reaction. Increasing temperature increases the rate of the Maillard reaction (Ajandouz et al., 2008) thus conditioning temperature along with hot pellet temperature would increase the temperature of the mash. Conditions created during diet formulation are common in current diet formulation trends for swine diets having high inclusions of byproduct ingredients and resulting high reducing sugars. Additionally, use of crystalline AA has become common to decrease soybean meal as a primary cost contributor to total diet cost. Utilization of both byproduct ingredients and crystalline AA are used to decrease diet cost. However, it is this combination that provides additional or ideal conditions for binding lysine through the Maillard reaction. This study aimed to determine if the Maillard reaction occurred through the pelleting process, however, this was not determined under the reported conditions. Further, AA digestibility in growing pigs was evaluated prior to this experiment (Dunmire et al., 2020). Dunmire et al., (2020) indicated no interaction of diet form, crystalline AA, or RS inclusion for SID of AA. As expected, there was a greater digestibility and growth performance in pigs fed pelleted diets.

In this experiment, there was an increase in feed intake for pigs fed high crystalline AA diets. While the authors are unsure of the cause of this response in nursery pigs, a few potential explanations include discrepancy in soybean meal NE value, added nutritional benefits of soybean meal, and absorptive capacity of free verses intact protein. First, soybean meal net (NE) estimations from book values may be underestimating the value of soybean meal therefore providing more energy than utilized in the formulation. When increasing crystalline AA and decreasing soybean meal concentration in diet formulation, it is hypothesized that the NE value is lower than expected which could potentially lead to an increase in feed intake to meet the pig's energy requirements. Lee et al., (2021) suggests the NE value of soybean meal should be updated to 2,233 kcal/kg for formulation where the NRC NE value of soybean meal is 2,087 kcal/kg. However, in this study NE of soybean meal used in formulation was 2,351 kcal/kg or 88% the value of corn. Therefore, this could potentially explain the response observed if the NE value of soybean meal is greater than 2,351 kcal/kg. Second, soybean meal has nutritional benefit beyond a desirable AA profile. Bioactive compounds such as isoflavones and flavonoid compounds are found in soybeans that possess anti-inflammatory, antiviral, and antioxidative properties (Smith and Dilger, 2018). It has been shown that nursery and growing pigs under immune stress had improved performance when fed increasing soybean meal (Boyd et al., 2010; Rochell et al., 2015). In a study where pigs were challenged with PRRSv, growth performance increased when pigs were fed soybean meal d 0 to 104 d post inoculation which was attributed to isoflavones, flavonoid compounds from soybean meal (Smith et al., 2020). Pigs in this study were considered late nursery pigs however still fit in the window of potential improved intake indicated by Smith et al. (2020). Thirdly, free AAs are absorbed and utilized rapidly where intact proteins are less available for absorption. However, protein synthesis may be better utilized by di- and tri-peptides

which could lead to greater whole body protein deposition. Wang et al., (2018) concluded that every 10 g/kg reduction of dietary CP resulted in a 3% reduction in protein ingredient inclusion where energy sources increased almost 3%. In this study, CP ranged from 17.0 to 24.7% in high crystalline AA × low RS and low crystalline AA × high RS mash fed diets, respectively. It is further discussed by Wang et al., (2018) that pigs fed very low protein diet resulted in poor performance from a deficiency in intact protein or excess of free AA. The di- and tri-peptides from intact protein has been positively correlated with digestive enzyme activity (Shimizu, 2004). Therefore, decreased body protein deposition and poor growth performance may be attributed to rapid absorption rates of free AA from excessive oxidation of AA (Yen et al., 2004). Thus, there was a maximum CP reduction of 7.7%. Additionally, in low protein diets, supplementing branched chain AA (BCAA) can increase the feed intake and skeletal muscle growth in piglets whereby increasing growth performance (Zheng et al., 2016). However, when BCAA causes an antagonism, reduced performance can be alleviated with valine and isoleucine supplementation (Powell et al., 2011; Wang et al., 2018). Valine:lysine and isoleucine:lysine usage in these diets was 72% and 60%, respectively in both high crystalline AA diets. Diets that were not supplemented with crystalline AA had valine and isoleucine ratios to lysine of 71 and 77 and 77 and 87%, respectively. Diets with the greatest amount of corn had the least amount of leucine, isoleucine, and valine. One potential explanation is BCAA antagonism, specifically excess leucine. The BCAA antagonism could have potentially occurred in this study when corn was removed and replaced by DDGS and bakery meal, which contain greater amounts if BCAA. Wang et al., (2018) suggests that the first four limiting AA (lysine, methionine, threonine, and tryptophan) should be supplemented when the CP reduction is below 3%. Further

334 supplementation with BCAA could maintain similar growth performance up to a 6% reduction in
335 CP.

336 In this experiment, pigs fed low RS diets had improved ADG and ADFI compared to
337 pigs fed high RS inclusion diets. There was an improvement in G:F and caloric efficiency for
338 pigs fed high RS inclusion diets compared to those fed low RS diets. However, these differences
339 did not translate into differences in BW. Dietary fiber was greatest in diets containing DDGS and
340 bakery meal with low crystalline AA × high RS were 6.17 and 6.12% in mash and pelleted diets,
341 respectively. Complex carbohydrates will solubilize in the intestinal tract leading to decreased
342 rate of feed passage, reduced feed intake, and proliferation of bacteria in the gastrointestinal tract
343 (Abdollahi et al.., 2013). It has been suggested that the reduction in growth performance in pigs
344 fed high fiber co-product could be ameliorated if diets are pelleted (Fry et al., 2012). However, in
345 this study diets containing byproduct ingredients (high RS) had reduced performance regardless
346 of feed form, refuting the suggestion that pelleting can ameliorate a reduction in performance
347 due to fiber.

348 In conclusion, this experiment was designed to determine if pelleting different swine
349 diets can differentially influence growth performance. Diets were formulated with low or high
350 crystalline AA and low or high reducing sugars provided by co-product ingredients, DDGS and
351 bakery meal to increase the chances of binding lysine via the Maillard reaction. Diets were
352 pelleted to a conditioning temperature of 86.4°C and average hot pellet temperature of 90.2°C.
353 There was no evidence of interactions between diet types, indicating that increasing amounts of
354 crystalline AA and RS did not increase the Maillard reaction enough to reduce growth
355 performance when pelleting diets by using the reported conditions. Diets formulated with 20%
356 DDGS and 15% bakery (high RS) resulted in decreased growth performance and caloric

efficiency compared with the corn-soybean meal-based diets. Pigs fed pelleted diets had improved feed intake and G:F. Pigs fed pelleted diets had similar ADG and an 8% improvement in G:F compared to those fed mash diets.

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Table 6.1 Diet composition (as-fed basis)[1,2]

Crystalline AA Reducing Sugars	Low Low	Low High	High Low	High High
Ingredient, %				
Corn	61.45	30.82	70.81	42.41
Soybean meal	33.55	27.92	24.07	16.34
Dried distillers' grain with solubles	---	20.00	---	20.00
Bakery meal	---	15.00	---	15.00
Poultry fat	1.55	3.08	0.85	2.25
Calcium carbonate	0.68	0.83	0.73	0.85
Monocalcium phosphate, 21%	1.50	1.10	1.60	1.23
Sodium chloride	0.60	0.60	0.60	0.60
L-Lysine-HCl	0.13	0.19	0.43	0.55
DL-Methionine	0.10	0.05	0.14	0.10
L-Threonine	0.05	0.02	0.19	0.17
L-Tryptophan	---	---	0.05	0.07
L-Valine	---	---	0.11	0.03
L-Isoleucine	--	---	0.04	0.01
Trace mineral premix[4]	0.15	0.15	0.15	0.15
Vitamin premix[5]	0.25	0.25	0.25	0.25
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible AA, %				
Lysine	1.10	1.10	1.10	1.10
Isoleucine:lysine	71	77	60	60
Leucine:lysine	144	168	123	143
Methionine:lysine	35	35	35	35
Methionine & cysteine:lysine	61	63	58	59
Threonine:lysine	65	65	65	65
Tryptophan:lysine	21.0	21.0	21.0	21.0
Valine:lysine	77	87	72	72
Total Lys, %	1.25	1.29	1.22	1.26
ME, kcal/kg	3,333	3,369	3,311	3,342
NE NRC, kcal/kg	2,555	2,555	2,555	2,555
SID Lys:NE, g/Mcal	4.31	4.31	4.31	4.31
CP, %	21.3	24.3	18.1	20.3
Ca, %	0.73	0.73	0.73	0.73
Standardized digestible P, %	0.45	0.45	0.45	0.45

[1]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA inclusion (low vs. high), reducing sugar inclusion (low vs. high), and diet form (mash vs. pellet).
[2]Experimental diet was formulated for 11.3 to 22.7 kg BW range.
[3]Quincy Farm Products, Quincy, Illinois.

[4]Provided per kg of premix: 73g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.
[5]Provided per kg of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.
[6]To ensure the ability to detect a difference in AA utilization, these diets were formulated to 85% of the recommended SID Lys requirement (NRC, 2012) of pigs.
[7]AA = amino acids. ME = metabolizable energy. NE = net energy.

Table 6.2 Chemical analysis of individual major ingredients (as-fed basis)[1]

Item, %	Corn	Soybean meal	DDGS	Bakery meal[2]
Dry matter	87.02 (88.31)	88.00 (89.98)	86.79 (89.31)	86.22 (86.05)
Crude protein	7.83 (8.24)	48.36 (47.73)	26.11 (28.15)	16.48 (17.08)
Crude fat	3.01 (3.48)	1.50 (1.52)	4.09 (7.5)	4.26 (4.13)
Crude fiber	1.74 (1.98)	3.36 (3.89)	7.85 (2.28)	4.69 (5.39)
Ash	1.39 (1.30)	7.29 (6.27)	4.57 (4.24)	6.69 (3.22)
Melanoidin,[4] abs	0.112	0.290	0.300	0.251
KOH protein solubility	75.48	45.35	23.59	39.62
Available lysine	0.26	3.00	0.87	0.57
Total AA	7.44	47.85	25.30	14.91
Indispensable AA				
Arginine	0.38 (0.37)	3.55 (3.45)	1.78 (1.20)	0.88 (0.96)
Histidine	0.21 (0.24)	1.29 (1.28)	0.75 (0.73)	0.40 (0.43)
Isoleucine	0.28 (0.28)	2.38 (2.14)	1.12 (1.05)	0.69 (0.64)
Leucine	0.86 (0.96)	3.74 (3.62)	3.08 (3.23)	1.46 (1.18)
Lysine	0.27 (0.25)	3.10 (2.96)	0.93 (0.79)	0.63 (0.54)
Methionine	0.17 (0.18)	0.67 (0.66)	0.53 (0.57)	0.26 (0.21)
Phenylalanine	0.37 (0.39)	2.53 (2.40)	1.26 (1.38)	0.84 (0.86)
Threonine	0.27 (0.28)	1.86 (1.86)	1.04 (1.02)	0.58 (0.46)
Tryptophan	0.06 (0.06)	0.66 (0.66)	0.19 (0.22)	0.16 (0.14)
Valine	0.36 (0.38)	2.43 (2.23)	1.38 (1.39)	0.89 (0.83)
Dispensable AA				
Alanine	0.55 (0.60)	2.08 (2.06)	1.84 (1.99)	0.96 (0.92)
Aspartic acid	0.51 (0.54)	5.43 (5.41)	1.78 (1.88)	1.18 (1.22)
Cystine	0.17 (0.19)	0.74 (0.70)	0.60 (0.53)	0.25 (0.23)
Glutamic acid	1.36 (1.48)	8.87 (8.54)	3.95 (4.49)	2.45 (2.57)
Serine	0.35 (0.38)	2.09 (2.36)	1.19 (1.22)	0.64 (0.67)
Tyrosine	0.20 (0.26)	1.79 (1.59)	0.96 (1.07)	0.81 (0.95)
Glycine	0.31 (0.31)	2.02 (1.99)	1.03 (1.07)	0.72 (0.81)
Proline	0.62 (0.71)	2.37 (2.53)	2.15 (2.16)	0.88 (0.90)

[1]Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories in Columbia, MO.

[2]Quincy Farm Products, Quincy, Illinois.

[3]Values in parentheses indicate values used in diet formulation. These values were acquired from the NRC and bakery meal values analyzed prior to diet formulation. The soybean meal NE value assigned was 88% of corn.

[4]Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.

Table 6.3 Chemical analysis of experimental diets (as-fed basis)[1,2]

	Mash				Pellets			
Crystalline AA	Low	Low	High	High	Low	Low	High	High
Reducing Sugars	Low	High	Low	High	Low	High	Low	High
Item, %								
Dry matter	87.70	87.76	87.44	87.86	86.72	87.20	86.85	87.07
Crude protein	20.53	24.70	17.02	20.37	22.05	23.74	19.33	20.26
Fat	3.34	6.17	3.09	5.00	3.92	6.12	3.48	5.72
Fiber	2.70	4.09	2.14	3.43	2.25	3.83	2.04	3.77
Ash	5.46	5.45	4.05	5.47	5.39	5.61	5.06	5.10
Melanoidin, abs[3]	0.095	0.145	0.087	0.120	0.089	0.125	0.082	0.128
KOH protein solubility	65.71	54.90	76.56	28.03	76.87	72.03	80.50	48.82
Available lysine	1.21	1.34	1.31	1.26	1.20	1.35	1.20	1.28
Total AA	20.03	22.91	19.59	19.17	19.63	23.45	17.18	19.53
Indispensable AA								
Arginine	1.32	1.46	1.27	1.09	1.32	1.48	1.08	1.12
Histidine	0.53	0.63	0.51	0.51	0.52	0.64	0.44	0.51
Isoleucine	0.92	1.07	0.88	0.84	0.90	1.06	0.75	0.85
Leucine	1.71	2.15	1.67	1.85	1.68	2.18	1.47	1.87
Lysine	1.24	1.39	1.33	1.30	1.23	1.39	1.22	1.31
Methionine	0.40	0.40	0.45	0.39	0.35	0.40	0.35	0.40
Phenylalanine	1.02	1.22	0.97	0.97	1.00	1.23	0.84	0.99
Threonine	0.79	0.91	0.86	0.89	0.78	0.94	0.79	0.88
Tryptophan	0.24	0.26	0.23	0.25	0.26	0.28	0.25	0.26
Valine	0.99	1.20	1.01	1.00	0.97	1.20	0.87	0.98
Dispensable AA								
Alanine	0.99	1.25	0.98	1.10	0.98	1.28	0.87	1.12
Aspartic acid	2.04	2.20	1.91	1.67	1.99	2.24	1.61	1.70
Cystine	0.36	0.38	0.35	0.32	0.34	0.42	0.29	0.35
Glutamic acid	3.71	4.00	3.48	3.25	3.56	4.16	3.02	3.32
Serine	0.86	0.99	0.82	0.81	0.85	1.02	0.74	0.83
Tyrosine	0.69	0.83	0.66	0.70	0.68	0.86	0.60	0.74
Glycine	0.84	0.95	0.80	0.77	0.82	0.98	0.69	0.79
Proline	1.13	1.40	1.13	1.21	1.13	1.44	1.01	1.24

[1]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA inclusion (low vs. high), reducing sugar inclusion (low vs. high), and diet form (mash vs. pellet).

[2]Samples were analyzed at the University of Missouri Agricultural Experiment Station. Chemical Laboratories in Columbia, MO.

Melanoidin values are the absorbance of the extract measured using spectrophotometer at 420 nm.

Table 6.4 Particle size of completed feed and feed processing and pellet quality of pelleted diets[1,2]

Crystalline AA Item Reducing Sugars	Low Low	Low High	High Low	High High
Item				
Mash diet particle size, µm	1380	1078	1335	900
Production rate, kg/hr	907.2	907.2	861.8	997.9
Conditioning temperature, °C	86.4	86.4	86.2	86.5
Hot pellet temperature, °C	90.2	89.3	91.9	89.4
Pellet durability index (PDI), %[3]	71.9	62.5	82.2	74.4
Energy Consumption, kWh/MT	10.9	9.7	10.8	10.4

[1]Treatments were pelleted using a 1-ton 30-horsepower pellet mill (Model PM1112-2, California Pellet Mill) equipped with a 4.4" × 34.9-mm (LD 8.1) pellet die.
[2]Pellets were sifted to remove fines to ensure no effect of pellet quality on pig performance.
[3]A 100-gram pellet sample was agitated with forced air for 60 seconds in the Holmen NHP100 to determine PDI.

Table 6.5 Main effects of the pelleting process on diet formulations with varying levels of crystalline amino acids (AA) and reducing sugars (RS) on nursery pig growth.[1,2]

	Form		AA		RS			*P*-value[3]		
Item	Mash	Pellet	Low	High	Low	High	SEM	Form	AA	RS
BW, kg										
d 0	11.3	11.4	11.4	11.3	11.4	11.3	0.19	0.720	0.719	0.782
d 18	21.9	21.7	21.8	21.8	22.1	21.6	0.26	0.586	0.986	0.191
d 0 to 18										
ADG, g	574	572	568	577	587	559	9.5	0.878	0.521	0.041
ADFI, g	962	878	900	940	960	881	12.2	0.001	0.024	0.001
G:F	0.60	0.65	0.63	0.62	0.61	0.64	0.006	0.001	0.073	0.019
Caloric efficiency, NE kcal/kg of gain[4]	4,297	3,938	4,062	4,173	4,191	4,044	43.1	0.001	0.073	0.019

[1]A total of 360 pigs (DNA 200 × 400; initially 11.3 kg)) were used in an 18-d growth trial with 5-pigs/pen with 3 barrows with 2 gilts or 3 gilts with 2 barrows in a completely randomized design.

[2]Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of crystalline AA inclusion (low vs. high), reducing sugar inclusion (low vs. high), and diet form (mash vs. pellet).

[3]Probability, P< for the main effects of feed form, crystalline amino acid inclusion, or reducing sugar inclusion.

[4]Caloric efficiency = (ADFI × NE, kcal/kg (1159 kcal/kg)/ADG.