EFFECTS OF THE MAILLARD REACTIONS ON CHEMICAL COMPOSITION AND AMINO ACID DIGESTIBILITY OF FEED INGREDIENTS AND ON PIG GROWTH PERFORMANCE

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT: Six experiments were conducted to evaluate the negative effects of heat damage on the nutritional composition and AA digestibility of feedstuffs fed to pigs, and also to determine the negative effects of feeding heat-damaged soybean meal (**SBM**) or heat-damaged distillers dried grains with solubles (**DDGS**) on growth performance of weanling pigs. In experiments 1, 2, 3, and 4, the primary objective was to determine the effects of heat treatment on the standardized ileal digestibility (**SID**) of AA in DDGS, canola meal, sunflower meal (**SFM**), and cottonseed meal (**CSM**) fed to pigs. The second objective was to develop regression equations that may be used to predict the concentration of SID AA in these ingredients from their nutrient composition. In Exp. 1, the SID of Lys was quadratically reduced ($P < 0.05$) from 66.8% in the non-autoclaved DDGS to 54.9, 55.3, and 51.9% in the DDGS that was autoclaved for 10, 20, or 30 min, respectively. The concentration of SID Lys may be best predicted by an equation that includes the concentration of acid detergent insoluble N (**ADIN;** $r^2 = 0.84$). In Exp. 2, autoclaving of canola meal reduced (quadratic, $P < 0.01$) the SID of CP and all AA. The concentration (%) of SID Lys in canola meal may be predicted by regression equations using the concentration (%) of reducing sugars ($r^2 = 0.96$) as the main predictor variable. Likewise, the concentrations of SID AA for most AA may also be predicted from the nutrient composition of canola meal. In Exp. 3, the SID of Lys in SFM was reduced (linear, $P < 0.05$) from 83.2 to 63.5% in non-autoclaved SFM or SFM autoclaved for 60 min at 130°C, respectively. The concentrations of Lys and reducing sugars in SFM may be used as good predictors ($r^2 = 0.85$) to estimate the concentration of SID Lys in SFM. In Exp. 4, the SID of Lys in CSM was greater ($P < 0.05$) in non-autoclaved CSM (66.2%) than in autoclaved (60 min at 130°C) CSM (54.1%). The equation ($r^2 = 0.68$) that best predicted the concentration of SID Lys in CSM includes the concentration ADIN. Conclusions from the first 4 experiments are that the SID of AA decreases

as a result of heat damage, but these reductions may be linear or quadratic depending on the type of ingredient. It is also concluded from these experiments that chemical composition may be used to predict the concentration of SID Lys in DDGS, canola meal, SFM, and CSM, but the predictor variables vary depending on the ingredient. Experiments 5 and 6 were conducted to investigate if adjustments in diet formulations based on either total analyzed AA or standardized ileal digestible (**SID**) AA may be used to eliminate negative effects of including heat-damaged soybean meal (**SBM**) or heat-damaged distillers dried grains with solubles (**DDGS**) in diets fed to weanling pigs. In Exp. 5, 4 corn-SBM diets were formulated. Diet 1 contained non-autoclaved SBM and this diet was formulated on the basis of analyzed AA concentrations and using SID values from the AminoDat® (2006) database. Three additional diets were formulated using autoclaved SBM. Diet 2 was formulated similar to Diet 1 except that the non-autoclaved SBM was replaced by the autoclaved SBM. Diet 3 was formulated by adjusting AA inclusion in the diet on the basis of analyzed total AA concentrations in the autoclaved SBM and published SID values (AminoDat®, 2006). Diet 4 also contained autoclaved SBM, but the formulation of this diet was adjusted on the basis of analyzed AA in the autoclaved SBM and SID values that were adjusted according to the degree of heat damage in this source of SBM. The G:F was greater ($P < 0.05$) for pigs fed Diet 1 compared with pigs fed the other diets. Pigs fed Diet 4 had greater ($P < 0.05$) G:F than pigs fed Diet 2. In Exp. 6, 4 diets containing corn, SBM (8.5%), and DDGS (non-autoclaved or autoclaved; 22%) were formulated using the concepts described for Exp. 5, except that heat-damaged DDGS, was used in the diets. Pigs fed Diet 1 had greater ($P < 0.05$) G:F than pigs fed the other diets, but no differences were observed for G:F among pigs fed diets containing autoclaved DDGS. Results demonstrate that the negative effects of heat damage may be ameliorated if the reduced concentration as well as the reduced digestibility of AA in heat-

damaged SBM is corrected. Diets for weaned pigs containing up to 22% of heat-damaged DDGS reduces performance of pigs compared with diets containing DDGS that has not been heat-damaged, but correction for the reduced concentration and the reduced digestibility of AA in heat-damaged DDGS may not be of practical importance for weaned pigs.

Key words: amino acid, digestibility, heat damage, pig

ACKNOWLEDGEMENTS

It is with immense gratitude that I thank my advisor, Dr. Hans H. Stein, whose guidance, support, encouragement, and patience throughout this part of my life enabled me to grow as a scientist. It was an honor for me to have had the privilege of working with him.

I thank my committee members, Dr. Jason Emmert, Dr. George C. Fahey, Jr., Dr. James Pettigrew, and Dr. John Thomson for the many ways you shared your knowledge with me, and for all of the suggestions that greatly contributed to this research. I also extend my gratitude to Dr. John Htoo who also advised me throughout the last 3 years. Financial support from Evonik Industries also was greatly appreciated.

I express my gratitude to our research manager and friend, Kate Horsman, our former and current post-docs, and to my fellow graduate students who directly contributed to the accomplishment of this dissertation. I consider it an honor to work with such a team. I also thank all the professors, laboratory personnel, the farm crew, and feed mill personnel for being available and ready to help when I needed.

I owe my deepest gratitude to my dad, Alfeno, my mom, Linei, and my sister, Isis, for their love, care, unrestricted support and prayers throughout my life. To my lovely wife, Juliana, I cannot find words to describe the appreciation for the wonderful woman that you are. I truly recognize how important you are, and without your love and support this would not have been accomplished. THANK YOU SO MUCH for being my helper. To my little daughter, Ester: your birth served as an extra encouragement for completing this dissertation.

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LIST OF ABBREVIATIONS

a*	Redness
AA	Amino acid
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
AEE	Acid hydrolyzed ether extract
AID	Apparent ileal digestibility
Ala	Alanine
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists` Society
Arg	Arginine
Asp	Aspartate
BW	Body weight
b*	Yellowness
°C	Degrees Celsius
Ca	Calcium
CDS	Condensed distillers solubles
CM	Canola meal
CO_2	Carbon dioxide
Corp.	Corporation
CP	Crude protein

CSM	Cottonseed meal
Cu	Copper
CV	Coefficient of variation
Cys	Cysteine
d	Days
DDGS	Distillers dried grains with solubles
DM	Dry matter
DMI	Dry matter intake
et al.	And others
Exp.	Experiment
FAOX	Fructosyl amino acid oxidase
FDNB	Fluorodinitrobenzene
Fe	Iron
FN3K	Fructosamine -3-kinase
FN6K	Fructosamine-6-kinase
g	Grams
G:F	Gain to feed ratio
Glu	Glutamate
Gly	Glycine
h	Hour
H	Hydrogen
HCl	Hydrochloric acid
HD	Heat damaged

His	Histidine
HMF	Hydroxymethylfurfural
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography
I	Iodine
IA	Iowa
ICP	Inductively coupled plasma
i.e.	That is
IL	Illinois
Ile	Isoleucine
IN	Indiana
Int.	International
IU	International units
K	Potassium
kcal	Kilocalories
kg	Kilograms
L*	Lightness
Lys	Lysine
Leu	Leucine
LLC	Limited liability company
M	Mole (concentration)
m	Meter
ME	Metabolizable energy

Met	Methionine
min	Minutes
mg	Milligrams
μmol	Micromoles
Mn	Manganese
MN	Minnesota
N	Nitrogen
N	Normal (concentration)
n	Sample size
Na	Sodium
NC	North Carolina
ND	North Dakota
NDF	Neutral detergent fiber
nm	Nanometer
NRC	National Research Council
OH	Ohio
OMIU	O-Methylisourea
P	Phosphorus
P	Probability
PC	Positive control
PDI	Protein dispersibility index
Phe	Phenylalanine
Pro	Proline

PUN	Plasma urea nitrogen
R^2	Coefficient of determination
RL	Reactive Lysine
RMSE	Root mean square error
rp	Coefficient of correlation
rpm	Revolutions per minute
RS	Reducing sugars
SAS	Statistical Analysis System
SBM	Soybean meal
SD	South Dakota
SFM	Sunflower meal
Se	Selenium
SE	Standard error
Ser	Serine
SID	Standardized ileal digestibility
Trp	Tryptophan
Tyr	Tyrosine
U.S.	United States
Val	Valine
vs.	Versus
wk	Week
Zn	Zinc

CHAPTER 1

INTRODUCTION

Feed costs account for the majority of the variable costs of swine production. Protein and energy are the main nutrients in swine diets and, thus, understanding their utilization by the animal is important to successful swine production. Many of the feed ingredients used in swine diets are processed in different ways. Among these ingredients, oilseed meals such as canola meal, sunflower meal, and cottonseed meal undergo heat processing to improve their nutritional quality and also to remove solvents that are commonly used during oil extraction. As such, these oilseed meals are exposed to varying degrees of heat, which, in excess, is deleterious to protein quality. Amino acids are required for growth and performance of pigs. Lysine, which is the first limiting AA in most swine diets, is particularly affected by heat processing of feed ingredients because it reacts with reducing sugars upon heat processing and initiates the Maillard reactions. Consequently, Lys that participates in the Maillard reactions becomes unavailable for protein synthesis in vivo and, therefore, reduced growth performance is expected under such conditions. Because of the variation in feed processing and the potential negative effects caused by heat processing on protein quality, it is important to develop strategies to evaluate the nutritional quality of protein and to determine the extent to which heat processing can damage feed proteins. A review of the literature regarding the Maillard reactions and their effects on feed ingredient utilization by pigs is provided in Chapter 2. In Chapters 3 to 5, we provide data on the digestibility of AA in distillers dried grains with solubles, canola meal, sunflower meal, and cottonseed meal as affected by heat damage, and we also provide suggestions for evaluating the protein quality of these feed ingredients. Performance of weaning pigs fed diets containing heat-

damaged soybean meal or heat-damaged distillers dried grains with solubles is described in Chapter 6, and different ways to ameliorate negative effects of heat damage also are evaluated.

CHAPTER 2

MAILLARD REACTIONS AND THEIR EFFECTS ON THE NUTRITIONAL QUALITY OF FEED INGREDIENTS FOR PIGS: REVIEW OF LITERATURE

INTRODUCTION

The nutritional value of feed ingredients may be reduced during storage and processing (Friedman, 1996). This is likely a consequence of a combination of heat and humidity that leads to the Maillard reaction, which starts with the condensation between an amino group of an AA or protein and a carbonyl group of a reducing sugar. Lysine is an essential AA that has an ε-amino group that easily condenses with the carbonyl group of a reducing sugar (Nursten, 2005). When the Maillard reaction occurs, Lys availability is reduced (Pahm et al., 2008; Boucher et al., 2009). During AA analysis, however, Lys is partially recovered leading to an overestimation of the available Lys. Because of this overestimation, standard AA analysis procedures may not be adequate to determine the amount of available Lys in feed ingredients that have been heat processed. Therefore, it is believed that analysis of reactive Lys is more accurate than standard Lys analysis (Boucher et al., 2009).

There are several methodologies developed for the calculation of reactive Lys. These procedures include the guanidination procedure (Rutherfurd et al., 1997), the furosine procedure (Desrosiers et al., 1989), the fluorodinitrobenzene (**FDNB**) difference method (Rao et al., 1963), and the sodium borohydride method (Hurrell and Carpenter, 1974). Despite their advantages and disadvantages, the 2 most commonly used procedures are the FDNB and homoarginine methods (Rutherfurd and Gilani, 2009). Because of the complexity of the Maillard reaction as well as the variety of methods used to determine reactive Lys in heat-damaged feed ingredients, the objectives of this literature review are to provide information describing the Maillard reaction,

factors that affect the rate of the reaction, and metabolism of Maillard reaction products *in vivo.* Objectives also include describing methodologies for estimation of reactive Lys in heat-damaged feed ingredients or diets.

LYSINE NOMENCLATURE AND FATE *IN VIVO*

The term, total Lys, refers to the concentration of reactive Lys plus the concentration of blocked Lys (Figure 2.1; Rutherfurd, 2010). Reactive Lys refers to the Lys that has not undergone Maillard reactions and the ε-amino group is not bound to other molecules. Blocked Lys is the Lys that was bound to Amadori products as a result of Maillard reactions. Acid hydrolysis of proteins during standard AA analysis, however, releases some of the blocked Lys from the Amadori product, which then is called regenerated Lys. This regenerated Lys appears in the same peak as reactive Lys in the chromatogram and, therefore, is included in the peak for total Lys. Therefore, in feed ingredients that have been heat processed, the concentration of Lys determined by standard AA analysis overestimates the concentration of reactive Lys that is available for protein synthesis by the pig, leading to formulation of diets that do not necessarily meet the Lys requirement of pigs, and possibly reducing growth or reproductive performance of pigs.

Reactive Lys has 2 fates *in vivo*: it may be absorbed and utilized by the animal, or it may pass through the gastrointestinal tract and be excreted in feces (Rutherfurd, 2010). This is in agreement with data reported by Pahm et al. (2009) who determined that the standardized ileal digestibility of reactive Lys in 12 sources of corn distillers dried grains with solubles (**DDGS**) was 67% on average. It was suggested that the reason for the relatively low digestibility of Lys may be that corn DDGS has a relatively high neutral detergent fiber (**NDF**) concentration, which

contributes to increased endogenous losses of Lys. Another reason may be that severe heat damage can lead to cross-linking of proteins, which is believed to impair digestion of Lys.

MAILLARD REACTION

The Maillard reaction is a series of reactions that starts with the condensation of an amino group of an AA with a carbonyl group of a reducing sugar (Figure 2.2; Mauron, 1981; Gerrard, 2002). This reaction was first described by Louis Maillard in 1912 when he observed the formation of brown pigments during heating of glucose and Lys. Because of the complexity of reactions, the series of reactions is normally divided into 3 main stages: initial, intermediate, and late stages of the Maillard reaction (Nursten, 2005).

Initial Stage

The initial stage of the Maillard reaction is characterized by formation of glycosylamine, which is later converted to Amadori compounds (de Kok and Rosing, 1994) in a series of reactions (Gerrard, 2002). The reaction starts with the condensation of an amino group of an AA or peptide with the carbonyl group of a sugar. The terminal amino group of all AA is susceptible to this reaction, as is the epsilon amino group of Lys. If AA are present in proteins, however, the terminal AA groups are used to form peptide bonds and they are, therefore, not available for condensation. The epsilon amino group of Lys, however, may condense with reducing sugars, and Lys is, therefore, often the AA that is most affected by Maillard reactions. Schiff bases are formed after dehydration of the condensation products. These Schiff bases may undergo sequential rearrangements (Amadori rearrangements) yielding the cyclic glycosylamine (Gerrard, 2002). After a protonation of the ring oxygen atom, glycosylamine is converted to Amadori compounds. All reactions up to the formation of Amadori compounds are reversible

depending on the conditions (pH, temperature, and rate of mutarotation) in which the reactions occur (Yaylayan and Huyghues-Despointes, 1994).

Intermediate Stage

The intermediate stage of the Maillard reaction includes 3 main reactions: sugar dehydration, sugar fragmentation, and AA degradation (Nursten, 2005). During sugar dehydration reactions, furfurals (under acid conditions) and reductones (under neutral or alkaline conditions) are the end-products. Dehydration of xylose yields furfural whereas dehydration of glucose yields hydroxymethylfurfural (**HMF**). During these reactions, 3 molecules of water are lost. In contrast, in the sugar dehydration reactions to form reductones, only 2 molecules of water are lost (Nursten, 2005).

Depending on the Amadori compound, sugar fragmentation can form various end products. There are 2 main mechanisms by which sugar fragmentation may occur: retroaldolisation and oxidative fission (Nursten, 2005). Some of the products from sugar fragmentation include glycolaldehyde, acetol, ethanol, pyruvic acid, lactic acid, formic acid, and formaldehyde among others (Nursten, 2005). Amino acid degradation or Strecker degradation occurs when α-AA are oxidized to form aldehydes, and these reactions involve the transfer of ammonia to other components in the system as well as liberation of CO_2 (Nursten, 2005).

Final Stage

The final stage of the Maillard reaction involves aldol condensations and an aldehydes-amine condensation reaction leading to the formation of polymeric compounds called melanoidins (Nursten, 2005). The aldol condensation reactions are initiated by the formation of aldehydes from the products formed in the intermediate stage of the Maillard reaction, with amines and carbonyl compounds (probably from lipid oxidation) serving as catalysts. Melanoidins that are

formed during this final stage contain 3 to 4% N and their composition may vary depending on the substrates by which they were formed. The nature of melanoidins is very complex and work has been conducted to isolate and purify melanoidins from foods (Silván et al., 2006). Thus, Lindenmeier et al. (2004) were able to identify one melanoidin structure that is formed from the reaction between the Lys side chains and acetylformoin. This melanoidin, called pronyl-L-Lys, was isolated from crust and crumbs of bread.

KINETICS OF THE MAILLARD REACTION

Some of the factors affecting the rate of Maillard reactions products formation are temperature, pH, type of substrate, and water activity. Each of these factors may affect the kinetics of the reactions in specific ways.

Temperature

The Maillard reaction can be initiated at temperatures similar to that of the human body. In fact, these reactions happen in vivo at 36˚C (Ledl and Schleicher, 1990). A fraction of hemoglobin, HbA_{1c}, which is normally present in high concentrations in diabetic patients, has a hexose bound at the N-terminal Val of the β-chain in the form of 1-amino-1-deoxyfructose. The formation of HbA_{1c} is a result of the Maillard reaction because in vitro incubation of another fraction of hemoglobin (HbA_0) with glucose yields HbA_{1c} through the addition of glucose to the N-terminal amino group of hemoglobin, which gives rise to the Schiff`s base and further Amadori compounds (Ledl and Schleicher, 1990).

Processing of foods and feed ingredients at high temperatures also may lead to the Maillard reaction. During production of DDGS, corn undergoes several steps under different temperatures (32 to 100˚C) that may lead to the formation of Maillard reaction products (Pahm et

al., 2008). The study of Maillard reaction products originating from food processing, however, is very complex due to the variety of conditions used in food processing and the many different products that are formed under each of these conditions (Argirova et al., 2010).

pH

Results of many experiments have indicated that increasing pH favors the Maillard reaction. The color formation in a Lys-glucose system was increased with a pH increase from 4.0 to 8.0 (Lee et al., 1984), and this conclusion was confirmed by Leahy and Reineccius (1989a) who observed that formation of pyrazines, which are heterocyclic compounds formed from Maillard reactions, was 500 times greater at pH 9.0 than at pH 5.0. The Maillard reaction itself also affects the pH (Delgado-Andrade et al., 2004). When heating glucose-Lys and glucose-methionine model systems, the pH decreased as heating time increased, which likely was a result of basic amino group disappearance at the early stages of the reaction. After formation of Amadori compounds, pH also plays an important role because it determines the pathway of fragmentation (intermediate stage) in which a low pH favors 1,2 enolisation leading to the formation of formaldehyde, glycolaldehyde, and glyceraldehyde. In contrast, a high pH favors 2,3 enolisation leading to the formation of other end-products that may include butanedione, isomaltol, and acetic acid (Nursten, 2005).

Type of Substrate

Alkylpyrazines, which are heterocyclic, N-containing compounds (Leahy and Reineccius, 1989b) may be among the end-products of the Maillard reactions (Hodge, 1953). In an attempt to investigate effects of both the type of AA and the type of sugar on the formation of pyrazines, Leahy and Reineccius (1989b) developed model systems in which 2 AA (Asp and Lys) were tested in combination with 3 sugars (glucose, fructose, and ribose). The formation of pyrazines

was evaluated after heating the solutions (pH = 9.0) at 95˚C for 2 h. Results of this study indicated that Lys systems yielded more pyrazines than Asp systems, especially when reacted with glucose. When comparing the effects of type of sugar, glucose yielded more pyrazines than fructose and ribose, regardless of the type of AA used in the system. In another study, Shibamoto and Bernhard (1977) observed that pentoses yielded more pyrazines than hexoses. These observations indicate that both the type of AA and the type of reducing sugar in a particular feed ingredient may have a direct effect on the rate of the Maillard reactions product formation and on the fate of the AA involved in it.

Water Activity and Relative Humidity

Water activity is the ratio of the water vapor pressure over a product to the water vapor pressure over pure water and can be converted to relative humidity if multiplied by 100 (Hahn-Hägerdal, 1986). The rate of Maillard reaction product formation is increased as water activity is decreased (van Boekel, 2001). This is likely due to the fact that as water activity decreases, the reactants become more concentrated. At a certain water activity, however, the increase in the reactant concentration prevents them from easily diffusing, which leads to a decrease in the rate of Maillard reactions. A maximum rate of reaction has been observed at water activities between 0.3 and 0.7, and as the relative humidity increases, the rate of reaction decreases (Eichner and Karel, 1972). The maximum rate of reaction occurs between 60 and 80% relative humidity, but the optimal relative humidity for the Maillard reaction depends on the food or model systems used (Acevedo et al., 2006).

METABOLISM OF MAILLARD REACTION PRODUCTS

A review of the metabolic transit of Maillard reaction products has been published (Faist and Erbersdobler, 2001). According to this review, Amadori products and advanced glycation end-products resulting from the Maillard reaction may have 3 fates *in vivo*: 1) absorption upon protein release by digestive enzymes or gut microbiota, 2) metabolism by bacteria in the gastrointestinal tract, and 3) excretion via feces and urine.

Schiff`s Bases

The utilization of Schiff`s bases by rodents is similar to that of free Lys (Finot and Magnenat, 1981), and when rats were fed ε-N-salicylidene-L-lysine and ε-N-benzylidine-L-lysine, the growth responses were similar to those obtained by rats fed free Lys. The reason for this observation is most likely that the Schiff`s bases are formed by reactions that are reversible under acidic conditions. Therefore, when feed containing Schiff`s bases reaches the stomach, Lys may be regenerated because of the acidic conditions in the stomach.

Amadori Compounds

Amadori compounds may be either excreted in feces or absorbed and excreted in urine (Erbersdobler et al., 1981). Absorption of ε-fructose-lysine occurs by passive diffusion and fecal excretion of ε-fructose-lysine has been shown in a narrow range between 1 and 3% of ingested protein bound ε-fructose-lysine (Faist and Erbersdobler, 2001). The excretion of ε-fructose-lysine, however, has shown some variability. Rats that were fed protein bound ε-fructose-lysine excreted 60% of total intake in the urine (Finot and Magnenat, 1981). In humans adults, only 3% of ingested protein ε-fructose-lysine was excreted via urine (Faist and Erbersdobler, 2001), whereas in human infants, the excretion of protein bound ε-fructose-lysine via urine corresponded to 16% of total intake (Niederweiser et al., 1975).

Amadori compounds may be degraded by bacteria (e.g., *Pseudomonas spp*. in soil; Gerhardinger et al., 1995), and incubation of protein-bound fructose-lysine with rat intestinal microorganisms for 48 h resulted in approximately 80% degradation (Erbersdobler et al., 1970). Amadori compounds cannot be utilized for protein synthesis in the body because formation of Amadori compounds is an irreversible process and Lys cannot be regenerated from Amadori compounds. The majority of the absorbed Amadori compounds are, therefore, excreted in urine.

Amadori compounds also may accumulate in different tissues of the body. Fructose-lysine accumulation in tissues was demonstrated by Finot and Magnenat (1981) who observed that the majority of accumulation occurs in the kidneys, although fructose-lysine may also accumulate in other tissues such as liver and pancreas.

Melanoidins

Melanoidins are partially digested and absorbed by the intestines (Faist and Erbersdobler, 2001; Tuohy et al., 2006). The absorbed melanoidins may be retained in the kidneys (Faist and Erbersdobler, 2001). Low molecular weight non-absorbed melanoidins appear to be degraded in the intestines while the high molecular weight non-absorbed melanoidins apparently are not degraded in significant amounts (O`Brien and Morrissey, 1989). Rats that were fed melanoidins formed by the reaction of Gly with ^{14}C-glucose excreted on average 0.96 and 92.6% of ingested radioactivity in the urine and feces, respectively, whereas 1.6% was retained in the carcass and another 1.5% was expired as $^{14}CO_2$ (Finot, 2005). These melanoidins were excreted unmodified in feces, which indicates that melanoidins are not metabolized by the microbes in the gastrointestinal tract. Valle-Riestra and Barnes (1970) also observed that melanoidins are partially absorbed in the small intestine of rats, but the majority (74%) is excreted in feces and only 3% is excreted in urine. Thus, in feed ingredients that have undergone advanced Maillard

reactions, Lys cannot be regenerated in the gastrointestinal tract and, therefore, cannot be utilized for protein synthesis.

AMADORI COMPOUND DEGRADING ENZYMES

Fructosyl amino acid oxidases (**FAOXs**) are enzymes present in fungi and bacteria that have the capacity to cleave the ketoamine bond in the Amadori compounds yielding the corresponding AA, glucosone, and H_2O_2 (Deppe et al., 2010; Lin and Zheng, 2011). Although these enzymes are present in fungi (i.e., *Achaetomiella*, *Achaetomium*, *Apergillus*, and *Fusarium*), bacteria (i.e., *Arthrobacte*r and *Pseudomonas*), and in yeast (i.e., *Debaryomyces* and *Pichia*), no evidence of the presence of FAOXs in mammalian organisms has been reported (Lin and Zheng, 2011). However, fructosamine 3-kinase (**FN3K**), which is another class of Amadori compound degrading enzymes, is present in mammals (Deppe et al., 2010). When fructosamine is the starting Amadori compound, FN3K phosphorylates the C3 of fructosamine, which yields fructosamine 3-phosphate. This compound then goes through an autocatalytic degradation that yields 3-desoxyglucosone, the original amino compound (i.e., amino compound that initially reacted with glucose), and inorganic phosphate (Deppe et al., 2010). A third class of Amadori compound degrading enzymes is called fructosamine 6-kinases (**FN6K**). These enzymes phosphorylate the C6 of fructosamine to form fructosamine 6-phosphate, which is degraded by the enzyme, deglycase, to form glucose 6-phosphate and a free amino compound (Deppe et al., 2010). The practical use of FAOXs has been limited because these enzymes only react with small glycated substrates (e.g., fructosyl amino acids or dipeptides), but an experiment conducted by Zheng et al., (2010) indicates that manipulation of FAOXs may be possible so that larger substrates also may be degraded by the enzyme. Nevertheless, commercial use of FAOXs in the

food industry to reduce the concentration of Amadori compounds in food preparations is not common.

PROTEIN DISPERSIBILITY INDEX

The degree of heat to which feed ingredients are exposed is correlated with the protein dispersibility index (**PDI**), which is the percent of total protein that disperses in water and has been primarily used as an indicator of minimum adequate heat processing of soy products (Reinitz, 1984; Marsman et al., 1995; Batal et al., 2000; Iwe et al., 2001; Palić et al., 2012). Soy products are routinely toasted to decrease the concentration of heat labile antinutritional factors, and the greater the PDI value, the less the degree of destruction of antinutritional factors (Reinitz, 1984). Conversely, a low PDI value (e.g., 20%, for soy flour) indicates a more complete destruction of antinutritional factors. Increasing time of autoclaving of soyflakes from 0 to 36 min (121°C) resulted in a linear decrease in PDI values and a concomitant increase in G:F of chicks, thus indicating that PDI can be used as an indicator of minimum adequate heat processing of soyflakes (Batal et al., 2000). For SBM, PDI values ranging from 15 to 30% indicate adequately heat processed SBM. Thus, it is expected that a PDI value for SBM of less than 15% may indicate excessive heat processing and, consequently, low protein quality. A study conducted to determine the degree of heat treatment in 5 sources of full-fat soybeans analyzed for PDI in 6 different laboratories, however, concluded that there was considerable variation in this analysis, thus suggesting a low precision of this method (Palić et al., 2012).

CHEMICAL EVALUATION OF REACTIVE LYS IN HEAT DAMAGED PROTEINS

Most of the chemical methods used to determine the amount of reactive Lys in heat-damaged proteins are based on specific reactions with the ε-amino group of Lys. Among these methods are the guanidination method, the furosine method, the difluorodinitrobenzene method, and the sodium borohydride method, which are the most common methods used to determine the concentration of reactive Lys in heat-damaged proteins (Moughan, 2003).

Guanidination Method

The guanidination procedure, in which feed or food proteins are guanidinated with O-methylisourea (**OMIU**), has been used for determination of reactive Lys (Figure 2.3; Fontaine et al., 2007; Pahm et al., 2008; Boucher et al., 2009). Guanidination results in a reaction between the ε-amino group of Lys and OMIU, which yields homoarginine (Rutherfurd and Moughan, 2007). In this reaction, only Lys that has not undergone Maillard reactions and, thus, has a free amino group will react with OMIU to form homoarginine (Pahm et al., 2010). Because homoarginine is acid stable, proteins can be hydrolyzed with HCl and the liberated homoarginine (using ion-exchange HPLC) is mathematically converted to Lys based on the molecular weight of homoarginine and Lys. Therefore, this Lys represents the reactive Lys. To ensure accuracy of this method, conversion of Lys to homoarginine needs to be complete. To achieve complete conversion of Lys to homoarginine, the guanidination reagent has to be adequately prepared and the incubation conditions need to be optimized. Optimum conditions may be achieved by varying pH and reaction time. Using an OMIU solution of 0.6 *M*, the transformation of Lys to homoarginine in soy products was shown to be most effective at a pH of 11.5 and reaction time of 2 d, but for DDGS, the optimum conditions are at a pH of 12.0 and a reaction time of 2.5 d (Fontaine et al., 2007; Pahm et al., 2010). Nyachoti et al. (2002) concluded that for canola meal

and barley, conversion of Lys to homoarginine via guanidination is best achieved using a OMIU solution of 0.5 *M* and a 6 d reaction time. The guanidination method may be used to estimate Lys damage in damaged proteins (Rutherfurd and Moughan, 2007). In damaged proteins containing early Maillard reaction products that may be partly converted back to Lys (under acid hydrolysis), total Lys overestimates reactive Lys, but because guanidination occurs prior to acid hydrolysis, the reactive Lys is converted to homoarginine before its exposition to acid. The reactive Lys is, therefore, represented by the amount of homoarginine in the sample (Pahm et al., 2008; 2010).

The guanidination procedure has been used to determine the concentrations of reactive Lys in blood meal, wheat, meat and bone meal, SBM, and cottonseed meal (Rutherfurd et al., 1997). The concentrations (mg/g) of reactive Lys in blood meal (88.0), wheat (3.1), meat and bone meal (34.6), and SBM (32.3) were somewhat similar to the concentrations of total Lys (89.1, 3.5, 36.5, and 32.3, respectively). Thus, it was suggested that such similarity may be a result of severe heat damage of the ingredients evaluated, which may cause structurally altered Lys to become acid stable. Consequently, regeneration of Lys upon acid hydrolysis may be impaired. These results indicate that the guanidination procedure may not accurately predict the concentration of reactive Lys in over-processed feed ingredients.

The concentration of reactive Lys determined by the guanidination procedure also has been reported in soy products (Fontaine et al., 2007). Soy products were autoclaved at 135°C in 3 min intervals from 0 to 30 min. Results indicated that the concentration of reactive Lys in SBM (47% CP), SBM (43% CP), and in full fat soybeans was less than the total concentration of Lys. It also was observed that increasing time of autoclaving linearly decreased the concentration of both the total Lys and the reactive Lys, but the decrease in the concentration of reactive Lys in

the 3 soy products was more accentuated, which indicates that the guanidination procedure to determine the concentration of reactive Lys is, indeed, more sensitive than standard AA analysis.

The main advantage of the guanidination method is that it provides results that are close to results given by in vivo tests. However, this is a time consuming method, which may take from 2 to 4 d for completion. There is also some variability in the absolute values that are obtained using this method (Meade et al., 2005).

Furosine Method

The furosine method may be used to determine the extent of early Maillard reactions (Figure 2.4; Krause et al., 2003). Formation of furosine is observed when Amadori compounds are acid hydrolyzed. Hydrolysis of Amadori compounds yields furosine, regenerated Lys, and pyridosine. Under standard AA analysis conditions (6 *M* HCl), the yield of furosine is assumed to be constant (32%; Pahm et al., 2008) although published data show a yield range from 20 to 42% (Krause et al., 2003; Nursten, 2005). Some of this variation may be due to the type of Amadori compounds present in the sample (Krause et al., 2003). The reaction of Lys with glucose forms ε-N-deoxyfructosyllysine, which under 6 *M* HCl for 24 h, yields 50% Lys, 20% furosine, and 10% pyridosine (Nursten, 2005). Hydrolysis of N-1-deoxy-D-tagatosylhippuryllysine yields 42% furosine (Krause et al., 2003). It may, therefore, be advantageous to determine the types of Amadori compounds present in a specific feed ingredient before using an assumed value for the yields of furosine to calculate the amount of reactive Lys.

If the concentration of furosine is determined, it is possible to calculate the concentration of unreactive Lys (Pahm et al., 2008). The concentration of reactive Lys, therefore, can be calculated by the difference between total Lys and unreactive Lys, in which total Lys corresponds to the sum of concentration of reactive and unreactive Lys.

The furosine procedure has been used to determine the concentration of reactive Lys in various feed ingredients. An increase in the concentration of furosine in combination with a decrease in the concentration of reactive Lys was observed for whey protein that was heat treated at temperatures that ranged from 75 to 121°C for 3 different time periods (Desrosiers et al., 1989). The concentration of unreactive Lys (0.28 g/100 g CP) in light colored wheat DDGS, however, was slightly greater than the concentration of unreactive Lys (0.24 g/100 g CP) in dark colored wheat DDGS (Cozannet et al., 2010). This is an interesting observation as one should expect a greater concentration of unreactive Lys in the dark colored wheat DDGS, considering that color may serve as an indicator of the degree of heat damage and Maillard reactions in a particular feed ingredient (González-Vega et al., 2011). Furthermore, Cozannet et al. (2011) observed that the sum of reactive Lys and unreactive Lys does not add to the total Lys in wheat DDGS. According to Cozannet et al. (2011), this observation indicates that determination of unreactive Lys by the furosine procedure may underestimate the real concentration of unreactive Lys and, therefore, overestimates the concentration of reactive Lys. However, these conclusions were based on the untested assumption that hydrolysis of unreactive Lys yields 32% furosine. As mentioned, it is possible that this value varies among feed ingredients and research to determine the furosine yield from unreactive Lys in different feed ingredients is, therefore, needed.

The advantage of the furosine procedure is that it may be used to quantify Lys in the initial stage of Maillard reactions, but it is assumed that intermediate and late Maillard reaction products cannot be degraded to form Lys (Meade et al., 2005).

Fluorodinitrobenzene Method

The procedures by which reactive Lys is measured using fluorodinitrobenzene **(FDNB)** can be divided into 2 methods: direct and difference methods (Hurrell and Carpenter, 1974). The

direct method consists of the conversion of reactive Lys to dinitrophenyl-Lys by reacting a feed ingredient with FDNB (Rutherfurd and Gilani, 2009). Because dinitrophenyl-Lys is a colored compound, it can be measured either by spectrophotometry or by reverse-phase HPLC (Pahm, 2008; Rutherfurd and Gilani, 2009). One disadvantage of this method is that during acid hydrolysis, FDNB may react with carbohydrates in the sample and this may lead to color deterioration, which may require use of correction factors. Hurrell and Carpenter (1974) used methoxycarbonyl chloride to correct for the loss of dinitrophenyl-Lys during acid hydrolysis. If the difference method is used (Figure 2.5; Roach et al., 1967), FDNB also is used to react with the reactive Lys. After acid hydrolysis, regenerated Lys, which does not react with FDNB, is estimated. Thus, reactive Lys is calculated by the difference between total Lys and regenerated Lys. Despite its disadvantages, the FDNB method has been used recently to evaluate the effects of rendering on protein quality of animal by-products (Pérez-Calvo et al., 2010). Rendered products from 2 processing plants were evaluated, and it was reported that the concentration of reactive Lys in a rendered product from one plant (processed at 150°C for 45 min) was 3.75% of CP, whereas the concentration of reactive Lys in a rendered product from another plant (processed at 140 °C for 167 min) was 4.20% of CP. The concentration of reactive Lys in the rendered products, regardless of the processing plant in which they were produced, was less than the concentration of total Lys. The FDNB procedure also was used to determine the concentration of reactive Lys in cake mix, and it was observed that the concentration of reactive Lys was reduced by 63.5% as a result of baking and toasting of the cake mix (Hurrel and Carpenter, 1977). This result agrees with the results observed by Pérez-Calvo et al. (2010), which indicates that the FDNB method is a sensitive indicator of heat damage in protein ingredients. Cereal products also have been evaluated by the FDNB method, and results from

this experiment revealed that the concentration of total Lys in 20 breakfast cereal products may have overestimated the concentration of reactive Lys (Torbatinejad et al., 2005). It was also observed that reactive Lys determined by both the FDNB method and the guanidination method had a high degree of correlation (0.99), which indicates that both methods may be used to determine the concentrations of reactive Lys in breakfast cereals.

The disadvantage of the direct and difference methods include the long acid hydrolysis step, and an additional disadvantage of the direct method is a slight overestimation of blocked Lys. It is also possible that samples that contain high concentrations of polysaccharides may yield results that are inaccurate (Meade et al., 2005).

Sodium Borohydride Method

When Maillard products are treated with sodium borohydride they become acid stable (Figure 2.6; Hurrell and Carpenter, 1974) because a covalent bond is formed between sodium borohydride and deoxyketosyl-Lys. As a consequence, Lys is not regenerated during acid hydrolysis and, therefore, the total Lys that is measured after AA analysis corresponds to the Lys that did not react with a reducing sugar. Thus, this method allows for the direct measurement of reactive Lys. This method has been used to determine the reactive Lys in SBM fed to growing pigs (Pahm, 2008). The concentration of reactive Lys determined by the sodium borohydride procedure in SBM was 1.14%, and this value was similar to the values for the concentration of reactive Lys determined by the guanidination and furosine methods (1.19 and 1.06%, respectively). The concentration of reactive Lys determined by the sodium borohydride procedure in a mixture of albumin and glucose was reduced as a result of heat damage and, as expected, the values for the concentration of reactive Lys in these mixtures was less than the values for the concentration of total Lys in each respective mixture (Hurrell and Carpenter,

1974). This observation confirms the sensitivity of the sodium borohydride procedure to evaluate heat-damaged proteins. Couch and Thomas (1976) compared the use of the sodium borohydride procedure to determine the concentration of reactive Lys in various proteins with the FDNB method. No differences in the concentration of reactive Lys in bovine serum albumin were observed between the 2 methods. Likewise, the concentration of reactive Lys in glandless cottonseed meal determined by the sodium borohydride procedure was not different from the concentration of reactive Lys determined by the FDNB method (Couch and Thomas, 1976). These observations indicate that there is a good agreement between the 2 methods for determination of reactive Lys; however, the sodium borohydride procedure has the advantage that it is less time consuming than the FDNB method (Couch and Thomas, 1976).

The advantage of the sodium borohydride method is that reactive Lys can be determined directly in heat-damaged proteins. The main disadvantage is that the reaction with sodium borohydride also may reduce the Schiff`s base of Lys, which may be biologically available to animals (Meade et al., 2005). However, more research is needed to determine if this results in significant inaccuracies in the estimates of reactive Lys.

PRACTICAL CONSEQUENCES OF HEAT DAMAGE

The concentration and digestibility of AA in feed ingredients and diets may be reduced due to heat treatment of feed ingredients (Martinez-Amezcua et al., 2007; Boucher et al., 2009). Distillers dried grains with solubles that were oven-dried at 50, 75, or 100˚C had reduced concentrations of reactive Lys (Pahm et al., 2008). When autoclaving DDGS for 45 min at 120˚C, the digestibility of AA was reduced, especially that of Lys (Martinez-Amezcua et al., 2007), and it was suggested that the reduction in the digestibility of AA other than Lys was a

result of the formation of Maillard reaction products that interfered with the absorption of other AA. Heat treatment of whey protein in the presence of lactose at temperatures that ranged from 75 to 121˚C also resulted in a decrease in availability of Lys from 75 to 45% (Desrosiers et al., 1989). When feeding broiler chicks a diet containing good quality soybean meal (**SBM**) or heat-damaged SBM, it was observed that chicks fed the heat-damaged SBM had a decrease in final BW, ADG, ADFI, and carcass weight compared with chicks fed the good quality SBM (Redshaw, 2010). These negative effects of heat damage on performance, however, were partially mitigated by adding crystalline AA to the diets. González-Vega et al. (2011) reported that the standardized ileal digestibility (**SID**) of Lys by pigs was reduced from 93% (non-heated SBM) to 89.3 and 84.2% when SBM was autoclaved for 15 and 30 min, respectively, at a temperature of 125˚C. In another experiment, Cozannet et al. (2010) observed that the SID of Lys in wheat DDGS was highly variable and that the samples with the lowest values for SID were darker and contained less Lys expressed as a percentage of CP than the samples with the greatest values for SID of Lys, thus indicating that color and the Lys:CP ratio may be used as indicators of heat damage in wheat DDGS. As observed by Stein and Shurson (2009) and confirmed by Cozannet et al. (2010), when feed ingredients are heat-damaged, the concentration of Lys is reduced whereas the concentration of CP remains relatively constant. Therefore, the concentration of SID Lys in wheat DDGS fed to pigs may accurately be predicted ($R^2 = 0.86$) from the Lys:CP ratio (Cozannet et al., 2010). Kim et al. (2012) determined the SID of CP and AA in 21 sources of corn DDGS and observed a positive correlation between the SID of Lys and the Lys:CP ratio, which further confirms the above theory. The effects of processing conditions of fish meal on protein digestibility by mink also were evaluated (Opstvedt et al., 2003). It was observed that protein digestibility was less in fish meal sources produced at higher temperatures

(> 100°C) than in fish meal sources produced at lower temperatures (< 100°C). Cysteine and Arg also have been shown to participate in the Maillard reactions (Ledl and Schleicher, 1990). Heat processing may cause oxidation of unsaturated lipids leading to formation of hydroperoxides (Meade et al., 2005). Hydroperoxidases may oxidize Cys, thus limiting its utilization by the animal. In feed ingredients that have been heat-damaged to a higher degree, pre-melanoidins also may react with Cys and Arg (Finot et al., 1990). Cysteine also may go through Strecker degradation reactions producing hydrogen sulfide, ammonia, and acetaldehyde (Mottram and Mottram, 2002). The products of these reactions serve as intermediates in the formation of aromatic compounds, such as thiazoles and disulfides, which are associated with the Maillard reactions (Mottram and Mottram, 2002). The participation of Arg in the Maillard reactions resulting from heat processing is associated with formation of cross-links with Lys through imidazopyridinium bridges (Ledl and Schleicher, 1990).

Heat damage also may cause losses in vitamins as observed by Ford et al. (1983). Results from their research clearly indicated that storage of whole milk powder at 60 and 70°C results in a reduction in the concentrations of vitamins B_6 and thiamine. At 60°C, however, the reduction is much less pronounced than at 70°C. These observations may be because, at higher temperatures, the Maillard reactions are favored, which was confirmed by an increase in lactulosyl-lysine (which is an intermediate of the Maillard reactions) as the concentrations of vitamins decreased (Ford et al., 1983).

There is, therefore, ample evidence that heat damage to feed ingredients may reduce the nutritional value of feed ingredients, specifically the concentration and digestibility of most AA and CP. Because many feed ingredients are heated during manufacturing or preparation, it is

necessary to evaluate the nutritional quality of these feed ingredients in a rapid and reliable manner to accurately use them in feeding programs.

CONCLUSIONS

Processing of feed ingredients involving heat often will result in Maillard reactions involving the condensation between the amino group of Lys or other AA and the carbonyl group of reducing sugars. Consequently, Lys becomes unavailable to pigs, thus reducing the digestibility of this AA. The Maillard reactions are a series of complex reactions that remain to be fully understood, although much is known about the initial and intermediate stages. Some enzymes present in fungi and bacteria hydrolyze Amadori reaction products, but little information is available regarding the practical use of these enzymes in feedstuffs. The majority of the early work conducted to determine the concentration of reactive Lys in feed ingredients has used the guanidination or FDNB methods, whereas a relatively large amount of research has been conducted using the furosine procedure, and the latter procedure has been suggested to determine the concentration of reactive Lys in commonly fed feed ingredients to pigs.

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FIGURES

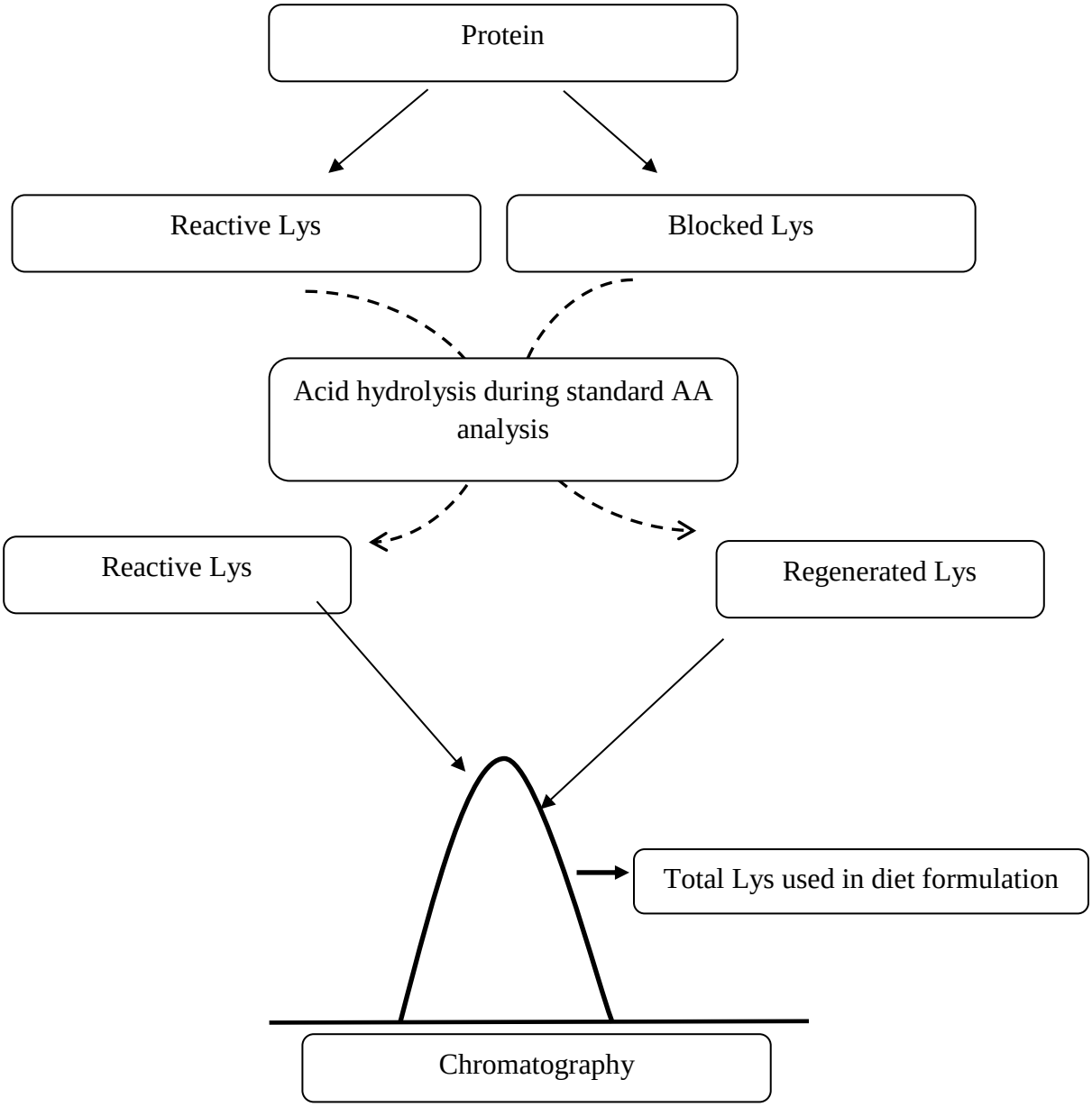

Figure 2.1. Lysine nomenclature.

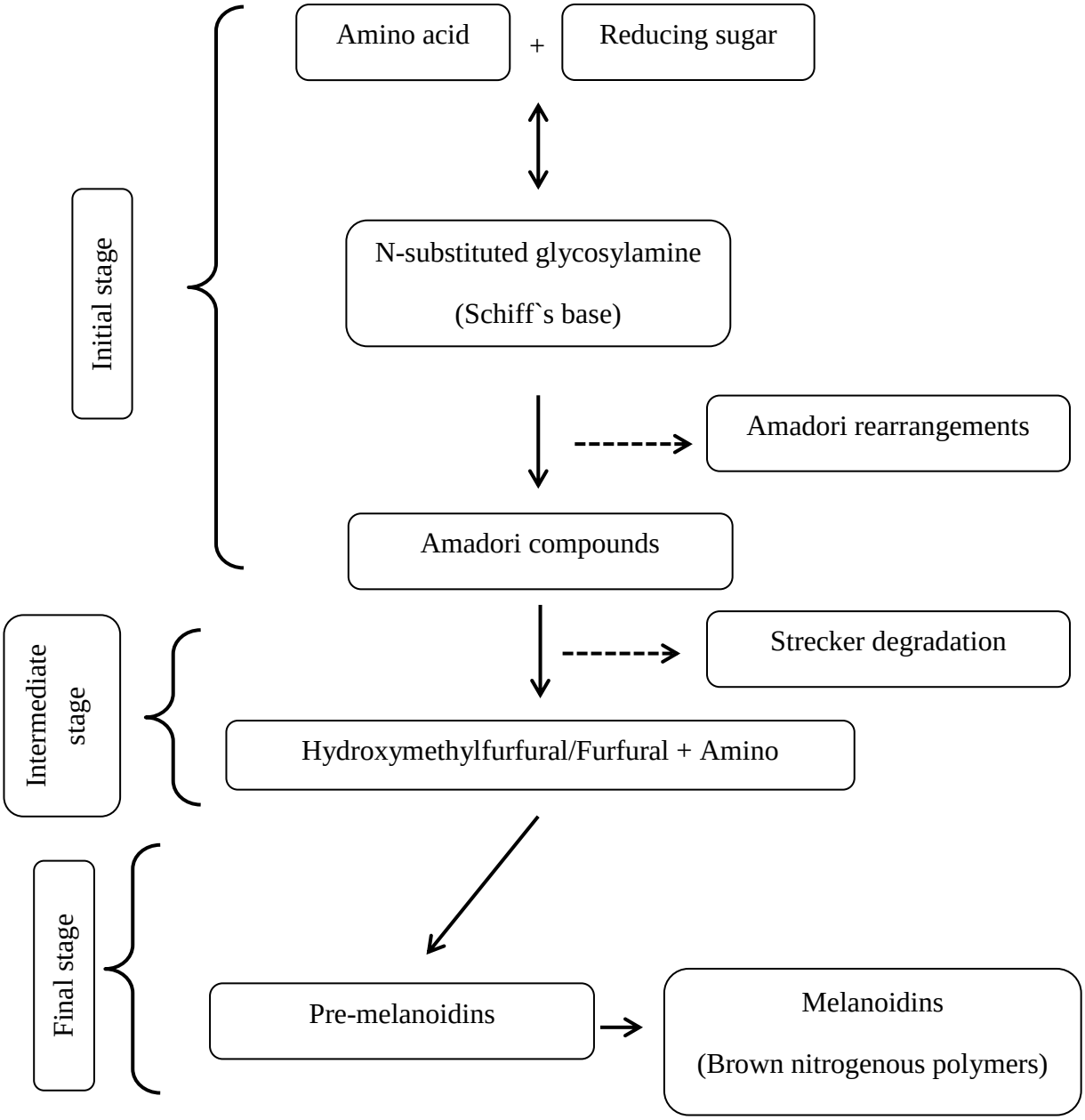

Figure 2.2. Overview of Maillard reactions, adapted from Purlis (2010).

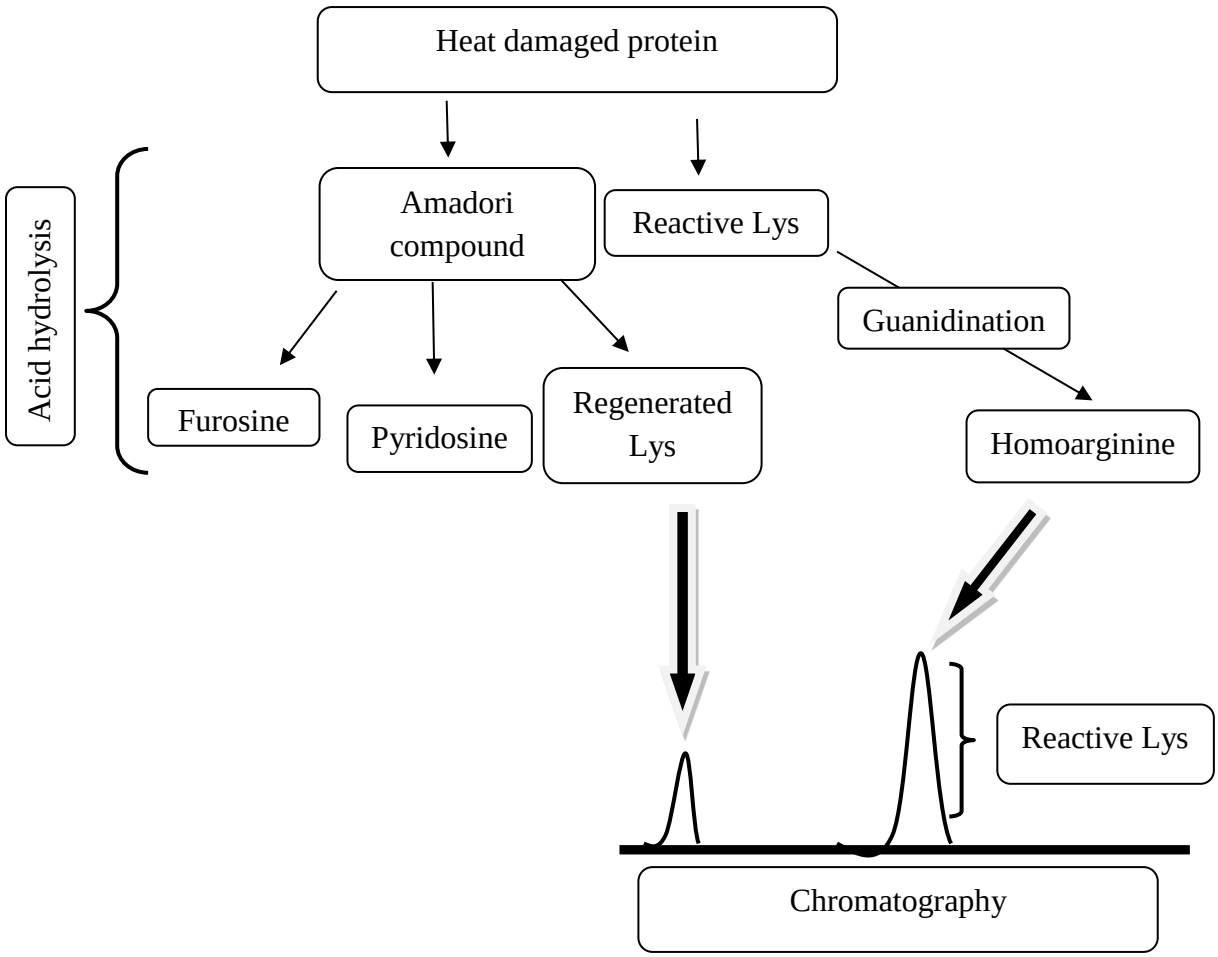

Figure 2.3. Principle for determination of reactive Lys in heat-damaged protein using the guanidination method.

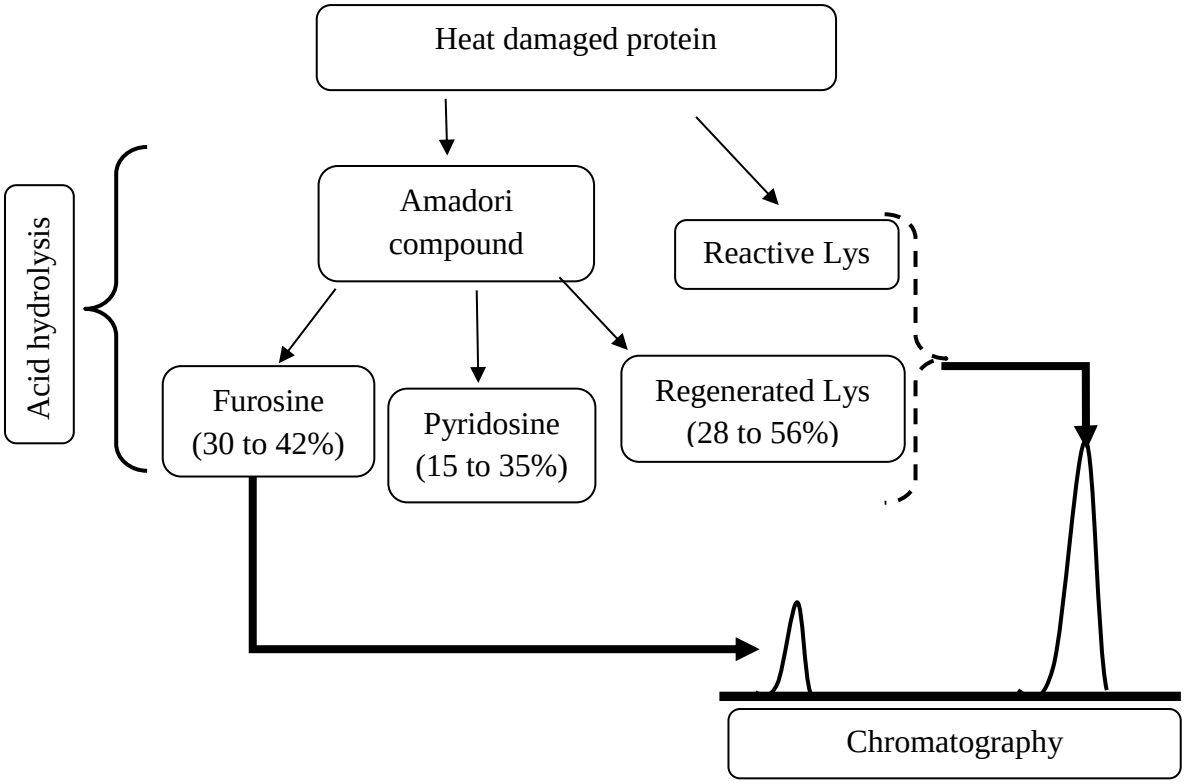

Figure 2.4. Principle for determination of reactive Lys in heat-damaged protein using the furosine method.

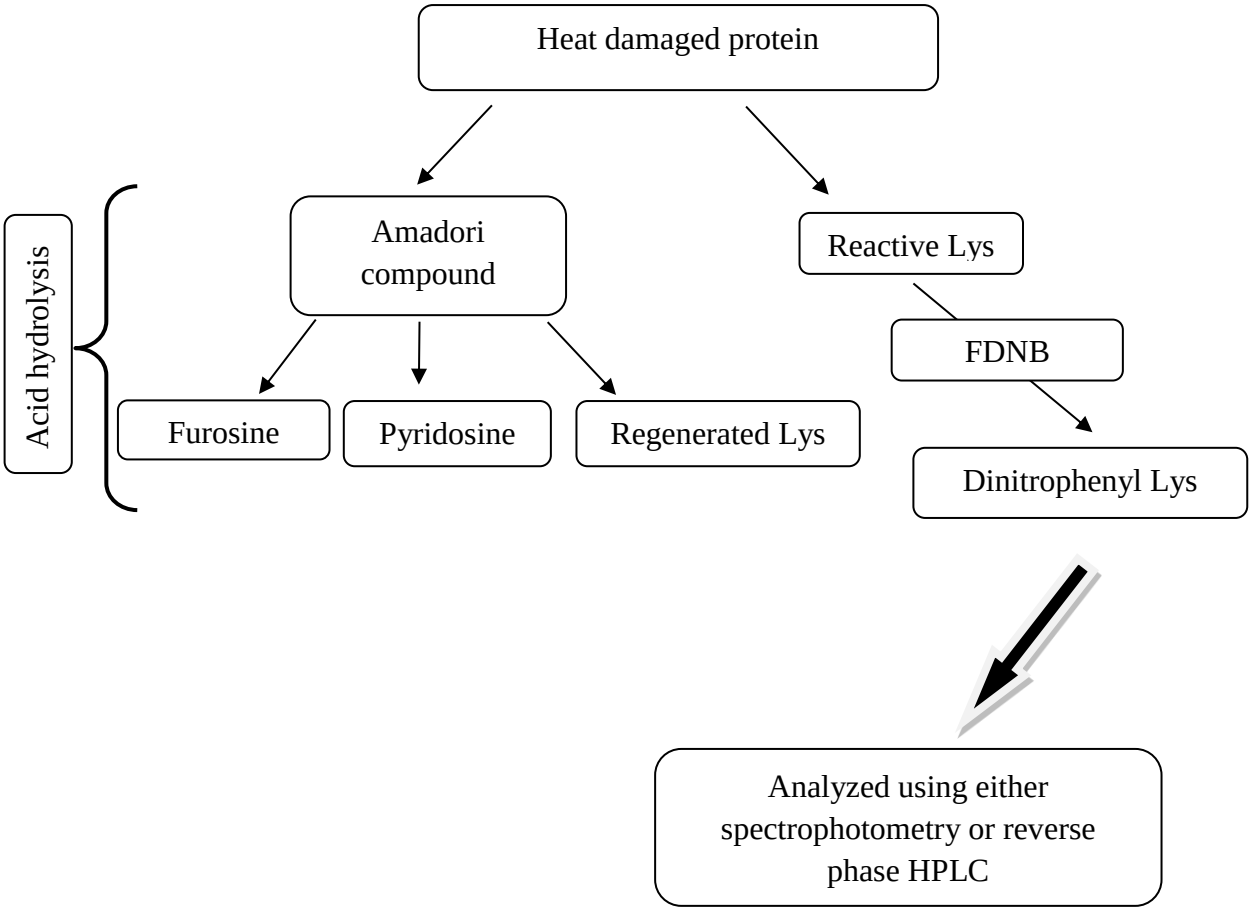

Figure 2.5. Principle for determination of reactive Lys in heat-damaged protein using the fluorodinitrobenze (FDNB) method.

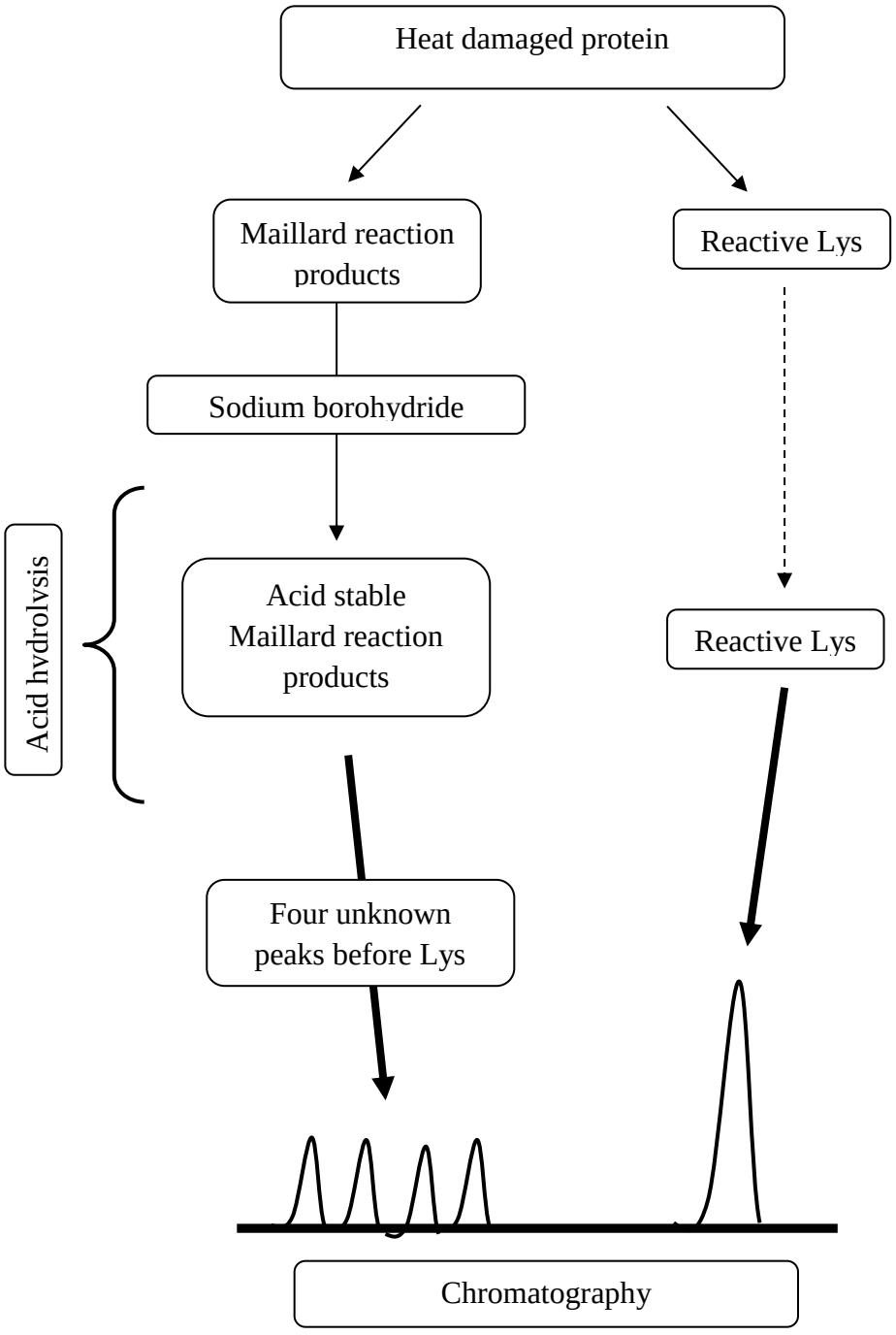

Figure 2.6. Principle for determination of reactive Lys in heat-damaged protein using the sodium borohydride method.

CHAPTER 3

AMINO ACID DIGESTIBILITY OF HEAT DAMAGED DISTILLERS DRIED GRAINS WITH SOLUBLES FED TO PIGS

ABSTRACT: The primary objective of this experiment was to determine the effects of heat treatment on the standardized ileal digestibility (**SID**) of AA in corn distillers dried grains with solubles (**DDGS**) fed to growing pigs. The second objective was to develop regression equations that may be used to predict the concentration of SID AA in corn DDGS. A source of corn DDGS was divided into 4 batches that were either not autoclaved or autoclaved at 130˚C for 10, 20, or 30 min. Four diets containing DDGS from each of the 4 batches were formulated with DDGS being the only source of AA and CP in the diets. A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. Ten growing pigs (initial BW: 53.5 ± 3.9 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 4 Youden square design with 5 diets and 4 periods in each square. The SID of CP decreased linearly ($P < 0.05$) from 77.9% in non-autoclaved DDGS to 72.1, 66.1, and 68.5% in the DDGS samples that were autoclaved for 10, 20, or 30 min, respectively. The SID of Lys was quadratically reduced ($P < 0.05$) from 66.8% in the non-autoclaved DDGS to 54.9, 55.3, and 51.9% in the DDGS autoclaved for 10, 20, or 30 min, respectively. The concentrations of SID Arg, His, Leu, Lys, Met, Phe, or Thr may be best predicted by equations that include the concentration of acid detergent insoluble N in the model (r^2 = 0.76, 0.68, 0.67, 0.84, 0.76, 0.73, or 0.54, respectively). The concentrations of SID Ile and Val were predicted (r^2 = 0.58 and 0.54, respectively) by the Lys:CP ratio, whereas the concentration of SID Trp was predicted (r^2 = 0.70) by the analyzed concentration of Trp. In conclusion, the SID of AA is decreased as a result of

heat damage and the concentration of SID AA in heat-damaged DDGS may be predicted by regression equations developed in this experiment.

Keywords: amino acids, distillers dried grains with solubles, heat damage, regression equations

INTRODUCTION

Production of corn distillers dried grains with solubles (**DDGS**) involves a drying step in which the temperature at the dryer inlet may be above 500°C, while the temperature at the dryer discharge may be above 100°C (Rosentrater et al., 2012). Application of heat to feed ingredients may initiate Maillard reactions, which decrease the concentration and digestibility of Lys and other AA (Pahm et al., 2008; Boucher et al., 2009; González-Vega et al., 2011). Lysine is particularly susceptible to undergo Maillard reactions because of its free amino group, which easily reacts with reducing sugars. If the amino group of an AA reacts with a reducing sugar to form early or advanced Maillard reaction products, it becomes unavailable to pigs (Rutherfurd and Moughan, 2007; Pahm et al., 2008). During the acid hydrolysis step of AA analysis, however, Lys that has reacted with reducing sugars is partially recovered, thus leading to an overestimation of available Lys. For this reason, determination of reactive Lys, color, and the Lys:CP ratio have been suggested as approaches to estimate the availability of Lys in DDGS (Fontaine et al., 2007; Pahm et al., 2008; Cozannet et al., 2010; Kim et al., 2012; Stein, 2012). Heat damage and Maillard reactions have been described in different sources of corn DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006; Pahm et al., 2008). However, if different sources of DDGS are used, it is difficult to distinguish between effects of heat damage and other factors influencing AA digestibility. Gradual increases in heating of a specific source of DDGS will result in reduced concentrations of reactive Lys (Fontaine et al., 2007; Pahm et al., 2008).

There is, however, no information about effects of increasing time of heating of a specific source of DDGS on *in vivo* AA digestibility, and on the changes in color, reactive Lys, and the Lys:CP ratio. Prediction of the concentration of digestible AA and CP from the concentration of reactive Lys and the Lys:CP ratio in corn DDGS that was purposefully heat-damaged have not been reported. Thus, the primary objective of this experiment was to determine effects of heat treatment on the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of AA in corn DDGS fed to growing pigs. A second objective was to develop regression equations that may be used to predict the concentration of SID AA in corn DDGS.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of G-performer boars and F-25 females (Genetiporc, Alexandria, MN).

Animals, Housing, and Experimental Design

Ten growing pigs (initial BW: 53.5 ± 3.9 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 4 Youden square design with 5 diets and 4 periods in each square. Pigs were individually housed in a controlled environment in pens (1.2 × 1.5 m) equipped with a feeder and a nipple waterer.

Diets and Feeding

Distillers dried grains with solubles was obtained from Poet Nutrition (Sioux Falls, SD) and analyzed for CP and AA. The DDGS was divided into 4 batches that were not autoclaved or autoclaved at 130˚C for 10, 20, or 30 min (Table 3.1). Four diets containing DDGS from each of the 4 batches were formulated, with DDGS being the only source of AA and CP in the diets

(Tables 3.2 and 3.3). A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. Diets were supplied with vitamins and minerals to meet or exceed the requirement estimates for growing pigs (NRC, 1998). Chromic oxide also was included (0.4%) in diets and used as an indigestible marker.

The amount of feed provided was calculated as 2.5 times the maintenance requirement of energy (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Pigs were fed once daily at 0800 h. At the beginning of each period, feed allowance was adjusted based on the BW of each pig. Water was available at all times.

Sample Collection

Each period consisted of 7 d. The initial 5 d were considered an adaptation period to the diet. On d 6 and d 7, ileal digesta were collected for 8 h using standard operating procedures. A plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were replaced whenever they were filled with digesta, or at least once every 30 min and immediately frozen at – 20^{o}C to prevent bacterial degradation of AA in the digesta.

Chemical Analyses

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized, finely ground, and analyzed. A sample of each diet and of each batch of DDGS was collected at the time of diet mixing. Diets, ingredients, and ileal samples were analyzed for AA by ion-exchange chromatography with postcolumn derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 *N* HCL for 24 h at 110°C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm.

Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000). Diets, ingredients, and ileal samples also were analyzed for DM (method 935.29; AOAC International, 2007), and for CP following the Dumas procedure (method 968.06; AOAC International, 2007). Diets and ileal samples were analyzed for chromium (method 990.08; AOAC International, 2007). Ingredients were analyzed for ADF (Method 973.18; AOAC International, 2007), NDF (Holst, 1973), for lignin (method 973.18 (A-D); AOAC International, 2007), ash (method 942.05; AOAC International, 2007), total reducing sugars (Dubois et al., 1956), and furosine as previously described (Kim et al., 2012). The concentration of ADIN in ingredients was determined as the concentration of N in the ADF fraction (method 990.03; AOAC International, 2007). Minolta L* (lightness), a* (redness), and b* (yellowness) values for each batch of DDGS were determined (8 mm aperture, D65 light source, and 0° observer, Minolta Camera Company, Osaka, Japan).

Calculations and Statistical Analysis

Values for AID and SID of CP and AA were calculated (Stein et al., 2007). The Lys:CP ratio in each DDGS sample was calculated by expressing the concentration of Lys in the sample as a percentage of the CP in the sample. The concentration of reactive Lys (%) was calculated by the following equation:

$$\text{Reactive Lys} = \text{total Lys (\%)} - [\text{furosine (\%)} \div 0.32 \times 0.40];$$

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). Normality of the data and the presence of outliers were evaluated using the UNIVARIATE procedure of SAS. One outlier was identified and removed from the data. The model included dietary treatment as fixed effect and pig and period as random effects. Linear and quadratic

effects of increasing time of heat treatment on the AID and SID of AA were analyzed by orthogonal polynomial contrasts. Correlations among predictor variables or between predictor variables and dependent variables were determined using the CORR procedure of SAS. The coefficients of correlation (r_p) were divided into 4 groups: no correlation ($P > 0.05$), low correlation ($r_p < 0.30$), moderate correlation ($0.60 > r_p \geq 0.30$), or high correlation ($r_p \geq 0.60$). Regression equations to estimate the relationship between the concentration of SID AA and color (L*, a*, or b*), or nutrient concentration were developed using the REG procedure in SAS. The pig was the experimental unit for all analyses and significance among means was assessed with an α level of 0.05.

RESULTS

Heat treatment did not change the concentrations of DM, ash, or CP in DDGS (Table 3.1). The concentration of total Lys, however, was 0.82% in non-autoclaved DDGS and 0.65, 0.73, and 0.68% in the DDGS that was autoclaved for 10, 20, and 30 min, respectively. The calculated concentration of reactive Lys was 0.80, 0.64, 0.72, and 0.67% for non-autoclaved DDGS and DDGS that was autoclaved for 10, 20, and 30 min, respectively. The Lys:CP ratio was 2.94 in non-autoclaved DDGS, whereas the Lys:CP ratio was 2.37, 2.75, and 2.51 in the batches that were autoclaved for 10, 20, and 30 min, respectively. The ADF concentration was 7.96% in non-autoclaved DDGS, whereas for autoclaved DDGS, the concentration of ADF was 11.05, 9.85, and 10.89% (10, 20, and 30 min, respectively). The concentration of ADIN in non-autoclaved DDGS was 0.12%, whereas the concentration of ADIN ranged from 0.42 to 0.55% in the autoclaved DDGS. Reducing sugar concentration was 0.78 in non-autoclaved DDGS, but DDGS that was autoclaved for 10, 20, and 30 min contained 0.60, 0.88, and 0.65% reducing

sugars, respectively. Lightness (L*) values were 43.90, 46.92, and 45.01 in the DDGS batches that were autoclaved for 10, 20, or 30 min, whereas L* in the non-autoclaved DDGS was 59.70. Yellowness (b*) values were 11.25, 14.51, and 12.01 in the batches of DDGS that were autoclaved for 10, 20, or 30 min, whereas b* in the non-autoclaved DDGS was 30.22.

Digestibility of CP and AA

The AID of CP decreased (linear, $P < 0.05$) from 64.4% in the non-autoclaved DDGS to 59.0, 52.2, and 55.7% in the DDGS that was autoclaved for 10, 20, or 30 min, respectively (Table 3.4). The AID of Ile, Lys, Met, Phe, and Asp was reduced (quadratic, $P < 0.05$) with increasing time of autoclaving, whereas the AID of all other AA was reduced (linear, $P < 0.05$) with increasing time of autoclaving. Lysine was the AA most affected by increasing time of autoclaving. The AID of Lys was reduced (quadratic, $P < 0.05$) from 61.2 to 48.0, 48.7, and 44.9% for the non-autoclaved DDGS and the DDGS that was autoclaved for 10, 20, or 30 min, respectively. The mean AID of indispensable AA was reduced (linear, $P < 0.05$) as time of autoclaving increased.

The SID of CP decreased (linear, $P < 0.05$) from 77.9 % in the non-autoclaved DDGS to 72.1, 66.1, and 68.5% in the DDGS that was autoclaved for 10, 20, or 30 min, respectively (Table 3.5). The SID of Lys was reduced (quadratic, $P < 0.05$) from 66.8% in the non-autoclaved DDGS to 54.9, 55.3, and 51.9% in the DDGS that was autoclaved for 10, 20, or 30 min, respectively.

The SID of Ile, Met, Phe, and Asp also was reduced (quadratic, $P < 0.05$) with increasing time of autoclaving, whereas the SID of all other AA was reduced (linear, $P < 0.05$) with increasing time of autoclaving. The mean SID of indispensable AA also was reduced (linear, $P < 0.05$) as time of autoclaving increased.

Coefficients of Linear Correlation and Regression Equations

Color measurements were correlated ($r_p > 0.70$; $P < 0.01$) with the concentrations of SID Arg, His, Ile, Leu, Lys, Met, Thr, and Trp (Table 3.6). The correlation between the concentration of SID Lys in corn DDGS and color L*, b*, or a* was 0.91 ($P < 0.01$).

The concentrations of SID AA were correlated ($r_p > 0.70$; $P < 0.01$) with the concentration of Lys:CP, except for the concentration of SID Thr, that was moderately correlated with the concentration of Lys:CP (Table 3.7). The concentration of SID AA was poorly correlated ($r_p < 0.50$) with the concentration of reducing sugars. The concentrations of SID AA were negatively correlated with the concentrations of ADF, NDF, lignin, and ADIN ($P < 0.01$). The concentration of SID of each AA also was well correlated ($r_p > 0.65$; $P < 0.01$) with its respective concentration of AA, except for SID Thr, which was moderately correlated ($r_p < 0.65$; $P < 0.01$) with the concentration of Thr.

The concentrations of digestible AA may be predicted by regression equations presented in Table 3.8. Color L* was generally a good predictor ($r^2 > 0.60$) of the concentration of SID AA, except for the concentration of SID Thr ($r^2 = 0.52$). The concentration of SID Arg or SID Lys were best predicted by equations that included the concentration of ADIN in the model ($r^2 = 0.76$ and 0.84, respectively). The concentrations of SID AA were predicted ($r^2 > 0.50$) by the concentrations of their respective AA, but the concentration of SID Thr was poorly ($r^2 = 0.36$) predicted by the concentration of Thr.

DISCUSSION

Composition of Distillers Dried Grains with Solubles

The concentrations of DM, ash, and CP were not affected by autoclaving DDGS and this observation supports results of González-Vega et al. (2011) who reported that autoclaving soybean meal did not change concentrations of DM, ash, or CP. Changes in the concentration of Lys observed for autoclaved DDGS in this experiment also agree with results of previous experiments in which the concentration of Lys was decreased by heat treatment (Fontaine et al., 2007; Martinez-Amezcua et al., 2007; Pahm et al., 2008; Boucher et al., 2009). Based on the concentration of Lys, these results indicate that autoclaving corn DDGS for 10 min was sufficient to cause heat damage, but increasing time of autoclaving to 20 and 30 min did not result in further decreases in Lys concentration. In contrast, in a previous experiment, the concentration of Lys in corn DDGS autoclaved for 0, 10, 20, or 30 min at 135°C was linearly decreased from 0.82 to 0.59% (Fontaine et al., 2007). These differences may be a result of differences in sample preparation and autoclaving procedures. Although temperature and time of autoclaving were similar between the 2 experiments, in this experiment, DDGS was autoclaved on an as-is basis in quantities of 2.5 kg per tray whereas Fontaine et al. (2007) ground DDGS to < 3 mm particle size and then autoclaved DDGS in 250 g quantities. The concentrations of furosine in DDGS used in this experiment were slightly less than the average concentration of furosine (0.02%; CV = 91.4%) measured in 21 sources of DDGS, and these differences may be a result of the high degree of variation when determining the concentration of furosine in DDGS (Kim et al., 2012). The concentration of reactive Lys in 33 sources of DDGS represents approximately 83% of the concentration of total Lys (Pahm et al., 2008), but as a result of the relatively low concentrations of furosine determined in DDGS used in this experiment, the

calculated concentration of reactive Lys in DDGS used in this experiment represented approximately 98% of the concentration of total Lys. Determination of reactive Lys using the furosine procedure was initially developed to be used in milk products under the assumption that Amadori compounds yield 32% furosine, 40% regenerated Lys, and 28% pyridosine (Erbersdobler and Somoza, 2007). These same assumptions have been used when determining the concentration of reactive Lys in DDGS (Pahm et al., 2008; Kim et al., 2012), but the differences observed among the present data and the data from Pahm et al. (2008) and Kim et al. (2012) indicate that these assumptions may not be true for DDGS. The reason for the reduced Lys:CP ratio in the autoclaved DDGS is that only the concentration of Lys, but not the concentration of CP, is reduced when heat damage occurs (Stein et al., 2009; Kim et al., 2012). The Lys:CP ratio also was reduced in soybean meal that was heat-damaged compared with unheated soybean meal (González-Vega et al., 2011). The concentration of Lys in the non-autoclaved DDGS was slightly greater than the average concentration of Lys (0.76%) in 39 sources of DDGS (Stein and Shurson, 2009). Likewise, the Lys:CP ratio was slightly greater in the unheated DDGS compared with the average value (2.77) reported by Stein and Shurson (2009). However, the concentrations of Lys and the Lys:CP ratio in autoclaved DDGS are close to the average values reported by Stein and Shurson (2009) for corn DDGS, which indicates that the heat damage caused by autoclaving simulated the varying degrees of heat damage caused by processing of DDGS in commercial production facilities.

The concentration of ADIN has been used as a predictor of heat damage in plant proteins and our results agree with these observations (Schroeder et al., 1996). The changes observed between the concentrations of reducing sugars in the unheated DDGS and the 3 autoclaved DDGS were expected because, in the initial steps of the Maillard reactions, reducing sugars react

with the ε-amino group of Lys to form fructoselysine and maltuloselysine, respectively (Erbersdobler and Hupe, 1991). Lightness (L*) of soybean meal subjected to heat damage is reduced (González-Vega et al., 2011). This may be attributed to the formation of advanced Maillard reaction products such as premelanoidins and melanoidins which, in some cases are characterized by a noticeable browning of the ingredients (Faist and Erbersdobler, 2001). Because Maillard reactions are associated with formation of brown color and reduced AA concentrations, it is expected that darker DDGS contains less AA than lighter DDGS. Among 10 different sources of wheat DDGS produced in European ethanol plants, 3 darker sources with L* < 50 had a reduced Lys:CP ratio compared with 7 lighter sources with L* > 50 (Cozannet et al., 2010). In corn DDGS, the concentration of Lys and the Lys:CP ratio is also less in darker DDGS than in lighter DDGS (Batal and Dale, 2006; Fastinger and Mahan, 2006).

Digestibility of CP and AA

Values for the AID of CP and AA in the unheated DDGS are in agreement with results from previous experiments (Fastinger and Mahan, 2006; Stein and Shurson, 2009). Results of previous experiments also indicated that the digestibility of Lys was more reduced by heat damage than the digestibility of other AA (Martinez-Amezcua et al., 2007; González-Vega et al., 2011). Values for the SID of CP and AA in the non-autoclaved DDGS were within the range of values for the SID of CP and AA in corn DDGS observed in previous experiments (Stein and Shurson, 2009). This observation is supported by the value for the Lys:CP ratio (2.94) determined for corn DDGS in this experiment, which also was similar to the average value (2.77) reported by Stein and Shurson (2009). For the autoclaved DDGS, however, values for the SID of CP and AA were less than those reported by Stein and Shurson (2009). Reductions observed for the SID of Lys with increasing time of autoclaving and the results from reducing sugars and the

Lys concentrations in autoclaved DDGS indicate that the conditions created by autoclaving of DDGS (i.e., heat, moisture, and pressure) were favorable to initiation of Maillard reactions, which renders Lys unavailable to the animals and, therefore, reduces Lys digestibility (Nursten, 2005; González-Vega et al., 2011). It appears, however, that the degree of heat damage among autoclaved DDGS (i.e., 10, 20, or 30 min) was not different and this observation is supported by the fact that the SID of Lys among these ingredients was not different. Reductions in the digestibility of AA other than Lys have been attributed to the formation of cross-linkages between protein chains (Tuohy et al., 2006). When these cross-links are formed, digestive enzymes such as trypsin have reduced access to the proteins, thus the ability of trypsin to hydrolyze peptide bonds is reduced. Exposure to excessive heat and pressure may also lead to AA racemization, thus converting L-AA to D-AA (Zagon et al., 1994). Proteolytic enzymes are unable to hydrolyze peptide bonds connecting D-AA and L-AA. Consequently, the digestibility is reduced not only for the D-AA, but also for the L-AA (Finot, 2005).

Coefficients of Linear Correlation and Regression Equations

Results of this experiment concur with results of previous experiments in which the concentration of SID Lys in different sources of corn DDGS or wheat DDGS was correlated with color L* (Batal and Dale, 2006; Fastinger and Mahan, 2006; Cozannet et al., 2010; 2011). These observations indicate that the concentration of SID Lys in DDGS may be predicted from values for color L* within the same source of DDGS (as for this experiment). The use of color as a predictor of SID AA in DDGS, however, is debatable (Shurson, 2011), because changes in the color of DDGS may not only occur as a result of heat damage (Ganesan et al., 2008; Liu, 2009; Kingsly et al., 2010). Condensed distillers solubles (**CDS**) are added to wet distillers grains in the production of DDGS, and because CDS are brown in color, the amount added to wet distillers

grain may affect the color of DDGS (Ganesan et al., 2008). Therefore, as CDS level is reduced the value for L* in DDGS increases (Kingsly et al., 2010). The CDS fraction of DDGS also contains reducing sugars, which may result in Maillard reactions and, therefore, increase browning of DDGS (Kingsly et al., 2010). Color of DDGS also is affected by particle size, and smaller particle size is associated with DDGS that is lighter, less red, and more yellow (Liu, 2009). Therefore, the regression equations using color to predict the concentration of SID AA in DDGS that were developed in this experiment should be used only for DDGS produced within a specific ethanol plant where variations in drying temperatures may exist, but the levels of CDS and particle size are constant. For feed manufacturers who use different sources of DDGS, however, the use of equations that include color is of limited value because changes in color may be a result of characteristics of DDGS not related to heat damage.

The relatively good correlations between the concentration of SID AA and the concentration of each AA in DDGS agree with results from Kim et al. (2012). Consequently, analyzed concentrations of most AA in DDGS may be used to predict the concentration of SID AA in DDGS although that is not the case for Thr. It has been observed that heat damage of feed ingredients is associated with an increase in ADF, lignin, and ADIN, which results in a decrease in N digestibility in cattle and sheep (Broesder et al., 1992). The concentrations of ADF and ADIN is increased in dark-colored DDGS, which suggests a greater degree of heat damage in such ingredients (Cromwell et al., 2003), and likely a lower digestibility of AA. The negative correlation between the concentration of SID AA and the concentrations of ADF, lignin, or ADIN observed in this experiment support the latter observations.

The concentration of SID Lys can accurately be predicted from the concentration of Lys. This observation agrees with Kim et al. (2012) who also concluded that the concentration of SID

Lys may be predicted from the concentrations of Lys. Prediction of the concentration of SID Lys in this study was slightly improved if the concentration of ADIN was used in the model. This indicates that the concentration of analyzed ADIN may be used to predict the concentration of SID Lys in DDGS.

In conclusion, results of this experiment confirmed that the concentration and digestibility of AA in DDGS is reduced as a result of heat damage. The concentrations of Lys and ADIN are good predictors of the concentration of most AA in heat-damaged DDGS, and the concentration of SID AA may accurately be predicted from regression equations developed in this experiment.

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TABLES

Table 3.1. Chemical composition of distillers dried grains with solubles

	Distillers dried grains with solubles			
		Autoclaved at 130°C		
Item	Non-autoclaved	10 min	20 min	30 min
DM, %	93.21	92.43	90.91	94.47
Ash, %	5.25	5.08	5.17	5.06
CP, %	27.91	27.44	26.51	27.05
Lys:CP ratio[1]	2.94	2.37	2.75	2.51
Furosine, %	0.015	0.009	0.006	0.008
Reactive Lys[2]	0.80	0.64	0.72	0.67
ADF, %	7.96	11.05	9.85	10.89
NDF, %	31.29	33.23	33.32	32.40
ADL, %	0.88	2.06	1.73	2.57
ADIN,[3] %	0.12	0.53	0.42	0.55
Reducing sugars, %	0.78	0.60	0.88	0.65
L*[4]	59.70	43.90	46.92	45.01
a*[4]	11.79	10.31	10.77	10.27
b*[4]	30.22	11.25	14.51	12.01
Indispensable AA, %				
Arg	1.24	1.10	1.19	1.10
His	0.71	0.67	0.70	0.67

Table 3.1. (Cont.)

Ile	0.97	0.91	0.96	0.93
Leu	2.92	2.82	2.89	2.78
Lys	0.82	0.65	0.73	0.68
Met	0.53	0.49	0.52	0.50
Phe	1.24	1.17	1.21	1.17
Thr	1.02	0.98	1.01	0.98
Trp	0.22	0.20	0.20	0.20
Val	1.26	1.19	1.26	1.23
All indispensable	10.93	10.18	10.67	10.24
Dispensable AA, %				
Ala	1.90	1.83	1.87	1.82
Asp	1.75	1.66	1.73	1.66
Cys	0.55	0.50	0.52	0.50
Glu	4.43	4.31	4.41	4.26
Gly	1.08	1.04	1.09	1.04
Pro	2.21	2.06	2.13	2.04
Ser	1.31	1.26	1.29	1.23
All dispensable	13.23	12.66	13.04	12.55

[1]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

[2]Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)]; Pahm et al., 2008.

[3]ADIN = acid detergent insoluble nitrogen.

[4]L* = lightness; a* = redness; b* = yellowness.

Table 3.2. Ingredient composition of experimental diets, as-fed basis

	Distillers dried grains with solubles diets				
		Autoclaved at 130°C			
Ingredient, %	Non-autoclaved	10 min	20 min	30 min	N-free diet
DDGS[1]	60.00	60.00	60.00	60.00	-
Cornstarch	27.00	27.00	27.00	27.00	66.40
Sucrose	10.00	10.00	10.00	10.00	20.00
Solka floc[2]	-	-	-	-	5.00
Soybean oil	-	-	-	-	4.00
Ground limestone	1.20	1.20	1.20	1.20	-
Dicalcium phosphate	0.60	0.60	0.60	0.60	3.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.40	0.40	0.40	0.40	0.30

[1]DDGS = distillers dried grains with solubles.

[2]Fiber Sales and Development Corp., Urbana, OH.

[3]Provided the following per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.3. Analyzed composition of experimental diets, as-fed basis

	Distillers dried grains with solubles				
		Autoclaved at 130°C			
Item	Non-autoclaved	10 min	20 min	30 min	N-free diet
DM, %	92.42	93.18	92.59	92.32	92.53
CP, %	14.96	15.59	14.62	15.76	0.28
Indispensable AA, %					
Arg	0.73	0.68	0.70	0.67	-
His	0.42	0.41	0.42	0.41	-
Ile	0.56	0.57	0.58	0.55	-
Leu	1.69	1.75	1.76	1.72	-
Lys	0.51	0.42	0.44	0.41	-
Met	0.31	0.29	0.29	0.28	-
Phe	0.71	0.73	0.74	0.72	-
Thr	0.61	0.61	0.62	0.60	-
Trp	0.13	0.12	0.12	0.12	-
Val	0.74	0.75	0.76	0.73	-
All indispensable	6.41	6.33	6.43	6.21	-
Dispensable AA, %					
Ala	1.11	1.14	1.15	0.12	-
Asp	1.05	1.04	1.06	1.03	-
Cys	0.33	0.32	0.31	0.31	-
Glu	2.60	2.69	2.70	2.65	-

Table 3.3. (Cont.)

Gly	0.64	0.65	0.66	0.64	-
Pro	1.30	1.24	1.27	1.23	-
Ser	0.78	0.79	0.79	0.78	-
All dispensable	7.81	7.87	7.94	6.76	-

Table 3.4. Apparent ileal digestibility of CP and AA in distillers dried grains with solubles subjected to increasing levels of heat treatment by weanling pigs[1]

	Distillers dried grains with solubles						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	10 min	20 min	30 min		Linear	Quadratic
CP, %	64.4	59.0	52.2	55.7	2.0	< 0.01	0.12
Indispensable AA, %							
Arg	79.3	71.9	72.0	69.1	1.7	< 0.01	0.06
His	73.9	66.8	65.9	66.0	1.3	< 0.01	0.23
Ile	72.3	66.2	66.6	63.1	1.4	< 0.01	0.02
Leu	84.0	80.7	80.4	79.1	0.8	< 0.01	0.12
Lys	61.2	48.0	48.7	44.9	1.9	< 0.01	0.02
Met	83.6	77.7	77.8	75.7	0.9	< 0.01	< 0.01
Phe	79.8	75.1	75.4	73.6	1.0	< 0.01	0.04
Thr	60.2	53.9	53.2	51.2	1.6	< 0.01	0.27
Trp	55.1	43.7	42.8	41.5	2.0	< 0.01	0.15

Table 3.4. (Cont.)

Val	71.9	65.7	65.5	62.6	1.4	< 0.01	0.08
Mean	75.6	70.0	69.9	67.8	1.1	< 0.01	0.07
Dispensable AA, %							
Ala	77.5	73.3	71.4	71.0	1.3	< 0.01	0.86
Asp	65.5	55.2	55.4	53.3	1.5	< 0.01	0.02
Cys	71.9	64.7	60.5	62.7	1.5	< 0.01	0.54
Glu	81.7	76.7	75.6	75.3	0.9	< 0.01	0.34
Gly	43.4	36.3	29.0	31.7	4.6	< 0.01	0.47
Ser	69.6	65.1	64.1	63.2	1.3	< 0.01	0.47
Mean	68.3	61.8	59.2	59.5	1.5	< 0.01	0.85

[1]Data are means of 8 observations, except for the distillers dried grains with solubles diet that was autoclaved for 10 min (n = 7).

[2]Linear and quadratic effects of time of autoclaving.

Table 3.5. Standardized ileal digestibility of CP and AA in distillers dried grains with solubles subjected to increasing levels of heat treatment by weanling pigs[1]

	Distillers dried grains with solubles						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	10 min	20 min	30 min		Linear	Quadratic
CP, %	77.9	72.1	66.1	68.5	2.0	< 0.01	0.25
Indispensable AA, %							
Arg	87.9	81.3	81.0	78.5	1.7	< 0.01	0.11
His	78.1	71.2	70.1	70.3	1.3	< 0.01	0.27
Ile	77.3	71.1	71.4	68.2	1.4	< 0.01	0.03
Leu	86.7	83.3	83.1	81.8	0.8	< 0.01	0.12
Lys	66.8	54.9	55.3	51.9	1.9	< 0.01	0.04
Met	86.1	80.4	80.5	78.5	0.9	< 0.01	< 0.01
Phe	83.7	79.0	79.2	77.5	1.0	< 0.01	0.05
Thr	70.2	64.0	63.1	61.3	1.6	< 0.01	0.33
Trp	65.9	55.5	54.5	53.2	2.0	< 0.01	0.20

Table 3.5. (Cont.)

Val	77.2	71.0	70.7	67.9	1.4	< 0.01	0.09
Mean	80.7	75.2	74.9	73.1	1.1	< 0.01	0.09
Dispensable AA, %							
Ala	83.3	79.1	77.1	76.8	1.3	< 0.01	0.89
Asp	72.3	62.1	62.1	60.2	1.5	< 0.01	0.03
Cys	77.7	70.8	66.8	68.9	1.5	< 0.01	0.55
Glu	85.2	80.1	79.0	78.7	0.9	< 0.01	0.33
Gly	73.4	66.0	58.1	61.7	4.6	< 0.01	0.39
Ser	76.9	72.4	71.3	70.5	1.3	< 0.01	0.48
Mean	78.1	71.7	69.0	69.4	1.5	< 0.01	0.91

[1]Data are means of 8 observations, except for the distillers dried grains with solubles diet that was autoclaved for 10 min (n = 7); Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI), which were determined by feeding pigs a N-free diet: CP, 21.93; Arg, 0.68; His, 0.19; Ile, 0.30; Leu, 0.50; Lys, 0.31; Met, 0.08; Phe, 0.30; Thr, 0.66; Trp, 0.15; Val, 0.43; Ala, 0.71; Asp, 0.77; Cys, 0.21; Glu, 0.98; Gly, 2.08; and Ser, 0.62.

[2]Linear and quadratic effects of time of autoclaving.

Table 3.6. Coefficients of linear correlation between color of distillers dried grains with solubles and the concentration (%) of standardized ileal digestible (SID) AA

	Color[1]		
Item	L*	b*	a*
SID Arg	0.84	0.84	0.87
SID His	0.83	0.83	0.82
SID Ile	0.79	0.79	0.81
SID Leu	0.79	0.79	0.82
SID Lys	0.91	0.91	0.91
SID Met	0.88	0.88	0.87
SID Phe	0.84	0.84	0.86
SID Thr	0.72	0.72	0.72
SID Trp	0.84	0.84	0.83
SID Val	0.77	0.77	0.79

[1]L* = lightness; a* = redness; b* = yellowness; all correlations are significant ($P < 0.01$).

Table 3.7. Coefficients of linear correlation between nutrient composition of distillers dried grains with solubles and the concentration (%) of standardized ileal digestible (SID) CP and AA

	Nutrient composition															
Item	Lys:CP	Reducing sugars	ADF	NDF	Lignin	ADIN[1]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
SID Arg	0.80*	0.50*	-0.86*	-0.60*	-0.62*	-0.87*	0.84*	-	-	-	-	-	-	-	-	-
SID His	0.72*	0.06ns	-0.81*	-0.69*	-0.48*	-0.82*	-	0.69*	-	-	-	-	-	-	-	-
SID Ile	0.76*	0.49*	-0.81*	-0.58*	-0.55*	-0.81*	-	-	0.70*	-	-	-	-	-	-	-
SID Leu	0.72*	0.45**	-0.81*	-0.54*	-0.65*	-0.82*	-	-	-	0.78*	-	-	-	-	-	-
SID Lys	0.83*	0.45**	-0.91*	-0.74*	-0.54*	-0.92*	-	-	-	-	0.89*	-	-	-	-	-
SID Met	0.78*	0.40**	-0.86*	-0.73*	-0.48*	-0.87*	-	-	-	-	-	0.75*	-	-	-	-
SID Phe	0.78*	0.47*	-0.85*	-0.63*	-0.56*	-0.86*	-	-	-	-	-	-	0.83*	-	-	-
SID Thr	0.58*	0.25ns	-0.70*	-0.55*	-0.56*	-0.73*	-	-	-	-	-	-	-	0.60*	-	-
SID Trp	0.71*	0.34ns	-0.81*	-0.70*	-0.52*	-0.83*	-	-	-	-	-	-	-	-	0.84*	-
SID Val	0.73*	0.45**	-0.78*	-0.59*	-0.50*	-0.78*	-	-	-	-	-	-	-	-	-	0.55*

[1]ADIN = acid detergent insoluble nitrogen.
* = $P < 0.01$; ** = $P < 0.05$; ns = not significant.

Table 3.8. Linear regression to predict the concentration (%) of standardized ileal digestible (SID) CP and AA from color, and nutrient concentrations (%) in corn distillers dried grains with solubles (DDGS) fed to pigs[1]

	Intercept			Independent variable				
Dependent variable	Estimate	SE	*P*-value	Variable	Estimate	SE	*P*-value	r^2
SID Arg	0.30	0.08	< 0.01	Color L*[2]	0.013	0.001	< 0.01	0.71
	-0.66	0.20	< 0.01	Arg	1.398	0.171	< 0.01	0.70
	1.16	0.02	< 0.01	ADIN[2]	-0.506	0.053	< 0.01	0.76
SID His	0.23	0.03	< 0.01	Color L*	0.006	0.0006	< 0.01	0.69
	-0.62	0.22	< 0.01	His	1.639	0.322	< 0.01	0.47
	0.58	0.01	< 0.01	ADIN	-0.203	0.025	< 0.01	0.68
SID Ile	0.34	0.05	< 0.01	Color L*	0.007	0.001	< 0.01	0.62
	-0.92	0.30	< 0.01	Ile	1.706	0.320	< 0.01	0.50
	0.15	0.08	0.08	Lys:CP	0.200	0.031	< 0.01	0.58
SID Leu	1.69	0.10	< 0.01	Color L*	0.014	0.002	< 0.01	0.62
	-2.23	0.69	< 0.01	Leu	1.623	0.243	< 0.01	0.61
	2.61	0.03	< 0.01	ADIN	-0.554	0.071	< 0.01	0.67

Table 3.8. (Cont.)

SID Lys	-0.20	0.05	< 0.01	Color L*	0.013	0.001	< 0.01	0.83
	-0.46	0.08	< 0.01	Lys	1.214	0.115	< 0.01	0.79
	0.60	0.02	< 0.01	ADIN	-0.463	0.037	< 0.01	0.84
SID Met	0.18	0.02	< 0.01	Color L*	0.004	0.0004	< 0.01	0.77
	-0.39	0.13	< 0.01	Met	1.53	0.252	< 0.01	0.56
	0.46	0.01	< 0.01	ADIN	-0.161	0.017	< 0.01	0.76
SID Phe	0.56	0.05	< 0.01	Color L*	0.008	0.0009	< 0.01	0.70
	-1.11	0.26	< 0.01	Phe	1.726	0.217	< 0.01	0.68
	1.08	0.01	< 0.01	ADIN	-0.304	0.034	< 0.01	0.73
SID Thr	0.32	0.06	< 0.01	Color L*	0.006	0.001	< 0.01	0.52
	-1.31	0.50	< 0.05	Thr	1.967	0.499	< 0.01	0.36
	0.75	0.02	< 0.01	ADIN	-0.247	0.043	< 0.01	0.54
SID Trp	0.002	0.01	0.91	Color L*	0.002	0.0002	< 0.01	0.70
	-0.24	0.04	< 0.01	Trp	1.767	0.219	< 0.01	0.70
	-0.04	0.03	0.19	Lys:CP	0.061	0.011	< 0.01	0.51

Table 3.8. (Cont.)

SID Val	0.46	0.07	< 0.01	Color L*	0.009	0.001	< 0.01	0.59
	-0.88	0.50	0.08	Val	1.438	0.400	< 0.01	0.31
	0.24	0.11	0.04	Lys:CP	0.246	0.042	< 0.01	0.54

[1] n = 31 observations; for all models $P < 0.01$.
[2] Color L* = lightness; ADIN = acid detergent insoluble nitrogen.

CHAPTER 4

EFFECTS OF HEAT TREATMENT ON THE APPARENT AND STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACIDS IN CANOLA MEAL FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to determine the effects of heat damage on the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of CP and AA in canola meal fed to growing pigs. The second objective was to develop regression equations to predict the concentration of SID AA from the nutrient composition of canola meal. Ten growing pigs (initial BW: 26.5 ± 0.7 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. One batch of canola meal was divided into 4 batches that were either not autoclaved or autoclaved at 130˚C for 20, 30, or 45 min. Four diets were formulated with canola meal being the only source of AA and CP. Each diet contained 1 of the 4 batches of canola meal. A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. The AID of CP and all AA was reduced (quadratic, $P < 0.01$) as a result of increasing time of autoclaving. Autoclaving of canola meal also reduced (quadratic, $P < 0.01$) the SID of CP and all AA. The concentration (%) of SID Lys in canola meal may be predicted by regression equations using the concentration (%) of reducing sugars ($r^2 = 0.96$). Likewise, the concentrations of SID AA for most AA may be predicted from the nutrient composition of canola meal. In conclusion, heat damage reduces both the concentration and the digestibility of AA in canola meal. Regression equations developed in this experiment may be used to predict the concentration of SID AA in canola meal.

Key words: amino acids, canola meal, digestibility, heat damage, pigs

INTRODUCTION

Canola meal, the product remaining after oil has been solvent extracted from canola, is the second most used protein source for feeding of poultry and livestock (Canola Council of Canada, 2009). The final step in producing canola meal involves desolventizing and toasting of the meal, which may last between 35 and 50 min and requires steam (i.e., moisture) and temperatures that vary from 95 to 115°C (Canola Council of Canada, 2009; Unger, 2011). Consequently, differences in processing of canola meal may result in variations in the nutritional composition of canola meal among different processing plants because Maillard reactions may occur as a result of the combination of heat and moisture in the presence of reducing sugars and AA (Nursten, 2005). These reactions result in a decrease in the concentration and digestibility of AA, and Lys is the AA most affected by heat damage (Carvalho et al., 2009; González-Vega et al., 2011; Newkirk, 2011). Conventional AA analysis may overestimate the concentration of Lys available for the pig in heat-damaged feed ingredients because some of the Lys that participates in the Maillard reactions is recovered during the acid hydrolysis step, although this Lys is not released *in vivo* (Williams et al., 2006). The use of reactive Lys determined by the furosine procedure has been suggested as an approach to evaluate the nutritional quality of corn DDGS (Pahm et al., 2008; Kim et al., 2012), but this hypothesis has not been tested in canola meal. Apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of CP and AA in canola meal have been determined (Stein et al., 2005; Woyengo et al., 2010; Trindade Neto et al., 2012), but effects of heat damage on the AID and SID of CP and AA in canola meal fed to growing pigs have not been determined. We hypothesized that heat damage decreases the

concentration and digestibility of AA in canola meal and that the concentration of SID AA in heat-damaged canola meal may be predicted from regression equations developed using the nutrient composition of heat-damaged canola meal. Therefore, the main objective of this experiment was to determine the effects of heat damage on the AID and SID of CP and AA in canola meal fed to growing pigs. The second objective was to develop regression equations to predict the concentration of SID AA in canola meal.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were the offspring of G-performer boars and F-25 females (Genetiporc, Alexandria, MN).

Animals, Housing, and Experimental Design

Ten growing pigs (initial BW: 26.5 ± 0.7 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were individually housed in a controlled environment in pens (1.2 × 1.5 m) that were equipped with a feeder and a nipple waterer.

Diets and Feeding

Canola meal was obtained from the University of Illinois Feed Mill (Champaign, IL) and divided into 4 batches that were either not autoclaved or autoclaved at 130˚C for 20, 30, or 45 min (Table 4.1). Four diets were formulated with canola meal being the only source of AA and CP in each diet (Tables 4.2 and 4.3). Each diet contained 1 of the 4 batches of canola meal. A N-free diet also was formulated and used to determine the basal endogenous losses of CP and AA in the pigs. Diets were supplied with vitamins and minerals to meet or exceed the requirement

estimates for growing pigs (NRC, 1998). Chromic oxide was included (0.4%) in the diets and used as an indigestible marker.

The amount of feed provided daily was calculated as 2.5 times the maintenance requirement of energy (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Pigs were fed once daily at 0800 h. At the beginning of each period, feed allowance was adjusted based on the BW of each pig. Water was available at all times.

Sample Collection

Each period lasted 7 d. The initial 5 d were considered an adaptation period to the diet. On d 6 and d 7, ileal digesta were collected for 8 h. A plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were replaced whenever they were filled with digesta, or at least once every 30 min, and immediately frozen at – 20^{o}C to prevent bacterial degradation of AA in the digesta.

Chemical Analyses

At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized, finely ground, and analyzed. A sample of each diet and of each batch of canola meal was collected at the time of diet mixing. Diets, ingredients, and ileal samples were analyzed for AA by ion-exchange chromatography with postcolumn derivatization with ninhydrin. Cysteine and Met were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 *N* HCL for 24 h at 110°C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide

octahydrate for 20 h at 110°C (Commission Directive, 2000). Diets, ingredients, and ileal samples were also analyzed for DM (Method 935.29; AOAC International, 2007), and for CP following the Dumas procedure (Method 968.06; AOAC International, 2007). Diets and ileal samples were also analyzed for chromium (Method 990.08; AOAC International, 2007). Ingredients were analyzed for ash (Method 942.05; AOAC International, 2007), ADF (Method 973.18; AOAC International, 2007), NDF (Holst, 1973), lignin (method 973.18 (A-D); AOAC International, 2007), furosine as described by Kim et al. (2012), total reducing sugars (Dubois et al., 1956), ADIN (method 990.03; AOAC International, 2007), Ca and P by inductively coupled plasma (ICP) spectroscopy (Method 985.01; AOAC International, 2007), total fat by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06; AOAC International, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN), and for total glucosinolates (Method ISO 9167-1:1992, Eurofins, Des Moines, IA). Minolta L* (lightness), a* (redness), and b* (yellowness) values for each batch of canola meal were determined (8 mm aperture, D65 light source, and 0° observer, Minolta Camera Co., Osaka, Japan).

Calculations and Statistical Analysis

The AID and SID values were calculated as previously described (Stein et al., 2007). The Lys:CP ratio in each canola meal sample was calculated by expressing the concentration of Lys in the sample as a percentage of the CP in the sample (Stein et al., 2009), and the concentration of reactive Lys was calculated as previously described using furosine to indicate the concentration of regenerated Lys (Kim et al., 2012). Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). The presence of outliers was evaluated using the UNIVARIATE procedure of SAS. The model included diet as a fixed effect and pig and period

as random effects. Linear and quadratic effects of increasing time of heat treatment on the AID and SID of AA were analyzed by orthogonal polynomial contrasts. Regression equations to estimate the concentration of SID AA were developed using the REG procedure in SAS. The pig was the experimental unit and significance among means was assessed with an α level of 0.05.

RESULTS

Pigs recovered well after the surgery. Feed intake was normalized within a week post-surgery. Pigs remained healthy during the experiment, and no feed refusals were observed.

The concentrations of ADF were 20.00, 23.63, 22.73, and 31.30% in non-autoclaved canola meal, and canola meal autoclaved for 20, 30, or 45 min, respectively (Table 4.1). The non-autoclaved canola meal contained 33.36% NDF, whereas the autoclaved canola meal contained 42.18, 41.95, and 46.88% NDF (20, 30, and 45 min, respectively). Non-autoclaved canola meal contained 8.02% lignin, but the concentrations of lignin in canola meals that were autoclaved for 20, 30, and 45 min were 10.74, 10.96, and 16.45%, respectively. The concentration of ADIN in the non-autoclaved canola meal was 0.37%, but autoclaving canola meal for 20, 30, or 45 min resulted in ADIN concentrations of 0.80, 0.88, and 1.75%, respectively. The concentration of reducing sugars in autoclaved canola meal was 4.20, 4.34, and 3.31% (20, 30, and 45 min, respectively), whereas the concentration of reducing sugars in non-autoclaved canola meal was 5.05%. The concentration of Lys was 1.36% in the canola meal autoclaved for 45 min vs. 1.92% in non-autoclaved canola meal. Non-autoclaved canola meal contained 0.016% furosine, but autoclaved canola meal contained 0.033, 0.033, and 0.025% furosine (20, 30, and 45 min, respectively). The concentration of reactive Lys was 1.90% in non-autoclaved canola meal, whereas canola meal that was autoclaved for 20, 30 and 45 min

contained 1.53, 1.47, and 1.33% reactive Lys, respectively. Lightness (color L*) was 52.88 in non-autoclaved canola meal, 47.19 in canola meal autoclaved for 20 min, 47.63 in canola meal autoclaved for 30 min, and 45.08 in canola meal autoclaved for 45 min.

The AID of CP and all AA was reduced (quadratic, $P < 0.01$) as a result of increasing time of autoclaving (Table 4.4). Autoclaving of canola meal also reduced (quadratic, $P < 0.01$) the SID of CP and all AA (Table 4.5).

A regression equation that uses the concentration (%) of reactive Lys as an independent variable may be used to predict the concentration (%) of SID Lys in heat-damaged canola meal (SID Lys = -1.66 + 1.60 × reactive Lys; $r^2 = 0.83$; Table 4.6). The concentration (%) of SID Lys in canola meal may be also calculated by regression equation using the concentration (%) of reducing sugars (SID Lys = -1.65 + 0.59 × reducing sugars; $r^2 = 0.97$). The concentration of lignin (%) may be used to predict the concentration (%) of SID Met (SID Met = 0.76 - 0.02 × lignin; $r^2 = 0.93$), whereas the concentrations of ADF in combination with the concentration of reducing sugars may be used to predict the concentration of SID Thr [SID Thr = 3.16 – (0.06 × ADF) – (0.15 × reducing sugars); $r^2 = 0.89$] and SID Trp [SID Trp = 0.99 – (0.018 × ADF) – (0.05 × reducing sugars); $r^2 = 0.88$].

DISCUSSION

Canola meal contains glucosinolates, which are antinutritional factors that may reduce feed intake because of their bitter taste, and which also may decrease growth performance (Seneviratne et al, 2010). Canola meal should not be included in diets of pigs at inclusion levels that result in dietary total concentration of glucosinolates that exceed 2 μmol/g (Arntfield and Hickling, 2011). Total glucosinolates in the non-autoclaved canola meal used in this experiment

was 1.58 μmol/g and even at relatively high inclusion levels of canola meal (42%) in the experimental diets, the calculated concentration of glucosinolates in the mixed diets (0.66 μmol/g) was well below the maximum recommended levels. Therefore, we did not expect any negative effects of glucosinolates on feed intake of pigs during the experiment.

During the desolventization and toasting processes of canola meal, injection of live steam and temperatures ranging from 95 to 115°C during an average of 30 min are commonly used (Canola Council of Canada, 2009). Processing, however, may not be consistent among processors and this may create some variability in the nutritional composition of canola meal (Spragg and Mailer, 2007). The concentration of Lys in different sources of canola meal ranges from 1.64 to 2.40% (Spragg and Mailer, 2007; Newkirk, 2011). Because some of this variation may be a result of heat damage and because the concentration of Lys in canola meal used in this experiment ranged from 1.36 to 1.92%, we believe that heat damage caused by autoclaving in this experiment is equivalent to heat damage that may be caused by processing conditions at different processing plants.

The concentrations of DM, CP, and AA in the non-autoclaved canola meal used in the experiment were in agreement with previously published values (Mariscal-Landín et al., 2008, NRC, 2012). The increase in analyzed ADF and lignin observed as a result of increased time of autoclaving also has been observed for Italian ryegrass (Miao et al., 1994). The analyzed concentrations of ADF and lignin may increase because some melanoidins, which are polymers originating from Maillard reactions, may be analyzed as ADF or lignin (Marlett and Johnson, 1985; Miao et al., 1994). Consequently, heat treatment of feed ingredients is expected to increase analyzed values of ADF, NDF, and lignin. Our results for the concentration of ADIN in heat-damaged canola meal are in agreement with previous observations in which the concentration of

ADIN increased as a result of heat damage (Cromwell et al., 1993; Schroeder et al., 1996; Seifdavati and Taghizadeh, 2012). This observation indicates that ADIN may be used as an indicator of heat damage in canola meal. The concentrations of reducing sugars and Lys were concomitantly reduced as time of autoclaving increased, and this was expected and clearly reflects the occurrence of Maillard reactions as a result of heat damage, in which the carbonyl group of reducing sugars reacts with the epsilon amino group of Lys to form Amadori compounds and other Maillard reaction products (Nursten, 2005). We observed that the concentration of furosine increased with autoclaving of canola meal up to 30 min, but the concentration of furosine was slightly reduced in canola meal that was autoclaved for 45 min. The reason for these observations is that furosine is a product of Amadori compounds subjected to acid hydrolysis (Boucher et al., 2009). Amadori compounds are formed during the early Maillard reaction stage, but if the reaction progresses to more advanced stages, the Amadori compounds are converted to advanced Maillard reaction products (Nursten, 2005). Therefore, autoclaving of canola meal for 45 min likely resulted in conversion of Amadori compounds to advanced Maillard reaction products, thus causing a reduction in the concentration of furosine. Although we observed a numerical decrease in the Lys concentration of canola meal as time of autoclaving increased, the concentration of CP remained unaffected regardless of the degree of heat damage. Thus, the calculated Lys:CP ratio also decreased as time of autoclaving increased. It has been suggested that the Lys:CP ratio may be used as an indicator of heat damage in feed ingredients and the current results support this assumption (Cozannet et al., 2010). A change in color of canola meal (i.e., less yellow and more brown) also has been observed after the desolventization and toasting processes (Newkirk et al., 2003), which is likely a result of the

formation of advanced Maillard reaction products such as pre-melanoidins and melanoidins that give a brown pigmentation to heat-damaged feeds (Nursten, 2005).

Values for the AID of CP and AA that were determined in this experiment for non-autoclaved canola meal are in agreement with previously published values (Fan and Sauer, 1995; Stein et al., 2005; NRC, 2012). The observation that the AID of most AA is reduced due to autoclaving is in agreement with data for autoclaved SBM (Fontaine et al., 2007; González-Vega et al., 2011). Values for the SID of AA in the non-autoclaved canola meal determined in this experiment are also in agreement with previously reported values (Sauvant et al., 2004; Stein et al., 2005; Woyengo et al., 2010; Rostagno et al., 2011). The reason the SID of Lys is reduced more than the SID of other AA is that in the presence of heat, moisture, and pressure, reducing sugars condense with the epsilon amino group of Lys (Nursten, 2005). This reaction initiates a series of other reactions. After Lys has reacted with reducing sugars, Amadori compounds are generated, and in more advanced stages, pre-melanoidins and melanoidins are generated, which reduces the digestibility of Lys. The observed reduction in SID of other AA may be associated with their direct participation in Maillard reactions (e.g., Cys and Arg) or with the formation of cross-links that impair digestibility (Finot et al., 1990; Ledl and Schleicher, 1990).

Regression equations developed in the experiment to predict the concentration of SID Lys in heat-damaged canola meal have a relatively high r^2, which indicates that variations are well explained by the models. The concentration of reactive Lys, calculated by the furosine procedure, is a good predictor for the concentration of SID Lys in heat-damaged canola meal. To our knowledge, this is the first time that the usefulness of reactive Lys, determined by the furosine procedure, to predict the concentration of SID Lys in canola meal fed to pigs is demonstrated. The concentration of SID Lys in DDGS is also accurately ($r^2 = 0.90$) predicted

from the concentration of reactive Lys (Kim et al., 2012). The concentration of analyzed reducing sugars is also a good predictor of the concentration of SID Lys in canola meal that has been heat-damaged. Regression equations developed in this experiment are valid if the same source of canola meal was used, as was the case in this experiment. Further research, however, needs to be conducted to determine if the use of reducing sugars as a predictor for the concentration of SID Lys in different sources of canola meal is applicable.

Conclusions

Results of this experiment confirm that the concentration and digestibility of AA in canola meal are reduced as a consequence of heat damage. This indicates that some of the variations in AA concentration and digestibility among different sources of canola meal fed to pigs are likely caused by differences in processing, specifically, the desolventization step, which likely causes Maillard reactions. Therefore, standardization of desolventization steps among processing plants may be beneficial to feed manufacturers and to the livestock industry, as it may create a product that is less variable in AA composition and digestibility. Regression equations developed in this experiment may be used to predict the concentration of SID AA in canola meal.

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TABLES

Table 4.1. Chemical composition of canola meal

	Canola meal			
		Autoclaved at 130°C		
Item[1]	Non-autoclaved	20 min	30 min	45 min
DM, %	90.87	89.44	89.78	88.42
Ash, %	7.54	7.47	7.66	7.55
CP, %	36.79	36.49	36.88	36.90
ADF, %	20.00	23.63	22.73	31.30
NDF, %	33.36	42.18	41.95	46.88
Lignin, %	8.02	10.74	10.96	16.45
ADIN,[1] %	0.37	0.80	0.88	1.75
Reducing sugars, %	5.05	4.20	4.34	3.31
AEE,[1] %	3.71	3.34	3.79	1.97
Total glucosinolates, μmol/g	1.58	-	-	-
Ca, %	0.64	0.61	0.65	0.62
P, %	1.05	1.03	1.02	0.99
Lys:CP ratio[2]	5.22	4.30	4.09	3.69
Furosine	0.016	0.033	0.033	0.025
Reactive Lys[3]	1.90	1.53	1.47	1.33
L*[4]	52.88	47.19	47.63	45.08
a*[4]	5.76	6.26	5.97	6.35

Table 4.1. (Cont.)

b*[4]	12.56	8.93	8.75	7.44
Indispensable AA, %				
Arg	2.22	1.96	1.90	1.74
His	0.97	0.92	0.90	0.91
Ile	1.41	1.40	1.32	1.35
Leu	2.60	2.55	2.50	2.55
Lys	1.92	1.57	1.51	1.36
Met	0.74	0.72	0.71	0.72
Phe	1.48	1.45	1.42	1.44
Thr	1.62	1.60	1.58	1.61
Trp	0.49	0.48	0.49	0.49
Val	1.81	1.79	1.70	1.72
All indispensable	15.26	14.44	14.03	13.89
Dispensable AA, %				
Ala	1.65	1.62	1.60	1.63
Asp	2.65	2.58	2.55	2.55
Cys	0.87	0.78	0.76	0.74
Glu	6.29	6.17	6.11	6.20
Gly	1.88	1.84	1.81	1.84
Pro	2.15	2.14	2.09	2.10
Ser	1.61	1.58	1.59	1.60
All dispensable	17.10	16.71	16.51	16.66

Table 4.1. (Cont.)

Total AA	32.36	31.15	30.54	30.55

[1]ADIN = acid detergent insoluble nitrogen; AEE = acid hydrolyzed ether extract.

[2]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

[3]Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)]; Pahm et al., 2008.

[4]L* = lightness; a* = redness; b* = yellowness.

Table 4.2. Ingredient composition of experimental diets, as-fed basis

	Canola meal				
		Autoclaved at 130°C			
Ingredient, %	Non-autoclaved	20 min	30 min	45 min	N-free diet
Canola meal	42.00	42.00	42.00	42.00	-
Cornstarch	42.15	42.15	42.15	42.15	66.40
Sucrose	10.00	10.00	10.00	10.00	20.00
Solka floc[1]	-	-	-	-	5.00
Soybean oil	3.40	3.40	3.40	3.40	4.00
Ground limestone	0.70	0.70	0.70	0.70	-
Monocalcium phosphate	0.65	0.65	0.65	0.65	3.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30	0.30

[1]Fiber Sales and Development Corp., Urbana, OH.

[2]Provided the following per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.3. Chemical composition of experimental diets, as-fed basis

	Canola meal				
		Autoclaved at 130°C			
Item	Non-autoclaved	20 min	30 min	45 min	N-free diet
DM, %	91.86	91.24	91.01	91.36	92.66
CP, %	17.17	15.24	15.09	15.04	0.19
Indispensable AA, %					
Arg	0.96	0.82	0.79	0.71	-
His	0.42	0.39	0.38	0.37	-
Ile	0.59	0.57	0.56	0.55	-
Leu	1.13	1.07	1.05	1.03	-
Lys	0.83	0.66	0.64	0.56	-
Met	0.31	0.28	0.27	0.27	-
Phe	0.64	0.61	0.60	0.58	-
Thr	0.71	0.67	0.65	0.65	-
Trp	0.21	0.20	0.19	0.20	-
Val	0.77	0.73	0.72	0.70	-
All indispensable	6.57	6.00	5.85	5.62	-
Dispensable AA, %					
Ala	0.73	0.68	0.67	0.66	-
Asp	1.18	1.10	1.07	1.05	-
Cys	0.38	0.33	0.32	0.29	-
Glu	2.77	2.63	2.57	2.53	-

Table 4.3. (Cont.)

Gly	0.82	0.77	0.75	0.74	-
Pro	0.95	0.90	0.86	0.85	-
Ser	0.72	0.67	0.65	0.65	-
All dispensable	7.55	7.08	6.89	6.77	-

Table 4.4. Apparent ileal digestibility of CP and AA in canola meal subjected to increasing levels of heat treatment by growing pigs[1]

	Canola meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	20 min	30 min	45 min		Linear	Quadratic
CP, %	59.75	48.55	51.32	22.66	2.5	< 0.01	< 0.01
Indispensable AA, %							
Arg	78.72	74.14	76.74	58.61	2.8	< 0.01	< 0.01
His	77.03	73.87	74.83	56.17	1.1	< 0.01	< 0.01
Ile	69.21	65.23	65.94	43.98	1.1	< 0.01	< 0.01
Leu	74.95	71.28	71.41	52.79	0.9	< 0.01	< 0.01
Lys	61.89	48.35	49.36	12.91	1.5	< 0.01	< 0.01
Met	81.14	77.83	78.14	64.05	0.6	< 0.01	< 0.01
Phe	74.28	71.57	72.09	53.01	1.3	< 0.01	< 0.01
Thr	63.17	59.17	59.21	36.21	1.2	< 0.01	< 0.01
Trp	66.28	64.03	63.13	44.06	1.1	< 0.01	< 0.01
Val	67.42	62.50	63.38	39.00	1.1	< 0.01	< 0.01

Table 4.4. (Cont.)

Mean	71.98	67.05	67.90	46.15	1.0	< 0.01	< 0.01
Dispensable AA, %							
Ala	62.98	54.18	56.26	27.75	2.1	< 0.01	< 0.01
Asp	63.93	55.38	56.60	27.69	1.2	< 0.01	< 0.01
Cys	69.41	65.19	64.57	40.21	1.2	< 0.01	< 0.01
Glu	79.11	76.52	76.56	60.61	1.0	< 0.01	< 0.01
Gly	46.68	34.23	36.01	-2.87	6.0	< 0.01	< 0.01
Ser	66.01	61.62	62.08	40.63	1.2	< 0.01	< 0.01
Mean	64.69	57.91	58.68	32.33	1.9	< 0.01	< 0.01

[1]Data are means of 10 observations.
[2]Linear and quadratic effects of time of autoclaving.

Table 4.5. Standardized ileal digestibility of CP and AA in canola meal subjected to increasing levels of heat treatment by growing pigs[1]

	Canola meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	20 min	30 min	45 min		Linear	Quadratic
CP, %	71.69	61.96	64.78	34.54	2.5	< 0.01	< 0.01
Indispensable AA, %							
Arg	84.64	81.02	83.86	66.56	2.8	< 0.01	< 0.01
His	81.34	78.48	79.55	61.04	1.1	< 0.01	< 0.01
Ile	75.42	71.61	72.43	50.61	1.1	< 0.01	< 0.01
Leu	80.25	76.84	77.07	58.58	0.9	< 0.01	< 0.01
Lys	68.17	56.24	57.43	20.81	1.5	< 0.01	< 0.01
Met	85.08	82.15	82.62	68.54	0.6	< 0.01	< 0.01
Phe	80.25	77.79	78.40	59.56	1.3	< 0.01	< 0.01
Thr	71.48	67.92	68.20	45.24	1.2	< 0.01	< 0.01
Trp	73.88	71.95	71.45	51.99	1.1	< 0.01	< 0.01

Table 4.5. (Cont.)

Val	74.04	69.44	70.39	46.24	1.1	< 0.01	< 0.01
Mean	77.85	73.44	74.44	53.02	1.0	< 0.01	< 0.01
Dispensable AA, %							
Ala	74.20	66.14	68.36	40.08	2.1	< 0.01	< 0.01
Asp	71.37	63.31	64.74	36.02	1.2	< 0.01	< 0.01
Cys	75.59	72.26	71.85	48.27	1.2	< 0.01	< 0.01
Glu	83.33	80.94	81.07	65.21	1.0	< 0.01	< 0.01
Gly	69.21	58.06	60.42	21.96	6.0	< 0.01	< 0.01
Ser	74.34	70.52	71.23	49.81	1.2	< 0.01	< 0.01
Mean	74.68	68.59	69.61	43.56	1.9	< 0.01	< 0.01

[1]Data are means of 10 observations; Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI), which were determined by feeding pigs a N-free diet: CP, 22.32; Arg, 0.62; His, 0.20; Ile, 0.40; Leu, 0.65; Lys, 0.57; Met, 0.13; Phe, 0.42; Thr, 0.64; Trp, 0.17; Val, 0.55; Ala, 0.89; Asp, 0.96, Cys, 0.26; Glu, 1.27; Gly, 2.01; and Ser, 0.65.

[2]Linear and quadratic effects of time of autoclaving.

Table 4.6. Linear regressions to predict the concentration (%) of standardized ileal digestible (SID) AA in canola meal fed to pigs[1]

Dependent variable	Intercept			Independent variables[2]								
	Estimate	SE	*P*-value	X_1	Estimate	SE	*P*-value	X_2	Estimate	SE	*P*-value	r^2
SID Arg	-0.20	0.16	0.23	RS	0.41	0.03	< 0.01	-	-	-	-	0.76
SID His	1.02	0.02	< 0.01	Lignin	-0.03	0.001	< 0.01	-	-	-	-	0.89
SID Ile	0.81	0.12	< 0.01	NDF	0.01	0.004	< 0.01	ADIN	-0.40	0.04	< 0.01	0.92
SID Leu	2.38	0.14	< 0.01	NDF	0.01	0.005	< 0.05	Lignin	-0.09	0.009	< 0.01	0.92
SID Lys	-1.66	0.19	< 0.01	RL	1.60	0.12	< 0.01	-	-	-	-	0.83
	-1.65	0.09	< 0.01	RS	0.59	0.02	< 0.01	-	-	-	-	0.96
SID Met	0.76	0.01	< 0.01	Lignin	-0.02	0.0007	< 0.01	-	-	-	-	0.93
SID Phe	1.80	0.05	< 0.01	ADF	-0.03	0.002	< 0.01	-	-	-	-	0.84
SID Thr	3.16	0.55	< 0.01	ADF	-0.06	0.01	< 0.01	RS	-0.15	0.07	< 0.05	0.89
SID Trp	0.99	0.15	< 0.01	ADF	-0.018	0.0028	< 0.01	RS	-0.05	0.02	< 0.05	0.88
SID Val	1.01	0.16	< 0.01	NDF	0.02	0.004	< 0.01	ADIN	-0.55	0.05	< 0.01	0.93

[1]n = 39 observations; for all models $P < 0.01$.

[2]ADIN = acid detergent insoluble nitrogen; RS = reducing sugars; RL = Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)].

CHAPTER 5

AMINO ACID DIGESTIBILITY OF HEAT DAMAGED SUNFLOWER MEAL AND COTTONSEED MEAL FED TO GROWING PIGS

ABSTRACT: Two experiments were conducted to determine the effects of heat damage, achieved by autoclaving, on the nutritional composition and on the standardized ileal digestibility (**SID**) of AA in sunflower meal (**SFM**), and cottonseed meal (**CSM**) fed to growing pigs. The second objective was to test the hypothesis that the concentration of SID AA in SFM and CSM may be predicted from the nutrient composition of the ingredients. In Exp. 1, ten growing pigs (initial BW: 23.1 ± 1.3 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. A common source of SFM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 20, 40, or 60 min. Four diets that contained each of the 4 batches of SFM were formulated, and SFM was the only source of CP and AA in the diets. A N-free diet that was used to determine the endogenous losses of CP and AA from pigs was also formulated. Each period consisted of 5 d of adaptation to the diets followed by 2 d of ileal digesta collection. The SID of Lys in SFM was reduced (linear, $P < 0.05$) from 83.2 to 63.5% in non-autoclaved SFM or SFM autoclaved for 60 min at 130°C, respectively. The concentrations of Lys and reducing sugars in SFM may be used as predictors ($r^2 = 0.85$) of the concentration of SID Lys in SFM. In Exp. 2, ten growing pigs (initial BW: 35.0 ± 1.5 kg) were surgically equipped with a T-cannula in the distal ileum and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. A source of CSM was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 15, 35, or 60 min. Four diets containing CSM as the only source of CP and AA were formulated. A N-free diet was also formulated and used as described

for Exp. 1. The SID of Lys in non-autoclaved CSM (66.2%) was greater ($P < 0.05$) than in autoclaved (60 min at 130°C) CSM (54.1%). The equation ($r^2 = 0.68$) that best predicted the concentration of SID Lys in CSM includes the concentration of ADIN. In both experiments, the SID of most AA was reduced (linear or quadratic, $P < 0.05$) as a result of heat damage. In conclusion, heat damage reduces the SID of AA in SFM and CSM, and the concentration of SID Lys in these ingredients may be predicted from the concentration of ADIN, Lys, or reducing sugars.

Key words: amino acids, cottonseed meal, digestibility, heat damage, pigs, sunflower meal

INTRODUCTION

Sunflower meal and cottonseed meal are alternative protein sources for swine diets. Solvent extraction of oil from sunflower and cottonseed involves application of heat and moisture to desolventize the meal. Cottonseed meal contains the antinutritional factor, gossypol, which may be deactivated by heat treatment of the meal. Processes involving heat and moisture may cause Maillard reactions (Nursten, 2005), and the application of heat to feed ingredients may decrease the concentration, digestibility, and utilization of Lys and other AA (Van Barneveld et al., 1994; Pahm et al., 2008; Boucher et al., 2009; González-Vega et al., 2011). Maillard reactions start with the condensation between an amino group of AA and the carbonyl group of a reducing sugar (Nursten, 2005). Amino acids that participate in the Maillard reactions may become unavailable to pigs and Lys is the AA most susceptible to participate in these reactions (Pahm et al., 2008). Conventional AA analysis of heat-damaged feed ingredients are believed to overestimate the concentration of Lys because Lys that has reacted with reducing sugars is partially recovered during the acid hydrolysis step although they are not available for

protein synthesis. This overestimation of the concentration of Lys in heat-damaged feed ingredients may result in inaccuracies in diet formulation, which ultimately may result in decreased performance of pigs. Determination of reactive Lys, color, and the Lys:CP ratio have been suggested as approaches to estimate the availability of Lys in heat processed feed ingredients (Moughan and Rutherfurd, 1996; Fontaine et al., 2007; Pahm et al., 2008; Kim et al., 2012). There is, however, limited information about the effects of heat processing on AA digestibility in sunflower meal and cottonseed meal, and to our knowledge, the use of chemical composition and physical characteristics either alone or in combination to predict the concentration of digestible AA in sunflower meal and cottonseed meal that have been heat-damaged have not been reported. Therefore, the primary objectives of these experiments were to determine the effects of heat damage on the apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of AA in sunflower meal and in cottonseed meal fed to growing pigs. A second objective was to develop regression equations that may be used to predict the concentration of SID AA in sunflower meal and cottonseed meal.

MATERIALS AND METHODS

The protocols for these experiments were reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiments were the offspring of G-performer boars mated to F-25 females (Genetiporc, Alexandria. MN).

Exp. 1 (AA Digestibility of Sunflower Meal)

Animals, Experimental Design, and Diets. Ten growing pigs (initial BW: 23.1 ± 1.3 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were placed

in pens (1.2 × 1.5 m) equipped with a nipple drinker and a feeder. Sunflower meal was sourced from Archer Daniels Midland (Enderlin, ND). The sunflower meal was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 20, 40, or 60 min. Four diets that contained each of the 4 batches of sunflower meal were formulated. Sunflower meal was the only source of CP and AA in the diets. A N-free diet used to determine the endogenous losses of CP and AA in the pigs also was formulated.

Feed allowance was calculated as 3 times the maintenance requirement for energy (i. e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Feed allowance was adjusted according to the BW of pigs at the beginning of each period. Feed was provided once daily at 800 h and water was available at all times.

Sample Collection. Each period consisted of 7 d. The initial 5 d were considered an adaptation period to the diet. Ileal digesta were collected on d 6 and 7 for 8 h by attaching a plastic bag to the cannula barrel and digesta flowing into the bag were collected. Bags were replaced whenever they were filled with digesta, or at least once every 30 min, and immediately stored at – 20^{o}C to prevent bacterial degradation of AA in the digesta.

Chemical Analyses. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized, finely ground, and analyzed. A sample of each diet and of each batch of sunflower meal was collected at the time of diet mixing. Diets, ingredients, and ileal samples were analyzed for AA by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6*N* HCL for 24 h at 110°C and quantified with the internal standard by measuring the absorption of reaction

products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm) after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000). Diets, ingredients, and ileal samples were also analyzed for DM (Method 935.29; AOAC International, 2007), and for CP following the Dumas procedure (Method 968.06; AOAC International, 2007), and diets and ileal samples were analyzed for chromium (Method 990.08; AOAC International, 2007). Each batch of sunflower meal was also analyzed for ash (Method 942.05; AOAC International, 2007), ADF (Method 973.18; AOAC International, 2007), NDF (Holst, 1973), lignin (method 973.18 (A-D); AOAC International, 2007), ADIN (method 990.03; AOAC International, 2007), total reducing sugars (Dubois et al., 1956), furosine as described by Kim et al. (2012), for Ca and P by inductively coupled plasma (ICP) spectroscopy (Method 985.01; AOAC International, 2007), and for total fat by acid hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06; AOAC International, 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). The Minolta L* (lightness) value for each batch of sunflower meal was also determined (8 mm aperture, D65 light source, and 0° observer, Minolta Camera Company, Osaka, Japan).

Calculations and Statistical Analysis. Values for AID and SID of CP and AA in each batch of sunflower meal were calculated (Stein et al., 2007), and the Lys:CP ratio in each batch was calculated by expressing the concentration of Lys in the sample as a percentage of the CP in the sample (Kim et al., 2012). The concentration of reactive Lys was calculated from the concentration of furosine as described by Kim et al. (2012). Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Normality of the data and the presence of outliers were evaluated using the UNIVARIATE procedure of SAS. The model included diet as a fixed

effect and pig and period as random effects. Linear and quadratic effects of increasing time of heat treatment on the AID and SID of AA were analyzed by orthogonal polynomial contrasts. Regression equations to estimate the concentration of SID AA were developed using the REG procedure in SAS. The forward selection method was used to choose the equations that best fit the data, but in the case of the regression equation to predict the concentration of SID Lys, the concentration of reactive Lys was forced into the model as an independent variable. The pig was the experimental unit and significance among means was assessed with an α level of 0.05.

Exp. 2 (AA Digestibility of Cottonseed Meal)

Animals, Experimental Design, and Diets. Ten growing pigs (initial BW: 35.0 ± 1.5 kg) were surgically equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a replicated 5 × 5 Latin square design with 5 diets and 5 periods in each square. Pigs were placed in pens (1.2 × 1.5 m) equipped with a nipple drinker and a feeder. Cottonseed meal was procured from Delta Oil Mill (Jonestown, MS). The cottonseed meal was separated into 4 batches that were either not autoclaved or autoclaved at 130°C for 15, 35, or 60 min. Four diets that contained each of the 4 batches of cottonseed meal were formulated, and cottonseed meal was the only source of CP and AA in the diets. A N-free diet that was used to determine the endogenous losses of CP and AA in the pigs was also formulated.

Feed allowance was calculated as 3 times the maintenance requirement of energy (i. e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Feed allowance was adjusted according to the BW of pigs at the beginning of each period. Feed was provided once daily at 0800 h and water was available at all times.

Ileal samples were collected from pigs as described for Exp. 1 and ileal samples, diets, and ingredients were processed and chemically analyzed as described for Exp. 1. Each batch of

cottonseed meal was also analyzed for free gossypol (Method Ba 8a-99; AOCS, 1998). Data were analyzed as described for Exp. 1.

RESULTS

Exp. 1 (Sunflower Meal)

The concentration of CP was 35.92% in the non-autoclaved sunflower meal whereas the sunflower meals that were autoclaved for 20, 40, or 60 min contained 36.55, 36.11, and 35.35% CP, respectively (Table 5.1). Non-autoclaved sunflower meal contained 21.60% ADF, but the ADF concentrations were 22.80, 19.87, and 24.12% in sunflower meals that were autoclaved for 20, 40, or 60 min, respectively. The concentration of NDF was 31.90, 34.88, 34.90, and 43.21% in non-autoclaved sunflower meal, and sunflower meals that were autoclaved for 20, 40, or 60 min, respectively. The concentration of lignin in the sunflower meals that were autoclaved for 20, 40, and 60 min was 5.42, 5.66, and 5.73%, respectively, and the concentration of lignin in non-autoclaved sunflower meal was 5.59%. Non-autoclaved sunflower meal contained 0.22% ADIN, and the concentration of ADIN in sunflower meal that was autoclaved for 20, 40, and 60 min was 0.18, 0.25, and 0.28%, respectively. Autoclaving of sunflower meal for 20, 40, or 60 min resulted in concentrations of reducing sugars of 4.43, 4.18, and 3.74%, respectively, but the concentration of reducing sugars in non-autoclaved sunflower meal was 4.64%. The concentrations of Lys and reactive Lys were 1.23 and 1.21% in non-autoclaved sunflower meal, 1.09 and 1.08% in sunflower meal autoclaved for 20 min, 1.08 and 1.05% in sunflower meal autoclaved for 40 min, and 0.98 and 0.94% in sunflower meals that were autoclaved for 60 min. Non-autoclaved sunflower meal contained 0.013% furosine, whereas the concentration of furosine was 0.012, 0.021, and 0.030% in sunflower meal autoclaved for 20, 40, and 60 min,

respectively. The concentration of Lys expressed as a percentage of the concentration of CP was 3.42 in the non-autoclaved sunflower meal whereas for the sunflower meals that were autoclaved for 20, 40, or 60 min, the concentration of Lys expressed as a percentage of the concentration of CP was 2.98, 2.99, and 2.77, respectively.

The AID of CP decreased (linear, $P < 0.01$) as the time of autoclaving increased (Table 5.4). Likewise, increasing the time of autoclaving decreased (linear, $P < 0.01$) the AID of all AA. The SID of CP was also reduced (linear, $P < 0.01$) by increasing the time of autoclaving (Table 5.5). For all AA, increasing the time of autoclaving reduced (linear, $P < 0.01$) the SID of AA. The concentration of SID Lys in sunflower meal may be predicted ($P < 0.01$) from the concentration (%) of analyzed Lys in combination with the concentration (%) of reducing sugars using the following equation: SID Lys (%) = -1.00 + 0.54 × Lys + 0.30 × reducing sugars (r^2 = 0.85; Table 5.6).

Exp. 2 (Cottonseed Meal)

The concentration of CP was relatively unaltered regardless of time of autoclaving (Table 5.1). The lignin concentration in non-autoclaved cottonseed meal was 5.52%, and cottonseed meals that were autoclaved for 15, 35, and 60 min contained 5.73, 6.49, and 6.68% of lignin, respectively. Non-autoclaved cottonseed meal contained 0.20% of ADIN, whereas cottonseed meals that were autoclaved for 15, 35, and 60 min contained 0.25, 0.29, and 0.27% of ADIN, respectively. Non-autoclaved cottonseed meal contained 3.59% reducing sugars, but cottonseed meals that were autoclaved for 15, 35, or 60 min contained 3.47, 1.76, or 2.31% reducing sugars, respectively. The Lys concentration in non-autoclaved cottonseed meal was 1.64%, whereas the concentration of Lys was 1.59, 1.52, and 1.52% for autoclaved cottonseed meal (15, 35, and 60 min, respectively). Non-autoclaved cottonseed meal contained 0.027% furosine, whereas

cottonseed meal autoclaved for 15, 35, and 60 min contained 0.040, 0.040, and 0.030% furosine, respectively. Non-autoclaved cottonseed meal contained 1.61% reactive Lys, but the concentration of reactive Lys in cottonseed meal that was autoclaved for 15, 35, and 60 min was 1.54, 1.47, and 1.48%, respectively.

The AID of all indispensable AA in cottonseed meal was quadratically decreased ($P < 0.01$) as time of autoclaving increased from 0 to 60 min (Table 5.7). Likewise, the SID of all AA in cottonseed meal was reduced (quadratic, $P < 0.01$) with increasing time of autoclaving (Table 5.8). The SID of Lys (54.42, 49.75, and 54.10%) in cottonseed meal autoclaved for 15, 35, and 60 min, respectively, was less ($P < 0.05$) than the SID of Lys in non-autoclaved cottonseed meal (66.21%).

For most AA, the SID AA (%) in cottonseed meal may be predicted from the concentration (%) of ADIN and from the concentration of other nutritional components, either alone or in combination (Table 5.9). The concentration (%) of SID Lys may be predicted using the following equation: SID Lys = 1.81 - 3.67 × ADIN, $r^2 = 0.68$;

DISCUSSION

Pigs maintained good health status throughout the experiments and pigs used in Exp. 1 consumed their diets well. At the end of period 3 in Exp. 2, some pigs refused to consume all of their daily allotments. It has been shown that free gossypol, which is an antinutritional factor in cottonseed meal, is toxic to animals and if present in diets in amounts greater than 100 mg/kg may cause depressed appetite (Tanksley and Knabe, 1981; Akande et al., 2010). Cottonseed meal used in this experiment, however, contained concentrations of free gossypol below detection levels and even at relatively high inclusion levels in the diets, the concentration of free gossypol

in the cottonseed meal diets was below 100 mg/kg. Diets used in the cottonseed meal experiment were also supplemented with ferrous sulfate, which has been reported to mitigate gossypol toxicity (Moreira et al., 2006). Nevertheless, after period 3, all pigs were fed regular commercial diets for 10 d and, when given the experimental diets for the subsequent experimental periods, no issues with feed consumption were observed.

Effects of Autoclaving on Nutrient Composition

The nutrient composition of non-autoclaved sunflower meal and cottonseed meal are in agreement with the values reported for these ingredients (Rostagno et al., 2011; NRC, 2012). Some variation in the nutritional composition among different sources of feed ingredients exists, and these variations may be caused by heat processing used during production of sunflower meal and cottonseed meal. As an example, heat damage increases the analyzed concentrations of ADF and lignin in hay because of the formation of Maillard products that are analyzed as lignin (Miao et al., 1994). The concentration of ADIN in orchardgrass and alfalfa also increases as length of exposure to heat increases, although the increase in ADIN concentration in orchardgrass was of a greater proportion than that observed for alfalfa (Goering et al., 1973). Heat processing of sunflower expellers at 150°C for different lengths of time also resulted in increased concentrations of ADIN (Schroeder et al., 1996). Our results for the concentrations of ADIN in sunflower meal support the above observations. In the Maillard reactions, reducing sugars and Lys are the primary substrates and, therefore, as the reactions progress to form Amadori compounds and other Maillard reaction products, it is expected that the concentration of substrates decreases (Nursten, 2005). The present results demonstrate this, as the concentrations of reducing sugars and Lys were reduced as the length of time sunflower meal and cottonseed meal were autoclaved increased. It is also expected that the concentration of furosine in heat-

damaged feed ingredients increases as the degree of heat damage increases because furosine is formed from acid hydrolyzed Amadori compounds formed during the early stages of the Maillard reactions (Nursten, 2005). Our data support this hypothesis in the case of sunflower meal, but that was not the case for cottonseed meal. The reason for these observations may be that the Maillard reactions in autoclaved sunflower meals that were used in this experiment did not progress to advanced stages, in which Amadori products are converted to melanoidins. In contrast, it appears that autoclaving of cottonseed meal resulted in formation of more advanced Maillard reactions. Heat damage of SBM does not affect the concentration of CP, although the concentration of Lys is reduced (González-Vega et al., 2011). As a consequence, the concentration of Lys expressed as a percentage of the concentration of CP can be used as an indicator of heat damage in feed ingredients. Thus, it is expected that the greater the degree of heat damage, is the lower the Lys:CP ratio will be (Cozannet et al., 2010; Skiba et al., 2011). Results observed in these experiments for both sunflower meal and cottonseed meal support this hypothesis.

Effects of Autoclaving on AA Digestibility

Values for the SID of CP and for the SID of AA determined for non-autoclaved sunflower meal are in close agreement with values reported by NRC (2012). Likewise, the SID of CP and SID of AA determined for non-autoclaved cottonseed meal concur with the SID values presented in NRC (2012). The SID of Lys in sunflower meal has been reported in a range from 75.8 to 80.0% , which is narrower than the range observed in this experiment, although it encompasses some of the values we observed (Jondreville et al., 2000; González-Vega and Stein, 2012). Values for the SID of Lys in cottonseed meal reported by NRC (2012) ranged from 52.15 to 73.85% and the SID of Lys determined for cottonseed meal in this experiment ranged from

49.75 to 66.21%. This indicates that some of the variation in the SID of Lys in commercial sources of sunflower meal and cottonseed meal may be a result of differences in heat processing of the meals. The observed decrease in the SID of AA in both sunflower and cottonseed meals resulting from increasing time of autoclaving was expected, and this also has been observed in DDGS and SBM (Fontaine et al., 2007; González-Vega et al., 2011). Heat processing reduces the digestibility of AA because AA and protein undergoes Maillard reactions to form insoluble complexes and cross-linking proteins (Nursten, 2005). These reactions, therefore, may yield AA and protein containing products that are less accessible to digestive enzymes. Consequently, the overall digestibility of CP and AA is reduced.

Regression Equations

The automatic search procedures for model selection, when developing regression equations, play a pivotal role on the selection of a "best" final model for a given data set (Kutner et al., 2004). In the present experiments, we used the forward selection method, which can be considered a simplified version of the forward stepwise selection method (Kutner et al., 2004). From the regression equations developed to predict the concentration of SID AA in sunflower meal, there was a clear pattern indicating that the concentrations of NDF in combination with the concentrations of AA were relatively accurate predictors. This observation, however, must be interpreted with caution as the concentration of NDF in different sources of sunflower meal may differ because of factors other than heat damage, such as genetic background of the feed ingredient. Thus, the equations developed from this experiment may be used to predict the concentration of SID AA within a source of sunflower meal if it is known that the only source of variation in the nutrient composition of sunflower meal is due to heat processing. Nevertheless,

the present results indicate that the concentration of NDF, AA, and the Lys:CP ratio in sunflower meal may serve as indicators of heat damage.

Regression equations developed to predict the concentration of SID AA in cottonseed meal indicate that the concentration of ADIN alone or combined with other nutrients may be used, although, a relatively low r^2 was calculated for most equations. Nevertheless, the SID Lys in cottonseed meal may be predicted from the concentration of ADIN, although a validation of the equation is required using other data sets.

The concentration of reactive Lys, calculated from the concentration of furosine, has been reported to be a predictor of the concentration of SID Lys in DDGS (Kim et al., 2012). Our results are in agreement with these observations, but reactive Lys was not shown to be the best predictor for the concentration of SID Lys in sunflower meal or cottonseed meal in these experiments.

Conclusions

Results of these experiments demonstrate that the concentrations of analyzed fiber components in sunflower meal and cottonseed meal are increased as a result of heat damage. As previously demonstrated, the concentrations and digestibility of AA is reduced as the degree of heat damage increases and Lys is the AA most affected by heat damage, but the AID and SID of all other AA may also be reduced by severe heat damage. Thus, heat processing of sunflower meal and cottonseed meal should be optimized to prevent reducing the digestibility of AA. Regression equations that use the concentrations of NDF, ADIN, and AA may be used to identify the nutritional quality of heat-damaged sunflower meal and cottonseed meal, but the practical use of the regression equations developed in the current work need to be validated.

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TABLES

Table 5.1. Chemical composition of sunflower meal and cottonseed meal used in Exp. 1 and 2, respectively

	Sunflower meal (Exp.1)				Cottonseed meal (Exp. 2)			
		Autoclaved at 130°C				Autoclaved at 130°C		
Item	Non-autoclaved	20 min	40 min	60 min	Non-autoclaved	15 min	35 min	60 min
DM, %	91.92	89.31	90.05	88.79	90.98	90.12	87.04	87.57
Ash, %	8.10	7.68	8.11	7.65	9.21	8.52	8.14	8.64
CP, %	35.92	36.55	36.11	35.35	41.68	42.20	42.23	42.26
ADF, %	21.60	22.80	19.87	24.12	18.78	16.89	17.92	17.96
NDF, %	31.90	34.88	34.90	43.21	26.19	27.12	27.99	29.83
Lignin, %	5.59	5.42	5.66	6.73	5.52	5.73	6.49	6.68
ADIN, %	0.22	0.18	0.25	0.28	0.20	0.25	0.29	0.27
Reducing sugars, %	4.64	4.43	4.18	3.74	3.59	3.47	1.76	2.31
Free gossypol, %	-	-	-	-	< 0.02	< 0.02	< 0.02	< 0.02
Total gossypol, %	-	-	-	-	0.65	0.60	0.48	0.36
AEE,[1] %	1.93	0.88	1.82	1.57	2.60	1.47	1.05	1.31
Ca, %	0.36	0.34	0.35	0.33	0.22	0.26	0.22	0.22
P, %	1.28	1.26	1.28	1.12	1.19	1.23	1.19	1.16
Lys:CP ratio[2]	3.42	2.98	2.99	2.77	3.93	3.77	3.60	3.60

Table 5.1. (Cont.)

Furosine	0.013	0.012	0.021	0.030	0.027	0.040	0.040	0.030
Reactive Lys[3]	1.21	1.08	1.05	0.94	1.61	1.54	1.47	1.48
L*[4]	53.83	51.74	49.65	51.84	51.34	48.02	48.03	45.56
Indispensable AA, %								
Arg	2.74	2.50	2.61	2.44	4.45	4.43	4.23	4.31
His	0.84	0.78	0.83	0.81	1.16	1.14	1.13	1.16
Ile	1.39	1.31	1.36	1.38	1.28	1.29	1.29	1.33
Leu	2.17	2.03	2.16	2.13	2.35	2.39	2.38	2.46
Lys	1.23	1.09	1.08	0.98	1.64	1.59	1.52	1.52
Met	0.75	0.69	0.74	0.72	0.61	0.63	0.62	0.64
Phe	1.56	1.45	1.55	1.53	2.14	2.17	2.17	2.23
Thr	1.24	1.17	1.25	1.21	1.27	1.31	1.30	1.34
Trp	0.51	0.46	0.44	0.43	0.50	0.51	0.49	0.48
Val	1.71	1.60	1.68	1.68	1.77	1.77	1.78	1.85
All indispensable AA	14.14	13.08	13.70	13.31	17.17	17.21	16.91	17.32
Dispensable AA, %								
Ala	1.49	1.39	1.48	1.47	1.56	1.59	1.58	1.63
Asp	3.04	2.84	3.03	2.97	3.54	3.61	3.54	3.54
Cys	0.55	0.51	0.52	0.47	0.66	0.66	0.63	0.64
Glu	6.41	5.97	6.40	6.29	7.75	7.91	7.87	8.05
Gly	1.98	1.84	1.96	1.94	1.70	1.72	1.71	1.76

Table 5.1. (Cont.)

Pro	1.48	1.40	1.47	1.46	1.57	1.55	1.55	1.62
Ser	1.41	1.32	1.43	1.37	1.68	1.74	1.71	1.76
All dispensable AA	16.36	15.27	16.29	15.97	18.46	18.78	18.59	19.00
Total AA	30.50	28.35	29.99	29.28	35.63	35.99	35.50	36.32

[1]AEE = acid hydrolyzed ether extract.

[2]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

[3]Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)]; Pahm et al., 2008.

[4]L* = lightness.

Table 5.2. Ingredient composition of experimental diets (as-fed basis), Exp. 1 and 2

	Sunflower meal (Exp.1)				Cottonseed meal (Exp.2)				
		Autoclaved at 130°C				Autoclaved at 130°C			
Ingredient, %	Non-autoclaved	20 min	40 min	60 min	Non-autoclaved	15 min	35 min	60 min	N-free[1]
Sunflower meal	42.00	42.00	42.00	42.00	-	-	-	-	-
Cottonseed meal	-	-	-	-	32.00	32.00	32.00	32.00	-
Cornstarch	42.00	42.00	42.00	42.00	41.97	41.97	41.97	41.97	67.00
Sucrose	10.00	10.00	10.00	10.00	20.00	20.00	20.00	20.00	20.00
Solka floc[2]	-	-	-	-	-	-	-	-	5.00
Soybean oil	3.40	3.40	3.40	3.40	3.50	3.50	3.50	3.50	4.00
Ground limestone	0.85	0.85	0.85	0.85	0.90	0.90	0.90	0.90	0.80
Monocalcium phosphate	0.65	0.65	0.65	0.65	0.50	0.50	0.50	0.50	1.60
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Ferrous sulfate	-	-	-	-	0.03	0.03	0.03	0.03	-
Magnesium oxide	-	-	-	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	-	-	-	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]A N-free diet was produced separately for Exp. 1 and 2.

[2]Fiber Sales and Development Corp., Urbana, OH.

[3]Provided the following per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadionenicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 5.3. Analyzed nutrient composition of experimental diets (as-fed basis), Exp. 1 and 2

Item	Sunflower meal (Exp.1)				Cottonseed meal (Exp. 2)			
		Autoclaved at 130°C				Autoclaved at 130°C		
	Non-autoclaved	20 min	40 min	60 min	Non-autoclaved	15 min	35 min	60 min
DM, %	91.39	90.90	90.91	90.70	92.66	91.93	91.98	92.40
CP, %	13.91	14.55	15.00	14.32	14.01	12.45	13.56	13.64
Indispensable AA, %								
Arg	1.21	1.12	1.19	1.00	1.49	1.28	1.30	1.37
His	0.38	0.35	0.38	0.33	0.39	0.33	0.34	0.36
Ile	0.61	0.59	0.62	0.56	0.44	0.38	0.39	0.42
Leu	0.98	0.94	1.01	0.90	0.80	0.72	0.74	0.80
Lys	0.54	0.50	0.50	0.41	0.57	0.47	0.47	0.48
Met	0.33	0.30	0.32	0.27	0.21	0.19	0.19	0.20
Phe	0.70	0.66	0.72	0.64	0.73	0.65	0.67	0.72
Thr	0.56	0.53	0.57	0.52	0.43	0.39	0.40	0.43

Table 5.3. (Cont.)

Trp	0.20	0.21	0.20	0.18	0.18	0.14	0.16	0.16
Val	0.75	0.72	0.77	0.69	0.61	0.52	0.54	0.58
All indispensable AA	6.26	5.92	6.28	5.50	5.85	5.07	5.20	5.52
Dispensable AA, %								
Ala	0.67	0.64	0.69	0.62	0.54	0.49	0.50	0.54
Asp	1.37	1.30	1.41	1.26	1.21	1.09	1.11	1.19
Cys	0.25	0.23	0.24	0.21	0.23	0.20	0.20	0.21
Glu	2.88	2.74	2.97	2.66	2.58	2.33	2.40	2.58
Gly	0.88	0.84	0.90	0.81	0.57	0.51	0.53	0.57
Pro	0.67	0.64	0.70	0.62	0.51	0.46	0.48	0.51
Ser	0.64	0.61	0.66	0.59	0.57	0.53	0.54	0.58
All dispensable AA	7.36	7.00	7.57	6.77	6.21	5.61	5.76	6.18
Total AA	13.62	12.92	13.85	12.27	12.06	10.68	10.96	11.70

[1]The concentrations (%) of DM and CP in the N-free diet were 92.16 and 0.58 in Exp. 1 and 91.94 and 0.24 in Exp. 2, respectively.

Table 5.4. Apparent ileal digestibility of CP and AA in sunflower meal subjected to increasing time of autoclaving by growing pigs (Exp.1)[1]

	Sunflower meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	20 min	40 min	60 min		Linear	Quadratic
CP, %	69.48	70.06	64.67	57.91	2.1	< 0.01	0.41
Indispensable AA, %							
Arg	87.95	87.15	84.63	81.02	1.0	< 0.01	0.88
His	81.54	80.13	78.53	71.63	1.2	< 0.01	0.23
Ile	81.64	81.44	79.96	75.02	1.1	< 0.01	0.56
Leu	82.05	81.70	80.32	75.30	1.1	< 0.01	0.53
Lys	73.88	71.29	64.87	51.40	2.1	< 0.01	0.66
Met	89.16	88.34	87.85	83.41	0.7	< 0.01	0.08
Phe	84.28	84.07	83.61	79.57	1.0	< 0.01	0.34
Thr	74.27	73.28	70.72	63.31	1.6	< 0.01	0.58
Trp	77.01	78.55	74.41	69.33	1.5	< 0.01	0.37

Table 5.4. (Cont.)

Val	80.63	80.29	78.65	73.26	1.2	< 0.01	0.56
Mean	82.01	81.14	79.26	73.34	1.1	< 0.01	0.43
Dispensable AA, %							
Ala	73.47	73.19	69.16	60.52	2.1	< 0.01	0.89
Asp	79.08	77.37	74.11	65.13	1.3	< 0.01	0.34
Cys	75.93	74.81	70.39	62.16	2.0	< 0.01	0.95
Glu	87.68	87.17	85.62	81.76	1.0	< 0.01	0.71
Gly	54.57	57.07	44.61	36.02	4.8	< 0.01	0.28
Ser	74.19	72.94	71.48	64.22	1.2	< 0.01	0.26
Mean	74.15	73.84	69.72	62.24	1.7	< 0.01	0.94

[1]Data are means of 10 observations.
[2]Linear and quadratic effects of time of autoclaving.

Table 5.5. Standardized ileal digestibility of CP and AA in sunflower meal subjected to increasing time of autoclaving by growing pigs (Exp. 1)[1]

	Sunflower meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	20 min	40 min	60 min		Linear	Quadratic
CP, %	82.66	82.61	76.84	70.62	2.1	< 0.01	0.35
Indispensable AA, %							
Arg	92.53	92.06	89.26	86.52	1.0	< 0.01	0.55
His	86.94	85.96	83.90	77.80	1.2	< 0.01	0.48
Ile	87.58	87.55	85.77	81.44	1.1	< 0.01	0.83
Leu	88.18	88.06	86.23	81.92	1.1	< 0.01	0.85
Lys	83.15	81.24	74.83	63.52	2.1	< 0.01	0.96
Met	92.80	92.32	91.58	87.82	0.7	< 0.01	0.25
Phe	89.80	89.89	88.95	85.56	1.0	< 0.01	0.68
Thr	84.63	84.17	80.84	74.38	1.6	< 0.01	0.96
Trp	85.39	86.49	82.75	78.58	1.5	< 0.01	0.40

Table 5.5. (Cont.)

Val	87.17	87.06	84.99	80.32	1.2	< 0.01	0.88
Mean	88.49	87.95	85.68	80.66	1.1	< 0.01	0.79
Dispensable AA, %							
Ala	84.88	85.08	80.19	72.76	2.1	< 0.01	0.69
Asp	85.37	83.96	80.18	71.92	1.3	< 0.01	0.62
Cys	84.55	84.14	79.33	72.35	2.0	< 0.01	0.78
Glu	91.61	91.28	89.41	85.99	1.0	< 0.01	0.99
Gly	73.76	77.06	63.36	52.92	3.9	< 0.01	0.17
Ser	83.17	82.31	80.14	73.89	1.2	< 0.01	0.56
Mean	83.89	84.02	79.07	71.94	1.7	< 0.01	0.59

[1]Data are means of 10 observations; Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI), which were determined by feeding pigs a N-free diet: CP, 20.08; Arg, 0.61; His, 0.22; Ile, 0.40; Leu, 0.66; Lys, 0.55; Met, 0.13; Phe, 0.42; Thr, 0.63; Trp, 0.18; Val, 0.54; Ala, 0.84; Asp, 0.94, Cys, 0.24; Glu, 1.24; Gly, 1.85; and Ser, 0.63.

[2]Linear and quadratic effects of time of autoclaving.

Table 5.6. Linear regressions to predict the concentration (%) of standardized ileal digestible (SID) AA in sunflower meal fed to pigs[1]

Dependent variable	Intercept			Independent variables[2]							RMSE[3]	Adjusted r^2
	Estimate	SE	*P*-value	Variable 1	Estimate	SE	*P*-value	Variable 2	Estimate	SE	*P*-value	
SID Arg	-0.99	0.30	< 0.01	Arg	0.89	0.15	< 0.01	Lys:CP	0.32	0.09	< 0.01	0.08	0.77
SID His	0.45	0.17	< 0.05	His	0.60	0.19	< 0.01	NDF	-0.07	0.001	< 0.01	0.03	0.67
SID Ile	0.64	0.29	< 0.05	Ile	0.59	0.29	< 0.01	NDF	-0.07	0.001	< 0.01	0.04	0.43
SID Leu	0.99	0.44	< 0.05	Leu	0.61	0.20	< 0.01	NDF	-0.01	0.003	< 0.01	0.07	0.49
SID Lys	-0.75	0.12	< 0.01	RL	1.49	0.11	< 0.01	-	-	-	-	0.07	0.83
	-1.00	0.12	< 0.01	Lys	0.54	0.31	0.08	RS	0.30	0.08	< 0.01	0.06	0.85
SID Met	0.20	0.08	< 0.05	Met	0.81	0.10	< 0.01	NDF	-0.003	0.001	< 0.01	0.01	0.81
SID Phe	0.46	0.26	0.08	Phe	0.73	0.16	< 0.01	NDF	-0.01	0.002	< 0.01	0.04	0.50
SID Thr	1.45	0.08	< 0.01	NDF	-0.01	0.002	< 0.01	-	-	-	-	0.06	0.48
SID Trp	-0.23	0.05	< 0.01	Trp	0.53	0.23	< 0.05	Lys:CP	0.12	0.04	< 0.01	0.02	0.81
SID Val	1.59	0.11	< 0.01	NDF	-0.03	0.005	< 0.01	Lignin	0.13	0.05	< 0.05	0.06	0.46

[1]n = 40 observations; for all models $P < 0.01$.
[2]RS = reducing sugars; RL = Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)].
[3]RMSE = root mean square error.

Table 5.7. Apparent ileal digestibility of CP and AA in cottonseed meal subjected to increasing time of autoclaving by growing pigs (Exp.2)[1]

	Cottonseed meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	15 min	35 min	60 min		Linear	Quadratic
CP, %	61.27	52.63	51.15	53.65	2.4	< 0.01	< 0.01
Indispensable AA, %							
Arg	82.20	77.29	74.63	77.84	1.3	< 0.01	< 0.01
His	75.51	67.87	64.44	68.09	1.2	< 0.01	< 0.01
Ile	63.95	54.47	51.94	57.87	1.6	0.04	< 0.01
Leu	66.82	60.22	57.84	63.19	1.5	0.15	< 0.01
Lys	59.06	45.81	41.14	45.63	1.8	< 0.01	< 0.01
Met	67.60	61.48	58.94	63.93	1.4	0.10	< 0.01
Phe	77.41	72.12	69.92	74.10	1.2	0.11	< 0.01
Thr	58.35	50.99	47.35	52.95	1.8	0.06	< 0.01
Trp	67.87	55.31	57.12	59.10	1.6	0.01	< 0.01

Table 5.7. (Cont.)

Val	67.43	58.61	56.39	62.01	1.5	0.05	< 0.01
Mean	71.17	63.88	61.13	65.53	1.2	< 0.01	< 0.01
Dispensable AA, %							
Ala	52.74	44.20	40.74	48.76	2.6	0.12	< 0.01
Asp	70.98	62.98	55.95	60.01	1.4	< 0.01	< 0.01
Cys	70.57	64.69	60.67	64.42	1.6	0.01	< 0.01
Glu	81.02	76.29	73.26	76.35	1.1	< 0.01	< 0.01
Gly	25.59	16.04	9.31	22.66	6.7	0.42	< 0.01
Ser	66.47	62.40	58.52	63.24	1.3	0.07	< 0.01
Mean	61.23	54.42	49.74	55.84	1.9	< 0.01	< 0.01

[1]Data are means of 10 observations.
[2]Linear and quadratic effects of time of autoclaving.

Table 5.8. Standardized ileal digestibility of CP and AA in cottonseed meal subjected to increasing levels of heat treatment by growing pigs (Exp. 2)[1]

	Cottonseed meal						
		Autoclaved at 130° C			SEM	*P*-value[2]	
Item	Non-autoclaved	15 min	35 min	60 min		Linear	Quadratic
CP, %	76.01	69.08	66.27	68.75	2.4	< 0.01	< 0.01
Indispensable AA, %							
Arg	88.39	84.44	81.67	84.55	1.3	< 0.01	< 0.01
His	80.92	74.22	70.61	73.94	1.2	< 0.01	< 0.01
Ile	70.71	62.23	59.51	64.93	1.6	0.04	< 0.01
Leu	73.14	67.18	64.63	69.49	1.5	0.13	< 0.01
Lys	66.21	54.42	49.75	54.10	1.8	< 0.01	< 0.01
Met	71.91	66.21	63.67	68.44	1.4	0.12	< 0.01
Phe	81.83	77.05	74.70	78.57	1.2	0.10	< 0.01
Thr	70.53	64.32	60.34	65.09	1.8	0.05	< 0.01
Trp	75.48	65.01	65.62	67.64	1.6	0.02	< 0.01

Table 5.8. (Cont.)

Val	74.28	66.59	64.08	69.20	1.5	0.05	< 0.01
Mean	75.34	68.20	65.49	69.56	1.4	0.01	< 0.01
Dispensable AA, %							
Ala	66.41	59.15	55.40	62.40	2.6	0.09	< 0.01
Asp	77.44	70.08	62.93	66.55	1.4	< 0.01	< 0.01
Cys	78.38	73.61	69.59	72.96	1.6	0.02	< 0.01
Glu	84.83	80.48	77.32	80.15	1.1	< 0.01	< 0.01
Gly	63.03	57.55	49.27	59.99	6.7	0.27	< 0.01
Ser	76.42	73.02	68.95	72.99	1.3	0.05	< 0.01
Mean	74.42	68.96	63.91	69.10	1.9	< 0.01	< 0.01

[1]Data are means of 10 observations; Values for standardized ileal digestibility were calculated by correcting apparent ileal digestibility values for basal endogenous losses (g/kg of DMI), which were determined by feeding pigs a N-free diet: CP, 22.29; Arg, 1.00; His, 0.23; Ile, 0.32; Leu, 0.55; Lys, 0.44; Met, 0.10; Phe, 0.35; Thr, 0.57; Trp, 0.15; Val, 0.45; Ala, 0.80; Asp, 0.84, Cys, 0.19; Glu, 1.06; Gly, 2.30; and Ser, 0.61.

[2]Linear and quadratic effects of time of autoclaving.

Table 5.9. Linear regressions to predict the concentration (%) of standardized ileal digestible (SID) AA in cottonseed meal fed to pigs[1]

Dependent variable	Intercept			Independent variables[2]								
	Estimate	SE	*P*-value	Variable 1	Estimate	SE	*P*-value	Variable 2	Estimate	SE	*P*-value	RMSE[3]	Adjusted r^2
SID Arg	-4.48	1.27	< 0.01	Arg	1.87	0.29	< 0.01	-	-	-	-	0.16	0.53
SID His	1.06	0.10	< 0.01	Lignin	0.06	0.03	0.04	ADIN	-2.18	0.39	< 0.01	0.04	0.62
SID Ile	5.49	1.49	< 0.01	Lys:CP	-0.92	0.32	< 0.01	ADIN	-4.95	1.30	< 0.01	0.07	0.40
SID Leu	0.78	0.44	0.08	NDF	0.06	0.02	< 0.01	ADIN	-3.22	0.77	< 0.01	0.11	0.30
SID Lys	-2.42	0.41	< 0.01	RL	2.17	0.27	< 0.01	-	-	-	-	0.09	0.65
	1.81	0.11	< 0.01	ADIN	-3.67	0.42	< 0.01	-	-	-	-	0.09	0.68
SID Met	0.21	0.11	0.06	NDF	0.01	0.005	< 0.01	ADIN	-0.78	0.19	< 0.01	0.03	0.28
SID Phe	1.01	0.33	< 0.01	NDF	0.05	0.02	< 0.01	ADIN	-2.38	0.57	< 0.01	0.08	0.30
SID Thr	0.56	0.29	0.06	NDF	0.03	0.01	0.05	ADIN	-1.75	0.50	< 0.01	0.07	0.23
SID Trp	-0.08	0.11	0.13	ADF	0.02	0.005	< 0.05	RS	0.004	0.005	< 0.05	0.02	0.28
SID Val	8.27	1.87	< 0.01	Lys:CP	-1.40	0.39	< 0.01	ADIN	-7.19	1.62	< 0.01	0.08	0.43

[1]n = 40 observations; for all models $P < 0.01$.

[2]ADIN = acid detergent insoluble nitrogen; RS = reducing sugars; RL = Reactive Lys (%) = [Lys (%) – (Furosine (%) ÷ 0.32 × 0.40)].

[3]RMSE = root mean square error.

CHAPTER 6

EFFECTS OF DIET FORMULATION ON PERFORMANCE OF WEANLING PIGS FED HEAT DAMAGED SOYBEAN MEAL OR HEAT DAMAGED DISTILLERS DRIED GRAINS WITH SOLUBLES

ABSTRACT: Two experiments were conducted to investigate if adjustments in diet formulations based on either total analyzed AA or standardized ileal digestible (**SID**) AA may be used to eliminate negative effects of including heat-damaged soybean meal (**SBM**) or heat-damaged distillers dried grains with solubles (**DDGS**) in diets fed to weanling pigs. In Exp. 1, 4 corn-SBM diets were formulated. Diet 1 contained non-autoclaved SBM, and this diet was formulated on the basis of analyzed AA concentrations and using SID values from the AminoDat® (2006) database. Three additional diets were formulated using autoclaved SBM, rather than the non-autoclaved SBM. Diet 2 was formulated similar to Diet 1, except that the non-autoclaved SBM was replaced by the autoclaved SBM. Diet 3 was formulated by adjusting AA inclusion in the diet on the basis of analyzed total AA concentrations in the autoclaved SBM and published SID values (AminoDat®, 2006). Diet 4 also contained autoclaved SBM, but the formulation of this diet was adjusted on the basis of analyzed AA in the autoclaved SBM and SID values that were adjusted according to the degree of heat damage in this source of SBM. Pigs (160; initial BW: 10.4 ± 1.3 kg) were allotted to 4 treatments with 8 replicate pens per treatment in a randomized complete block design. The final BW on d 21 for pigs fed either Diet 3 or Diet 4 was less ($P < 0.05$) than the final BW of pigs fed Diet 1, but greater ($P < 0.05$) than the final BW of pigs fed Diet 2. The ADG of pigs fed Diet 1 was greater ($P < 0.05$) than the ADG of pigs fed the other diets, but pigs fed either Diet 3 or Diet 4 had greater ($P < 0.05$) ADG than pigs fed Diet 2. The G:F was greater ($P < 0.05$) for pigs fed Diet 1 compared with pigs fed

the other diets. Pigs fed Diet 4 had greater ($P < 0.05$) G:F than pigs fed Diet 2. In Exp. 2, 144 pigs (initial BW: 9.9 ± 1.5 kg) were allotted to 4 diets with 8 replicate pens per diet. The 4 diets contained corn, SBM (8.5%), and DDGS (autoclaved or not autoclaved; 22%), and were formulated using the concepts described for Exp. 1, except that heat-damaged DDGS but not heat-damaged SBM, was used in the diets. Pigs fed Diet 2 or Diet 4 had greater ($P < 0.05$) ADFI than pigs fed Diet 1, but no differences in ADFI were observed among pigs fed the diets containing autoclaved DDGS. Pigs fed Diet 1 had greater ($P < 0.05$) G:F than pigs fed the other diets, but no differences were observed for G:F among pigs fed diets containing autoclaved DDGS. Results demonstrate that the negative effects of heat damage may be ameliorated if the reduced concentration as well as the reduced digestibility of AA in heat-damaged SBM is corrected.

Diets for weaned pigs containing up to 22% of heat-damaged DDGS reduces performance of pigs compared with diets containing DDGS that has not been heat-damaged, but correction for the reduced concentration and the reduced digestibility of AA in heat-damaged DDGS may not be of practical importance for weaned pigs.

Key words: distillers dried grains with solubles, soybean meal, weaned pigs

INTRODUCTION

Successful feed formulation and nutrition of farm animals requires accurate information about the nutritional value of the feed ingredients used. Whereas several nutritional criteria are routinely considered in feed ingredients evaluation, the ability to assess the impact of heat damage that occurs during processing of particular ingredients has received less attention. The digestibility of AA by growing pigs in certain ingredients decreases gradually with increasing

degree of heat treatment (Fontaine et al., 2007; Pahm et al., 2008; González-Vega et al. 2011). If this reduction is not considered in feed formulation, use of heat-damaged ingredients may result in reduced animal performance. Whereas AA analysis of raw materials by rapid methods such as NIR may identify AA losses in heat-damaged ingredients, which may be considered in diet formulation, adjustment of diets for changes in AA digestibility is less trivial. Lack of adjustments in diets containing heat-damaged ingredients, however, may result in reduced performance of pigs. It is, therefore, necessary that the degree of heat damage in a feed ingredient is accounted for in diet formulation. Adjustments of values for the standardized ileal digestibility (**SID**) of AA in heat-damaged feed ingredients, to ameliorate reduced performance of chicks fed heat-damaged SBM, have been investigated (Evonik, 2010). In contrast, effects of formulating diets containing heat-damaged soybean meal (**SBM**) or heat-damaged distillers dried grains with solubles (**DDGS**) to pigs on the basis of adjustments of the SID of AA according to the degree of heat damage and its effects on performance have not been determined. We hypothesized that the negative effects of feeding heat-damaged SBM or DDGS to weanling pigs may be reduced if values for the SID AA used in diet formulation are adjusted according to the degree of heat damage of the ingredients. Therefore, the objectives of the present experiments were to investigate if adjustments in diet formulations based on either total analyzed AA or SID AA may be used to eliminate negative effects of including heat-damaged SBM or DDGS in diets fed to weanling pigs.

MATERIALS AND METHODS

Protocols for the experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in both experiments were the offspring of G-performer boars mated to F-25 females (Genetiporc, Alexandria, MN).

Exp. 1, Use of Heat Damaged Soybean Meal

Diet formulation. Dehulled SBM was procured from Solae (Gibson City, IL) and separated into 2 batches. One batch was not autoclaved, whereas the other batch was autoclaved at 125°C for 60 min (Table 6.1). Four corn-SBM diets were formulated to contain similar concentrations of CP and GE (Table 6.2). Diet 1 contained the non-autoclaved SBM and this diet was formulated on the basis of analyzed AA concentrations and using SID values from the AminoDat® (2006) database. Values for the SID of AA in SBM in the AminoDat® (2006) database are the average of SID values from 95 digestibility experiments. Three additional diets were formulated using the autoclaved SBM rather than the non-autoclaved SBM. Diet 2 was formulated exactly like Diet 1, except that the non-autoclaved SBM was replaced by the autoclaved SBM. Values for the SID AA used in the formulation of Diet 2 were the same values used in the formulation of Diet 1. Diet 3 was formulated by adjusting AA inclusion in the diet on the basis of analyzed total AA concentrations in the autoclaved SBM and published SID values (AminoDat®, 2006). Adjustments for the SID AA in Diet 3 were achieved by adding increased quantities of crystalline Lys, Met, Thr, and Trp compared with Diets 1 and 2. Diet 4 also contained autoclaved SBM, but the formulation of this diet was adjusted on the basis of analyzed AA in the autoclaved SBM and SID values that were adjusted according to the degree of heat damage in this source of SBM. Crystalline Lys, Met, Thr, and Trp were also added to diet 4, but in greater amounts than

in Diet 3. The calculated SID Lys for Diets 1, 2, 3, and 4 were 1.00, 0.88, 0.95, and 1.00%, respectively.

Animals, Experimental Design, and Housing. A total of 160 pigs (initial BW: 10.4 ± 1.3 kg) weaned at approximately 21 d of age and fed a common phase 1 diet for 14 d after weaning, were allotted to 4 dietary treatments with 8 replicate pens per treatment in a randomized complete block design. Four replicates of each treatment consisted of a pen with 3 barrows and 2 gilts whereas the other 4 replicates of each treatment consisted of a pen with 2 barrows and 3 gilts. Pigs were fed the treatment diets for 21 d. Pigs were housed in an environmental controlled room in pens (1.2 × 1.2 m) with fully slatted floors. Feed and water were available at all times.

Sample Analyses, Performance Measurements, and Data Processing. Ingredients and diets were analyzed for DM by drying in an oven at 103°C for 4 h (Method 935.29; AOAC International), Ca and P (Method 985.01; AOAC International, 2007), ADF (Method 973.18; AOAC International, 2007), NDF (Holst, 1973), CP according to the Dumas procedure (Method 968.06; AOAC International, 2007), and AA by ion-exchange chromatography with post-column derivatization with ninhydrin. Cysteine and Met were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 *N* HCL for 24 h at 110°C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive, 2000).

The individual BW of pigs was recorded at the beginning of the experiment and every 7-d thereafter. The amount of feed provided to each pen was recorded daily. On the last day of the

experiment, feeders were weighed and emptied at 700 h, and at 1300 h blood samples from the heaviest barrow and the heaviest gilt in each pen were collected via jugular venipuncture in EDTA tubes. Tubes were stored on ice and centrifuged (2,000 rpm at 5°C for 15 min). Plasma was then collected from the centrifuged tubes and analyzed for plasma urea nitrogen (**PUN**) on an Olympus AU680 Chemistry Analyzer (Olympus Life Science Research Europa GmbH, Sauerbruchstr., Munich, Germany). At the conclusion of the experiment, values for ADG, ADFI, and G:F for each 7-d period and for the overall experimental period were calculated. The average value for PUN for each pen was used in the statistical analysis. The ratio of SID Lys/kg of BW gain was calculated by the following equation:

SID Lys/BW gain = SID Lys intake (g/d) / ADG (kg).

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The UNIVARIATE procedure of SAS was used to verify normality of the data and to test for the presence of outliers. The model included dietary treatment as the fixed effect, whereas replication was included as the random effect. The pen was the experimental unit and significance among means was assessed using an alpha level of 0.05.

Exp. 2, Use of Heat Damaged Distillers Dried Grains with Solubles

Diet formulation. Distillers dried grains with solubles was sourced from Poet LLC (North Manchester, IN) and separated into 2 batches. One batch was not autoclaved, whereas the other batch was autoclaved at 125°C for 60 min (Table 6.1). Four diets based on corn, SBM (8.5%), and DDGS (22%) were formulated following the same concepts as described for Exp. 1 (Table 6.3). Adjustments for the concentration of SID AA in Diet 3 were achieved by adding crystalline Lys, Met, Thr, Trp, Val, and Ile to a diet that was otherwise similar to Diet 2. Crystalline Lys,

Met, Thr, Trp, Val, and Ile were also added to Diet 4, but in greater quantities than in Diet 3. The calculated SID Lys for Diets 1, 2, 3, and 4 were 1.00, 0.95, 0.97, and 1.00%, respectively.

Animals, Experimental Design, and Housing. A total of 144 pigs (initial BW: 9.9 ± 1.5 kg) that were weaned at approximately 21 d of age and fed a common phase 1 diet for 14 d were allotted to 4 dietary treatments with 8 replicate pens per treatment in a randomized complete block design. Four replicates of each treatment consisted of a pen with 3 gilts and 2 barrows whereas the other 4 replicates of each treatment consisted of a pen with 2 gilts and 2 barrows. Pigs were fed the dietary treatments for 21 d. Pigs were housed and fed treatment diets as outlined for Exp. 1.

Sample Analysis, Performance Measurements, and Data Processing. Ingredients and diets were analyzed as described for Exp. 1. Performance measurements and data processing were also similar to those described for Exp. 1, except that PUN analysis was not performed.

RESULTS

Exp. 1, Use of Heat Damaged Soybean Meal

The concentration of CP was 47.78 and 46.47% in the non-autoclaved SBM and the autoclaved SBM, respectively (Table 6.1). The concentration of NDF was 10.28% in the non-autoclaved SBM vs. 34.94% in the autoclaved SBM. The non-autoclaved SBM contained 2.85% Lys, whereas the autoclaved SBM contained 2.58% Lys. The concentration of Lys:CP was 6.09 for the non-autoclaved SBM and 5.40 for the autoclaved SBM. The concentration of CP in Diets 1, 2, 3, and 4 were 19.75, 19.85, 19.48, and 20.01%, respectively (Table 6.4). The concentration of Lys was 1.13% in Diet 1, 0.97% in Diet 2, 1.03% in Diet 3, and 1.10% in Diet 4.

No differences in initial BW of pigs were observed among dietary treatments (Table 6.6). Pigs that were fed Diet 2, however, had reduced ($P < 0.05$) BW on d 7 compared with pigs fed

the other diets, but the BW on d 7 for pigs fed Diet 4 was not different from the BW of pigs fed Diet 1. Likewise, the BW on d 7 of pigs fed Diet 3 was not different from that of pigs fed Diet 4. A similar trend for the BW of pigs on d 14 was observed. The final BW on d 21 for pigs fed either Diet 3 or Diet 4 was less ($P < 0.05$) than the final BW of pigs fed Diet 1, but greater ($P < 0.05$) than the final BW of pigs fed Diet 2.

The ADG (d 0 to 7, and d 7 to 14) of pigs fed Diet 1 was greater ($P < 0.05$) than the ADG of pigs fed either Diet 2 or Diet 3, but not different from the ADG of pigs fed Diet 4. The ADG from d 14 to 21 and the overall ADG of pigs fed Diet 1 was also greater ($P < 0.05$) than the ADG of pigs fed the other diets, but pigs fed either Diet 3 or Diet 4 had greater ($P < 0.05$) ADG than pigs fed Diet 2.

Pigs fed Diet 3 or Diet 4 had greater ($P < 0.05$) ADFI (d 0 to 7) than pigs fed Diet 2, but there was no difference in ADFI between pigs fed Diet 1 or Diet 3. From d 7 to 14, d 14 to 21, or d 0 to 21, pigs fed Diet 3 or Diet 4 tended ($P = 0.08$) to consume more feed than pigs fed the other diets.

The G:F (d 0 to 7) was greater ($P < 0.05$) for pigs fed Diet 1 than for pigs fed the other diets. Pigs fed Diet 3 or Diet 4 had greater ($P < 0.05$) G:F compared with pigs fed Diet 2. Likewise, the G:F (d 7 to 14, d 14 to 21, and overall) was greater ($P < 0.05$) for pigs fed Diet 1 compared with pigs fed the other diets. Pigs fed Diet 4 had greater ($P < 0.05$) G:F during the entire experiment than pigs fed Diet 2.

Pigs that were fed Diet 1 required less ($P < 0.05$) SID Lys:BW gain (g/kg) than pigs fed the other diets. There were no differences on the SID Lys:BW gain among pigs fed Diets 2, 3, or 4.

The concentration of PUN was less ($P < 0.05$) in pigs fed Diet 1 than in pigs fed Diet 2 or Diet 3, but not different form the PUN in pigs fed Diet 4. Pigs fed Diet 2 had the greatest ($P < 0.05$) concentration of PUN among all dietary treatments.

Exp. 2, Use of Heat Damaged Distillers Dried Grains with Solubles

The analyzed ADF concentration was 8.53% in non-autoclaved DDGS, whereas autoclaved DDGS contained 15.47% ADF (Table 6.1). The Lys:CP ratio in non-autoclaved DDGS was 3.05, but 2.24 in autoclaved DDGS. The Lys concentration in Diet 1 was 1.11%, whereas the concentration of Lys was 1.06, 1.10, and 1.08% for Diets 2, 3, 4, respectively (Table 6.5).

No differences were observed for initial BW among dietary treatments (Table 6.7), but pigs fed Diets 1 or 4 tended ($P = 0.06$) to have a greater BW on d 7 than pigs fed Diets 2 or 3. The G:F from d 0 to 7 was greater ($P < 0.05$) for pigs fed Diet 1 or Diet 4 than for pigs fed Diet 2, but the G:F of pigs fed Diet 3 was not different from the G:F of pigs fed Diets 2 or 4.

From d 7 to 14, no differences in growth performance were observed among treatments, except that pigs fed diets containing autoclaved DDGS had greater ($P < 0.05$) ADFI than pigs fed the positive control diet. Likewise, no differences in performance were observed from d 14 to 21 among dietary treatments. For the entire period (d 0 to 21), it was observed that pigs fed Diets 2 or 4 had greater ($P < 0.05$) ADFI than pigs fed Diet 1, but no differences in ADFI were observed among pigs fed the diets containing autoclaved DDGS. Pigs fed Diet 1 had greater ($P < 0.05$) G:F than pigs fed the other diets. No differences were observed for the SID Lys:BW gain (g/kg) among dietary treatments.

DISCUSSION

Ingredients

Soybean meal is the protein source most utilized in diets fed to pigs, but because inactivation of antinutritional factors in SBM requires heat processing, some variation in the nutritional value of different sources of SBM may exist (Stein et al., 2008). The concentrations of DM, CP, and indispensable AA in the non-autoclaved SBM used in this experiment are in agreement with values reported by Fontaine et al. (2007) and González-Vega et al. (2011). The concentration of Lys is reduced in heat-damaged ingredients whereas the concentration of CP remains relatively constant during heat damage (Fontaine et al., 2007; González-Vega et al., 2011). The reduction in the concentration of Lys in heat-damaged feed ingredients is likely a result of Maillard reactions (Pahm et al., 2008). Because the concentration of Lys, but not the concentration of CP, is reduced during heat damage, it has been suggested that the Lys:CP ratio may be used as an indicator of heat damage in feed ingredients (Stein et al., 2009; Cozannet et al., 2010; Kim et al., 2012). Reductions in the Lys:CP ratio as a result of heat damage in SBM that were observed in this experiment have also been observed in previous experiments (Fontaine et al., 2007; González-Vega et al., 2011). Increased NDF in the autoclaved SBM compared with the non-autoclaved SBM is in agreement with observations by Hussein et al. (1995). Some of the products from the Maillard reactions form a "lignin-like matrix", which is analyzed as fractions of NDF (Hussein et al., 1995). Thus, it is likely that the observed increase in the analyzed concentration of ADF in autoclaved DDGS compared with non-autoclaved DDGS was also a result of this artifact. The protein fraction of SBM is mainly composed of globulins and albumins, which are susceptible to heat damage and, therefore, Maillard reactions may increase the concentrations of insoluble N, NDF, and ADF (Hussein et al., 1995). The concentration of

NDF was increased from 14.3% in non-autoclaved SBM to 17.1% in SBM autoclaved at 127°C for 10 min (Sadeghi et al., 2006). These observations indicate that the concentration of NDF within a source of SBM may also serve as an indicator of heat damage. When SBM is heat processed, the combination of heat, reducing sugars, and the "free" amino groups of proteins and AA may initiate Maillard reactions (Fontaine et al., 2007; González-Vega et al., 2011). Lysine that reacts with reducing sugars through Maillard reactions becomes unavailable to pigs (Nursten, 2005, Pahm et al., 2008). In heat-damaged feed ingredients, however, Lys that initially reacted with reducing sugars is partially recovered under traditional AA analysis (Pahm et al., 2008). The analyzed concentration of total Lys in heat-damaged feed ingredients is, therefore, believed to overestimate the concentration of reactive Lys, which is the Lys that can potentially be used for protein synthesis by the pig. The concentration of reactive Lys and the digestibility of Lys in heat-damaged SBM is reduced (Fontaine et al., 2007; González-Vega et al., 2011) compared with SBM that is not heat-damaged. Thus, if heat-damaged SBM or DDGS is used in diet formulation assuming the same concentration and digestibility of Lys and other AA as in non-heat damaged SBM, diets that are deficient in digestible AA may be formulated.

Diets

The Diets 2 used in the experiments were formulated to simulate a situation where a batch of heat-damaged SBM or DDGS is treated as regular (not heat-damaged) SBM or DDGS. Thus, in these diets, the concentrations of analyzed AA from SBM or DDGS that are not heat-damaged were used. The concentrations of digestible AA in these diets, therefore, were likely overestimated because the concentration and the digestibility of most AA in autoclaved SBM and DDGS is reduced compared with SBM or DDGS that has not been autoclaved (Cozannet et al., 2010; González-Vega et al., 2011). This observation is supported by the reduced

concentrations of analyzed AA in Diet 2 in both experiments compared with concentrations in the diets containing non-autoclaved SBM or DDGS. When formulating Diet 3, the reduced concentrations of AA, but not the reduced digestibility, was corrected by addition of increased quantities of crystalline Lys, Met, and Thr (Exp. 1), or increased quantities of crystalline Lys, Thr, Met, Trp, Val, and Ile (Exp. 2). Thus, Diet 3 in both experiments simulated a situation where ingredients were analyzed for concentrations of AA prior to diet formulation. However, in Diet 4, additional crystalline AA were used to compensate for the reduced concentrations and the reduced digestibility of AA in the heat-damaged SBM or DDGS. Diet 4, therefore, simulated a situation where it was recognized that SBM or DDGS was heat-damaged, and that expected reductions in AA digestibility were taken into account in diet formulations. It was expected that this approach would result in concentrations of SID AA in Diet 4 that were similar to concentrations in Diet 1. To achieve this, a greater concentration of total Lys in Diet 4 was necessary. For Exp. 1, the analyzed total concentration of Lys in Diet 4 was slightly less than expected (1.10 vs. 1.18%). An analysis of free AA in the diets was, therefore, performed and it was confirmed that crystalline AA were accurately supplemented to the diets. The AA concentrations in the autoclaved SBM (analyzed by NIR) used in diet formulation were slightly greater than the corresponding values analyzed via wet chemistry (e.g., Lys, 2.68 vs. 2.51%, [data not shown]). Thus, it is likely that the difference between calculated and analyzed total AA in Diet 4 was a result of an overestimation of these AA (by NIR analysis) in heat-damaged SBM, thereby indicating the necessity for further improvement in the analysis. In diets for Exp. 2, the concentration of analyzed total Lys was also less than the calculated concentrations. In particular, the concentration of total Lys in Diet 4 turned out to be close to that in Diet 3 (1.08 vs.

1.10%) and approximately 0.08% less than the calculated value. This was unexpected and may have partly contributed to the observed performance of pigs in Exp. 2.

Performance

The difference in the final BW observed between pigs fed Diet 1 and Diet 2 at the end of Exp. 1 was expected because of the reduced concentration and digestibility of AA in Diet 2. Supplementation of practical diets with crystalline AA is a common practice, but because of the overestimation of the concentration of Lys in feedstuffs that have been heat-damaged, the quantity of digestible AA added to diets containing such ingredients may not meet the pig`s requirement, thus, leading to decreased performance. This is likely the reason for the reduced performance of pigs fed Diet 2. However, the fact that pigs fed Diet 4 had greater final BW, ADG, and G:F than pigs fed Diet 2 indicates that by taking both the reduced concentration of AA and the reduced digestibility of AA in heat-damaged SBM into account in diet formulation, the negative effects of heat damage may be ameliorated. To our knowledge, this is the first time an experiment has been conducted to evaluate the effects of heat damage and the use of different diet formulation approaches on performance of weanling pigs fed heat-damaged SBM. A similar experiment, however, was conducted with broiler chicks (Evonik, 2010). Performance of broilers fed diets containing autoclaved SBM was less than the performance of broilers fed diets containing non-autoclaved SBM, but performance of broilers fed diets containing autoclaved SBM was restored to the same level as that observed for chicks fed diet containing non-autoclaved SBM when the reduced concentration and digestibility of AA in the negative control diet was taken into account in diet formulations. Thus, results of the present experiment with pigs are in agreement with the data observed in broilers. These results confirm the need for nutrient evaluation of each individual batch of SBM before diet formulation.

One of the possible reasons why performance of pigs fed Diet 4 in Exp. 2 was not improved to the same level as that of pigs fed Diet 1 may be that the SID values used to formulate this diet were determined from experiments using growing pigs whereas weaned pigs were used in this experiment. It has been reported that the digestibility of AA is considerably less for weaned pigs compared with growing pigs (Mariscal-Landin et al., 2008). It is also possible that the impact of heat damage on the digestibility of AA may be greater in weaned pigs compared with growing pigs but, to our knowledge, this has not been demonstrated. Another factor that may have contributed to the lack of a response to adjustments in diet formulation in Exp. 2 is that the differences in Lys concentration among diets were less than in Exp. 1 because of the relatively low inclusion level of DDGS in the diets. In the present experiment, DDGS was included in diets at a level of 22% to be consistent with what is recommended for weaned pigs (National Swine Nutrition Guide, 2010). The small differences in Lys concentration among diets is likely the reason we did not observe differences in ADG among treatments. The differences in G:F among treatments is likely a result of the increased ADFI of pigs fed diets containing autoclaved SBM or DDGS compared with pigs fed the non-autoclaved ingredients. The reasons for the greater ADFI in pigs fed diets containing autoclaved DDGS may be that diets were more palatable because of the formation of Maillard reaction products (Ames, 1998) or because pigs were trying to compensate for the Lys deficiency in the diets. The latter assumption is supported by the fact that SID Lys:BW gain was the same among dietary treatments.

As a consequence of the reduced concentration and digestibility of AA in heat-damaged SBM, an imbalance of AA may be created in pigs fed diets containing SBM that has been heat-damaged, and some AA may have been absorbed in quantities that are less than the requirement. Protein synthesis, therefore, may have been limited by the concentrations of the limiting AA.

Amino acids that are absorbed in excess of what is used for protein synthesis will be catabolized and the amino group will be used in the synthesis of urea, which then is excreted via the urine (Klindt et al., 2006). Thus, the differences observed in PUN between pigs fed Diet 1 and Diet 2 were expected because in the diet containing non-autoclaved SBM, AA were expected to be more balanced, therefore, leading to increased protein synthesis and less PUN. The PUN concentrations observed for pigs fed Diet 4 were not different from those of pigs fed Diet 1, which confirms the need for adjustments in total AA concentrations and SID AA values according to the degree of heat damage in SBM. This observation also indicates that if such adjustments are accomplished, not only protein synthesis, but also performance, may be improved if heat-damaged SBM is included in diets.

Conclusions

Results from this experiment demonstrate the negative effects of excessive heating of SBM on performance of weaned pigs. Results also demonstrate that the negative effects of heat damage may be ameliorated if the reduced concentration as well as the reduced digestibility of AA in heat-damaged SBM is corrected. Diets containing heat-damaged SBM, therefore, need to contain greater concentrations of Lys, Met, and Thr compared with diets containing non-heat damaged SBM. Diets for weaned pigs containing up to 22% of heat-damaged DDGS reduce performance of pigs compared with diets containing DDGS that has not been heat-damaged, but correction for the reduced concentration and the reduced digestibility of AA in heat-damaged DDGS may not be of practical importance for weaned pigs.

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TABLES

Table 6.1. Analyzed nutrient composition of ingredients (as-fed basis), Exp. 1 and 2[1]

	Exp. 1			Exp. 2		
Item	Corn	Non-autoclaved SBM[2]	Autoclaved SBM[3]	Corn	Non-autoclaved DDGS	Autoclaved DDGS[3]
DM, %	89.5	90.7	85.0	88.2	92.4	88.5
CP, %	8.54	47.78	46.47	7.89	27.91	26.82
ADF, %	2.09	4.56	4.49	1.85	8.53	15.47
NDF, %	8.93	10.28	34.94	9.75	31.93	29.65
Ca, %	0.00	0.31	0.29	0.01	0.05	0.05
P, %	0.23	0.61	0.57	0.24	0.89	0.87
Lys:CP[4]	-	6.09	5.40	-	3.05	2.24
Indispensable AA, %						
Arg	0.40	3.44	3.09	0.39	1.27	1.05
His	0.23	1.23	1.15	0.22	0.73	0.64

Table 6.1. (Cont.)

Ile	0.28	2.14	2.07	0.26	1.01	0.94
Leu	1.00	3.62	3.44	0.91	3.07	2.86
Lys	0.26	2.91	2.51	0.24	0.85	0.60
Met	0.17	0.67	0.63	0.16	0.56	0.50
Phe	0.41	2.38	2.25	0.37	1.30	1.20
Thr	0.29	1.89	1.78	0.28	1.05	0.97
Trp	0.06	0.65	0.62	-	-	-
Val	0.37	2.23	2.16	0.36	1.35	1.25
Dispensable AA, %						
Ala	0.60	2.05	1.95	0.58	1.99	1.85
Asp	0.56	5.39	5.08	0.52	1.82	1.66
Cys	0.18	0.67	0.58	0.18	0.57	0.49
Glu	1.49	8.43	7.99	1.39	4.69	4.37
Gly	0.32	2.02	1.93	0.31	1.12	1.04
Pro	0.74	2.34	2.25	0.69	2.32	1.99

Table 6.1. (Cont.)

Ser	0.40	2.40	2.24	0.38	1.35	1.23

[1]SBM = soybean meal; DDGS = distillers dried grains with solubles.
[2]This source of SBM was also included in diets fed to pigs in Exp. 2.
[3]Autoclaved at 125°C for 60 min.
[4]Calculated by expressing the concentration of Lys in each ingredient as a percentage of the concentration of CP (Stein et al., 2009).

Table 6.2. Ingredient and nutrient composition of diets used in Exp. 1 (as-fed basis)

	Diets[1]			
	Non-autoclaved SBM[2]	Autoclaved SBM - 125°C, 60 min		
	Diet 1	Diet 2	Diet 3	Diet 4
Ingredient, %				
Non-autoclaved SBM	31.50	-	-	-
Autoclaved SBM	-	31.50	31.50	31.50
Ground corn	61.93	61.93	61.93	61.93
Soybean oil	2.00	2.74	2.00	2.29
Corn starch	2.00	2.00	1.84	1.42
Dicalcium phosphate	0.94	0.94	0.94	0.94
Ground limestone	1.00	1.00	1.00	1.00
Salt	0.20	0.20	0.20	0.20
L-Lys	0.08	0.08	0.18	0.24
L-Thr	-	-	0.02	0.06
DL-Met	0.05	0.05	0.08	0.10
L-Trp	-	-	0.01	0.02
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30
Calculated nutrients,[4] %				
CP	20.42	19.89	20.01	20.12
Total Lys	1.14	1.14	1.13	1.18

Table 6.2. (Cont.)

SID Lys	1.00	1.00 (0.88)	1.00 (0.95)	1.00
SID Met	0.33	0.33 (0.31)	0.35 (0.34)	0.36
SID Met + Cys	0.61	0.61 (0.56)	0.61 (0.59)	0.61
SID Thr	0.67	0.67 (0.61)	0.66 (0.63)	0.67
SID Trp	0.21	0.21 (0.19)	0.21 (0.20)	0.21
SID Ile	0.76	0.76 (0.70)	0.74 (0.70)	0.70
SID Val	0.84	0.84 (0.78)	0.82 (0.78)	0.78
SID Leu	1.56	1.56 (1.46)	1.53 (1.46)	1.46
Ca, %	0.71	0.71	0.71	0.71
Available P, %	0.32	0.32	0.32	0.32

[1]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA.

[2]SBM = soybean meal.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4]SID = standardized ileal digestible; Values in parentheses were calculated taking into account the negative effect of heat damage on the SID of AA.

Table 6.3. Ingredient and nutrient composition of diets used in Exp. 2 (as-fed basis)

	Diets[1]			
	Non-autoclaved DDGS[2]	Autoclaved DDGS - 125°C, 60 min		
	Diet 1	Diet 2	Diet 3	Diet 4
Ingredient, %				
Non-autoclaved DDGS	22.00	-	-	-
Autoclaved DDGS	-	22.00	22.00	22.00
Ground corn	62.20	62.20	62.20	62.20
Soybean meal	8.50	8.50	8.50	8.50
Soybean oil	1.464	1.464	1.464	1.593
Corn starch	2.00	2.00	1.95	1.67
Dicalcium phosphate	0.98	0.98	0.98	0.98
Ground limestone	1.13	1.13	1.13	1.13
Salt	0.15	0.15	0.15	0.15
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30
L-Lys	0.695	0.695	0.715	0.754
L-Thr	0.181	0.181	0.186	0.210
DL-Met	0.146	0.146	0.160	0.184
L-Trp	0.098	0.098	0.101	0.110
L-Val	0.091	0.091	0.092	0.130
L-Ile	0.060	0.060	0.065	0.091

Table 6.3. (Cont.)

Calculated nutrients,[4] %				
CP	16.02	15.97	16.01	16.13
Total Lys	1.13	1.13	1.13	1.15
SID Lys	1.00	1.00 (0.95)	1.00 (0.97)	1.00
SID Met	1.13	1.13 (1.11)	1.13 (1.12)	1.15
SID Met + Cys	0.38	0.38 (0.36)	0.38 (0.38)	0.40
SID Thr	0.60	0.60 (0.56)	0.60 (0.57)	0.60
SID Trp	0.63	0.63 (0.60)	0.63 (0.61)	0.63
SID Ile	0.21	0.21 (0.20)	0.21 (0.20)	0.21
SID Val	0.54	0.54 (0.51)	0.54 (0.51)	0.54
SID Leu	0.68	0.68 (0.64)	0.68 (0.64)	0.68
Ca, %	1.36	1.36 (1.30)	1.34 (1.30)	1.30
Available P, %	0.70	0.70	0.70	0.70

[1]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA.

[2]DDGS = distillers dried grains with solubles.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alphatocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide, 1.0 mg, and nicotinic acid, 43.0 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[4]SID = standardized ileal digestible; Values in parentheses were calculated taking into account the negative effect of heat damage on the SID of AA.

Table 6.4. Analyzed nutrient composition of diets used in Exp. 1 (as-fed basis)

	Diets[1]			
	Non-autoclaved SBM[2]	Autoclaved SBM - 125°C, 60 min		
	Diet 1	Diet 2	Diet 3	Diet 4
Item				
GE, kcal/kg	4,106	4,041	4,059	4,058
DM, %	90.27	89.23	89.29	89.36
CP, %	19.75	19.85	19.48	20.01
ADF, %	2.74	2.71	2.79	2.98
NDF, %	8.92	17.75	18.88	17.68
Ca, %	0.74	0.70	0.75	0.75
P, %	0.55	0.50	0.52	0.54
Indispensable AA, %				
Arg	1.31	1.20	1.19	1.20
His	0.53	0.51	0.51	0.51
Ile	0.84	0.82	0.82	0.85
Leu	1.73	1.73	1.74	1.73
Lys	1.13	0.97	1.03	1.10
Met	0.36	0.33	0.36	0.38
Phe	0.99	0.99	0.98	0.98
Thr	0.77	0.76	0.79	0.79
Trp	0.24	0.23	0.24	0.25

Table 6.4. (Cont.)

Val	0.93	0.91	0.90	0.95
Dispensable AA, %				
Ala	1.01	1.00	1.01	1.00
Asp	2.02	1.99	1.96	1.99
Cys	0.32	0.29	0.29	0.29
Glu	3.53	3.50	3.49	3.49
Gly	0.83	0.82	0.81	0.82
Pro	1.19	1.19	1.19	1.18
Ser	0.99	0.99	0.97	0.95

[1]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA.

[2]SBM = soybean meal.

Table 6.5. Analyzed nutrient composition of diets used in Exp. 2 (as-fed basis)

	Diets[1]			
	Non-autoclaved DDGS[2]	Autoclaved DDGS - 125°C, 60 min		
	Diet 1	Diet 2	Diet 3	Diet 4
Item				
GE, kcal/kg	4,071	4,054	4,077	4,064
DM, %	89.17	88.51	88.34	88.44
CP, %	15.72	15.27	15.44	16.01
ADF, %	3.58	4.47	5.44	5.03
NDF, %	13.52	14.33	14.00	13.62
Ca, %	0.74	0.63	0.68	0.70
P, %	0.60	0.58	0.57	0.57
Indispensable AA, %				
Arg	0.80	0.76	0.75	0.78
His	0.40	0.39	0.38	0.40
Ile	0.61	0.58	0.60	0.65
Leu	1.52	1.53	1.50	1.56
Lys	1.11	1.06	1.10	1.08
Met	0.41	0.39	0.41	0.44
Phe	0.71	0.71	0.70	0.73
Thr	0.72	0.69	0.72	0.72

Table 6.5. (Cont.)

Trp	0.22	0.22	0.21	0.23
Val	0.77	0.75	0.76	0.82
Dispensable AA, %				
Ala	0.94	0.95	0.93	0.96
Asp	1.17	1.15	1.13	1.18
Cys	0.28	0.27	0.27	0.28
Glu	0.61	0.60	0.59	0.61
Gly	1.15	1.10	1.08	1.13
Pro	0.73	0.73	0.70	0.73
Ser	0.94	0.95	0.93	0.96

[1]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA.

[2]DDGS = distillers dried grains with solubles.

Table 6.6. Performance of weanling pigs fed diets containing non-autoclaved or autoclaved soybean meal (SBM)[1]

	Diets[2]					
	Non-autoclaved SBM	Autoclaved SBM - 125°C, 60 min			SEM	*P*-value
Item	Diet 1	Diet 2	Diet 3	Diet 4		
d 0 to 7						
Initial BW, kg	10.47	10.43	10.44	10.44	0.45	0.52
ADG, kg	0.353[a]	0.231[c]	0.295[b]	0.331[ab]	0.020	< 0.01
ADFI, kg	0.590[b]	0.563[b]	0.619[ab]	0.663[a]	0.030	0.04
G:F	0.595[a]	0.410[c]	0.459[b]	0.500[b]	0.017	< 0.01
Final BW, kg	13.02[a]	12.06[c]	12.52[b]	12.75[ab]	0.54	< 0.01
d 7 to 14						
ADG, kg	0.526[a]	0.384[c]	0.451[b]	0.481[ab]	0.019	< 0.01
ADFI, kg	0.861[b]	0.864[b]	0.906[ab]	1.008[a]	0.044	0.08
G:F	0.614[a]	0.460[b]	0.480[b]	0.480[b]	0.019	< 0.01
Final BW, kg	16.70[a]	14.87[c]	15.68[b]	16.13[ab]	0.66	< 0.01
d 14 to 21						

Table 6.6. (Cont.)

ADG, kg	0.571[a]	0.437[c]	0.510[b]	0.510[b]	0.027	< 0.01
ADFI, kg	0.989	1.085	1.099	1.099	0.051	0.18
G:F	0.579[a]	0.432[b]	0.464[b]	0.464[b]	0.01	< 0.01
Final BW, kg	20.71[a]	18.00[c]	19.26[b]	19.70[b]	0.82	< 0.01
Overall (d 0 to 21)						
ADG, kg	0.490[a]	0.351[c]	0.418[b]	0.440[b]	0.018	< 0.01
ADFI, kg	0.813[b]	0.838[ab]	0.873[ab]	0.921[a]	0.038	0.08
G:F	0.600[a]	0.442[c]	0.471[bc]	0.478[b]	0.012	< 0.01
SID Lys:BW gain, g/kg	16.49[b]	18.30[a]	18.46[a]	19.59[a]	0.43	< 0.01
PUN,[3] mg/dL	11.19[c]	17.88[a]	14.44[b]	12.81[bc]	0.70	< 0.01

[a-c]Means within the same row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are means of 8 observations per treatment.

[2]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA. The SID Lys level in diets was 0.99 (Diet 1), 0.81 (Diet 2), 0.87 (Diet 3), and 0.93% (Diet 4).

[3]PUN = plasma urea nitrogen.

Table 6.7. Performance of weanling pigs fed diets containing non-autoclaved or autoclaved distillers dried grains with solubles (DDGS)[1]

	Diets[2]					
	Non-autoclaved DDGS	Autoclaved DDGS - 125°C, 60 min			SEM	*P*-value
Item	Diet 1	Diet 2	Diet 3	Diet 4		
d 0 to 7						
Initial BW, kg	9.89	9.94	9.89	9.93	0.52	0.50
ADG, kg	0.214	0.170	0.182	0.215	0.02	0.06
ADFI, kg	0.569	0.612	0.563	0.604	0.05	0.35
G:F	0.383[a]	0.278[b]	0.324[ab]	0.363[a]	0.03	0.03
Final BW, kg	11.39	11.15	11.15	11.44	0.63	0.06
d 7 to 14						
ADG, kg	0.395	0.435	0.433	0.442	0.03	0.32
ADFI, kg	0.807[b]	0.969[a]	0.945[a]	1.030[a]	0.07	0.02
G:F	0.490	0.454	0.466	0.433	0.02	0.21
Final BW, kg	14.16	14.19	14.18	14.54	0.77	0.23

Table 6.7. (Cont.)

d 14 to 21						
ADG, kg	0.546	0.528	0.501	0.535	0.03	0.39
ADFI, kg	1.086	1.189	1.155	1.219	0.07	0.17
G:F	0.505	0.451	0.436	0.446	0.02	0.08
Final BW, kg	17.99	17.89	17.69	18.28	0.95	0.36
Overall (d 0 to 21)						
ADG, kg	0.385	0.378	0.372	0.397	0.02	0.46
ADFI, kg	0.821[b]	0.923[a]	0.887[ab]	0.950[a]	0.06	0.03
G:F	0.472[a]	0.413[b]	0.423[b]	0.422[b]	0.01	0.02
SID Lys:BW gain, g/kg	20.86	22.19	22.65	22.47	0.75	0.32

[a-b] Means within the same row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are means of 8 observations per treatment.

[2]Diet 3 = diet was formulated taking into account the negative effects of heat damage on the concentration of AA; Diet 4 = diet was formulated taking into account the negative effects of heat damage on the concentration and on the digestibility of AA.

GENERAL CONCLUSIONS

Heat processing during manufacture of distillers dried grains with solubles (DDGS), canola meal, sunflower meal, and cottonseed meal is detrimental to their nutritional value because not only the concentration, but also the digestibility of most AA is reduced.

Fiber components, such as ADF, NDF, and lignin, give some indication that a feed ingredient has been heat-damaged, but these indications are not consistent among all ingredients used in this research. As the degree of heat damage increased, the analyzed concentrations of NDF in canola meal, sunflower meal, and cottonseed meal increased, but that was not the case for DDGS. Increasing the degree of heat damage also resulted in an increase in the concentration of ADF in DDGS, canola meal, and sunflower meal, but not in cottonseed meal. The concentration of analyzed lignin was also affected by heat damage, in which the greater the degree of heat damage, the greater the concentration of lignin in the feed ingredients evaluated. The concentration of calculated reactive Lys using the furosine procedure also provides information about the degree of heat damage. In the current research, the concentration of reactive Lys corresponded to approximately 98% of the concentration of total Lys. Regression analyses indicated that there is a good correlation between the concentration of standardized ileal digestible Lys and the concentration of reactive Lys. Equations developed in these experiments, however, need further validation using different sources of DDGS, canola meal, sunflower meal, and cottonseed meal. Alternatively, the Lys:CP ratio gives a good indication that a feed ingredient has been heat-damaged, and this was consistent among all feed ingredients evaluated in this research. Thus, it appears that the protein quality of DDGS, canola meal, sunflower meal, and cottonseed meal can be determined by the Lys:CP ratio.

Among all AA, the digestibility of Lys was the most negatively affected by heat damage in DDGS, canola meal, sunflower meal, and cottonseed meal. Heat damage also decreased the digestibility of all AA for all feed ingredients evaluated in this research, although this reduction was in some cases, linear, and in others, quadratic.

Performance of weanling pigs fed heat-damaged soybean meal or DDGS was reduced compared with pigs that were feed non-heat- damaged soybean meal or DDGS. If the concentration of standardized ileal digestible AA in diets containing heat-damaged soybean meal or DDGS is adjusted by supplementation of with crystalline AA, performance of weaned pigs may be partially ameliorated.

In conclusion, this research indicates that the chemical composition of feed ingredients is affected by heat damage, but the Lys:CP ratio was the parameter that showed more consistent changes among the feed ingredients evaluated. Thus, determination of Lys:CP ratio is recommended to identify heat-damaged feed ingredients. If heat-damaged feed ingredients are used in diets for weaned pigs, it is necessary to adjust the concentrations of standardized ileal digestible AA in the formulation, by supplementing crystalline AA, to compensate for the losses in the concentrations and digestibility of AA.