UTILIZATION OF AMINO ACIDS IN CORN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) BY PIGS AND POULTRY AND THE USE OF REACTIVE LYSINE PROCEDURES TO EVALUATE DDGS QUALITY

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ABSTRACT

Five experiments were conducted to investigate aspects of AA digestibility in distillers dried grains with solubles (DDGS) and the use of in vitro procedures to predict Lys utilization in DDGS by growing pigs and poultry. In Exp. 1, the effect of regional source of DDGS (IL, MN, KY), addition of solubles to DDGS (DDG vs. DDGS) and type of facility producing DDGS (ethanol plants vs. a beverage plant) on the standardized ileal digestibility (SID) of AA was investigated using ileal cannulated pigs. Results showed that SID of AA among DDGS sources within a region varies as much as DDGS sourced across regions. The SID for Lys in DDGS from a beverage plant was greater ($P < 0.05$) than the SID of Lys in DDGS from dry-grind ethanol plants. Most AA in DDG had a greater ($P < 0.05$) SID compared with DDGS. In Exp. 2, optimal conditions for guanidination to convert unbound (i.e., reactive) Lys to homoarginine of DDGS were initially established to improve the accuracy of measuring the amount of reactive Lys in DDGS by homoarginine procedure. Optimum conditions for guanidination of DDGS and ileal digesta were at 3 and 1 d of incubation, respectively, using 0.6 *M* O-methylisourea with a pH between 11 and 12. In Exp. 3, the quantity of reactive Lys in 33 sources of corn DDGS was measured and was used to predict the concentration of SID Lys in DDGS fed to growing pigs. The effect of solubles in DDGS on the concentration of reactive Lys was also investigated using 4 ratios of condensed distillers solubles (CDS) to wet distillers grain (WDG) that was freeze-dried, or oven-dried at 50, 75, or 100°C. Only 74.1% of total Lys was reactive in the 33 sources of DDGS. The concentration of SID Lys in DDGS was correlated with the concentration of reactive Lys ($r^2 = 0.70$, $P < 0.05$). It was concluded that reactive Lys may be used as an in vitro procedure to predict the

SID of Lys in DDGS. Addition of CDS was found to exacerbate the negative effects of heating on the concentration of total Lys and reactive Lys in DDGS. The implication of this is that addition of CDS during DDGS production should not be excessive. In Exp. 4, it is hypothesized that the conventional procedure to measure SID of Lys in DDGS fed to pigs may overestimate the amount of digestible Lys because this procedure does not completely distinguish between reactive Lys and unreactive Lys, although only reactive Lys is bioavailable. Ileal digestibility of Lys in DDGS estimated using reactive Lys or conventional ileal digestibility procedure was, therefore, measured using cannulated growing pigs. The average SID of reactive Lys was 66.9%, which is close to the average SID of total Lys (66.5%). However, the concentration of SID reactive Lys (3.9 g/kg) was lower ($P < 0.05$) than the concentration of SID total Lys (5.1 g/kg). It was concluded that the conventional procedure may over-estimate the concentration of digestible Lys in DDGS, and measurement of digestible reactive Lys may more accurately estimate Lys available to the pig. In Exp. 5, the concentration of standardized digestible (SDD) Lys in DDGS was measured using cecectomized roosters and these values were compared the relative bioavailable Lys in DDGS measured in chicks. Two in vitro procedures (reactive Lys and Hunterlab color score) were also used to predict the concentration of SDD Lys and bioavailable Lys in DDGS. Concentration of SDD Lys among DDGS sources varied ($P < 0.05$), but the concentration of relative bioavailable Lys were similar among DDGS sources. The average concentration of SDD Lys in DDGS was not different from the concentration of bioavailable Lys (0.47% and 0.53%, respectively). The concentration of SDD Lys in DDGS was correlated ($r^2 = 0.84$, $P < 0.05$) with the concentration of reactive Lys. Lighter color of DDGS was associated with a greater ($r^2 = 0.90$, $P < 0.05$)

concentration of bioavailable Lys. In conclusion, the concentration of SDD Lys in DDGS is closely related with the concentration of bioavailable Lys. Values for reactive Lys and the degree of lightness (L score) in DDGS may be used to estimate the concentration of SDD Lys and bioavailable Lys in DDGS fed to poultry.

Keywords: Amino acid, digestibility, distillers dried grains with solubles, pigs, reactive Lys

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LIST OF ABBREVIATIONS

a (score)	Degree of redness, Hunterlab scale
AA	Amino acid
ADF	Acid detergent fiber
AID	Apparent ileal digestibility
Ala	Alanine
ANF	Antinutritional factor(s)
AOAC	Association of Official Analytical Chemists
Arg	Arginine
Asp	Aspartate
b (score)	Degree of yellowness, Hunterlab scale
BW	Body weight
^{o}C	Degree Celsius
CA	California
Ca	Calcium
CDS	Condensed distillers solubles
cm	Centimeter(s)
CP	Crude protein
Cu	Copper
CV	Coefficient of variation
Cys	Cystine
d	Day(s)

DDG	Distillers dried grains
DDGS	Distillers dried grains with solubles
DE	Digestible energy
DM	Dry matter
Exp.	Experiment
FDNB	Fluorodinitrobenze sulfonic acid
Fe	Iron
g	Gram(s)
GE	Gross energy
Glu	Glutamate
Gly	Glycine
h	Hour(s)
HCl	Hydrochloride/ Hydrochloric acid
His	Histidine
HPLC	High performance liquid chromatography
IL	Illinois
Ile	Isoleucine
IN	Indiana
IU	International unit
kg	Kilogram
KY	Kentucky
L (score)	Degree of lightness, Hunterlab scale
Leu	Leucine

Lys	Lysine
M	Meter(s)
M	Molar
ME	Metabolizable energy
Met	Methionine
mg	Milligram(s)
mL	Milliliter(s)
mmol	Millimole(s)
MN	Minnesota
Mn	Manganese
MO	Missouri
MR	Maillard reaction
MW	Molecular weight
µg	Microgram(s)
Na	Sodium
NaOH	Sodium hydroxide
NC	North Carolina
ND	North Dakota
NDF	Neutral detergent fiber
NRC	National Research Council
OH	Ohio
OM	Organic matter
P	Phosphorus

PA	Pennsylvania
Pro	Proline
r^2	Coefficient of determination
RFA	Renewable fuels resources
RMSE	Root mean square error
SAS	Statistical analysis system
SD	South Dakota
Se	Selenium
SEM	Standard error of the mean
Ser	Serine
SID	Standardized ileal digestibility
Thr	Threonine
TID	True ileal digestibility
TNBS	Trinitrobenze sulfonic acid
Trp	Tryptophan
US	United States of America
VA	Virginia
Val	Valine
WDG	Wet distillers grains
WI	Wisconsin
x *g*	Multiplied by gravity (force)
Zn	Zinc

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CHAPTER 1

Introduction

Corn distillers dried grains with solubles (**DDGS**) is a by-product from the wet mill and dry grind ethanol extraction process that is obtained by drying 2 product streams, namely the wet distillers grains (**WDG**) and the condensed distillers solubles (**CDS)** in a 75:25 ratio (AAFCO, 2006). The availability of dry grind ethanol by-products has increased in recent years and approximately 17% of total US corn production in 2006 was used as feedstock for ethanol production (RFA, 2007).

The use of DDGS as feedstuff for non-ruminant animals has traditionally been limited due to its less consistent nutrient composition relative to other protein sources such as soybean meal. However, DDGS produced from newer dry-grind ethanol plants appear to have a better nutrient composition than the traditional DDGS from beverage plants (Spiehs et al., 2002). Drying of DDGS prolongs its shelf life and improves its handling during transport. However, the drying process can reduce the feeding value of DDGS as a result of the interaction between Lys and reducing sugars. Therefore, it is important to understand the factors that affect the quality and the procedures that can be used to measure the nutritional value of DDGS.

Ileal AA digestibility and AA bioavailability values are necessary to establish the protein quality and optimize the feeding value of DDGS for livestock. However, rapid methods may be needed to routinely evaluate DDGS for correctness of processing to minimize the impact of its nutrient variation on animal performance. The sources of variation in DDGS composition can be due to differences in plant design, drying temperature, and amount of CDS added to the WDG during drying (Spiehs et al., 2002).

This variation has been suggested to cause differences in the standardized ileal digestibility (**SID**) of AA, particularly Lys, by growing pigs (Stein et al., 2006).

Among the rapid methods that may be used as a routine analysis for DDGS quality are procedures that measure of the amount of Lys that is not bound to reducing sugars; this is known as the reactive Lys. The reactive Lys procedure has been used to estimate the bioavailable Lys in a number of protein sources (Hurrell and Carpenter, 1981). It is not known if the reactive Lys concentration in DDGS is also related to Lys digestibility in pigs.

The objectives of the work presented in this dissertation are to determine the variability of SID by growing pigs in DDGS from different sources. The second objective is to investigate if there is a relationship between the reactive Lys concentration and the SID Lys concentration in DDGS and to evaluate the use of reactive Lys as a procedure to rank the quality of DDGS from different sources. The final objective is to evaluate if the digestible Lys concentration is similar to the concentration of bioavailable Lys in DDGS fed to poultry.

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CHAPTER 2

Distillers dried grains with solubles fed to pigs and poultry and methods in protein quality evaluation: Review of literature

Production of Distillers Dried Grains with Solubles (DDGS)

Majority of the DDGS produced in the US is from the dry milling of grains and only a smaller percentage from the wet milling process (RFA, 2007). The dry milling process has been described (Rausch and Belyea, 2006) and involves at least 5 steps: grinding, liquefaction, saccharification, fermentation, and distillation (Figure 2.1). The feedstock is initially ground and mixed with water, followed by steam-cooking. Alpha-amylase is added to hydrolyze the starches into glucose, maltose, and branched, short chains of sugars called α-limit dextrins. The slurry then becomes semi-liquid as a result of starch gelatinization and enzymatic hydrolysis. The α-limit dextrins are converted to glucose by the action of glucoamylase in the saccharification step. Glucoamylase catalyses the hydrolysis of α-1,4 glycosidic bonds and progressively removes single glucose molecule from both ends of amylose. Glucoamylase can also cleave the branching point (α-1,6 bonds) of amylopectin, but at a lower rate than the hydrolysis of α-1,4 bonds. To completely hydrolyze the α-limit dextrins, further cooking at 60^{o}C for about 5 h is needed and the pH is adjusted to 4 to5 to maximize the activity of the enzyme. The slurry is converted to an almost liquid mash and enters the fermentation step that converts glucose into ethanol though the addition of a live yeast, typically *Saccharomyces ceriviciae*.

The mash that remains after ethanol has been extracted is called whole stillage, which is composed of the residual proteins, fat, fiber, unfermented starch, sugars, yeast cell proteins, vitamins, minerals, and water. The whole stillage is centrifuged to remove most of the liquid phase, leaving behind most of the solids called wet distillers grains (**WDG**). The liquid that is recovered from centrifugation is called the thin stillage, which contains some protein, AA, fat, low molecular weight sugars, vitamins and minerals. Most of the thin stillage is water, which may be concentrated by evaporation to form the condensed distillers solubles (**CDS**). This increases the DM of CDS to about 50% (Belyea et al., 1998), and it may be further dried to 88% DM. The dried WDG, called dried distillers grains (**DDG**) contains mostly proteins and fiber, whereas the CDS contains mostly residual sugars (Wu, 1994). The residual fat is present both in CDS and DDG (Belyea et al., 1998). During drying, CDS is added back to WDG and the amount added is believed to vary (Knott et al., 2004). This contributes to the differences in the composition of DDGS.

Composition of Dry Grind Ethanol Co-products

The composition of most nutrients is 3 times greater in DDGS than in the original grain source as a result of the removal of most starch after fermentation. The typical composition of ethanol by-products is summarized in Table 2.1. The shift in DDGS production from the beverage industry to the ethanol industry has changed the composition of traditional DDGS. On a DM basis, corn DDGS contains about 30% CP, 3440 kcal DE, 9% crude fat, 0.20% calcium, 0.8% phosphorus, 37% NDF, and 17% ADF (NRC, 1998). The concentration of Lys, Met, Thr, and Trp in DDGS is typically about 0.6, 0.5, 1.0, and 0.27% (NRC, 1998). Newer batches of DDGS appear to have a higher

Lys, Thr, and Trp concentrations than previously published values (Spiehs et al., 2002). However, the standardized ileal digestibility (**SID**) values of most AA in DDGS is lower compared with corn and this may be due to the high NDF concentration in DDGS, which can exert a negative effect on AA digestibility (Lenis et al., 1996). The fat concentration in newer batches of DDGS appears to be greater than previously published values (NRC, 1998). Recent studies (Stein et al., 2006; Pedersen et al., 2007) indicate that the DE and ME concentrations in newer batches of DDGS is higher than previously predicted by NRC (1998) and this may be due to the higher concentration of crude fat in newer corn varieties. The P digestibility in DDGS is reported to be higher than in corn because of the improved digestibility of phytate phosphorus after fermentation (Pedersen et al., 2007).

Application of Heat During Dry Milling and DDGS Production

Heat processing has been applied to various feedstuffs including field peas (Stein and Bohlke, 2007) soybean meal (Vandergrift et al., 1983; Hancock et al., 1990) and cottonseed meal (Kuiken and Trant, 1952). The most important benefits of proper heat processing is improvement in nutrient and energy digestibility, improved storage life, and elimination of anti-nutritional factors (**ANF**). Moderate heating can partially denature the proteins, which may increase the access of digestive enzymes (Mauron, 1982; Swaisgood and Catignani, 1991). Several steps in the dry grind ethanol facilities involve application of heat. In the liquefaction stage, the jet-cooking process can raise the temperature of the mash to 90 to 100^{o}C for several minutes (Bothast and Schilcher, 2005). In the saccharification stage, the temperature of the mash is kept at about 60^{o}C, which is the optimum for the action of glucoamylase at a pH of 4 to 5. In the dehydration of thin

stillage to CDS, a temperature of 100^{o}C is used. In the drying of WDG, the temperature may range from 90 to 315^{o}C. The temperature and time may vary depending on the dryer design.

The consequences of heat application include the initiation of the Maillard reaction (**MR**). Other forms of heat damage such as caramelization, which only involves the oxidation of sugars (Heyns and Klier, 1968), may occur as well. Formation of enzymatically resistant bonds between ε-NH_2 groups of Lys and carboxyl groups of Glu and Asp can also occur (Bjarnason and Carpenter, 1969). These indigestible peptides can block the transport of AA across the intestinal wall (Ford, 1973). Other negative effects of heating include the conversion of some of the L-forms of AA into D-forms (racemization) and formation of lysinoalanine (Hayase et al., 1973; Sternberg et al., 1975; Meade et al., 2005).

Maillard Reaction

The pathways of MR and its physiological and nutritional consequences have been described (Hodge, 1953; Mauron, 1981). The initial steps involve a covalent binding of the aldehyde group of reducing sugars and the ε-NH_2 groups of Lys. The reaction is driven mainly by the presence of substrates and sufficient temperature and moisture. The reaction has been classified into early, advanced, and late Maillard stages (Mauron, 1981), but it is a continuous process and all phases are probably occurring at the same time. Different MR products are produced at each stage, with Amadori products being produced in the early phase and premelanoidins and melanoidins in the late phase. The advanced stage is variable because it depends on the pH of the solution. Formation of furfurals such as 5-hydroxy methyl-2 furaldehyde is favored when the pH is

< 3, whereas formation of reductones and dehydroreductones will predominate when the pH is >7 (Hodge, 1953). Otherwise, carbonyls and dicarbonyls are formed. Regardless of the advanced MR product formed, all pathways will eventually proceed to the formation of melanoidins.

Factors Affecting the Rate of Maillard Reaction

Temperature. High temperature is not necessary for MR to proceed because even prolonged storage at room temperature can initiate the reaction (Hurrell and Carpenter, 1981). Temperature and water interact to initiate MR and when water activity is low, higher temperature is needed for MR to occur (Labuza and Saltmarch, 1981; Petriella et al., 1985).

Moisture and Water Activity. No reaction takes place when the reactive groups are not dissolved in water (Swaisgood and Catignani, 1991). However, when the water content is above 90%, MR is also least likely to occur because the substrates may not be in contact with each other (Wolfrom and Rooney, 1953; Lea and Hannan, 1949). It appears that MR is fastest at 30% moisture or at a water activity of 0.65 to 0.70 (Leah and Hannan, 1949).

pH. The reaction generally has a minimum pH requirement of 3 (Lea and Hannan, 1949) and the rate of reaction is increased at pH above 6 (Ashoor and Zent, 1984). At a pH of less than 3 and greater than 9, other non-enzymatic interactions (i.e. sugar-sugar and protein-protein) compete with the MR, slowing the MR rate.

Type of Substrate. Glucose, xylose, and arabinose, appear to be more susceptible to MR than other sugars, whereas fructose is not susceptible at all (Lewis and Lea, 1950). Maillard reaction is also affected by the type of AA in the protein, with Lys, Gly, Trp and

Tyr as the most susceptible, whereas His, Thr, Asp, Arg, Glu, and Cys being least susceptible (Ashoor and Zent, 1984).

Negative Effects of Heating on AA in Feedstuffs

Reduction in Concentration of Lys. Application of heat is known to reduce the concentration of Lys in DDGS and other feedstuffs (Craig and Broderick, 1981; Martinez Amezcua and Parsons, 2007). During MR, the loss of analyzed Lys is due to the transformation of Lys to advanced MR products such as premelanoidins, and melanoidins (Mauron, 1981).

Reduction in Digestible Lys. Feedstuffs that have been subjected to over-processing frequently have a lower N digestibility (e.g., Craig and Broderick, 1981). Autoclaving and oven-drying have been reported to reduce the true ileal digestibility of Lys and other AA in DDGS fed to poultry (Martinez Amezcua and Parsons, 2007). The reduction in digestibility of AA may be due to the reduced efficiency of digestive enzymes (Hansen and Millington, 1979), formation of resistant cross-links between AA (Mottu and Mauron, 1967; Bjarnason and Carpenter, 1969; Hurrell et al., 1976), and increased endogenous N losses (Knipfel, 1981). Amadori compounds may reduce the absorption of other AA, particularly Thr, Pro, and Gly by blocking their transport (Erbersdobler et. al., 1981; Rerat et al., 2002). Advanced MR products may also bind to the side chains of other AA and thereby inhibit their normal absorption (Hurrell and Finot, 1985).

Reduction in Bioavailable Lys. A consequence of heat processing of feedstuffs is the reduction in Lys bioavailability (Batterham et al., 1986b; Johns et al., 1987; Moehn et al., 2005). This can occur even when the digestibility is slightly increased, as seen when

legumes are processed (Van Barneveld et al., 1994). Heat processing affects the bioavailability of AA more than its digestibility (Batterham et al., 1990; Van Barneveld et al., 1994) but digestibility and availability of AA in the same source of DDGS has not been measured in pigs. One of the causes for the reduction in the efficiency of utilization of absorbed Lys in heated proteins may be due to the unavailable Amadori compounds that are absorbed in the SI (Ford and Shorrock, 1971; Erbersdobler et al., 1981; Finot and Magnenat, 1981).

Overestimation of analyzed Lys in Early MR. Analysis of heated protein containing Amadori compounds can over-estimate the Lys due to the release of Lys from the Amadori compound during acid hydrolysis step of AA analysis (Erbersdobler, 1977). Because these released Lys from Amadori compounds are chemically identical to Lys that has not undergone Maillard reaction, both AA appear as a single peak in the chromatogram.

Variation in DDGS Composition, Digestibility, and Availability

Within and across plants, variation in nutrient composition in DDGS has been documented (Spiehs et al., 2002; Knott et al., 2004). The composition of WDG vary less than that of CDS (Knott et al., 2004) and the composition of CDS varies in the concentration of fat, total P, CP and AA (Wu et al., 1985; Wu; 1989; Belyea et al., 1998). The sources of variation in chemical composition of DDGS and ethanol by-products are grain source and grind size, amount of yeast proteins, fermentation and distillation condition, drying equipment and conditions, and the amount of CDS added to WDG (Carpenter, 1970). Particle size distribution may also affect the composition of DDGS

because it affects the efficiency of fermentation and subsequently, the concentration of residual components (Rausch et al., 2005).

The SID of Lys in DDGS is more variable than the SID of other AA (Table 2.2). Several factors affect the AA digestibility of feedstuffs including DM intake, feed processing, amount of CP and AA in the diet, fiber, fat, and ANF (Gabert et al., 2001). The extent of processing and the amount of structural fibers are 2 factors that likely contribute to the variability in digestibility of AA in DDGS.

The relative bioavailability of Lys in DDGS fed to poultry may vary from 66 to 100% (Combs and Bossard, 1968, 1969; Parsons et al., 1983; Lumpkins and Batal, 2005) and the wide variability may be due to drying of DDGS or the differences in the type and source of DDGS used in those studies. This may be due to differences in the temperature used to dry DDGS as observed in heat treatments of other feedstuffs (Hancock et al., 1990; Van Barneveld et al., 1994; Zhang and Parsons, 1996).

In Vivo Methods to Evaluate Protein Quality

Relative Bioavailability. Biological methods to estimate the efficiency of AA utilization include AA availability studies such as the slope-ratio assay, net protein ratio, net protein utilization, and protein efficiency ratio (Moughan, 2003). Bioavailability of AA in proteins include measures of digestion, absorption and utilization of AA at the cellular level. Determination of Lys bioavailability is usually obtained by slope ratio assay and parallel line assay (Campbell, 1966; Littell et al., 1997). Slope ratio assay has been used to assess the Lys bioavailability in DDGS (Combs and Bossard, 1969; Lumpkins and Batal, 2005) as well as other protein and animal sources (Batterham et al., 1984; 1986a). Validity of slope ratio assay depends on the linearity of response of the

animal performance (gain, feed efficiency) to the reference AA and the test AA and both curves should have a common intercept (Littell et al., 1997). The advantage of the bioavailability procedure is that it accounts for only the digested AA that is retained by the animal. The possible pitfalls in bioavailability assays include the inaccuracy in designing the specific AA-deficient diet, failure to ensure that the response is solely due to the effect of the test AA, and variation in the response of individual animals due to differences in nutritional and health status (Batterham, 1992). The repeatability of this procedure may vary as a result of differences in genotype, energy and AA balance of the test diets, and the type of response being measured such as N retention, weight gain, and feed efficiency (Adeola et al., 1994). The SE can be high (10% or more) in bioavailability studies and the procedure is tedious because only 1 AA can be measured for bioavailability at a time (Batterham, 1992; 1993).

Ileal AA Digestibility. The digestibility of a protein encompasses the process of enzymatic cleavage of peptide bonds in the proteins into short peptides and free AA and the absorption of these end-products from the intestinal lumen (Fuller, 2003). The principles and methodologies in ileal AA digestibility in pigs have been reviewed (Low, 1982; Sauer and Ozimek, 1986; Sibbald, 1987; Sauer et al., 1989; Stein et al., 2007). The individual animal response that is measured in digestibility study (i.e., extent of disappearance of AA in the digesta within the same treatment group) tend to vary less than the response being measured in bioavailability studies because availability is affected by both feed and animal factors (Batterham, 1992). As a result, ileal digestibility studies are more repeatable and the SE is generally lower than that of

bioavailability studies. The digestibility procedure is also less tedious because all AA can be measured for ileal AA digestibility in a single study.

Ileal digestibility of AA is more accurate than total tract digestibility because values for total tract digestibility are affected by the microbial fermentation in the large intestine (Sauer et al., 1989). Unless the animal is sacrificed to collect ileal digesta, it is necessary to attach a cannula in the distal ileum of pigs to collect the digesta. In poultry species, surgical removal of the ceca may be done to minimize the effect of microbial fermentation (Parsons, 1985).

Ileal AA digestibility procedures have been used to evaluate the AA quality in DDGS using pigs (Fastinger and Mahan, 2006; Stein et al., 2006) and poultry (Batal and Dale, 2006). Ileal digesta collection is conducted using different methods described in detail by Gabert et al. (2001). The T-cannulation method is widely accepted and has been used in many DDGS ileal digestibility studies (Fastinger and Mahan, 2006; Stein et al., 2006). Values for apparent ileal digestibility (**AID**) have some disadvantages because they are not always additive in mixed diets (Stein et al., 2005). These inaccuracies are a consequence of the fact that the AA in the ileal digesta are a mixture of unabsorbed AA from the ingested feed and unabsorbed AA from the endogenous secretions into the stomach and small intestine (Fuller, 1991). The nitrogenous components of endogenous origin include mucuproteins, digestive enzymes, bile, and desquamated epithelial cells (Moughan and Schuttert, 1991).

Methods have been developed to estimate the endogenous N losses including feeding of a N-free diet (Carlson and Bayley, 1970), low protein casein diet (Nyachoti et al., 1997), feeding of a diet labeled with isotopes (Souffrant et al., 1986), feeding

different diets with increasing level of N and regressing the digestibility to zero N intake (Fan et al., 1995), or guanidinating the diet (Schmitz et al., 1991). Regardless of the method, a degree of uncertainty inherent to the procedure is present (Nyachoti et al., 1997). Endogenous AA losses can be classified into basal and specific endogenous AA losses. The difference between basal and specific losses is that basal endogenous AA losses is the obligatory AA losses that are a result of the normal turn-over of intestinal secretions along the intestinal lumen, whereas specific endogenous AA losses are the additional endogenous AA losses that are induced by the feed ingredient (Stein et al., 2007). Apparent ileal digestibility (**AID**) is the calculated digestibility of AA that has not been corrected for endogenous AA losses. When AID values have been corrected for basal endogenous AA losses, then the values are termed standardized ileal digestibility (**SID**). True ileal digestibility values are AID values that have been corrected for both basal and specific endogenous AA losses (Stein et al., 2007).

Relationship Between Ileal Digestibility and Availability

Several studies indicate a close agreement between Lys digestibility and Lys bioavailability in a number of feedstuffs (Leibholz, 1985; Moughan and Smith, 1985). However, for heated proteins, ileal digestibility may overestimate availability (Batterham et al., 1990). Thus, the bioavailability of Lys in unheated proteins is close to the ileal digestibility of Lys, but this is not the case in heated proteins. This discrepancy has been reported in pigs but not in poultry (Table 2.3; Parsons, 1996). The reason for this difference observed in pigs may be due to the reduced efficiency of utilization of digested Lys in heated proteins (Wiseman et al. 1991; Van Barneveld et al.,1994).

In summary, both ileal digestibility and relative bioavailability procedures are established methods to evaluate the extent of utilization of AA, but both procedures have limitations. In unheated proteins, values for AA bioavailability are similar to values for ileal digestibility. However, for heated proteins, values for ileal digestibility may be less accurate than values for bioavailability.

In Vitro Procedures in Protein Quality Evaluation.

In vitro procedures to measure AA digestibility are relatively inexpensive and less time-consuming than most in vivo digestibility and availability procedures. Because these procedures can provide an estimate of the quality of a given feedstuff, they are useful as a routine quality control tool in the food and feed industries.

In-vitro Enzymatic Digestion Procedures. This method involves the use of single (e.g. pepsin) or multiple enzyme preparations (e.g., porcine pancreatin) to hydrolyze the peptide bonds in the protein. After enzymatic digestion, the amount of liberated AA is estimated by measuring the change in pH of the solution due to peptide bond cleavage (Hsu et al., 1977) or by separation of low molecular weight products of digestion by dialysis (Gauthier, 1982). Multi-enzyme assays have been reported to predict the DM, nitrogen, CP, OM, and to a certain extent, AA digestibility in single and compound feeds (Furuya et al., 1979; Boisen and Fernandez, 1991).

One of the disadvantages of enzyme-based procedures is that it may overestimate in vivo ileal digestibility when the feedstuff contains high amounts of ANF and structural carbohydrates (Van der Meer and Perez,1992; Cone and van der Poel, 1993). These components have a negative effect on in vivo AA digestibility because they increase the endogenous AA losses (Graham et al., 1986; Li et al., 1994; Gilani et al., 2005) and may

increase digesta passage (Wilfart et al., 2007), which reduces the time for enzymatic digestion in the small intestine. The effects of these ANF do not occur in vitro, which may lead to inaccuracies in the results. Correction factors are also needed if vitro AA digestibility data were to predict the SID of AA because in vitro AA digestibility is considered as true ileal AA digestibility values (Boisen and Eggum, 1991; Cone and Van der Poel, 1993).

Reactive Lys Procedure and Bioavailable Lys

While enzymatic procedures simulate the conditions in the GIT to estimate digestibility, the reactive Lys procedure estimates the bioavailability of Lys by measuring the amount of Lys with free ε-NH_2 group. This procedure has been used to assess AA quality in heated food and feedstuffs (Carpenter and Booth, 1973; Sibbald, 1987; Moughan, 1999; Ravindran and Bryden, 1999). Various studies have proposed the use of different chemicals to modify the free ε-NH_2 group of Lys in heated proteins (Table 2.4).

Fluorodinitrobenzene Method. The FDNB method (Carpenter et al., 1957) involves the use of 1-fluoro-2,4-dinitrobenze or FDNB solution to impart color to the Lys that has not undergone binding with reducing sugars after heating. After incubation, the Lys with unbound ε-NH_2 group is transformed to colored dinitrophenyl or trinitrophenyl Lys. The sample is acid hydrolyzed for 24 h and color intensity is measured quantitatively. The FDNB-reactive Lys retains the color, whereas the unreactive Lys that contained bound ε-NH_2 remain colorless, allowing the quantification of reactive Lys using a spectrophotometer. However, during acid hydrolysis, FDNB can occasionally react with other components in the sample, such as carbohydrates, which can cause the color of FDNB-reactive Lys to deteriorate (Holsinger and Posati, 1975; Bodwell, 1976).

This method has also been reported to indiscriminately overestimate the reactive Lys in heated proteins in the presence of Amadori compounds (Bujard and Finot, 1978; Finot and Hurrell, 1985).

Homoarginine Procedure. Guanidination of proteins with O-methylisourea (Mauron and Bujard, 1964) involves the incubation of the protein with O-methylisourea to initiate a guanidination reaction (Geschwind and Li, 1957; Habeeb, 1972). During guanidination, the unbound Lys, but not the bound Lys, is substituted at the ε-NH_2 with a guanidino group from O-methylisourea (Figure 2.2), forming 2-amino-6-guanidohexanoic acid or homoarginine (Kimmel, 1967). Thus, the amount of Lys that is converted to homoarginine represents the reactive Lys concentration in the sample (Figure 2.3). Guanidination is specific for ε-NH_2 group of Lys and does not react with the α-NH_2 group (Hughes et al., 1949). The homoarginine procedure can measure the reactive Lys in proteins that have undergone early and advanced stages of MR and non-MR type of Lys damage (Finot and Mauron, 1972; Hurrell and Carpenter, 1974; Rutherfurd and Moughan, 1990). However, the guanidination step takes 1 to 5 d to complete and guanidination of unbound Lys has to be ensured to avoid underestimating reactive Lys. Therefore, the guanidination conditions have to be optimized for each type of protein (Bujard and Finot, 1978; Maga, 1981). Reactions of free ε-NH_2 groups of Lys with components other than O-methylisourea such as dehydroalanine (Hurrell and Carpenter, 1981) can underestimate the reactive Lys using this procedure. This can be avoided by keeping the pH below 12 during guanidination.

Digestibility of Reactive Lys. A combination of the homoarginine procedure and the ileal digestibility procedure may increase the accuracy of the ileal digestibility

measurement. This method involves measurement of the concentration of reactive Lys in diets and digesta from pigs, which will allow for the calculation of digestible reactive Lys (Moughan and Rutherfurd, 1996; Rutherfurd et al., 1997). Rutherfurd and Moughan, (1997) compared the conventional ileal digestibility method with the ileal digestible reactive Lys procedure and they reported differences particularly for heated proteins (Table 2.5)

Trinitrobenzesulfonate (TNBS) Procedure. Trinitrobenzesulfonate reacts with the free ε-NH_2 of Lys to yield trinitro-phenyl Lys (Okuyama and Satake, 1960). The TNBS procedure is relatively easy to conduct and fast to complete. However, TNBS procedure also suffers from the same limitation as the FDNB method in presence of carbohydrates and the reactive Lys can be over-estimated in the presence of Amadori compounds (Finot and Hurrell, 1985).

Furosine Method. Furosine (ε-*N*-[2-furosyl methyl]-L-lysine; Erbersdobler and Zucker, 1966) is a basic AA that was first detected as a product of hydrolysis of Amadori products. Because Amadori products (Figure 2.4) can hydrolyze into 40% regenerated Lys, 32% furosine, and 28% pyridosine, measuring the concentration of furosine using HPLC allow for the calculation of regenerated Lys (Finot and Mauron, 1969; Finot et al., 1977; Bujard and Finot, 1978). Measurement of both total Lys (i.e., sum of concentration of reactive and unreactive or regenerated Lys) regenerated Lys from furosine values allows for the estimation of the reactive Lys by calculating the difference between Total Lys and regenerated Lys (Figure 2.5).

The Lys in Amadori products are not bioavailable (Erbersdobler et al., 1970). Thus, the amount of furosine has been used as an index of quality of processed milk and

milk products, pasta (Resmini and Pellegrino, 1990), in cereals (Guerra Hernández-Corzo et al., 1999) and bread (Ramírez-Jiménez et al., 2001). The furosine method is highly sensitive in determining Amadori products (Erbersdobler et al., 1987; Pellegrino et al., 1995) but it is not able to detect non-Maillard type of Lys damage.

SUMMARY

The variation in ileal Lys digestibility of DDGS fed to pigs and poultry may be related to the damage to Lys during drying. However, the extent of heat damage in DDGS has not been studied. There is also a possibility that the ileal digestible Lys concentration may be overestimated if Amadori products are present in DDGS. The use of a reactive Lys procedure to evaluate the processing of DDGS should be explored because it may be related to the ileal digestibility and bioavailability of Lys. Drying of DDGS can cause a reduction in bioavailable Lys that is greater than the reduction in the concentration of ileal digestible Lys. It is not known if the concentration of ileal digestible Lys can be used to predict the concentration of bioavailable Lys in DDGS.

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Table 2.1. Composition of distillers dried grains with solubles (DDGS) and condensed distillers solubles (CDS), DM basis

	Corn DDGS[1]	Wheat DDGS[3]	Barley DDGS[5]	NRC 1998 (swine)		
				Corn DDG	Corn DDGS	Corn CDS
DM, %	88.70	91.2	93.6	94.00	93.0	92.0
CP, %	31.20	40.4	41.5	26.38	29.8	29.0
GE, kcal/kg	5465	4349	-	-	-	-
DE, kcal/kg swine	4140[2]	4019[4]	-	3297	3441	3614
ME kcal/kg swine	3897[2]	-	-	2888	3032	3201
Calcium, %	-	-	-	0.11	0.22	0.32
P, %	0.89	0.76	0.70	0.43	0.80	1.10
Starch, %	7.31	-	-	-	-	-
Ether Extract, %	11.5[2]	6.3	8.4	8.4	9.0	9.9
ADF, %	12.65	18.2	22.0	18.6	17.5	8.2
NDF, %	42.27	40.9	38.0	42.9	37.2	27.0
Indispensable AA, %						
Arg	1.07	1.43	1.30	0.96	1.22	0.98
His	0.73	0.93	0.58	0.67	0.74	0.72
Ile	1.01	1.24	1.18	1.01	1.11	1.32
Leu	3.10	2.56	2.36	2.80	2.76	2.45

Table 2.1. Cont.

Lys	0.74	0.63	0.85	0.79	0.67	0.89
Met	0.57	0.42	0.3	0.46	0.54	0.55
Phe	1.34	1.85	1.61	1.05	1.44	1.50
Thr	0.98	1.24	1.20	0.66	1.01	1.12
Trp	0.20	-	-	0.21	0.27	0.25
Val	1.37	1.65	1.69	1.32	1.40	1.63
Dispensable AA, %						
Ala	1.80	1.48	1.43	-	-	-
Asp	1.85	1.92	2.08	-	-	-
Cys	0.62	0.57	0.49	-	-	-
Glu	3.65	9.81	6.94	-	-	-
Gly	0.98	1.62	1.27	-	-	-
Pro	1.96	4.11	3.43	-	-	-
Ser	1.07	1.88	1.48	-	-	-
Tyr	0.94	-	0.95	-	-	-

[1]Average values from Fastinger and Mahan, 2006, and Stein et al., 2006, except for DE and ME values.

[2]Pedersen et al., 2007.

[3]Näsi, 1985, and Lan et al., 2007.

[4]Widyaratne and Zijlstra, 2007.

[5]Näsi, 1985.

Table 2.2. Mean, SD, and CV of total, standardized ileal digestibility (SID), %, and SID concentration, % in corn distillers dried grains with solubles[1]

	Total AA, %			SID values, %			SID concentration, %		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
CP, %	27.68	1.25	4.5	70.15	2.1	2.9	19.25	1.02	5.3
Indispensable AA, %									
Arg	1.07	0.09	8.2	78.2	3.0	3.8	0.84	0.08	9.6
His	0.73	0.07	9.5	73.5	2.3	3.1	0.54	0.05	9.9
Ile	1.01	0.07	6.5	73.1	3.5	4.8	0.74	0.06	7.9
Leu	3.10	0.14	4.7	81.0	3.3	4.0	2.51	0.12	4.9
Lys	0.74	0.11	15.4	55.0	7.6	13.9	0.41	0.11	25.6
Met	0.57	0.07	13.0	79.8	3.6	4.5	0.46	0.06	14.0
Phe	1.34	0.07	5.3	78.3	2.6	3.4	1.05	0.06	6.1
Thr	0.98	0.06	6.3	67.2	4.1	6.1	0.66	0.07	10.4
Trp	0.20	0.04	22.2	76.7	3.5	4.6	0.15	0.04	23.4
Val	1.37	0.09	6.2	71.6	3.3	4.6	0.98	0.07	6.7
Dispensable AA, %									
Ala	1.80	0.09	5.2	75.7	3.7	4.8	1.36	0.11	8.2
Asp	1.85	0.16	8.7	66.3	4.7	7.1	1.23	0.16	13.0
Cys	0.62	0.11	18.0	69.8	4.8	6.8	0.44	0.09	21.7
Glu	3.65	0.33	9.1	75.9	5.2	6.8	2.78	0.39	14.2
Gly	0.98	0.05	4.7	58.4	4.7	8.0	0.57	0.07	11.4
Pro	1.96	0.10	5.3	68.0	9.2	13.6	1.34	0.22	16.3
Ser	1.07	0.07	7.0	75.0	4.5	6.0	0.80	0.08	10.3
Tyr	0.94	0.06	6.7	80.0	2.7	3.4	0.75	0.06	8.5

[1]Data from Fastinger and Mahan, 2006 (n = 5), and Stein et al., 2006 (n = 10).

Table 2.3. Comparison of digestibility and availability of lysine (%) in feedstuffs for poultry and swine[1]

Feedstuff	Poultry[2]		Swine[3]	
	Digestibility	Availability	Digestibility	Availability
Soybean meal	91	82	89	90
Overcooked soybean meal	69	70	-	-
Cottonseed meal	67	-	66	29
Meat meal	79	72	78	68
Poultry by-products	75	66	-	-
Field peas	-	-	96	92
Overcooked field peas	-	-	84	47

[1]From Parsons, 1996.

[2]Digestibility determined using cecectomized adult cockerels and availability determined using slope-ratio. chick growth assays. Values taken from Parsons (1986), Han and Parsons (1990), and Parsons et al. (1991).

[3] Digestibility determined using ileal cannulated pigs and availability determined using slope-ratio assay (Batterham, 1992, 1993).

Table 2.4. Studies that evaluated the effectivity of reactive Lys procedures in predicting the bioavailability of Lys in protein sources

Reactive Lys procedure	Test protein	Authors
Fluorodinitrobenze	Peanut flour	Creamer et al., 1976
	Milk	Mottu and Mauron, 1967
	Fish flour	Morrison and McLaughland, 1972
	Casein	Stahmann and Woldergeorgis, 1975
	Meat	Hurrell and Carpenter, 1974
Trinitrobenzesulfonic acid	Meat	Hurrell and Carpenter, 1974
	Cowpeas	Tella and Ashton, 2006
	Albumin	Hurrell and Carpenter, 1979, 1981
O-methylisourea	Meat	Hurrell and Carpenter, 1974
	Milk	Creamer et al., 1976; Hurrell and Carpenter, 1981; Maga, 1981; Rutherfurd and Moughan, 1997, 2005
	Field peas	Rutherfurd and Moughan, 1997
	Casein	Moughan and Rutherfurd, 1996
Dye binding	Albumin	Hurrell et al., 1979
Borohydride	Albumin	Hurrell and Carpenter, 1981
	Meat	Hurrell and Carpenter, 1974
Furosine	Albumin	Hurrell and Carpenter, 1974
	Meat	Hurrell and Carpenter, 1974

Table 2.5. Comparison of ileal digestibility of Lys using conventional method and reactive Lys method in selected proteins[1,2]

	Lysine digestibility, % of sample		Lysine digestibility, g/kg of sample	
	Conventional	Reactive	Conventional	Reactive
Blood meal	96.3	96.7	85.9	85.1
Wheat meal	92.6	92.1	3.2	2.9
Meat and bone meal	88.9	91.5	32.5	31.6
Soybean	94.5	96.5	30.6	31.2
Dried corn	80.5	84.3	2.6	1.9
Heated skim milk powder	69.1	94.0	19.8	16.6
Cottonseed meal	62.1	71.9	12.9	10.3
Alfalfa-based	74.2	86.3	14.4	10.8

[1]From Rutherfurd and Moughan , 1997.

[2]Lysine digestibility was calculated using true ileal lysine digestibility method using rats. Diets and digesta from the ileal Lys digestibility study were analyzed for ileal digestibility of reactive Lys using guanidination method.

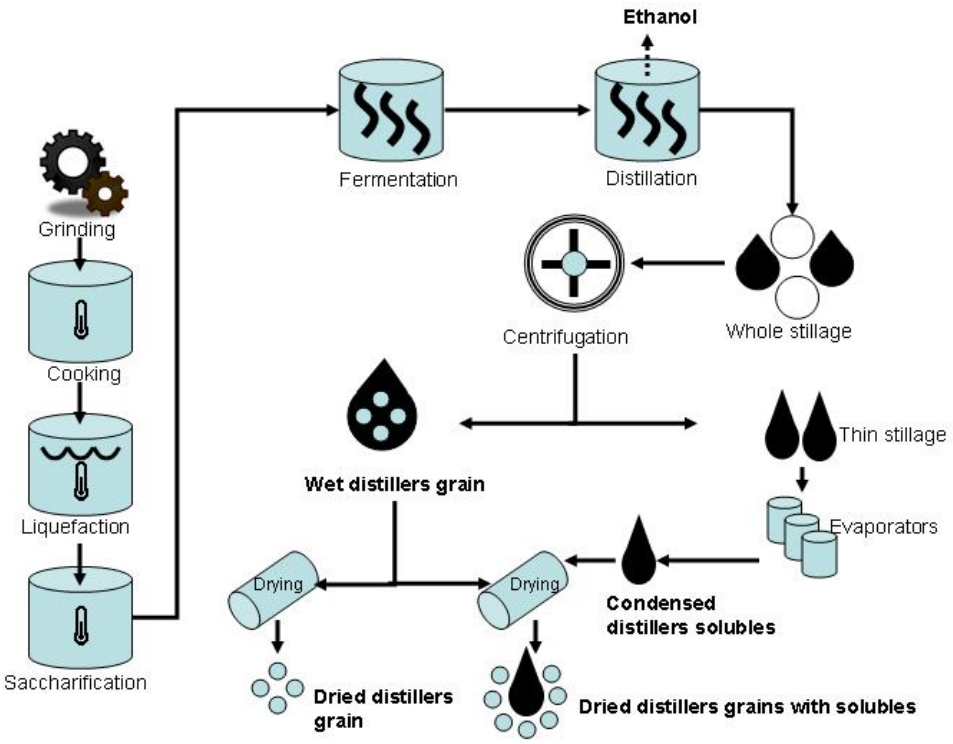

Figure 2.1. Process flow in a typical dry grind ethanol plant.

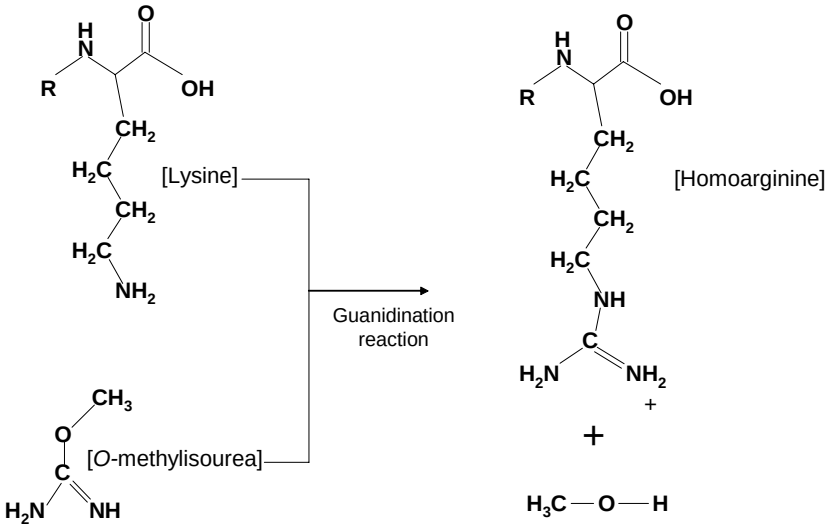

Figure 2.2. Guanidination of lysine using O-methylisourea

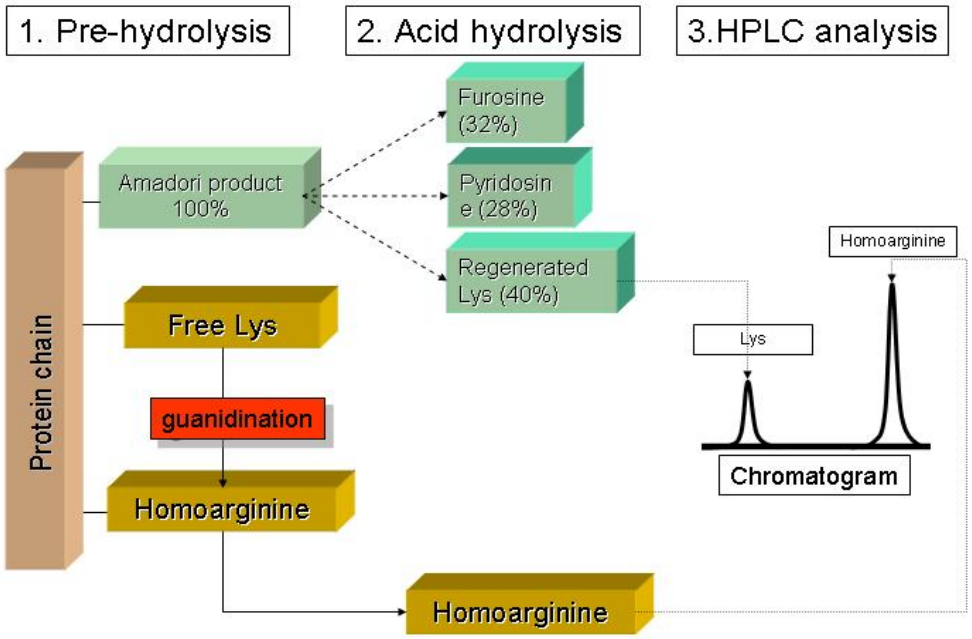

Figure 2.3. Principle of homoarginine procedure to measure reactive Lys in food and feed.

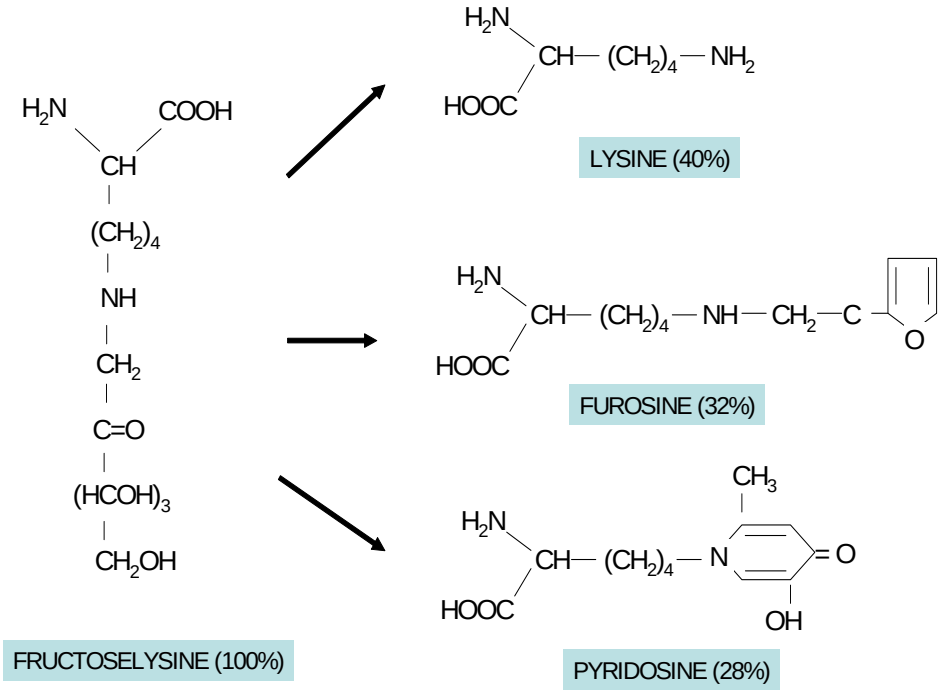

Figure 2.4. Acid hydrolysis products of fructoselysine.

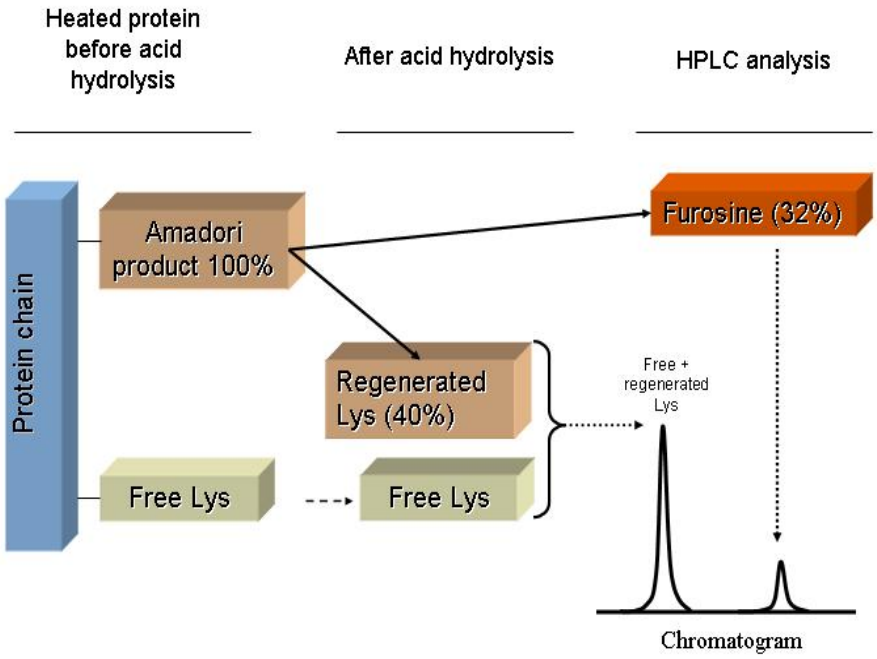

Figure 2.5. Principle of furosine analysis

CHAPTER 3

Factors affecting ileal amino acid digestibility of distillers dried grains with solubles fed to growing pigs

ABSTRACT

The objectives of the study were to compare the ileal digestibility of AA in distillers dried grains with solubles (DDGS) sourced from different regions (IL, MN, KY), compare the effect of adding solubles to DDGS on AA digestibility, and to compare the effect of type of facility producing DDGS (ethanol plants vs. a beverage plant) on AA digestibility. In Exp. 1, five samples of DDGS were sourced from MN (MN1, MN2), IL (IL1, IL2), and from KY. All samples were from ethanol plants ($DDGS_{ethanol}$). The apparent (AID) and standardized (SID) ileal AA digestibility were measured using 12 cannulated growing pigs that were allotted in a replicated 4 x 6 Youden square design. In Exp. 2, six $DDGS_{ethanol}$ sources, 1 sample of distillers dried grain (DDG) and 1 sample of DDGS from a beverage plant ($DDGS_{beverage}$) were used to compare the values for AID and SID of AA between $DDGS_{ethanol}$ and $DDGS_{beverage}$, and between DDG and $DDGS_{ethanol}$. Nine cannulated growing pigs were used in a 7 x 9 Youden square design. In both experiments, a N-free diet was included to determine the basal endogenous ileal AA losses. In Exp. 1, results showed that DDGS from MN2 and IL2 had a greater ($P < 0.01$) SID of Lys than MN1, IL1, and KY. Except for Leu and Glu, however, no differences in SID for any of the other AA were observed among the 5 DDGS sources. Contrast analyses showed that DDGS from MN had a greater ($P < 0.05$) SID for Lys compared with DDGS from KY. Thus, ileal AA digestibility among DDGS sources

within a region can vary as much as DDGS sourced across regions. In Exp. 2, the SID for Lys in $DDGS_{beverage}$ was greater ($P < 0.01$) than in $DDGS_{ethanol}$, but the SID for His was lower in $DDGS_{beverage}$ compared with $DDGS_{ethanol}$. However, $DDGS_{beverage}$ and $DDGS_{ethanol}$ had similar values for SID of CP and all other AA. On the other hand, DDG had a greater ($P < 0.05$) SID for most AA compared with $DDGS_{ethanol}$, and this suggests that the solubles added to DDGS may be less digestible than the DDG component. This study also showed that the starch concentration and the SID for Lys were more variable than the other components and this indicates that the efficiency of starch extraction in ethanol plants varies. The variability in the SID of AA in DDGS may be due to differences in the fermentation process, to the extent of heat damage during drying, and to the amount of solubles added to the DDG.

Keywords: amino acids, digestibility, distillers dried grains with solubles, distillers dried grains, pigs

INTRODUCTION

Distillers dried grains with solubles (**DDGS**) is a by-product of the ethanol industry that is produced after alcohol is extracted from the grain or grain mixture. It contains at least three-fourths of the solids of the resultant whole stillage (AFFCO, 2006), which is blended with the condensed solubles (syrup) during drying. If the coarse grain fraction of the whole stillage is dried without the solubles added to it, the product is called distillers dried grains (**DDG**).

The digestibility of AA in DDGS can vary depending on the location of the plant that produced it (Whitney et al., 2000; Fastinger and Mahan, 2006). However, the DDGS used in these studies were sourced from ethanol plants located only in the upper Midwest. Another digestibility study involved 10 samples of DDGS that were sourced from across the Midwestern region, but from a single branded product (Stein et al., 2006). Currently, there are no data on the AA digestibility of DDGS randomly sourced from the entire Midwestern region.

The amount of solubles added to the DDG may also contribute to the variability of DDGS composition (Goodson and Fontaine, 2004). However, no study has been conducted to compare the digestibility of DDG and DDGS. Recently built plants (built after 1990) were reported to produce DDGS with a greater AA digestibility than plants that were built earlier than 1990 (Whitney et al., 2000). However, no study has been conducted to compare the AA digestibility of DDGS from dry grind ethanol plant and a beverage plant.

Thus, the objective of the study was to compare the digestibility of AA in DDGS sourced from ethanol plants located in the Northern (i.e., MN), Central (i.e., IL), or Southern (i.e., KY) corn belt. The second objective was to compare the AA digestibility of DDG and DDGS. The third objective was to compare the AA digestibility of DDGS produced from 2 types of alcohol extraction facility (i.e., dry grind ethanol plants vs. an alcoholic beverage plant). The last objective was to measure the variability of nutrient composition, and AA digestibility in DDGS.

MATERIALS AND METHODS

Two digestibility experiments were conducted using ileal cannulated growing pigs. In Exp. 1, the apparent (**AID**) and standardized (**SID**) ileal digestibility of CP and AA in DDGS obtained from the Southern, Central, and Northern regions were compared. In Exp. 2, the AID and SID values in DDG were compared with AID and SID in DDGS. The DDG and DDGS were obtained from 2 separate dry grind ethanol plants. The digestibility of AA in DDGS from a beverage plant (**$DDGS_{beverage}$**) was also compared with the digestibility of DDGS produced by dry grind ethanol plants (**$DDGS_{ethanol}$**). Data from DDGS sources used in both experiments were analyzed to calculate the variability in AA concentration and digestibility among DDGS sources.

Animals, Housing, and Experimental design

In Exp. 1, twelve barrows (initial BW: 37.0 ± 5.6 kg) were allotted to a replicated 4 x 6 Youden square design (Anderson and McLean, 1974) with 4 periods and 6 diets. In Exp. 2, nine barrows (initial BW: 76.0 ± 9.2 kg) were used in a 7 x 9 Youden square design with 7 periods and 9 diets. All barrows originated from the matings of SP-1 boars to Line 13 sows (Ausgene Intl. Inc., Gridley, IL) and were surgically fitted with a T-cannula in the distal ileum following the procedure of Stein et al. (1998). The pigs were housed in an environmentally controlled room (22^{o}C) with fully slatted pens (1.2 x 1.8 m). A feeder and a nipple drinker were installed in each pen. Both experiments were conducted at South Dakota State University (Brookings, SD), and experimental protocols were approved by the Institutional Animal Care and Use Committee at South Dakota State University (#05-A017).

Ingredients, Diets, Feeding

Twelve sources of DDGS and a source of DDG were used in the study (Table 3.1 and 3.2). In Exp. 1, a N-free diet and 5 diets based on the 5 DDGS sources were formulated (Tables 3.3 and 3.4). Two of the DDGS samples were from MN (**MN1**, **MN2**), 2 were from IL (**IL1**, **IL2**), and 1 sample was from KY (**KY**). All sources of DDGS were from fuel ethanol plants. In Exp. 2, a N-free diet, 1 diet based on DDG, 1 diet based on $DDGS_{beverage}$, and 6 diets based on $DDGS_{ethanol}$ were formulated (Tables 3.3 and 3.5). The $DDGS_{ethanol}$ were sourced from IL (2), IN (1), WI (1), ND (1), and MN (1). A 1- kg sample of each source of DDG and DDGS was collected as soon as the samples arrived and stored at 4^{o}C until analyzed.

All diets in Exp. 1 and 2, except the N-free diet, were formulated to contain DDGS or DDG as the only source of CP and AA. All diets contained cornstarch, sugar, vitamins, and minerals to meet or exceed current requirement estimates (NRC, 1998). The N-free diet contained cornstarch, sugar, vitamins, minerals, and synthetic fiber (Solka Floc, Fiber Sales and Development Corp., OH). The calculated ME (NRC, 1998) in the diet containing DDG was 3,150 kcal ME/kg, whereas the DDGS-containing diets were calculated to contain 3,080 kcal ME/kg. The N-free diet was calculated to contain 3,807 kcal ME/kg.

In both experiments, feed was provided in the amount of 3 times the estimated daily ME requirement for maintenance (i.e., 106 kcal of ME per kg of $BW^{0.75}$, NRC, 1998). The daily feed allowance was equally divided into 2 meals at 0800 and 1700.

Sample and Data Collection

The initial 5d of each period was considered an adaptation period to the diet. The ileal digesta were collected on d 6 and 7 from 0800 till 1700. A 225-mL plastic bag (Gerber baby bottle bag, Gerber Products Company, Fremont, MI) was fitted to the barrel of the cannula and was kept in place with an auto-locking cable tie. The bags were replaced when filled, and the digesta were transferred to 2-L plastic pitchers and immediately frozen at -20^{o}C. The ileal samples collected during each 2-d period were pooled within pig and period and comprised a single observation. The opening of the cannula was capped at the end of every collection-day and a zinc oxide-based cream (Desitin, Pfizer, New York, NY) was applied to the skin around the cannula when needed. All samples were freeze-dried and ground prior to chemical analyses.

Chemical Analyses

The DDG and DDGS samples, diets, and ileal digesta were analyzed for DM (procedure 4.1.06; AOAC, 2000). All samples were analyzed for CP and AA at Degussa Analytical Nutrition Laboratory in Hanau, Germany. Amino acids were analyzed by cation exchange chromatography (method 994.12; AOAC, 1995). Analysis for Met and Cys were performed by initially oxidizing the samples with performic acid (Llames and Fontaine, 1994). Tryptophan was measured after hydrolysis in 4 *M* barium hydroxide at 110°C for 20h (Llames and Fontaine, 1994). Tyrosine was not measured. The diets were analyzed for Ca (procedure 4.8.03; AOAC; 2000) and P (procedure 3.4.11; AOAC, 2000). The DDG and DDGS samples were also analyzed for ADF and NDF (procedure 4.6.03; AOAC, 2000) and starch (Xiong et al., 1990). All samples of diets and ileal digesta were

analyzed for Cr (procedure 9.2.39; AOAC, 2000) after nitric acid - perchloric acid wet ash sample preparation.

Digestibility Calculations, Statistical Analysis

The AID, basal ileal endogenous losses ($\mathbf{IAA_{end}}$), and SID of CP and AA were calculated for DDG and each source of DDGS as described by Stein et al. (2007). Data were analyzed using the MIXED procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC). The pig was the experimental unit. Pig and period were considered random effects and diet was the fixed effect. Least square means were calculated and separated using the PDIFF option of SAS. In Exp. 1 the CONTRAST option of SAS was used to compare $DDGS_{ethanol}$ from different locations. The CONTRAST option was also used in Exp. 2 to compare the DDG vs. the DDGS sources, and $DDGS_{beverage}$ vs. $DDGS_{ethanol}$. In all analyses, a probability of $P < 0.05$ was considered significant.

RESULTS

Experiment 1

The mean CP, NDF, ADF, and starch concentration of DDGS in Exp. 1 were 26.46, 28.31, 10.99, and 7.29%, respectively (Table 3.1). The DDGS from MN1 had a greater CP and AA concentration compared with other sources, whereas DDGS from KY had the lowest concentration of CP and all AA except Arg. The mean Lys concentration of DDGS sources was 0.75%, and concentration of the other indispensable AA ranged from 0.21% (Trp) to 3.07% (Leu).

The AID of Arg ($P < 0.05$), Lys ($P < 0.01$), Leu ($P < 0.05$), and Trp ($P < 0.05$) varied among DDGS sources (Table 3.6) but the AID of CP and all other AA were

similar among sources. The mean AID of Lys was 63.1%, and ranged from 60.3 to 67.4%. The AID of Lys was greater ($P < 0.05$) in DDGS from MN2 than from sources MN1, IL1, and KY. The AID of Lys in DDGS from MN2, however, was similar to IL2. The mean AID for indispensable AA ranged from 60.1% (Trp) to 83.2% (Met). Contrast analyses showed that DDGS from MN had a greater AID for Arg ($P < 0.05$) and Lys ($P < 0.05$) than DDGS from KY, whereas DDGS from IL had a greater ($P < 0.01$) AID for Leu compared with DDGS from MN.

The SID of Leu ($P < 0.01$), Lys ($P < 0.01$), and Glu ($P < 0.05$) varied among the 5 DDGS sources (Table 3.7), but the SID for CP and all other AA did not differ among sources. The mean SID of Lys was 68.5% and varied from 65.8 to 72.8%. The SID of Lys was greater ($P < 0.05$) for DDGS from MN2 than from MN1, IL1, and KY, but similar to DDGS from IL2. Contrast analysis showed that DDGS from IL had a greater ($P < 0.01$) SID of Leu than from DDGS from MN, whereas the DDGS from KY has a greater ($P < 0.05$) SID of Arg and Leu compared with DDGS from MN. In contrast, DDGS from MN had a greater ($P < 0.05$) SID of Lys than DDGS from KY. However, for all other AA, SID values were not influenced by the region in which the DDGS was produced.

Experiment 2

The mean CP, NDF, ADF, and starch concentration in the 6 $DDGS_{ethanol}$ sources were 25.54, 29.13, 11.61, and 8.55%, respectively (Table 3.2). The CP, NDF, ADF, and starch concentration in $DDGS_{beverage}$ were 25.55. 31.67, 11.64, and 7.32%, respectively, and were within the range of values observed in $DDGS_{ethanol}$. The concentration of CP, NDF, and ADF in DDG were 28.77, 37.29, and 18.19%, respectively. These were

greater than in $DDGS_{ethanol}$ and $DDGS_{beverage}$. However, starch concentration (3.83%) was lower in DDG compared with both DDGS sources.

The $DDGS_{beverage}$ had a greater concentration of Lys and Arg and a lower concentration of Leu compared with $DDGS_{ethanol}$. However, DDG contained greater concentrations of all AA, except for Arg, Trp, and Gly, than $DDGS_{ethanol}$.

The AID for CP and all AA varied ($P < 0.01$) among $DDGS_{ethanol}$ sources (Table 3.8). Sources 3 and 5 had a greater ($P < 0.01$) AID for Lys (69.3 and 70.0%, respectively) than sources 1, 2, 4, and 6 (51.1, 44.4. 58.9, 60.1%, respectively). The mean AID for the other indispensable AA varied from 59.6% (Trp) to 82.1% (Met).

The AID of CP and all AA except Gly and Pro were greater ($P < 0.01$) in DDG than in $DDGS_{ethanol}$. The AID of Lys in DDG was 73.4%, compared with a mean of 59.0% in $DDGS_{ethanol.}$ The AID of indispensable AA in DDG varied from 63.0 (Trp) to 87.5% (Met). In $DDGS_{beverage}$, the AID for Lys (64.7%) was greater ($P < 0.01$) than in $DDGS_{ethanol}$. However, the AID for His (72.9%), Ala (71.9%), Cys (65.6%), and Gly (30.0%) were lower ($P < 0.01$) in $DDGS_{beverage}$ than in $DDGS_{ethanol}$. The CP and all other AA had AID values that were similar for $DDGS_{ethanol}$ and $DDGS_{beverage}$. The range of AID for other indispensable AA in $DDGS_{beverage}$ was from 59.4% (Trp) to 82.3% (Leu).

The SID of CP and all AA also varied among $DDGS_{ethanol}$ sources (Table 3.9). The mean SID for Lys was 64.8%. Sources 3 and 5 had a greater ($P < 0.01$) SID for Lys (73.8 and 74.5%, respectively) compared with sources 1, 2, 4, and 6 (56.8, 51.4, 63.7, and 68.7%, respectively). The mean SID for indispensable AA varied from 68.6% (Trp) to 84.0% (Leu).

The SID of CP and all AA except Gly and Pro were greater ($P < 0.05$) in DDG than in $DDGS_{ethanol}$. The mean SID of indispensable AA in DDG varied from 71.8% (Trp) to 89.2% (Met). The DDG had a greater ($P < 0.01$) SID of Lys (77.9%) compared with $DDGS_{ethanol}$ (64.8%). The SID of Lys for $DDGS_{beverage}$ (69.3%) was also greater ($P < 0.01$) than for $DDGS_{ethanol}$. However, a lower ($P < 0.01$) SID for His and Cys were obtained in $DDGS_{beverage}$ compared with $DDGS_{ethanol}$. The SID for CP and all other AA were similar for DDGS from beverage and ethanol plants.

The CV for nutrient composition and for AID and SID in the 11 sources of $DDGS_{ethanol}$ that were used in Exp. 1 and 2 are summarized in Table 3.10. The CV for starch concentration of $DDGS_{ethanol}$ was 22.6%, but the CV of CP, NDF, and ADF was only 4.1, 6.9, and 10.0%, respectively. The AA concentration in $DDGS_{ethanol}$ had a CV of less than 8.0% for all AA, except for Lys, which had a CV of 10.1%. When expressed on a g/kg basis, the CV of AID and SID of all AA were greater than if expressed on a percentage basis. Among the indispensable AA, the AID and SID for Lys, Arg, and Trp, were the most variable. When expressed on a g/kg basis, the AID for Lys, Arg, and Trp had a CV of 19.9, 14.7, and 14.0%, respectively. On the other hand, the SID of Lys had a CV of 18.0%, whereas Arg and Trp had a CV of 14.2 and 12.2%, respectively.

DISCUSSION

Comparison of DDGS from MN, IL, and KY

The nutrient composition and the AID and SID of CP and AA in DDGS obtained in Exp. 1 were similar to previously reported values (Fastinger and Mahan, 2006; Stein et al., 2006). The differences in the SID of Lys, Leu, and Glu among the 5 DDGS sources

as well as the differences in the SID for Lys, Leu, and Arg between IL, MN, and KY indicate that both inter - plant variation and inter - region variation in DDGS AA digestibility exist. However, the differences are not consistent and only limited to Lys, Leu, and Arg digestibility. The differences may be due to plant design, processing procedures, and corn growing conditions which can affect the starch content of corn (Mathew et al., 1999), and subsequently corn DDGS nutrient composition. Based on these data, it is concluded that the region in which DDGS is produced is not a major contributor to the variability in nutrient concentration and AA digestibility.

Composition of DDG, $DDGS_{ethanol}$, and $DDGS_{beverage}$.

The concentrations of CP and AA in $DDGS_{ethanol}$ and $DDGS_{beverage}$ in this study were similar to reported values (Cromwell et al., 1993; Fastinger and Mahan, 2006; Stein et al., 2006), but the CP and AA concentration of DDG used in this study was greater than the values reported by NRC (1998). The greater CP concentration in DDG than in $DDGS_{ethanol}$ and $DDGS_{beverage}$ may be a result of the lower CP concentration in distillers solubles than in DDGS. Thus, addition of solubles may have diluted the CP concentration in DDGS. It was shown that the CP in the solubles can be as low as 16.8% (Larson et al., 1993), whereas the CP of DDG in this study was 28.77% (as fed basis). It was also reported that the AA concentration of barley distillers solubles was at least 50% lower than in barley DDG (Näsi, 1985).

The NDF concentration of $DDGS_{ethanol}$ and $DDGS_{beverage}$ used in this study was lower than previously reported values (NRC, 1998; Fastinger and Mahan, 2006; Stein et al., 2006). This may be a result of the use of more effective enzymes during fermentation because the use of cellulytic enzymes may reduce the NDF concentration in DDGS (Näsi,

1985). The ADF and starch concentrations of both DDGS sources were within the range of values that have been reported (Belyea et al., 2004; Stein et al., 2006). However, the DDG in this study had a concentration of NDF that was close to reported values (NRC, 1998).

Ileal AA digestibility in $DDGS_{ethanol}$, and $DDGS_{beverage}$

Except for Lys, His, and a few dispensable AA, the lack of differences in SID values between $DDGS_{ethanol}$ and $DDGS_{beverage}$ indicate that DDGS from these two alcohol extraction facilities are similar. This conclusion is supported by the similarity in the nutrient composition of the 2 sources of DDGS. The AID and SID of CP and AA in DDGS from both sources were similar to reported values (Fastinger and Mahan, 2006; Stein et al., 2006). However, the values for AID of AA in DDGS from both sources were greater than the values reported by NRC (1998). The SID of AA in DDGS from these facilities were also greater than the values reported for the true ileal AA digestibility of DDGS (NRC, 1998). This suggests that the DDGS source used for NRC (1998) data may be less digestible than current DDGS sources. The greater SID of Lys in $DDGS_{beverage}$ than in $DDGS_{ethanol}$ indicates that the $DDGS_{beverage}$ that was used in this study may have been less heat damaged than $DDGS_{ethanol}$. Although it was suggested that AA in DDGS produced in plants built before 1990 is less digestible than DDGS produced in newer plants, this difference may not be related to whether the DDGS originates from an ethanol or a beverage plant.

Ileal AA Digestibility in DDG

The greater SID of CP and AA in DDG than in $DDGS_{ethanol}$ may be due to a greater AA digestibility in the whole stillage component than in the solubles. This

observation concurs with Näsi (1985) who reported that the AID for CP was lower in solubles than in DDG. It was also reported that the digestibility of Lys and Met by cecectomized turkeys in distillers solubles was only 51 and 21%, respectively (Belyea et al., 1998), which further indicates that the digestibility in solubles is low. Therefore, it is likely that DDG is a more valuable source of digestible AA than DDGS.

Variability in Composition and Ileal Digestibility of Nutrients in DDGS$_{ethanol}$

The CV for CP in DDGS$_{ethanol}$ was similar to the value of 5.1% reported by Goodson and Fontaine (2004). Except for Lys, the CV of AA concentrations is similar to the reported CV of AA in DDGS (Degussa, 2006). Spiehs et al. (2002) reported that in DDGS, the CV of Lys (17.3%) and Met (13.6%) were greater than for other AA. In the current study, however, the CV of Met was low. The CV of CP and AA is comparable to reported CV of co-products such as corn gluten meal and corn germ meal (Degussa, 2006). The variation in NDF and ADF in DDGS may be related to the use of cellulytic enzymes during fermentation and to the drying of DDGS. In soybean meal, sugar-AA interactions that are formed during heat treatment (Maillard reaction) can form a lignin-like matrix, which may increase the analyzed concentration of ADF and NDF (Hussein et al., 1995). The observed variability in starch concentration in DDGS indicates some differences among ethanol plants in the efficiency of fermenting starch to alcohol.

Variable amounts of protein and non-protein nitrogen originating from fermented corn as well as from yeast may be present in DDGS (Wilson, 1987; Larson et al., 1993). However, it has not been established how these components affect the AA digestibility of DDGS. Nevertheless, the CV of AID and SID of indispensable AA in DDGS$_{ethanol}$ was considered low.

The greater variability in the SID of Lys compared with other AA may be related to the degree of heating of DDGS, and the amount of solubles added to the DDGS. It is possible that the presence of solubles in DDGS increased the heat damage to Lys, thereby causing a lower digestibility of Lys in DDGS compared with DDG. Corn distillers solubles and DDGS contain high amounts of glucose compared with DDG, which contains more xylose and less glucose (Wu, 1994). Thus, DDGS is potentially more susceptible to heat damage by Maillard reactions than DDG. Additional variability is caused by the solubles fraction in DDGS because within an ethanol plant, the nutrient composition of solubles can vary as a result of variation in the amount of steepwater added to fermentors, starch purity, and temperature of the steepwater and evaporators (Belyea et al., 1998).

The variability of SID Lys is almost twice as high when expressed as g/kg rather than on a percentage basis. The reason for this observation is that SID Lys in g/kg takes into account the decline in both total Lys and the digestibility of Lys that occurs as a result of heat damage. Thus, it is more appropriate to express the CV for SID Lys in g/kg than on a percentage basis.

In conclusion, AA digestibility of DDGS among plants within a region can vary as much as among plants across regions and there is no evidence that DDGS produced in certain region has greater or lower digestibility of AA than DDGS produced in other regions. However, inter-plant differences in AA digestibility indicate the need to screen the quality of DDGS from multiple plant sources prior to use in livestock feed. The ileal AA digestibility of $DDGS_{beverage}$ is comparable to $DDGS_{ethanol}$ for most indispensable AA. However, the greater SID Lys in $DDGS_{beverage}$ indicates that some $DDGS_{ethanol}$ may be

more heat damaged than the $DDGS_{beverage}$ used in this study. The solubles in DDGS appear to negatively affect AA digestibility. The lower ileal AA digestibility in $DDGS_{ethanol}$ than in DDG shows that DDG is a more valuable source of digestible AA than $DDGS_{ethanol}$. The variability in composition and AA digestibility of DDGS may be related to the variation in the fermentation process during alcohol extraction, to the process used to dry the DDGS, and to the amount of solubles added to the DDG during DDGS processing.

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Table 3.1. Analyzed composition (%) of distillers dried grains with solubles (DDGS) from ethanol plants located in MN, IL, and KY, Exp. 1[1]

	DDGS source[2]					
Item	MN1	MN2	IL1	IL2	KY	Mean
CP, %	28.30	26.91	24.45	26.10	26.52	26.46
NDF, %	26.54	29.48	27.97	28.50	29.05	28.31
ADF, %	11.57	12.63	9.87	10.16	10.70	10.99
Starch, %	6.78	5.39	10.35	6.84	7.07	7.29
Indispensable AA, %						
Arg	1.26	1.09	1.13	1.22	1.19	1.18
His	0.74	0.69	0.68	0.70	0.70	0.70
Ile	1.08	0.98	0.90	0.91	0.99	0.97
Leu	3.31	3.21	2.83	3.00	3.00	3.07
Lys	0.79	0.73	0.73	0.74	0.75	0.75
Met	0.52	0.48	0.46	0.52	0.53	0.50
Phe	1.38	1.30	1.19	1.25	1.27	1.28
Thr	1.09	1.01	0.92	0.98	0.95	0.99
Trp	0.23	0.20	0.20	0.22	0.22	0.21
Val	1.42	1.28	1.20	1.22	1.28	1.28
Dispensable AA, %						
Ala	2.08	2.02	1.80	2.05	1.89	1.97
Asp	1.90	1.71	1.61	1.68	1.71	1.72

Table 3.1. (Cont.)

Cys	0.50	0.48	0.47	0.48	0.51	0.49
Glu	4.79	4.63	4.18	4.47	4.52	4.52
Gly	1.11	1.02	0.98	1.06	1.04	1.04
Pro	2.22	2.17	1.98	2.16	2.17	2.14
Ser	1.36	1.30	1.17	1.27	1.18	1.26

[1]All values adjusted to 88.0 % DM.

[2]MN1 and MN2 were sourced from MN, IL1 and IL2 were sourced from IL, and KY was sourced from KY.

Table 3.2. Analyzed composition (%) of distillers dried grains (DDG) and distillers dried grains with solubles from ethanol plants ($DDGS_{ethanol}$) and a beverage plant ($DDGS_{beverage}$), Exp. 2[1]

Item	$DDGS_{ethanol}$ source							DDG	$DDGS_{beverage}$
	1	2	3	4	5	6	Mean		
CP, %	24.99	24.75	25.64	25.97	26.13	25.77	25.54	28.77	25.55
NDF, %	30.26	28.29	26.67	29.44	26.70	33.39	29.13	37.29	31.67
ADF, %	12.18	13.08	11.41	10.33	10.24	12.43	11.61	18.19	11.64
Starch, %	11.44	7.46	6.96	8.09	7.56	9.78	8.55	3.83	7.32
Indispensable AA, %									
Arg	1.08	0.95	1.11	1.13	1.16	1.02	1.08	1.15	1.17
His	0.62	0.56	0.63	0.63	0.63	0.62	0.62	0.68	0.62
Ile	0.91	0.87	0.96	0.94	0.95	0.97	0.93	1.08	0.94
Leu	2.89	2.85	2.98	2.93	2.82	3.00	2.91	3.69	2.76
Lys	0.63	0.54	0.73	0.74	0.74	0.65	0.67	0.81	0.81

Table 3.2. (Cont.)

Met	0.46	0.46	0.48	0.46	0.47	0.51	0.47	0.56	0.48
Phe	1.22	1.22	1.27	1.26	1.23	1.26	1.24	1.52	1.28
Thr	0.91	0.90	0.98	0.99	0.97	0.96	0.95	1.10	0.90
Trp	0.19	0.18	0.20	0.21	0.22	0.21	0.20	0.22	0.22
Val	1.21	1.15	1.28	1.25	1.28	1.30	1.25	1.39	1.23
Dispensable AA, %									
Ala	1.81	1.80	1.84	1.88	1.84	1.87	1.84	2.16	1.73
Asp	1.63	1.56	1.71	1.76	1.75	1.69	1.68	1.86	1.64
Cys	0.49	0.44	0.46	0.43	0.46	0.42	0.45	0.54	0.48
Glu	4.28	4.17	4.33	4.36	4.34	4.35	4.31	5.06	4.44
Gly	0.99	0.93	0.99	1.01	1.05	0.99	0.99	1.00	0.98
Pro	2.08	1.97	2.04	2.03	2.01	2.07	2.03	2.50	2.26
Ser	1.17	1.17	1.23	1.25	1.20	1.18	1.20	1.45	1.13

[1]All values adjusted to 88.0 % DM.

Table 3.3. Ingredient composition (%) of diets, Exp. 1and 2

Ingredient	N-free diet	With DDG[1] or DDGS[1]
DDG or DDGS	-	66.70
Cornstarch	81.22	27.07
Dextrose	9.00	3.00
Soybean oil	3.00	1.00
Cellulose [2]	3.00	-
Limestone	-	1.35
Dicalcium phosphate	2.40	-
Salt	0.40	0.40
Chromic oxide	0.30	0.30
Micromineral premix [3]	0.15	0.15
Vitamin premix [4]	0.03	0.03
Potassium carbonate	0.30	-
Magnesium oxide	0.10	-
Total	100.00	100.00

[1]DDG = Distillers dried grains; DDGS = Distillers dried grains with solubles.

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

[3]Provided the following quantities of microminerals per kilogram of complete diet: Cu, 26.0 mg as copper sulfate; Fe, 125.0 mg as iron sulfate; I, 0.31 mg as potassium iodate; Mn, 26.0 mg as manganese sulfate; Se, 0.30 mg as sodium selenite; and Zn, 130 mg as zinc oxide.

[4]Provided the following quantities of vitamins per kilogram of complete diet; vitamin A, 10,990 IU as vitamin acetate; vitamin B12, 0.044 mg; vitamin D3, 1,648 as D-

activated animal sterol; vitamin E, 55 IU as DL-α-tocopheryl acetate; vitamin K3, 4.4 mg as menadione dimethylpyrimidinol bisulfite; biotin, 0.17 mg; D-pantothenic acid, 33 mg as calcium pantothenate; folic acid, 1.1 mg; niacin, 55 mg; pyridoxine, 3.3 mg as pyridoxine hydrochloride; riboflavin, 9.9 mg; and thiamin, 3.3 mg as thiamine mononitrate.

Table 3.4. Analyzed composition of diets containing distillers dried grains with solubles (DDGS) from ethanol plants located in MN, IL, and KY, Exp. 1[1]

Item	N-free	DDGS source				
		MN1	MN2	IL1	IL2	KY
CP, %	0.20	18.53	18.20	15.61	17.14	17.58
Ca, %	0.40	0.44	0.47	0.47	0.47	0.40
P, %	0.31	0.44	0.46	0.46	0.51	0.50
Indispensable AA, %						
Arg	-	0.82	0.72	0.71	0.80	0.76
His	-	0.49	0.46	0.41	0.47	0.44
Ile	-	0.73	0.66	0.57	0.62	0.61
Leu	-	2.16	2.14	1.77	1.93	1.93
Lys	-	0.52	0.49	0.47	0.49	0.48
Met	-	0.33	0.32	0.28	0.33	0.33
Phe	-	0.90	0.86	0.75	0.82	0.82
Thr	-	0.70	0.67	0.58	0.61	0.62
Trp	-	0.16	0.13	0.13	0.14	0.14
Val	-	0.95	0.86	0.76	0.83	0.81
Dispensable AA, %						
Ala	-	1.36	1.34	1.12	1.22	1.22
Asp	-	1.25	1.14	1.02	1.09	1.11
Cys	-	0.33	0.31	0.29	0.32	0.33
Glu	-	3.12	3.08	2.62	2.87	2.91
Gly	-	0.74	0.68	0.63	0.69	0.67
Pro	-	1.47	1.44	1.26	1.36	1.39
Ser	-	0.86	0.85	0.73	0.77	0.80

[1]All values adjusted to 88.0% DM.

[2]MN1 and MN2 were sourced from MN, IL1 and IL2 was sourced from IL, and KY was sourced from KY.

Table 3.5. Analyzed composition of diets containing distillers dried grains (DDG), distillers dried grains with solubles from ethanol plants ($DDGS_{ethanol}$) and from beverage plant ($DDGS_{beverage}$), Exp. 2[1]

	N-free	$DDGS_{ethanol}$ source						DDG	$DDGS_{beverage}$
Item		1	2	3	4	5	6		
CP, %	0.24	16.49	15.13	16.61	17.40	16.79	16.64	18.83	16.89
Ca, %	0.37	0.47	0.44	0.37	0.63	0.50	0.43	0.41	0.45
P, %	0.34	0.46	0.45	0.42	0.53	0.48	0.48	0.26	0.44
Indispensable AA, %									
Arg	-	0.70	0.58	0.75	0.75	0.78	0.65	0.76	0.77
Ile	-	0.58	0.55	0.62	0.61	0.63	0.60	0.72	0.59
Leu	-	1.90	1.78	2.00	1.93	1.92	1.88	2.43	1.81
Lys	-	0.42	0.34	0.52	0.51	0.53	0.44	0.55	0.54
Met	-	0.29	0.27	0.31	0.29	0.30	0.30	0.35	0.30
Phe	-	0.80	0.76	0.86	0.83	0.84	0.79	1.00	0.84
Thr	-	0.60	0.55	0.65	0.65	0.66	0.61	0.71	0.60

Table 3.5. (Cont.)

Trp	-	0.13	0.11	0.13	0.14	0.14	0.13	0.14	0.15
Val	-	0.77	0.72	0.82	0.81	0.83	0.80	0.92	0.78
Dispensable AA, %									
Ala	-	1.18	1.11	1.24	1.24	1.26	1.17	1.41	1.13
Asp	-	1.05	0.97	1.12	1.14	1.17	1.07	1.2	1.08
Cys	-	0.31	0.27	0.29	0.29	0.31	0.29	0.35	0.32
Glu	-	2.79	2.58	2.86	2.84	2.93	2.72	3.31	2.94
Gly	-	0.64	0.58	0.66	0.67	0.71	0.63	0.66	0.65
Pro	-	1.35	1.22	1.34	1.29	1.32	1.27	1.6	1.48
Ser	-	0.77	0.71	0.84	0.83	0.83	0.77	0.92	0.79

[1]All values adjusted to 88.0% DM.

Table 3.6. Apparent ileal digestibility (%) of CP and AA in distillers dried grains with solubles (DDGS) from ethanol plants from MN, IL, and KY, Exp. 1[1]

	DDGS source[2]								Contrast		
Item	MN1	MN2	IL1	IL2	KY	Mean	SEM	*P*- value	IL vs. MN	IL vs. KY	MN vs. KY
CP	70.1	71.2	68.0	71.8	69.2	70.1	1.79	0.315	0.588	0.646	0.364
Indispensable AA, %											
Arg	82.5^{y}	81.3^{xy}	78.2^{x}	81.0^{xy}	78.4^{x}	80.3	1.24	0.047	0.056	0.297	0.012
His	77.1	76.8	76.2	78.2	74.7	76.6	1.24	0.274	0.801	0.064	0.090
Ile	76.8	76.3	74.6	76.3	74.3	75.7	1.53	0.47	0.367	0.419	0.123
Leu	81.0^{x}	82.0^{xy}	84.7^{z}	84.3^{yz}	83.6^{yz}	83.1	1.16	0.026	0.002	0.440	0.054
Lys	61.8^{xy}	67.4^{z}	61.2^{xy}	64.7^{yz}	60.3^{x}	63.1	2.30	0.009	0.270	0.143	0.020
Met	84.3	83.7	82.4	82.8	82.6	83.2	1.08	0.452	0.110	1.000	0.184
Phe	80.5	80.1	79.3	80.0	79.6	79.9	1.31	0.928	0.548	0.926	0.555
Thr	66.6	65.3	64.2	66.0	64.2	65.2	2.11	0.749	0.583	0.628	0.347
Trp	64.3^{z}	56.4^{x}	57.3^{x}	62.8^{yz}	59.5^{xy}	60.1	2.52	0.011	0.867	0.798	0.690

Table 3.6. (Cont.)

Val	74.7	74.1	73.5	75.2	72.9	74.1	1.67	0.708	0.949	0.336	0.303
Dispensable AA, %											
Ala	78.0	78.6	78.4	79.2	77.8	78.4	1.27	0.895	0.635	0.444	0.697
Asp	67.1	65.4	66.1	68.5	66.3	66.7	2.04	0.684	0.463	0.564	0.985
Cys	66.9	66.1	67.4	72.2	68.4	68.2	2.21	0.097	0.040	0.467	0.306
Glu	79.8	80.3	82.7	82.8	81.1	81.3	1.21	0.084	0.006	0.157	0.336
Gly	56.5	54.6	48.4	56.7	48.3	52.9	3.20	0.079	0.250	0.194	0.029
Pro	60.0	55.4	49.8	58.3	47.6	54.2	6.04	0.236	0.360	0.214	0.051
Ser	72.3	72.1	71.5	73.0	71.8	72.1	1.73	0.956	0.977	0.789	0.804

[x-z] Means within the same row without a common superscript letter differ ($P \leq 0.05$).

[1]Values are least square means of 7 observations per treatment.

[2]MN1 and MN2 were sourced from MN, IL1 and IL2 were source from IL, and KY was sourced from KY.

Table 3.7. Standardized ileal digestibility (%) of CP and AA of distillers dried grains with solubles (DDGS) from ethanol plants from MN, IL, and KY, Exp. 1[1, 2]

	DDGS source[3]								Contrast		
Item	MN1	MN2	IL1	IL2	KY	Mean	SEM	*P*- value	IL vs. MN	IL vs. KY	MN vs. KY
CP	81.6	82.9	81.7	84.3	81.3	82.4	1.79	0.543	0.590	0.311	0.553
Indispensable AA, %											
Arg	88.4	91.9	89.3	90.6	92.0	90.4	1.18	0.133	0.079	0.262	0.013
His	80.9	80.8	80.7	82.1	78.9	80.7	1.54	0.369	0.576	0.062	0.140
Ile	80.6	80.6	79.5	80.9	78.9	80.1	1.53	0.763	0.741	0.371	0.241
Leu	83.2^{x}	84.2^{xy}	87.3^{z}	86.7^{yz}	86.1^{yz}	85.5	1.16	0.010	0.001	0.384	0.032
Lys	66.8^{xy}	72.8^{z}	66.8^{xy}	70.1^{yz}	65.8^{x}	68.5	2.30	0.009	0.354	0.143	0.028
Met	86.6	86.0	85.0	85.1	84.9	85.5	1.08	0.543	0.157	0.848	0.174
Phe	85.1	84.9	84.8	85.1	84.6	84.9	1.31	0.997	0.971	0.782	0.755
Thr	75.1	74.3	74.5	75.9	73.9	74.7	2.11	0.914	0.735	0.479	0.656
Trp	73.6	67.9	68.7	73.5	70.2	70.8	2.52	0.076	0.817	0.648	0.785

Table 3.7. (Cont.)

Val	79.3	79.2	79.2	80.5	78.2	79.3	1.67	0.825	0.633	0.300	0.504
Dispensable AA, %											
Ala	83.0	83.6	84.4	84.7	83.3	83.8	1.27	0.722	0.219	0.337	0.963
Asp	73.5	72.5	74.0	75.9	73.5	73.9	2.04	0.607	0.182	0.428	0.758
Cys	72.1	71.7	73.3	77.6	73.6	73.7	2.21	0.106	0.027	0.339	0.350
Glu	82.9^{x}	83.5^{xy}	86.3^{z}	86.2^{yz}	84.4^{xy}	84.7	1.21	0.034	0.002	0.112	0.253
Gly	86.3	87.0	83.4	88.6	81.2	85.3	3.20	0.329	0.806	0.143	0.093
Pro	122.6	119.3	122.8	125.9	113.8	120.9	6.04	0.358	0.395	0.047	0.158
Ser	80.0	79.9	80.6	81.6	80.1	80.4	1.73	0.899	0.390	0.541	0.929

[x - z]Means within the same row without a common superscript letter differ ($P \leq 0.05$).

[1]Values are least square means of 8 observations per treatment.

[2]Standardized ileal digestibilities were calculated by correcting the apparent ileal digestibilities for the basal endogenous CP and AA losses (g/kg DMI), which were as follows: CP, 24.23; Arg, 0.87; His, 0.21; Ile, 0.32; Leu, 0.53; Lys, 0.30; Met, 0.09; Phe, 0.47; Thr, 0.68; Trp, 0.17; Val, 0.50; Ala, 0.76; Asp, 0.91; Cys, 0.19; Glu, 1.10; Gly, 2.51; Pro, 10.45; and Ser, 0.75.

[3]MN1 and MN2 were sourced from MN, IL1 and IL2 were sourced from IL, and KY was sourced from KY.

Table 3.8. Apparent ileal digestibility (%) of distillers dried grains (DDG) and distillers dried grains with solubles (DDGS) from ethanol and beverage plant, Exp. 2[1]

	$DDGS_{ethanol}$ source									$DDGS_{ethanol}$		Contrast	
Item	1	2	3	4	5	6	Mean	DDG	$DDGS_{beverage}$	SEM	*P* <	DDG vs. $DDGS_{ethanol}$	$DDGS_{beverage}$ vs. $DDGS_{ethanol}$
CP	56.7^{y}	53.0^{x}	65.1^{u}	60.7^{z}	66.7^{u}	65.8^{u}	61.3	66.7	59.5	1.42	0.001	0.001	0.222
Indispensable AA, %													
Arg	68.3^{y}	62.9^{x}	77.0^{uv}	70.9^{yz}	78.6^{v}	73.8^{zu}	71.9	75.0	71.5	1.63	0.001	0.006	0.857
His	75.9^{y}	67.0^{x}	80.4^{u}	75.2^{y}	80.6^{u}	78.9^{z}	76.3	81.3	72.9	1.10	0.001	0.001	0.001
Ile	73.8^{yz}	65.4^{x}	77.2^{u}	71.2^{y}	78.5^{u}	76.5^{zu}	73.8	80.4	74.0	1.26	0.001	0.001	0.764
Leu	83.6^{z}	75.2^{x}	83.6^{z}	79.1^{y}	85.2^{z}	86.0^{u}	82.1	84.4	82.3	1.06	0.001	0.006	0.888
Lys	51.1^{y}	44.4^{x}	69.3^{u}	58.9^{z}	70.0^{u}	60.1^{z}	59.0	73.4	64.7	1.57	0.001	0.001	0.001
Met	81.0^{y}	71.8^{x}	85.2^{z}	79.1^{y}	83.8^{z}	83.4^{z}	80.7	87.5	80.8	1.10	0.001	0.001	0.943
Phe	77.8^{z}	70.9^{x}	81.4^{u}	75.1^{y}	81.8^{u}	80.5^{u}	77.9	83.4	77.8	1.11	0.001	0.001	0.939
Thr	64.5^{yz}	56.0^{x}	68.9^{uv}	63.1^{y}	71.6^{v}	67.5^{zu}	65.3	71.4	63.7	1.36	0.001	0.001	0.191

Table 3.7. (Cont.)

Trp	59.2^{y}	48.6^{x}	62.3^{yz}	59.5^{y}	66.2^{z}	61.6^{y}	59.6	63.0	59.4	1.56	0.001	0.018	0.938
Val	73.1^{y}	64.6^{x}	76.3^{u}	70.6^{y}	77.4^{u}	76.2^{z}	73.0	77.5	71.5	1.26	0.001	0.001	0.132
Dispensable AA, %													
Ala	73.3^{y}	66.5^{x}	77.7^{z}	71.9^{y}	78.9^{z}	77.5^{z}	74.3	78.1	71.9	1.14	0.001	0.001	0.015
Asp	61.5^{y}	54.5^{x}	66.5^{z}	61.2^{y}	67.6^{z}	65.9^{z}	62.9	68.6	62.7	1.45	0.001	0.001	0.946
Cys	71.2^{yz}	62.4^{x}	74.2^{u}	70.4^{y}	74.8^{u}	73.8^{z}	71.1	77.2	65.6	1.32	0.001	0.001	0.001
Glu	79.4^{u}	71.4^{x}	81.7^{v}	76.7^{y}	82.8^{v}	82.6^{v}	79.1	84.6	79.0	1.05	0.001	0.001	0.929
Gly	27.6^{x}	27.0^{x}	44.9^{z}	36.6^{y}	51.5^{z}	44.9^{z}	38.7	40.0	30.0	3.36	0.001	0.395	0.004
Pro	-17.8^{x}	-0.4^{xy}	17.4^{yu}	0.2^{y}	22.9^{u}	18.3^{yu}	6.8	11.5	11.9	8.56	0.001	0.311	0.228
Ser	70.5^{y}	63.8^{x}	75.2^{z}	69.1^{y}	76.7^{z}	74.7^{z}	71.6	76.2	70.0	1.14	0.001	0.001	0.075

$^{u-z}$Means within a row and within $DDGS_{ethanol}$ sources without a common superscript letter differ, ($P \leq 0.05$).

[1]Values are least square means of 7 observations.

Table 3.9. Standardized ileal digestibility (%) of distillers dried grains (DDG) and distillers dried grains with solubles from ethanol plants ($DDGS_{ethanol}$) and beverage plant ($DDGS_{beverage}$), Exp. 2[1, 2]

	$DDGS_{ethanol}$ sources									$DDGS_{ethanol}$ sources		Contrast	
Item	1	2	3	4	5	6	Mean	DDG	DDGS beverage	SEM	*P* <	DDG vs. $DDG_{ethanol}$	$DDGS_{beverage}$ vs. $DDGS_{ethanol}$
CP	67.5^{x}	64.8^{x}	76.1^{z}	70.8^{y}	77.3^{z}	74.4^{z}	71.8	76.4	70.3	1.41	0.001	0.001	0.432
Indispensable AA, %													
Arg	76.3^{xy}	72.9^{x}	85.7^{u}	78.4^{yz}	86.2^{u}	81.2^{z}	80.1	82.8	79.0	1.59	0.001	0.028	0.450
His	79.1^{y}	70.7^{x}	83.3^{z}	78.5^{y}	83.7^{z}	83.4^{z}	79.8	84.4	76.2	1.1	0.001	0.001	0.004
Ile	$77.4y^{z}$	69.2^{x}	79.9^{zu}	74.7^{y}	81.8^{u}	81.3^{u}	77.4	83.3	77.7	1.28	0.001	0.001	0.581
Leu	85.4^{z}	77.0^{x}	84.9^{z}	80.9^{y}	87.0^{zu}	88.6^{u}	84.0	86.0	84.1	1.07	0.001	0.022	0.619
Lys	56.8^{y}	51.4^{x}	73.8^{v}	63.7^{z}	74.5^{v}	68.7^{u}	64.8	77.9	69.3	1.57	0.001	0.001	0.004
Met	83.1^{y}	73.9^{x}	86.8^{z}	81.2^{y}	85.8^{z}	86.5^{z}	82.9	89.2	82.8	1.1	0.001	0.001	0.808
Phe	82.1^{z}	75.3^{x}	85.0^{u}	79.2^{y}	85.8^{u}	84.2^{zu}	81.9	87.0	81.9	1.12	0.001	0.001	0.744
Thr	71.6^{y}	63.8^{x}	75.3^{z}	69.7^{y}	78.0^{z}	76.0^{z}	72.4	77.5	70.8	1.36	0.001	0.001	0.520

Table 3.9. (Cont.)

Trp	68.7^{y}	59.7^{x}	70.9^{yz}	68.3^{y}	75.0^{z}	68.7^{y}	68.6	71.8	67.9	1.58	0.001	0.050	0.990
Val	77.0y^{z}	68.7^{x}	79.3^{zu}	74.3^{y}	80.9^{u}	81.8^{u}	77.0	80.7	75.2	1.28	0.001	0.003	0.367
Dispensable AA, %													
Ala	77.4^{y}	70.9^{x}	81.8^{z}	75.8^{y}	82.8^{z}	82.4^{z}	78.5	81.8	76.3	1.15	0.001	0.001	0.067
Asp	67.9^{y}	61.4^{x}	72.2^{z}	67.1^{y}	73.3^{z}	73.1^{z}	69.2	74.3	69.0	1.46	0.001	0.001	0.838
Cys	75.6^{y}	67.5^{x}	78.7^{z}	75.1^{y}	79.2^{z}	80.6^{z}	76.1	81.2	69.9	1.33	0.001	0.001	0.001
Glu	82.2^{z}	74.3^{x}	84.1^{z}	79.4^{y}	85.3^{u}	86.4^{u}	82.0	87.0	81.6	1.06	0.001	0.001	0.965
Gly	53.6^{x}	56.6^{x}	72.9^{zu}	61.2^{xy}	75.7^{y}	66.3^{yz}	64.4	66.1	56.4	3.25	0.001	0.427	0.009
Pro	32.1^{x}	57.6^{yz}	75.0^{z}	51.5^{xy}	75.3^{z}	54.2^{y}	57.6	55.3	59.8	8.32	0.002	0.734	0.754
Ser	77.0^{y}	70.8^{x}	81.2^{z}	75.1^{y}	82.7^{z}	81.1^{z}	78.0	81.8	76.4	1.14	0.001	0.001	0.266

[x-u]Means within a row and within $DDGS_{ethanol}$ sources without a common superscript letter differ, ($P \leq 0.05$).

[1]Values are least square means of 7 observations.

[2]Standardized ileal digestibilities were calculated by correcting the apparent ileal digestibilities for the basal ileal endogenous CP and AA losses (g/kg DMI), which were as follows: CP, 20.12; Arg, 0.64; His, 0.15; Ile, 0.24; Leu, 0.39; Lys, 0.27; Met, 0.07; Phe, 0.39; Thr, 0.48; Trp, 0.14; Val, 0.34; Ala, 0.55; Asp, 0.77; Cys, 0.16; Glu, 0.87; Gly, 1.90; Pro, 7.66; and Ser, 0.57.

Table 3.10. Summary of CV in composition, apparent (AID) and standardized (SID) ileal digestibility of CP and AA of 11 sources of distillers dried grains with solubles from ethanol plants ($DDGS_{ethanol}$) used in Exp. 1 and 2[1]

	Composition, %		AID, %		AID,g/kg		SID, %		SID, g/kg	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
CP	26.0	4.1	65.3	9.3	169.5	12.18	76.6	8.6	198.8	11.30
NDF	28.8	6.9	-	-	-	-	-	-	-	-
ADF	11.3	10.0	-	-	-	-	-	-	-	-
Starch	8.0	22.6	-	-	-	-	-	-	-	-
Indispensable AA										
Arg	1.1	7.9	75.7	8.1	8.5	14.7	84.8	7.8	9.5	14.2
His	0.7	7.9	76.4	4.9	5.0	10.3	79.9	4.5	5.2	10.0
Ile	1.0	6.0	74.6	4.9	7.1	9.4	78.6	4.7	7.5	9.2
Leu	3.0	5.2	82.6	3.8	24.6	6.2	84.7	3.9	25.3	6.3
Lys	0.7	10.1	60.8	12.6	4.3	19.9	66.5	10.7	4.7	18.0
Met	0.5	5.8	81.8	4.6	4.0	8.7	84.1	4.5	4.1	8.7

Table 3.10. (Cont.)

Phe	1.3	4.0	78.8	4.1	9.9	6.5	83.3	3.9	10.5	6.3
Thr	1.0	5.5	65.3	6.0	6.3	9.5	73.5	5.3	7.1	9.0
Trp	0.2	7.2	59.8	7.9	1.2	14.0	69.6	5.9	1.4	12.2
Val	1.3	5.5	73.5	4.8	9.3	8.9	78.0	4.7	9.8	8.9
Dispensable AA										
Ala	1.9	5.4	76.2	5.2	14.5	9.0	80.9	5.4	15.4	9.2
Asp	1.7	5.2	64.6	6.3	11.0	9.8	71.3	5.9	12.1	9.2
Cys	0.5	6.1	69.8	5.6	3.3	7.3	75.0	5.2	3.5	6.9
Glu	4.4	4.3	80.1	4.3	35.3	6.6	83.2	4.3	36.6	6.6
Gly	1.0	4.8	45.2	23.5	4.6	27.0	73.9	17.2	7.5	20.6
Pro	2.1	4.1	28.3	96.4	5.9	99.8	86.4	40.6	18.0	43.3
Ser	1.2	5.2	71.9	4.8	8.8	7.5	79.1	4.4	9.7	7.4

CHAPTER 4

Guanidination of lysine in distillers dried grains with solubles (DDGS) and in ileal digesta of pigs fed DDGS

ABSTRACT

An experiment was conducted to determine the optimum conditions for guanidination of Lys in distillers dried grains with solubles (DDGS) and in ileal digesta from cannulated pigs. To determine the optimum incubation time for guanidination, a DDGS sample was guanidinated in 0.6 *M* O-methylisourea reagent at pH 11.4 for 1, 3, 6, or 9 d. Ileal digesta from pigs fed a diet containing DDGS was also incubated in 0.6 *M* O-methylisourea for 1, 3, or 6 d. To determine the effect of the pH of O-methylisourea on the extent of conversion of Lys to homoarginine, DDGS and ileal digesta samples were guanidinated in 0.6 *M* O-methylisourea at a pH of 9, 10, 11, 12, or 13, with an incubation time of 3 d. Results showed that in DDGS, the conversion of Lys to homoarginine was greater ($P < 0.05$) when DDGS was incubated for 3 d than for 1 d. However, there was no increase in Lys conversion if the incubation time exceeded 3 d. The conversion of Lys to homoarginine in ileal digesta samples was similar after 1, 3, and 6 d of incubation. Result also showed that Lys conversion was maximized at a pH between 11 and 12. However, the conversion was reduced at pH 13. In conclusion, the optimum conditions for guanidination of DDGS and ileal digesta is at 3 and 1 d of incubation, respectively, using 0.6 *M* O-methylisourea with a pH between 11 and 12.

Keywords: amino acids, digestibility, distillers dried grains with solubles, pigs, reactive Lys

INTRODUCTION

During drying of distillers dried grains with solubles (**DDGS**), the reducing sugars can react with the ε–NH_2 group of Lys, forming early and advanced Maillard products. This may increase the variation in the ileal digestibility of Lys in DDGS fed to pigs (Stein et al., 2006). The Lys that is not bound to a reducing sugar is called reactive Lys (Hurrell and Carpenter, 1981). This is the Lys that is readily digested by pigs. In contrast, the Lys that has an ε – NH_2 group that is bound to a reducing sugar is called unreactive Lys. Some unreactive Lys may be absorbed but not utilized in vivo (Finot and Magnenat, 1981), but most of the unreactive Lys is not digestible. A method to measure the amount of reactive Lys in a feedstuff is the homoarginine procedure (Mauron and Bujard, 1964). This procedure involves a guanidination reaction where the reactive Lys, but not the unreactive Lys, is modified by substitution of the ε–NH_2 with a guanidino group from O-methylisourea, forming 2-amino-6-guanidohexanoic acid or homoarginine (Kimmel, 1967). Thus, the amount of Lys that is converted to homoarginine represents the reactive Lys concentration in the sample. By guanidination of Lys in a diet containing DDGS and in the ileal digesta of pigs fed a diet containing DDGS, the concentration of digestible reactive Lys can be calculated (Moughan and Rutherfurd, 1996; Rutherfurd et al., 1997). However, there are no data on the concentration of reactive Lys in DDGS.

The extent of the conversion of Lys to homoarginine is affected by the type of protein that is used (Maga, 1981). Thus, there is variation among feedstuffs in the conversion of Lys to homoarginine (Ravindran et al., 1996). The incubation time and the

pH of O-methylisourea may also affect the conversion of Lys to homoarginine during guanidination (Maga, 1981; Rutherfurd and Moughan, 1990). Therefore, it is necessary to determine the optimum guanidination conditions for each protein source.

The objective of this experiment was to determine the conditions for guanidination (i.e., incubation time and pH of O-methylisourea) that will maximize the conversion of Lys to homoarginine in DDGS and in ileal digesta of pigs fed diets based on DDGS.

MATERIALS AND METHODS

Preparation of O-Methylisourea Reagent

Preparation of the O-methylisourea reagent was based on the procedure by Rutherfurd and Moughan (1990). For every 100 mL of the reagent, 20.6 g of barium hydroxide (Fisher Scientific International, Inc., Pittsburgh, PA) was dissolved in 69.0 mL of degassed water and the solution was slowly heated to 95^{o}C. The solution was then mixed with 10.4 g of O-methylisourea hydrogen sulfate (Sigma-Aldrich Inc., St. Louis, MO) and cooled to 25^{o}C by gently stirring the solution. The solution was centrifuged twice in a Jouan CR 412 centrifuge (Winchester, VA) at 4200 x *g* for 15 min. The supernatant was retained whereas the solid phase was discarded. The supernatant, which had an initial pH between 12 and 12.5, was adjusted to the desired pH by adding drops of 1.0 *M* HCl or 1.0 *M* NaOH. The supernatant was then adjusted to a final volume of 100 mL by adding degassed water.

Guanidination of DDGS and Ileal Digesta at Varying Incubation Time

A sample of DDGS was obtained from a dry grind ethanol plant in Minnesota. A 0.5 g sample of DDGS was added to each of 8 (25-mL capacity) test tubes. Six

milliliters of 0.6 *M* O-methylisourea reagent (pH 11.4) was then added to each tube. Duplicate samples were incubated for 1, 3, 6, or 9 d. Six additional test tubes were prepared with 0.5 g of dried ileal digesta that were collected from pigs fed diets based on DDGS. The digesta samples were mixed with 6 mL of 0.6 *M* O-methylisourea (pH 11.4) and incubated (in duplicates) for 1, 3, or 6 d. During incubation, all DDGS and ileal digesta samples were continuously stirred using an automatic test tube tumbler. Samples were air-dried after incubation and 0.2 g sub-samples were collected for homoarginine analysis.

Guanidination of DDGS and Ileal Digesta with Varying pH of O-Methylisourea

Five test tubes were prepared with 0.5 g of DDGS and an additional 4 test tubes were prepared with 0.5 g of dried ileal digesta. The same sources of DDGS and ileal digesta were used in this experiment as in the previous. Five batches (20 mL each) of 0.6 *M* O-methylisourea reagents were prepared and adjusted to a final pH of 9, 10, 11, 12, or 13. The supernatants collected from these 5 solutions had an initial pH that ranged from 12 to 12.5. Drops of 1 *M* HCl were added to 4 of the supernatants to attain a pH of 9, 10, 11, or 12, whereas 1 *M* NaOH was added to the 5th supernatant to increase the pH to 13. Each of these solutions was then added to the test tubes containing DDGS. However, only the reagents with pH 10, 11, 12, and 13 were used to guanidinate the 4 ileal digesta samples. All samples were incubated for 3 d. The samples were stirred continuously at 20^{o}C using an automatic test tube tumbler. Samples were air-dried after incubation and 0.2 g sub-samples were collected for homoarginine analysis.

Homoarginine Analysis

The homoarginine analysis of DDGS and ileal digesta was performed by initially hydrolyzing the samples with 30 mL of 6.0 *N* HCl followed by refluxing for 24 h at 110°C (procedure 4.1.11; AOAC, 2000). The samples were then derivatized and analyzed for homoarginine using cation exchange chromatography. Norleucine (Sigma-Aldrich Inc., St. Louis, MO) was used as an internal standard. Ninhydrin (Trione**,** Pickerings Laboratories, Mt View, CA) was used to react with the primary and secondary amines including homoarginine at 130°C. The color change was detected using a Spectrasystem UV3000 spectrophotometer (Thermo Separation Products, Riviera Beach, FL) at 570 nm. Homoarginine was eluted after Arg and quantified using a standard of homoarginine hydrochloride (Fisher Scientific International Inc., Pittsburgh, PA).

Calculations

Calculation of the conversion of Lys to homoarginine was based on the amount of untransformed Lys (i.e., Lys that was not converted to homoarginine after guandination; unreactive Lys) and homoarginine (Rutherfurd and Moughan, 1990):

Lys conversion rate, % =

(mmol homoarginine /[mmol homoarginine + mmol Lys]) x 100 [1]

Statistical Analyses

Conversion rates of Lys to homoarginine in DDGS and ileal digesta were compared using the MIXED procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC). The optimum days of guanidination was identified as the incubation time that resulted in the greatest conversion of Lys to homoarginine in DDGS and ileal digesta. A probability of $P < 0.05$ was considered significant. The optimum pH for guanidination was

determined by plotting the conversion rates of DDGS and ileal digesta against their respective Lys conversion and determining the peak on the graph.

RESULTS

The effect of incubation time on the conversion of Lys to homoarginine is shown in Table 4.1. After 1 d of incubation, 78.3% of Lys in DDGS was converted to homoarginine. After 3 d of incubation, Lys conversion was increased to 82.5% ($P < 0.05$). However, the Lys conversion in DDGS after 3 d of incubation was similar to the Lys conversion after 6 and 9 d of incubation. In contrast, the ileal digesta samples that were incubated for 1, 3 and 6 d were all similar in Lys conversion rates (72.8, 75.4, and 76.7%, respectively).

Guanidination of DDGS and ileal digesta using 0.6 *M* O-methylisourea with varying pH showed that maximum Lys conversion for DDGS and ileal digesta (84.1 and 77.7%, respectively) occurred between pH 11 and 12 for both samples (Figure 4.1). However, at pH 13, the Lys conversion rate of DDGS and ileal digesta was reduced to 16.8 and 11.2%, respectively.

DISCUSSION

In the guanidination of proteins for reactive Lys analysis, optimizing the rate of Lys conversion is important because it increases the accuracy of the determination of reactive Lys. Any Lys that is not guanidinated is assumed to be unreactive. Some of the reactive Lys may not be guanidinated if the conditions are not optimal, leading to an underestimation of the reactive Lys concentration in the sample. The rapid increase in

the Lys conversion during the initial 3 d of incubation was similar to results obtained for casein (Maga, 1981). It appears that 3 d is the minimum guanidination time for DDGS because there was no difference in the Lys conversion from 3 to 9 d of incubation. An optimum incubation time of 3 d was also reported for cottonseed protein (Ravindran et al., 1996). The maximum Lys conversion rate for DDGS (83.0%) obtained in this study is similar to the Lys conversion in guanidinated canola meal and barley (88.0 and 84.6% respectively) obtained by Nyachoti et al. (2002). However, a conversion rate of only 36.1% was reported for cottonseed protein (Ravindran et al., 1996) but that was obtained under conditions where the pH and the molar concentration of O-methylisourea was lower than in this study. It is also possible that the cottonseed used in the study had a greater concentration of Lys that was bound as Maillard products (Almquist and Halloran, 1975).

The lack of a difference in Lys conversion in ileal digesta between d 1 and 3 suggests that the ileal digesta of pigs contains highly accessible protein-bound and free Lys. This may be due to the digestive enzymes that acted on the DDGS in the gastrointestinal tract, because they may have increased the surface contact of the O-methylisourea with the free $\varepsilon - NH_2$ groups of Lys. Despite the rapid conversion of Lys to homoarginine in ileal digesta, the highest conversion rate was at 76.7% only. The lower conversion rate of ileal digesta compared with DDGS may be due to the greater concentration of unreactive Lys in the digesta because most of the reactive Lys may already have been absorbed, thus increasing the ratio of the unreactive to reactive Lys.

The optimum pH for DDGS guanidination was shown to be between 11 and 12. For cottonseed, a pH of 12.5 resulted in the greatest conversion rate if a 3-d incubation

with 0.4 *M* O-methylisourea was used (Ravindran et al., 1996). The lower pH needed for ileal digesta to attain maximum Lys conversion may be related to less structural hindrance for O-methylisourea to the free Lys in the ileal digesta compared with DDGS. The greater conversion of Lys in DDGS between pH 11 and 12 may be related to the net charge of Lys at this pH range. The $\varepsilon - NH_2$ of Lys is reactive with O-methylisourea only when it is deprotonated (Beardsley and Reilly, 2002) and at pH 12, Lys is fully deprotonated. This makes the Lys negatively charged and more susceptible to addition of a guanidino group. When the pH of O-methylisourea was increased to 13, however, the Lys conversion rate was drastically reduced. Coagulation and aggregation of the proteins in DDGS and ileal digesta may have occurred at pH 13, which may have contributed to the low Lys conversion. It was also suggested that the changes in the tertiary structure of the protein at higher pH makes it more difficult for O-methylisourea to access the Lys residues (Ravindran et al., 1996).

In conclusion, the optimum conditions for DDGS and ileal digesta guanidination is 3 d and 1 d of incubation, respectively, if a 0.6 *M* O-methylisourea with a pH between 11 and 12 is used. Using 0.6 *M* O-methylisourea with a pH above 12 will reduce the conversion of Lys to homoarginine.

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Table 4.1. Effect of guanidination time (d) on Lys to homoarginine conversion in distillers dried grains with solubles (DDGS) and ileal digesta from pigs fed diets based on DDGS[1]

	Guanidination time, d					
	1	3	6	9	SEM	*P*-value
DDGS						
Unreactive Lys[2], mmol/kg	8.3	7.0	6.6	6.2	0.39	0.085
Homoarginine[3], mmol/kg	32.2	33.6	33.2	32.2	1.49	0.873
Rate of Lys conversion, %[d]	78.3[x]	82.5[y]	83.0[y]	82.9[y]	0.69	0.043
Ileal digesta						
Unreactive Lys[2], mmol/kg	9.1	8.4	8.1	-	0.24	0.117
Homoarginine, mmol/kg[3]	24.6	26.6	24.9	-	0.89	0.328
Rate of Lys conversion, %[4]	72.8	75.4	76.7	-	0.63	0.113

[x,y]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Data are means of duplicate analyses.

[2]Lysine that was not transformed to homoarginine after guanidination.

[3]Homoarginine that was produced from guanidination of Lys with O-methylisourea.

[4]Conversion (%) from Lys to homoarginine.

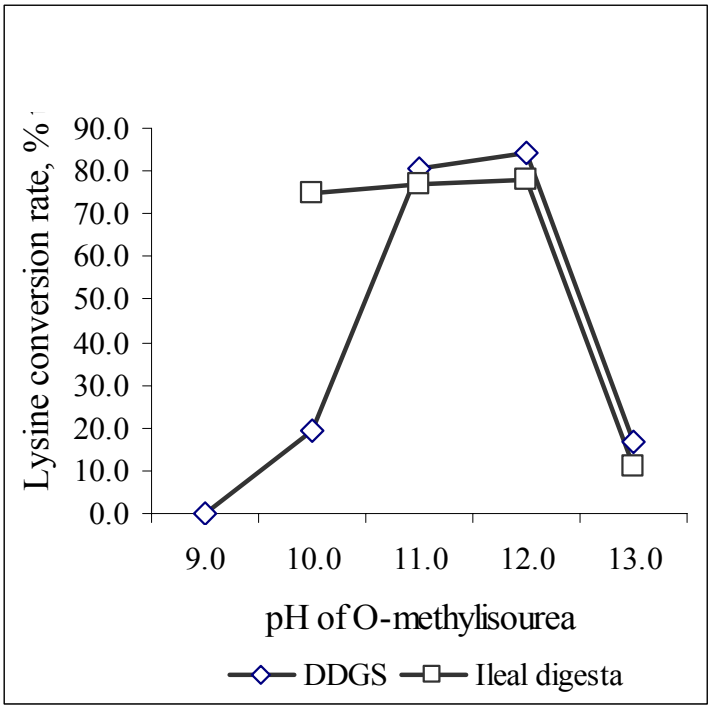

Figure 4.1. Effect of varying pH of 0.6 *M* O-methylisourea on the conversion of Lys to homoarginine in distillers dried grains with solubles (DDGS) and ileal digesta from pigs fed diets based on DDGS.

CHAPTER 5

Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs

ABSTRACT

A study was conducted to measure the reactive Lys concentration in 33 sources of corn distillers dried grains with solubles (DDGS). In Exp. 1, reactive Lys was measured using 2 procedures: the homoarginine procedure and the furosine procedure. The concentration of reactive Lys was then correlated with the concentration of standardized ileal digestible (SID) Lys in DDGS fed to growing pigs. In Exp. 2, a factorial experiment was conducted using 4 ratios of condensed distillers solubles (CDS) and wet distillers grain (WDG). The ratios (wt/wt) of CDS to WDG were 0:100, 20:80, 40:60, and 100:0. These samples were designated as DDG, $DDGS_{20}$, $DDGS_{40}$, and CDS, respectively, after drying. Four sub-samples from each combination were freeze-dried or oven-dried at 50, 75, or 100°C. All sub-samples were analyzed for total Lys and for reactive Lys using the homoarginine procedure. Results of Exp. 1 showed that only 74.1% of total Lys was reactive if measured by homoarginine procedure, and 83.5% was reactive if measured by the furosine procedure. The concentration of SID Lys in DDGS was correlated with the concentration of reactive Lys by homoarginine procedure (r^2=0.70, $P < 0.05$) and by the furosine procedure (r^2=0.66, $P < 0.05$). In Exp. 2, the concentration of total Lys and reactive Lys was reduced ($P < 0.05$) when addition of CDS or drying temperature of the samples was increased, but the reduction was greater ($P < 0.05$) when both CDS addition and drying temperature were increased at the same time. After oven-drying at 100°C,

total Lys was 75.7% reactive in DDG, but only 27.6% and 10.2% reactive in $DDGS_{20}$ and $DDGS_{40}$, respectively. In conclusion, reactive Lys is correlated with the concentration of SID Lys in DDGS, and addition of CDS exacerbates the negative effects of heating on the concentration of total Lys and reactive Lys in DDGS.

Keywords: amino acids, digestibility, distillers dried grains with solubles, pigs, reactive Lys

INTRODUCTION

Wet distillers grains (**WDG**), dried distillers grains (**DDG**), and condensed distillers solubles (**CDS**) are co-products of the dry-grind ethanol industry. The WDG and CDS are typically blended and dried together to produce distillers dried grains with solubles or **DDGS (**AAFCO, 2006). The binding of reducing sugars to the free NH_2 group of Lys via the Maillard reaction may be responsible for the variability in ileal Lys digestibility in DDGS by growing pigs (Fastinger and Mahan, 2006; Stein et al., 2006). Only the Lys that has not undergone binding with reducing sugars (reactive Lys) is bioavailable to the animal, whereas the bound Lys (unreactive Lys) is not bioavailable (Finot and Magnenat, 1981).

Reactive Lys may be measured using the homoarginine procedure that transforms the unbound Lys to homoarginine through a guanidination reaction (Kimmel, 1967). The unreactive Lys may be measured using the furosine procedure (Finot et al., 1981), and the concentration of reactive Lys can be subsequently calculated by subtracting the unreactive Lys from the total Lys in the sample.

It is hypothesized that the concentration of reactive Lys is correlated with the concentration of standardized ileal (**SID**) digestible Lys in DDGS fed to pigs and that both CDS and heat-drying can reduce the concentration of reactive Lys in DDGS. The objective of this study was to measure the concentration of reactive Lys in 33 samples of corn DDGS and correlate the concentration of reactive Lys with the concentration of SID Lys. A second objective was to measure the effect of the addition of CDS and of drying temperature on the concentration of reactive Lys in DDGS and on the CV of the concentration of AA in DDGS.

MATERIALS AND METHODS

Experiment 1

A total of 33 samples of corn DDGS were analyzed for reactive Lys using the homoarginine and the furosine procedures. All DDGS sources had been previously analyzed for chemical composition and the SID of AA had been measured using cannulated growing pigs (Table 5.1). The concentration of reactive Lys in DDGS measured by both procedures was then correlated with the concentration of SID Lys. Samples were also analyzed for total Lys, which is the sum of reactive Lys and unreactive Lys. The concentration of reactive Lys in the samples was also compared with the concentration of total Lys to estimate the extent of the formation of unreactive Lys in DDGS after drying.

Reactive Lys Analysis. In proteins that have undergone Maillard reaction, the Lys-sugar complex in the form of Amadori products is the major form of unreactive Lys. The Lys-sugar complex, although resistant to enzymatic digestion in the small intestine,

is split during the acid hydrolysis step of AA analysis (Finot and Magnenat, 1981). During this step, the Amadori product is converted to 3 AA products, namely regenerated Lys, furosine, and pyridosine (Finot et al., 1968), whereas reactive Lys remains as Lys. The regenerated Lys is structurally identical to reactive Lys, but regeneration of Lys does not occur in the animal because of the relatively mild conditions in the intestinal tract (Finot et al., 1968). Thus, the regenerated Lys is not available to the animal. As a result, the Lys that appears in the chromatogram in a conventional AA analysis, called total Lys, is composed of the reactive Lys and the regenerated Lys. Therefore, conventional methods of AA analysis cannot distinguish reactive Lys and unreactive Lys separately, leading to a potential over-estimation of the Lys concentration in heated proteins. To overcome this limitation, several methods have been developed to estimate the reactive Lys such as the homoarginine procedure and the furosine procedure.

Homoarginine Procedure. This method chemically transforms the reactive Lys, but not the bound Lys, to homoarginine through a guanidination reaction using O-methylisourea before samples are acid-hydrolyzed (Kimmel, 1967). This separates the reactive Lys from unreactive Lys in the chromatogram during AA analysis because the reactive Lys appears as homoarginine, whereas the regenerated (unreactive) Lys appears as Lys. The amount of homoarginine is then converted to Lys on a molar basis to calculate the amount of reactive Lys.

In Exp. 1, all samples were guanidinated using conditions that have previously been determined to result in the most effective conversion of reactive Lys to homoarginine in DDGS (i.e., 0.6 *M* O-methylisourea and incubation for 3 d at a pH of 11.4; Pahm et al., unpublished data). The guanidinating solution was prepared using a

procedure adopted from Rutherfurd and Moughan (1990). To prepare 100 mL of the O-methylisourea solution, 20.6 g of barium hydroxide (Fisher Scientific International, Inc., Pittsburgh, PA) was added to 69.0 mL of degassed water (distilled water that was boiled for 30 min.) followed by heating to 95°C. The solution was mixed with 10.4 g of O-methylisourea hydrogen sulfate (Sigma-Aldrich Inc., St. Louis, MO) and cooled to 25°C. The solution was centrifuged twice in a Jouan CR 412 centrifuge (Winchester, VA) at 4200 x *g* for 15 min. The supernatant was retained, whereas the solid phase was discarded. The supernatant, which had an initial pH between 12 and 12.5, was adjusted to pH 11.4 by adding drops of 1.0 *M* HCl. The supernatant was adjusted to a final volume of 100 mL using degassed water. Six milliters of the O-methylisourea reagent was added to 0.2 g of each sample in a 25-mL flask. Samples were stirred for 12 h using a magnetic stirrer (MultiMagnestir 1278, Lab-line Instruments, Melrose Park, IL). Samples were then incubated for 60 h at 20°C followed by air-drying and analysis for homoarginine using HPLC. In the HPLC analysis, samples were initially acid-hydrolyzed by adding 30 mL of 6 *N* HCl to each sample followed by refluxing for 24 h at 110°C (procedure 4.1.11; AOAC, 2000). The concentration of homoarginine and other AA was determined using an HPLC system (Pickerings Laboratories, Mt. View, CA.). Homoarginine hydrochloride (Sigma Chemicals, St. Louis, MO) was used as a standard to quantify the area of the homoarginine peak in the chromatograph. The reactive Lys was calculated based on the amount of homoarginine in the samples. Homoarginine was transformed to Lys on a molar basis using the following equation:

Reactive Lys, % of sample:

= homoarginine, %/MW homoarginine x MW Lys

The efficiency of the conversion of Lys to homoarginine was calculated using the following equation:

Lys conversion rate, %

= 100 x [mmol homoarginine / (mmol homoarginine + mmol Lys)]

The amount of Lys recovered after guanidination was quantified using the following equation:

Lys recovery, %

= 100 x (reactive Lys + unreactive Lys) / total Lys in unguanidinated sample

Furosine Procedure. This procedure is used to calculate the concentration of reactive Lys based on the concentration of furosine in heated proteins. Furosine is one of the AA that is generated from Amadori compounds when Maillard-reacted proteins are acid-hydrolyzed during AA analysis (Finot et al., 1968). Amadori products in milk yield 32% furosine, 40% regenerated Lys, and 28% pyridosine (Bujard and Finot, 1978). Because of this nearly fixed proportion of breakdown products of Amadori compounds, the total concentration of regenerated Lys can be calculated if the concentration of furosine is analyzed.

In the furosine procedure, 0.2 g of each DDGS sample was acid-hydrolyzed in 30 mL of 0.6 *N* HCL followed by 24-h refluxing (procedure 4.1.11; AOAC, 2000). Each acid-hydrolyzed sample was analyzed for the concentration of furosine using HPLC. In this study, ε-*N*-2-furoymethyl-lysine (Neosystems Laboratory, Strasbourg, France) was used to identify the furosine peak in the chromatogram. Furosine was quantified at 570 nm. The conversion factors used to calculate for the Amadori product, unreactive Lys,

and subsequently, reactive Lys in DDGS were based on previous studies in milk products (Finot et al. 1981). Thus, the Amadori product was calculated as follows:

Amadori product, % =

Furosine, % / (32/100)

The regenerated Lys, which is assumed to be 40% of the Amadori product in DDGS, was then calculated:

Regenerated Lys, % =

Amadori product, % x (40/100)

In the furosine procedure, the Lys peak that appears in the chromatogram is identical to total Lys, which is the sum of concentrations of reactive Lys and regenerated Lys. Thus, the amount of regenerated (i.e., unreactive) Lys that was calculated based on the furosine concentration can be subtracted from the amount of total Lys to calculate the amount of reactive Lys:

Reactive Lys, % =

Total Lys – regenerated Lys

Statistical Analysis. Each of the 33 DDGS sources was the experimental unit. The Proc REG procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC) was used to correlate CP, Lys, and reactive Lys with the SID concentration of Lys. A paired dependent t-test in SAS was used to compare the concentration of reactive Lys that was estimated by the furosine procedure and homoarginine procedure, respectively.

Experiment 2

Samples and Experimental Design. Ten kilograms of CDS and WDG were obtained from a dry-grind ethanol plant in SD. Two additional samples (1 kg each) were

prepared by mixing CDS and WDG using a ratio (wt/wt, as-is basis) of CDS to WDG of 20:80 and 40:60. Four 25g-subsamples from CDS, WDG, WDG with 20% CDS, and WDG with 40% CDS were collected and dried by freeze-drying or oven-dried at 50, 75, or 100^{o}C for 5 h. After drying, the samples were designated as follows: **CDS** for dried CDS, **DDG** for dried WDG, **DDGS$_{20}$** for dried WDG with 20% CDS, and **DDGS$_{40}$** for dried WDG with 40% CDS. A 4 x 4 factorial arrangement was used, with the first factor being the amount of CDS in the fresh sample (100, 40, 20, or 0%), whereas the second factor was the drying temperature (freeze-drying, 50, 75, or 100^{o}C). The experimental unit was the sample and a total of 16 treatments were used, with 2 replications per treatment.

Drying, Sample Analysis. Samples were oven-dried using a Thelco 130DM oven dryer. During drying, samples were evenly spread in 11.25 x 11.25 cm aluminum pans. A commercial freeze-dryer (Dura Dry MP, FTS Systems, Inc., Stone Ridge, NY) was used to freeze-dry the samples. Samples were ground in a coffee grinder and were stored at -20^{o}C until analyzed. Samples were analyzed for CP and for total Lys using the conventional procedure and for concentration of reactive Lys using the homoarginine procedure.

Calculations and Statistical Analysis. To equalize the differences in initial total Lys and initial reactive Lys among samples, the total Lys as percent of initial total Lys and the reactive Lys as percent of initial reactive Lys were calculated after oven-drying. To determine the extent of formation of unreactive Lys after oven-drying, the reactive Lys as percent of total Lys was also calculated.

Data were analyzed as a 4 x 4 factorial experiment, with a total of 16 treatment combinations of CDS addition and drying temperature. The experimental unit was the dried sample with 2 replicates per sample. The effect of CDS addition, drying temperature, and CDS x drying temperature on the concentration of CP, total Lys and reactive Lys were analyzed using Proc MIXED of SAS with CDS addition, drying temperature, and their interaction as fixed effects. All directly measured data (CP, total Lys and reactive Lys) as well as the calculated data on total and reactive Lys as percentage of initial values were compared using the PDIFF option of SAS and in all analyses, a probability of $P < 0.05$ was considered significant.

RESULTS

Experiment 1

The concentration of reactive Lys in all 33 DDGS samples was lower ($P < 0.05$) than the concentration of total Lys (mean = 0.85%) in DDGS samples (Table 5.2) and this was true regardless of the procedure that was used to measure reactive Lys. The reactive Lys concentration was 83.5% (0.71 % vs. 0.85%) of total Lys if calculated from the data based on the furosine procedure and only 74.1% (0.63% vs. 0.85%) if calculated from the data using the homoarginine procedure. The CV of reactive Lys was 17.34% if using the homoarginine procedure, but only 6.67% if using the furosine procedure. The concentration of SID Lys was correlated with the concentration of reactive Lys measured by the homoarginine procedure, with an $r^2 = 0.70$ ($P < 0.05$). However, the r^2 was 0.66 ($P < 0.05$) if measured by the furosine procedure. The concentration of SID Lys was

correlated with the CP in the samples with an $r^2 = 0.22$ ($P < 0.08$) and with the concentration of total Lys with an r^2 of 0.60 ($P < 0.05$; Table 5.3).

Experiment 2

Composition of Unheated Samples. Before oven-drying, the concentration of CP and AA was greater in DDG than in CDS (Table 5.4). This resulted in a lower concentration of CP and AA in $DDGS_{20}$ and in $DDGS_{40}$ compared with DDG. The concentration of reactive Lys, as percent of total Lys before oven-drying, was 80.9% in DDG, 79.0% in $DDGS_{20}$, 83.3% in $DDGS_{40}$, and only 50.0% in CDS.

Effect of CDS and Drying Temperature on the Concentration of CP and Total Lys. The concentration of total Lys was reduced ($P < 0.05$) when CDS concentration was increased (Table 5.5). Drying temperature, however, did not affect the CP in the samples.

The concentration of total Lys as percent of initial concentration of Lys (i.e., total Lys in freeze-dried samples) was reduced ($P < 0.05$) when drying temperature or CDS addition was increased. However, there was an interaction ($P < 0.05$) between the effect of CDS addition and drying temperature. At a drying temperature of 50°C or lower, the total Lys as percent of initial concentration of Lys did not differ widely among samples. However, at 100°C, the least ($P < 0.05$) reduction in reactive Lys as percent of initial concentration of Lys was observed when samples contained no CDS. Thus, 91.4% of the total Lys in DDG was still present. In contrast, the greatest ($P < 0.05$) reduction in total Lys as percent of initial concentration of Lys was observed when samples contained the greatest addition of CDS to DDGS. Thus, only 33.6% of the initial total Lys in $DDGS_{40}$ was recovered after drying at 100°C.

Effect of CDS and Drying Temperature on the Concentration of Reactive Lys. Among freeze-dried samples, the concentration of reactive Lys in DDG (0.89%) was greater ($P < 0.05$) than in $DDGS_{20}$ (0.80%) and in $DDGS_{40}$ (0.70%). The concentration of reactive Lys in CDS (0.22%) was the lowest ($P < 0.05$) among samples. The concentration of reactive Lys was reduced ($P < 0.05$) when CDS addition or drying temperature was increased.

The concentration of reactive Lys as percent of initial concentration of reactive Lys (i.e., reactive Lys in freeze-dried samples) was reduced ($P < 0.05$) when drying temperature or CDS addition was increased. However, there was an interaction ($P < 0.05$) between the effect of CDS addition and drying temperature. At a drying temperature of 50°C, the reactive Lys as percent of initial concentration of reactive Lys did not differ among samples. However, at 100°C, the least ($P < 0.05$) reduction in reactive Lys as percent of initial concentration of reactive Lys was observed when samples contained no CDS. Thus, 75.7% of the initial reactive Lys in DDG was still present after drying at 100°C. In contrast, greatest ($P < 0.05$) reduction in reactive Lys as % of initial concentration of reactive Lys was observed when samples that contained the most CDS were dried at 100°C and in $DDGS_{40}$, only 10.2% of initial reactive Lys was present after the sample has been dried at 100°C.

The concentration of reactive Lys as percent of total Lys in samples was reduced ($P < 0.05$) when drying temperature, CDS addition, or both, was increased. However, there was an interaction ($P < 0.05$) between the effect of the amount of CDS addition and drying temperature. Before oven-drying, the percent of reactive Lys in total Lys did not vary among samples. However, after drying at 100°C, only 25.2% of the total Lys in

$DDGS_{40}$ and only 43.0% of the total Lys in $DDGS_{20}$ was reactive. These values were lower ($P < 0.05$) than in DDG, where 67.1% of the total Lys was reactive after drying at 100°C.

Coefficient of Variability of AA. The CV of most AA except Lys increased with increasing addition of CDS (Table 5.6). The greatest CV of most AA was observed in CDS. The CV of reactive Lys was 70.96% in $DDGS_{40}$, but only 36.58% in CDS. Similarly, the CV of total Lys was 43.81% in $DDGS_{40}$, but only 23.08% in CDS.

DISCUSSION

Several steps in the dry grind ethanol extraction process may reduce the concentration of total Lys, reactive Lys, and SID Lys in DDGS. In the liquefaction stage, the jet-cooking process can raise the temperature of the mash to between 90 and 100°C for several minutes (Bothast and Schilcher, 2005). In the saccharification stage, the temperature of the mash is kept at about 60°C, whereas a temperature of 32°C is maintained for 48-72 h during fermentation stage (Bothast and Schilcher, 2005). In the dehydration of thin stillage to condensed distillers solubles, at least100°C of heat is used to evaporate water. Thus, even before drying, some of the reducing sugars and AA in the mash can potentially interact and initiate sugar-Lys binding. The relatively low concentration of reactive Lys in total Lys in freeze-dried samples of both DDG (80.9%) and CDS (50.0%) suggest that Lys-sugar binding can occur before DDGS is dried.

During drying of WDG, the temperature can range from 127 to 621°C (Shurson and Noll, 2005). The cause of Lys deterioration in DDGS is often attributed to addition of solubles to WDG (Cromwell et al., 1993; Martinez Amezcua and Parsons, 2007). This

is likely because WDG contains most of the total Lys, whereas CDS contains most of the low molecular weight sugars (Wu, 1994).

The reactive Lys procedure has previously been used to measure heat damage in protein sources (Carpenter and Booth, 1973; Hurrell and Carpenter, 1974). The furosine method has been used to measure heat damage in several milk and cereal products that were moderately heated (Erbersdobler and Somoza, 2007), whereas the homoarginine procedure has been used to evaluate the reactive Lys in feedstuffs that have undergone mild to severe heating (Rutherfurd and Moughan, 1997). These procedures have, however, not been previously used to measure the extent of heat damage in DDGS. The difference between the concentration of reactive Lys measured by the furosine procedure and the homoarginine procedure in DDGS is similar to differences measured in previous studies in other protein sources (Carpenter and Booth, 1973; Möller et al., 1977). In the furosine procedure, there are uncertainties about the correct value of the conversion factor used to calculate the unreactive and reactive Lys from furosine values (Campos-Gimenez et al., 2004). These factors may be affected by the type of sugars and AA in the Amadori product (Krause et al., 2003). Conversion factors may also vary depending on whether the Amadori product is in free-form or protein-bound form (Desrosiers et al., 1989). In the present study, the Amadori products in DDGS were assumed to be protein-bound and result in 40% regenerated Lys and 32% furosine upon acid hydrolysis. These values are obtained in heated milk products (Finot et al., 1981) but the conversion factors have not been measured directly in DDGS.

The furosine method is suited for proteins that have undergone moderate heating and when Amadori products are the sole source of unreactive Lys (Erbersdobler and

Somoza, 2007; Chiang, 1983). The homoarginine procedure appears to be effective in measuring reactive Lys in proteins that have undergone moderate or severe heating (Hurrell and Carpenter, 1981).

An important observation in Exp. 1 is that only 74.1 to 83.5 % of the total Lys in DDGS is reactive, whereas 16.5 to 25.9% is unreactive. The presence of variable amounts of unreactive Lys in DDGS may explain the variability in the ileal digestibility of Lys (Fastinger and Mahan, 2006; Stein et al., 2006) and in the bioavailability of Lys (Combs and Bossard, 1968, 1969; Lumpkins and Batal, 2005) observed in previous studies. The better correlation of SID Lys with reactive Lys than with total Lys or CP indicates that the concentration of reactive Lys is a better indicator of the concentration of digestible Lys in DDGS than total Lys or CP. The unreactive Lys is poorly digested in the small intestine (Finot and Magnenat, 1981; Erbersdobler and Faist, 2001) and may also reduce the absorption of reactive Lys, possibly via blockage of the absorption sites (Lee et al., 1977; Sherr et al., 1989). Maillard products may also reduce the action of pancreatic and intestinal enzymes (Hansen and Millington, 1979; Öste et al., 1986, 1987) through increased steric hindrance of the cleavage sites in the protein (Kilara and Sharkasi, 1986). Therefore, greater concentration of reactive Lys in DDGS is correlated with greater concentration of SID Lys in DDGS.

Results of Exp. 2 demonstrate that addition of CDS exacerbates the effect of drying temperature on the concentration of total and reactive Lys in DDGS because the reduction in total and reactive Lys was increased as CDS addition and drying temperature were increased. The increased formation of unreactive Lys when both CDS and WDG were dried together is most likely due to increased binding of reducing sugars in CDS

with reactive Lys in WDG. This hypothesis is supported by the less drastic reduction in the concentration of reactive Lys when the 2 samples were dried separately.

In a study involving soy protein concentrate and increased concentration of glucose, the reactive Lys loss increased by 25 fold when the samples were heated at 130°C, but only by 5 fold when samples were heated at 100°C (Wolf et al., 1977). In the present study, only 10% of the initial concentration of reactive Lys was recovered when WDG that contained 40% CDS was dried at 100°C, compared with a recovery of reactive Lys of 75.2% when WDG was dried at the same temperature but without CDS. These results indicate that heating of proteins in the presence of sugars increases the loss of reactive Lys. Similar observations were made in previous studies (Yoong et al., 1994; Evangelisti et al., 1999). The increase in CV of AA when CDS was added to the samples demonstrates that the variability in the AA concentration in DDGS is due to variation in the amount of CDS added to the WDG and to variation in drying temperature.

In conclusion, the concentration of reactive Lys in DDGS is correlated with the concentration of SID Lys in growing pigs. This procedure may, therefore, be used to assess the quality of DDGS. Increased drying temperature exacerbates the negative effect of CDS addition on the concentration of reactive Lys in DDGS.

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Table 5.1. Mean CP and AA composition, and standardized ileal digestibility (SID) of AA in 33 sources of corn distillers dried grains with solubles (DDGS), Exp. 1[1,2]

	Total AA, %			SID AA, %			SID AA concentration, %		
	Mean	SD	CV	mean	SD	CV	mean	SD	CV
CP, %	30.78	1.65	5.36	73.07	5.52	7.56	22.48	1.98	8.79
Indispensable AA, %									
Arg	1.31	0.12	8.94	81.39	5.38	6.60	1.07	0.14	13.34
His	0.82	0.07	8.86	77.80	4.47	5.75	0.63	0.06	9.79
Ile	1.14	0.08	7.24	75.45	4.71	6.24	0.86	0.08	8.89
Leu	3.54	0.29	8.09	83.81	3.84	4.58	2.97	0.29	9.91
Lys	0.85	0.10	12.05	62.08	7.24	11.67	0.53	0.09	17.16
Met	0.62	0.09	13.71	82.17	3.94	4.79	0.51	0.07	13.77
Phe	1.50	0.11	7.26	81.21	3.86	4.76	1.22	0.11	8.66
Thr	1.20	0.23	19.29	71.16	5.14	7.22	0.86	0.21	24.82
Trp	0.23	0.04	15.20	69.32	8.17	11.79	0.16	0.02	14.30

Table 5.1. (Cont.)

Val	1.52	0.11	7.09	74.80	4.80	6.42	1.14	0.09	8.20
Dispensable AA, %									
Ala	2.15	0.16	7.58	78.27	4.55	5.81	1.69	0.19	11.53
Asp	2.06	0.15	7.14	68.86	4.77	6.93	1.42	0.13	9.19
Cys	0.60	0.13	22.07	73.70	4.27	5.79	0.44	0.09	20.42
Glu	4.84	0.70	14.46	80.57	5.63	6.99	3.93	0.77	19.58
Gly	1.16	0.07	5.77	64.02	11.17	17.44	0.74	0.15	20.14
Pro	2.33	0.16	6.73	73.28	22.91	31.26	1.72	0.60	34.72
Ser	1.31	0.14	10.62	75.83	5.29	6.98	1.00	0.16	15.66

[1]Summarized from Stein et al. (2005, 2006), Pahm et al. (2006a, b), and Urriola et al. (2007).

[2]As fed basis.

Table 5.2. Reactive Lys and total Lys concentration in 33 sources of corn distillers dried grains with solubles, Exp. 1 (DM basis)

	Mean	Low	High	CV
Reactive Lys				
Homoarginine procedure %[1]	0.63[x]	0.31	0.82	17.34
Furosine procedure, %	0.71[y]	0.35	0.97	6.67
Total Lys, %	0.85[z]	0.56	1.03	12.25
SEM	0.02	ND[2]	ND	ND
P-value	< 0.01	ND	ND	ND

[x-z]Values within a column that do not contain a common superscript letter are different, $P < 0.05$.

[1]The mean conversion of Lys to homoarginine was 78.85% and the mean recovery of Lys after guanidination was 93.32 ± 6.5.

[2]ND = not determined.

Table 5.3. Correlation r^2 between standardized ileal digestible Lys concentration and CP, total Lys, or reactive Lys in 33 sources of corn distillers dried grains with solubles, Exp. 1

Measured variable	Regression equation	r^2	RMSE	*P*-value
CP	-0.1284 + 0.0217(CP)	0.22	0.082	< 0.08
Total Lys	-0.051 + 0.682 (total Lys)	0.60	0.059	< 0.01
Reactive Lys procedure				
Homoarginine	0.093 + 0.694 (Reactive Lys)	0.70	0.051	< 0.01
Furosine	0.023 + 0.637 (Reactive Lys)	0.66	0.054	< 0.01

Table 5.4. Initial CP and AA concentration in samples before oven-drying, Exp. 2 (DM basis)

Item	DDG[1]	$DDGS_{20}$[1]	$DDGS_{40}$[1]	CDS[1]
CP, %	35.93	33.33	28.00	14.61
Indispensable AA, %				
Arg	1.67	1.52	1.39	0.64
Ile	1.38	1.21	1.05	0.31
Leu	4.48	3.86	3.27	0.69
Phe	1.89	1.65	1.42	0.34
Thr	1.20	1.05	0.96	0.36
Val	1.97	1.72	1.52	0.50
Total Lys	1.10	1.00	0.84	0.42
Reactive Lys[2]	0.89	0.79	0.70	0.21
Reactive Lys as % total Lys	80.90	79.00	83.30	50.00
Dispensable AA, %				
Ala	2.70	2.42	2.17	0.80
Asp	2.65	2.38	2.25	0.90
Glu	6.69	5.89	5.30	2.05
Gly	1.34	1.22	1.15	0.58
Tyr	1.49	1.30	1.14	0.30
Ser	1.97	1.72	1.54	0.51
Pro	2.98	2.58	2.27	0.61

[1]Dried samples with the following composition: DDG = distillers dried grains; CDS = condensed distillers solubles; $DDGS_{20}$= wet distillers grains with 20% CDS; $DDGS_{40}$= wet distillers grains with 40% CDS. The DM was obtained by freeze-drying samples.

[2]Reactive Lys measured by the homoarginine procedure.

Table 5.5. Effect of drying and addition of condensed distillers solubles (CDS) on CP, total Lys, and reactive Lys in samples, Exp. 2 (DM basis)[1]

Item	CP, %	Total Lys		Reactive Lys		
		%	% of initial	%	% of initial	% of total Lys
Freeze-drying						
DDG	34.6^{v}	1.1^{l}	100.0^{t}	0.89^{s}	100.0^{s}	80.9^{v}
$DDGS_{20}$	32.2^{u}	1.0^{op}	100.0^{t}	0.80^{wr}	100.0^{s}	79.2^{v}
$DDGS_{40}$	28.0^{z}	0.84st	100.0^{t}	0.70^{v}	100.0^{s}	83.3^{v}
CDS	14.6^{y}	0.42^{u}	100.0^{t}	0.22^{y}	100.0^{s}	51.9^{z}
50°C						
DDG	34.5^{v}	1.11^{l}	101.3^{to}	0.84^{rs}	94.7^{rs}	75.7^{u}
$DDGS_{20}$	32.3^{u}	1.03^{q}	103.0^{o}	0.76^{w}	95.7^{rs}	73.9^{u}
$DDGS_{40}$	28.3^{z}	0.84^{t}	100.5^{t}	0.63^{u}	89.8^{wr}	74.6^{u}
CDS	13.5^{x}	0.36^{z}	86.1^{w}	0.20^{y}	92.7^{r}	54.3^{z}
75°C						
DDG	35.2^{v}	1.05^{q}	95.6^{s}	0.81^{wr}	91.1^{r}	77.2^{v}
$DDGS_{20}$	32.1^{u}	0.78^{r}	77.8^{u}	0.45^{z}	56.5^{u}	57.8^{z}
$DDGS_{40}$	28.6^{z}	0.53^{w}	63.2^{z}	0.21^{y}	29.9^{z}	39.5^{y}
CDS	12.9^{x}	0.34^{z}	81.1^{v}	0.18^{y}	83.4^{w}	52.7^{z}
100°C						
DDG	35.2^{v}	1.01^{p}	91.4^{r}	0.67^{uv}	75.7^{v}	67.1^{u}
$DDGS_{20}$	32.2^{u}	0.51^{v}	51.0^{y}	0.22^{y}	27.6^{y}	43.0^{y}

Table 5.5. (Cont.)

$DDGS_{40}$	28.4^{z}	0.28^{y}	33.6^{x}	0.08^{x}	10.2^{x}	25.2^{x}
CDS	12.9^{x}	0.22^{x}	52.4^{y}	0.07^{x}	35.3^{z}	34.3^{y}
SEM	0.34	0.01	2.39	0.18	0.72	2.96
P-value						
Drying	< 0.50	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
CDS	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Drying x CDS	< 0.06	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[o-z.]Values within a column that do not contain a common superscript letter are different, $P < 0.05$.

[1]Dried samples with the following composition: DDG = distillers dried grains; CDS = condensed distillers solubles; $DDGS_{20}$= wet distillers grains with 20% CDS; $DDGS_{40}$ = wet distillers grains with 40% CDS.

Table 5.6. Coefficient of variation of CP and AA concentrations in samples that were freeze-dried or oven-dried at 50, 75, or 100°C, Exp. 2[1]

Item	DDG[2]	$DDGS_{20}$[2]	$DDGS_{40}$[2]	CDS[2]
CP, %	1.21	1.79	1.84	6.03
Indispensable AA, %				
Arg	2.14	3.77	8.74	9.68
Ile	2.26	2.76	5.36	11.12
Leu	1.23	2.32	4.70	11.06
Phe	1.49	2.62	5.65	10.53
Thr	1.49	2.15	5.75	10.49
Val	2.90	3.11	6.46	10.91
Reactive Lys[3]	11.20	49.15	76.96	36.58
Total Lys	2.23	28.29	43.81	23.08
Dispensable AA, %				
Ala	1.39	2.45	4.66	10.81
Asp	4.46	5.67	5.71	8.78
Glu	1.19	2.59	3.81	9.34
Gly	1.25	2.05	4.87	9.53
Pro	1.91	3.29	5.85	11.35
Ser	1.39	2.21	5.32	11.26
Tyr	1.91	13.70	6.24	10.20

[1]Samples were not analyzed for Met, Trp, and His.

[2]Dried samples with the following composition: DDG = distillers dried grains; CDS = condensed distillers solubles; $DDGS_{20}$= wet distillers grains with 20% CDS; $DDGS_{40}$ = wet distillers grains with 40% CDS.

[3]Reactive Lys measured by homoarginine procedure.

CHAPTER 6

Standardized ileal digestibility of reactive lysine in distillers dried grains with solubles fed to growing pigs

ABSTRACT

Distillers dried grains with solubles (DDGS) are produced by drying a mixture of wet distillers grains and condensed solubles. During this process, some of the ε-NH_2 groups in Lys may be bound to reducing sugars through the Maillard reaction. This Lys is called unreactive Lys, whereas Lys that is not bound to reducing sugars is called reactive Lys. It has been suggested that the conventional procedure to measure standardized ileal digestibility (SID) of Lys in DDGS may over-estimate the amount of digestible Lys in DDGS because this procedure does not distinguish between reactive and unreactive Lys, although only the reactive Lys is bioavailable to animals. By measuring the SID of only the reactive Lys, it is expected that the estimation of digestible Lys will be more accurate. The objective of this experiment, therefore, was to test the hypothesis that the SID of reactive Lys is lower than the SID calculated using the conventional procedure. Pigs fitted with ileal cannula were fed diets containing each of 12 sources of DDGS and the SID for Lys was measured using standard procedures. Diets and ileal digesta samples were also guanidinated with O-Methylisourea and analyzed for the concentration of homoarginine. It was assumed that only the reactive Lys would be transformed to homoarginine, whereas the unreactive Lys would not. This procedure, therefore, allows for a separation of reactive and unreactive Lys, and the SID of reactive Lys could be calculated. Results showed that Lys in DDGS is only 76% reactive on average. The

mean SID of reactive Lys was 66.9%, which is close to the mean SID of total Lys (66.5%). However, the concentration of SID reactive Lys (3.9 g/kg) was lower ($P < 0.05$) than the concentration of SID total Lys (5.1 g/kg). Thus, 24% of the digestible Lys that was calculated using the conventional procedure was unreactive Lys. The implication of this is that the conventional procedure overestimates the concentration of digestible Lys in DDGS, and measurement of reactive Lys may more accurately estimate how much Lys is available to the pig.

Keywords: amino acids, digestible reactive Lys, distillers dried grains with solubles, pigs

INTRODUCTION

Distillers dried grains with solubles (**DDGS**) is a co-product from the dry-grind ethanol industry that is increasingly being used as a feed ingredient for pigs. During processing of DDGS, the heat-sensitive AA such as Lys that contain free ε-NH_2 groups can react with reducing sugars via the Maillard reaction (Maillard, 1912), binding the Lys in the form of early Maillard or Amadori products. The Lys in Amadori products, called unreactive Lys, is unavailable to the animal (Erbersdobler and Faist, 2001). In contrast, the Lys with a free ε-NH_2 group, called reactive Lys, is considered bioavailable (Hurrell and Carpenter, 1981). To optimally use DDGS and other by-products with low AA digestibility, AA in feedstuffs are better expressed on an ileal digestible AA basis because it gives a close estimate of the amount of bioavailable AA, particularly for unheated proteins (Moughan and Smith, 1985; Batterham et al., 1990). For heated proteins, however, conventional ileal AA digestibility appears to over-estimate the

amount of digestible Lys because of the presence of unreactive Lys that may be partly digested (Finot and Magnenat, 1981). By measuring the ileal digestibility of only the Lys that is reactive (ileal digestible reactive Lys) rather than the ileal digestibility of total Lys (i.e., the sum of reactive an unreactive Lys), the amount of ileal digestible Lys in heated feedstuffs may be more closely estimated (Rutherfurd and Moughan, 1990). Only 73% of the total Lys in 33 samples of DDGS is reactive (Pahm et al., unpublished). This indicates that considerable heat damage may have taken place during the production of DDGS. It is expected, therefore, that a proportion of Lys that is measured as digestible Lys in conventional AA digestibility calculations is actually unavailable to the pig. Therefore, the objective of this study was to test the hypothesis that the amount of standardized ileal digestible (**SID**) reactive Lys is lower than the amount of SID total Lys in DDGS fed to pigs.

MATERIALS AND METHODS

Samples

The sources of DDGS, experimental diets, and ileal digesta were obtained from previous studies that were conducted to measure the ileal AA digestibility of DDGS using cannulated growing pigs (Pahm et al., unpublished data). Two experiments (Exp. 1 and 2) were previously conducted: in Exp. 1, the ileal AA digestibility was measured in 5 sources of DDGS and in Exp. 2, the ileal AA digestibility was measured in 7 sources of DDGS. Thus, 12 DDGS sources, 12 diets containing each of the DDGS source, and the ileal digesta of pigs fed with each 12 diets were used. The DDGS sources included 1 source of DDGS from a beverage plant, and 11 sources from dry grind ethanol plants.

Two N-free diets from the previous experiments and the digesta from pigs fed N-free diets were also included in the study. All diets contained Cr as an indigestible marker.

Diets

There were 8 pigs per diet for DDGS sources 1 through 5, and 7 pigs per diet for DDGS sources 6 through 12. All diets from the previous experiments, except the N-free diets, were formulated to contain DDGS as the only source of CP, reactive Lys, and other AA. All diets contained cornstarch, sugar, vitamins, and minerals to meet or exceed NRC (1998) requirements for pigs, whereas the N-free diet contained cornstarch, sugar, vitamins, minerals, and synthetic fiber.

Reactive Lys Analysis in Samples

Diets and ileal digesta were analyzed for reactive Lys concentration using the homoarginine procedure. In this procedure, the amount of reactive Lys is measured by chemically transforming the Lys with free ε-NH_2 group into homoarginine through a guanidination reaction with O-methylisourea (Kimmel, 1967). The unreactive Lys appears as Lys in the chromatogram, whereas the reactive Lys appears as homoarginine, allowing the separate measurement of the 2 AA. In a conventional AA analysis, however, both reactive Lys and unreactive Lys appear as a single Lys peak in the chromatogram, thereby not allowing a distinction between reactive Lys and unreactive Lys.

Guanidination conditions for DDGS and ileal digesta of pigs fed DDGS were based on a previous study (Pahm et al. unpublished) where 0.6 *M* O-methylisourea at a pH of 11.4 was used to guanidinate the DDGS and ileal digesta samples for 3d at 20^{o}C. Six mL of the O-methylisourea reagent was added to 0.2 g of each sample in a 25-mL flask and stirred for 12 h using a magnetic stirrer (MultiMagnestir 1278, Lab-line

Instruments, Melrose Park, IL). Samples were then incubated for 60 h at 20°C, air-dried and then analyzed for homoarginine after acid-hydrolysis with 30 mL of 6 *N* HCl followed by refluxing for 24 h at 110°C (procedure 4.1.11; AOAC, 2000). The concentration of homoarginine and other AA was measured using an HPLC system (Pickerings Laboratories, Mt. View, CA.) The reactive Lys was calculated based on the amount of homoarginine in the samples.

Digestibility Calculations, Data Collection

The apparent ileal digestibility (**AID**) and SID of reactive Lys were calculated as previously described (Stein et al., 2007). Because DDGS was the only source of reactive Lys in the diet, the calculated AID and SID of reactive Lys in the diet represent the AID and SID of reactive Lys in DDGS. The concentration of digestible reactive Lys (g/kg DDGS) was subsequently calculated by multiplying the AID or SID value by the amount of reactive Lys in the sample (Stein et al., 2007).

The AID and SID of other AA in the guanidinated samples were also measured to evaluate the effect of guanidination on the ileal AA digestibility of AA other than Lys. The recovery of total Lys (i.e., the sum of reactive and unreactive Lys) in DDGS, diets and ileal digesta of pigs for the guanidinated samples were also calculated and compared with the concentration of total Lys.

Statistical Analysis

Least square means of AID and SID of reactive Lys and other AA for each DDGS source were calculated using the MIXED procedure of SAS (SAS Inst. Inc. Cary, NC) with DDGS source as the fixed effect and pig and period as random effects. The AID and SID of reactive Lys and other AA were compared with the previously calculated AID

and SID of total Lys and other AA using a T-test in SAS. In all analyses, a probability of $P < 0.05$ was considered significant.

RESULTS

The concentration of total Lys in the 12 DDGS samples (Table 6.1) ranged from 5.6 to 8.8 g/kg (mean: 7.6 g/kg), whereas the concentration of reactive Lys ranged from 3.1 to 7.0 g/kg (mean: 5.8 g/kg). The CV of reactive Lys (20.0%) is greater than the CV of total Lys (11.7%).

Most sources of DDGS had a similar AID for reactive Lys and for total Lys (Table 6.2). The DDGS sources 11 and 12, however, had a greater ($P < 0.05$) AID for reactive Lys than for total Lys, whereas DDGS sources 2, 3, and 4 had a lower ($P < 0.05$) AID for reactive Lys than for total Lys. The AID for reactive Lys in DDGS ranged from 37.4 to 71. 6% (mean: 60.1%), whereas the AID for total Lys ranged from 41.5 to 69.9% (mean: 60.9%). All DDGS sources had a lower ($P < 0.05$) concentration of AID reactive Lys (g/kg DDGS) than the concentration of AID total Lys. The concentration of AID reactive Lys ranged from 1.2 to 4.2 g/kg (mean: 3.5 g/kg), whereas the concentration of AID total Lys ranged from 2.3 to 5.7 g/kg (mean: 4.7 g/kg).

Most of the DDGS sources had a similar SID for reactive Lys and total Lys (Table 6.3), but DDGS sources 1 and 11 had a greater ($P < 0.05$) SID for reactive Lys than for total Lys. In contrast, source 2 had a lower ($P < 0.05$) SID for reactive Lys than for total Lys. The SID for reactive Lys in DDGS ranged from 48.8 to 77.5% (mean: 66.9%), whereas the SID for total Lys ranged from 48.6 to 74.4% (mean: 66.5%). In all sources of DDGS, the concentration of SID reactive Lys (g/kg) was lower ($P < 0.05$) than

the concentration of SID total Lys. The concentration of SID reactive Lys in DDGS ranged from 1.5 to 4.5 g/kg (mean: 3.9 g/kg), whereas the concentration of SID total Lys ranged from 2.7 to 6.1 g/kg (mean: 5.1 g/kg).

Guanidination of DDGS did not change the total concentration of most AA except for Arg, Thr, Asp, and Ser that had a lower ($P < 0.05$) concentration in guanidinated DDGS compared with non-guanidinated DDGS (Table 6.4). Guanidination of diets and ileal digesta of pigs did not change the AID values of any AA. Similarly, guanidination of diets and ileal digesta did not change the SID values of AA except for Ile, Asp, Gly, and Ser, where greater ($P < 0.05$) SID values were calculated for the guanidinated DDGS compared with non-guanidinated DDGS.

The mean recovery of Lys (i.e. sum of reactive and unreactive Lys) in guanidinated samples relative to unguanidinated samples were 98.9 ± 8% for DDGS, 94.0 ± 3.8% for diets with DDGS, and 93.7 ± 3.7% for ileal digesta of pigs fed diets with DDGS.

DISCUSSION

The reduction in nutritive value in feedstuffs after heat processing can be due to the binding of Lys with other components such as reducing sugars (Adrian, 1974) and AA (Bjarnason and Carpenter, 1970) leading to a reduced Lys utilization by animals (Carpenter and Booth, 1973). The extent of Lys binding in heated proteins can be measured through the determination of reactive Lys (Hurrell and Carpenter, 1981; Friedman, 1982). Reactive Lys analysis of DDGS shows that only 76% of the total Lys in DDGS is reactive Lys. This suggests that part of the Lys in DDGS may be unreactive. The bound, unreactive Lys may have been produced during the drying procedure in the

ethanol plant. The formation of unreactive Lys in DDGS has been demonstrated to be affected by the amount of solubles added to the wet distillers grains and by drying temperature (Pahm et al., unpublished data).

Traditionally, the amount of analyzed reactive Lys in proteins is considered to represent the amount of bioavailable Lys (e.g., Carpenter, 1960) and therefore, is expected to be fully absorbable in the small intestine. However, this study shows that this may not be true for DDGS. Of the 76% of the total Lys that is reactive in DDGS, only a portion is digested, with a mean SID reactive Lys of 67.0%. This result is in agreement with previous studies showing that not all reactive Lys in heated proteins is absorbed (Desrosiers et al., 1989; Moughan et al., 1996). Therefore, the measurement of reactive Lys in DDGS may not always accurately predict the quantity of Lys that is absorbed by the animal.

The inability of some of the reactive Lys in DDGS to be digested is probably related to several factors, such as the high amount of NDF in DDGS, which may increase the endogenous reactive Lys loss. It was reported that fiber can increase the endogenous N loss through increased mucosal cell sloughing and mucus production (Schneeman et al., 1982) leading to increased specific endogenous Lys losses (Schulze et al., 1994). The increase in specific endogenous Lys loss will increase the total endogenous reactive Lys loss because the Lys in both basal and specific endogenous AA is reactive. It is also possible that some of the reactive Lys in DDGS is associated within the structural fiber and is less accessible to digestive enzymes. The DDGS used in this study contained high amounts of NDF (mean of 30%, data not shown), which may have reduced the enzymatic digestion of reactive Lys. This hypothesis is supported by the fact that addition of

purified NDF leads to a decreased N digestibility due to increased ileal losses of both endogenous and exogenous protein (Schulze et al., 1994).

Another reason for the relatively low digestibility of reactive Lys in DDGS is the extent of heat application during drying of DDGS. The CV of 20% for SID of reactive Lys indicates that some of the samples have undergone severe heating, which may have formed early and advanced Maillard products. Maillard products can affect the digestibility of reactive Lys by competitively inhibiting the absorption of Lys (Sherr et al., 1989), or block the cleavage of protein-bound Lys by inhibition of carboxypeptidases (Hansen and Millington, 1979).

During severe heating, protein aggregation and cross-linking may occur in heated feedstuffs, which may limit the digestion of the reactive Lys through increased steric hindrance at the site of cleavage along the protein chain, thereby trapping the reactive Lys in indigestible peptides (Boctor and Harper, 1967; Valle-Riestra and Barnes, 1970; Kilara and Sharkasi, 1986; Desrosiers et al., 1989).

The similarity in the SID of reactive Lys (mean: 66.9%) to SID of total Lys (mean: 66.5%) was attributed to the considerable amount (67%, data not shown) of unreactive Lys in diet with DDGS that was not recovered in the terminal ileum and was assumed to have been digested by pigs. In this study, the SID for total Lys is closer to the SID for reactive Lys as the digestibility of unreactive Lys increases. However, as the digestibility of unreactive Lys decreases, the AID and SID of reactive Lys tend to be greater than the AID and SID of total Lys.

The result herein is different from some previous studies, where the SID of reactive Lys is greater than the SID of total Lys. This has been shown in a number of

feedstuffs including milk products, field peas, and cottonseed meal, but not in blood meal, meat and bone meal, and soybean meal (Rutherfurd and Moughan, 1997; Rutherfurd et al., 1997).

The result of this study is similar to the data obtained by Hurrell et al. (1976), who reported that unreactive Lys had a similar ileal digestibility as the reactive Lys. It was also reported that as much as 70% of free-form early Maillard products were absorbed by rats but then eliminated through the urine (Finot and Magnenat, 1981; Finot and Furniss, 1988). However, for protein-bound early Maillard products, only about 12% may be absorbed (Finot and Magnenat, 1981). Fermentation of wheat flour with baker's yeast has been shown to increase the concentration of free AA by as much as 93% (Erbas et al., 2005). It is possible that during starch fermentation of the grain to extract ethanol, free AA and reducing sugars have been formed, subsequently producing free-form early Maillard compounds during drying. Although early Maillard products are present in variable quantity in DDGS, it is not known if it is in free-form or protein-bound form (Pahm et al., unpublished data). It is also possible that during drying of DDGS, Lys-AA cross-linking have formed. It was reported that during heating, Lys can cross-link with other AA forming unreactive Lys cross-linked peptides that can be absorbed as much as the reactive Lys in the small intestine (Hurrell et al., 1976).

Only 76.3% of the concentration of SID for total Lys was reactive (3.9 g/kg SID reactive Lys vs. 5.1 g/kg SID total Lys). This observation is in agreement with data from previous studies where the amount of true ileal digestible Lys in heated proteins, including skim milk and field peas, over-estimates the true ileal digestible reactive Lys (Rutherfurd and Moughan, 1997; Rutherfurd et al., 1997). The over-estimation of SID

reactive Lys was mainly due to the lower concentration of reactive Lys in DDGS because the values of SID total Lys and SID reactive Lys were similar (66.5% vs. 66.9%). This over-estimation of digestible total Lys in DDGS does not occur if the unreactive Lys is totally indigestible, because the resulting SID for total Lys will be lower.

The lower concentration of SID reactive Lys (in g/kg DDGS) compared with the concentration of SID total Lys obtained in this study implies that the concentration of SID total Lys in DDGS may be greater than the Lys that is actually available for utilization by pigs. This would imply that concentration of bioavailable Lys will be over-estimated by the conventional SID procedure for Lys. Traditionally, it is assumed that all AA that are absorbed by the animal are bioavailable. However, the absorption of unreactive Lys by pigs fed DDGS may lead to reduced efficiency of utilization of digested total Lys because the absorbed unreactive Lys is not bioavailable (Erbersdobober, 1977; Erbersdobler et al., 1981). For heat processed feedstuffs, it has been reported that the availability may be lower than the digestibility of AA (Batterham, 1992, 1993).

The use of ileal AA digestibility is a well accepted method of evaluation of AA digestibility in many feedstuffs. However, for heated feedstuffs, formulating on a digestible total AA basis does not appear to maintain the performance of animals compared to unheated feedstuffs (van Barneveld et al., 1994). This implies that when using DDGS as a substitute for a high quality protein source such as soybean meal, caution should be used because the efficiency of utilization of SID Lys (i.e., bioavailability) is expected to be lower due to the absorption of unreactive Lys from

DDGS. Formulating on a SID reactive Lys can potentially minimize for this over-estimation because of the correction for presence of digestible unreactive Lys

The homoarginine procedure was chosen for this study because of its ability to detect unreactive Lys in both early and advanced Maillard phases (Carpenter and Booth, 1973). There are, however, drawbacks to this procedure. It was reported that guanidination may not always lead to a full recovery of total Lys (Hurrell and Carpenter, 1981). In this study, a high recovery of total Lys (i.e., sum of reactive and unreactive Lys) was obtained in DDGS, in diets containing DDGS, and in ileal digesta from pigs fed DDGS-based diets. The AID and SID of AA other than Lys were also not affected by guanidination, suggesting that the guanidination of diets and digesta samples only affected the Lys. The guanidination step is time-consuming because it takes 3 d to complete the procedure before HPLC analysis can be conducted. The determination of SID reactive Lys is tedious because both diets and digesta samples need to be guanidinated. However, the similarity in the AID and SID of total Lys compared with the AID and SID of reactive Lys would imply that in the measurement of digestible reactive Lys in DDGS, it may be necessary to guanidinate the DDGS, but not the ileal digesta if the SID for total Lys has already been measured. The SID for reactive Lys can be estimated by simply multiplying the coefficients of AID and SID of total Lys in DDGS with the concentration of reactive Lys in DDGS.

In conclusion, the quantity of SID total Lys in DDGS appears to be over-estimated because of the presence of unreactive Lys in DDGS that may be partly absorbed by pigs. Formulating on a digestible reactive Lys basis may be considered to correct the over-estimation of Lys digestibility.

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Table 6.1. Concentration (g/kg) of total Lys and reactive Lys in 12 sources of distillers dried grains with solubles, DM basis

DDGS source	Total Lys	Reactive Lys
1	8.6	6.3
2	8.1	6.8
3	8.1	6.7
4	7.6	5.5
5	8.3	7.0
6	8.8	6.5
7	6.6	4.1
8	5.6	3.1
9	8.0	6.7
10	7.8	6.0
11	7.7	5.9
12	6.6	4.7
mean	7.6	5.8
SD	0.9	1.2
CV	11.7	20.0

Table 6.2. Comparison of apparent ileal digestibility (AID) of total Lys and AID of reactive Lys in 12 sources of distillers dried grains with solubles, DM basis[1]

DDGS source	AID Lys , %				AID Lys concentration, g/kg			
	Total	Reactive	SE	*P*- value	Total	Reactive	SE	*P*-value
1	61.8	63.4	0.87	0.10	5.3	4.0	0.10	< 0.01
2	61.2	54.3	0.90	<0.01	5.0	3.7	0.07	< 0.01
3	64.9	61.2	0.80	0.01	5.3	4.1	0.04	< 0.01
4	67.4	65.8	0.47	0.01	5.1	3.6	0.04	< 0.01
5	60.3	58.7	0.82	0.11	5.0	4.1	0.06	< 0.01
6	64.7	64.7	0.97	1.00	5.7	4.2	0.07	< 0.01
7	50.9	49.8	1.90	0.60	3.4	2.1	0.10	< 0.01
8	41.5	37.4	2.18	0.10	2.3	1.2	0.10	< 0.01
9	69.6	70.0	0.88	0.60	5.6	4.7	0.06	< 0.01
10	58.8	59.5	0.87	0.50	4.6	3.6	0.04	< 0.01
11	69.9	71.6	0.11	<0.01	5.3	4.3	0.02	< 0.01
12	60.2	65.1	1.70	0.02	4.0	3.0	0.09	< 0.01
Mean	60.9	60.1	-	-	4.7	3.5	-	-

[1]Each value is least square mean of 8 observations for each DDGS in source 1 through 5 and 7 observations for each DDGS in source 6 through 12.

Table 6. 3. Comparison of standardized ileal digestibility (SID) of total Lys and SID of reactive Lys in 12 sources of distillers dried grains with solubles, DM basis[1]

DDGS source	SID Lys, %				SID Lys concentration, g/kg			
	Total	Reactive	SE	*P*-value	Total	Reactive	SE	*P*-value
1	66.8	70.1	0.87	0.008	5.8	4.4	0.09	< 0.01
2	66.8	61.3	0.90	0.002	5.4	4.2	0.05	< 0.01
3	70.3	68.2	0.80	0.072	5.7	4.6	0.03	< 0.01
4	72.8	72.5	0.47	0.538	5.5	4.0	0.04	< 0.01
5	65.8	65.2	0.82	0.518	5.5	4.6	0.06	< 0.01
6	69.1	69.6	0.93	0.599	6.1	4.5	0.07	< 0.01
7	56.6	57.8	1.91	0.534	3.8	2.4	0.10	< 0.01
8	48.6	48.0	2.18	0.807	2.7	1.5	0.10	< 0.01
9	74.1	75.8	0.95	0.118	6.0	5.0	0.07	< 0.01
10	63.5	65.1	0.89	0.112	5.0	4.0	0.05	< 0.01
11	74.4	77.5	0.10	<0.01	5.7	4.6	0.03	< 0.01
12	68.8	71.8	1.71	0.123	4.6	3.3	0.08	< 0.01
Mean	66.5	66.9	-	-	5.1	3.9	-	-

[1]Each value is a mean of 8 observations for each DDGS in sources 1 through 5 and 7 observations for each DDGS in sources 6 through 12.

Table 6.4. Amino acid concentration, apparent (AID) and standardized (SID) ileal digestibility of AA in 12 sources of distillers dried grains with solubles, DM basis[1]

AA	Total AA, %		SE	*P*-value	AID, %		SE	*P*- value	SID, %		SE	*P*- value
	Guani-dinated	Non-guani-dinated			Guani-dinated	Non-guani-dinated			Guani-dinated	Non-guani-dinated		
Arg	1.4	1.3	0.02	0.010	74.1	75.4	0.04	0.551	83.6	84.3	0.68	0.299
Ile	1.1	1.1	0.01	1.000	73.8	74.6	0.19	0.556	81.1	78.5	0.42	< 0.01
Leu	3.2	3.4	0.14	0.265	81.9	82.5	0.69	1.580	84.0	84.6	1.44	0.677
Phe	1.4	1.4	0.04	0.731	80.0	78.7	0.34	1.280	82.9	83.1	1.09	0.866
Thr	1.0	1.1	0.02	<0.01	65.9	65.1	0.20	0.579	73.9	73.2	0.43	0.158
Val	1.5	1.5	0.05	0.207	73.9	73.3	0.27	0.472	77.9	77.8	0.40	0.783
Ala	2.1	2.1	0.10	0.625	77.0	75.8	0.06	0.614	81.5	80.5	0.58	0.101
Asp	2.1	1.9	0.06	0.027	64.6	64.5	0.82	0.822	73.0	71.1	0.64	0.012
Glu	5.0	5.0	0.29	0.910	78.9	80.0	0.59	1.894	82.1	83.0	1.68	0.571
Gly	1.1	1.2	0.04	0.290	49.4	44.0	<0.01	0.753	74.6	72.4	0.93	0.034
Ser	1.5	1.4	0.04	0.003	71.8	71.4	0.61	0.736	79.9	78.9	0.40	0.027

[1] Each value is the mean of 12 observations.

Table 6.5. Recovery of total Lys in guanidinated and unguanidinated samples of distillers dried grains with solubles (DDGS), diets with DDGS, and ileal digesta of pigs fed with diets with DDGS, DM basis[1,2]

	Total Lys in DDGS, g/kg			Total Lys in diet, g/kg			Total Lys in digesta, g/kg		
DDGS Source	Guanidi-nated	Non-guanidi-nated	Recovery %	Guanidi-nated	Non-guanidi-nated	Recovery %	Guanidi-nated	Non-guanidi-nated	Recovery %
1	8.3	8.6	96.5	5.7	5.9	96.6	4.2	4.9	85.7
2	8.3	8.1	102.5	5.1	5.3	96.2	4.3	4.4	97.7
3	8.4	8.1	103.7	5.3	5.6	94.6	4.3	4.7	91.5
4	7.1	7.6	93.4	5.2	5.6	92.9	4.0	4.2	95.2
5	8.6	8.3	103.6	5.3	5.5	96.4	4.5	4.8	93.8
6	8.0	8.8	90.9	5.8	6.1	95.1	4.3	4.4	97.7
7	5.8	6.6	87.9	4.1	4.8	85.4	5.3	5.8	91.4
8	4.9	5.6	87.5	3.5	3.9	89.7	4.6	5.0	92.0
9	8.5	8.0	106.3	5.4	5.9	91.5	4.7	4.8	97.9

Table 6.5. (Cont.)

10	7.8	7.8	100.0	5.6	5.8	96.6	5.3	5.5	96.4
11	8.0	7.0	114.3	5.6	6.0	93.3	5.0	5.3	94.3
12	6.5	6.5	100.0	5.0	5.0	100.0	4.7	5.2	90.4
Mean	7.5	7.6	98.9	5.1	5.5	94.0	4.6	4.9	93.7
SD	1.2	0.9	8.0	0.7	0.6	3.8	0.4	0.5	3.7

[1]Each value is a mean of 8 observations for each DDGS in sources 1 through 5 and 7 observations for each DDGS in sources 6 through 12.

[2]Total AA in guanidinated samples = sum of reactive and unreactive Lys.

CHAPTER 7

Standardized amino acid digestibility in cecectomized roosters and lysine bioavailability in chicks fed distillers dried grains with solubles

ABSTRACT

This study was conducted to compare the concentration of standardized digestible (SDD) Lys and relative bioavailable Lys in 7 sources of corn DDGS. A second objective of the study was to evaluate the use of 2 in vitro methods, reactive Lys and color score, to predict the concentration of SDD Lys and bioavailable Lys in DDGS. Seven sources of DDGS were fed to cecectomized roosters by crop intubation and digestibility of AA was measured using the total excreta collection method. To measure the relative bioavailable Lys in DDGS, a standard curve (r^2=0.96) was initially constructed from the weight gain of 9-d old chicks fed a Lys-deficient basal diet or diets containing increasing concentration of L-Lys HCl. Seven additional diets were formulated by adding each of the 7 sources of DDGS to the basal diet, and total weight gain of chicks fed each diet was measured. The total weight gain of chicks fed each DDGS-containing diet was then compared with the standard curve to calculate the relative bioavailability of Lys in each source of DDGS. The 7 DDGS sources were analyzed for reactive Lys using the guanidination procedure, and they were also measured for Hunterlab color score for degree of lightness, redness, and yellowness. Results showed that the mean concentration of SDD Lys was 61.4%. The SDD for Lys among the 7 DDGS sources varied ($P < 0.05$). However, differences in the concentration of relative bioavailable Lys among 7 DDGS sources was not observed. Difference between the concentration of SDD

Lys and concentration of bioavailable Lys was not observed in 5 of 7 sources of DDGS. The concentration of SDD Lys was correlated ($r^2 = 0.84$, $P < 0.05$) with the concentration of reactive Lys in DDGS. Lighter color or greater Hunterlab L scores was associated with a greater ($r^2=0.90$, $P < 0.05$) concentration of bioavailable Lys in DDGS. In conclusion, the concentration of SDD Lys in DDGS is closely related with the concentration of bioavailable Lys. Values for reactive Lys may be used to estimate the concentration of SDD Lys, whereas Hunterlab L may be used to estimate the concentration of bioavailable Lys in DDGS.

Keywords: amino acids, availability, chicks, distillers dried grains with solubles, standardized digestibility, roosters

INTRODUCTION

Distillers dried grains with solubles **(DDGS)** is a co-product from the dry milling of grains and is the remaining component of the grain kernel after the starch has been fermented by yeast to produce ethanol. Heat processing is needed to reduce the moisture concentration of wet distillers grains, but it may reduce the utilization of heat sensitive AA such as Lys (Cromwell et al., 1993). Lysine and reducing sugars in DDGS can interact leading to the initiation of the Maillard reaction (Stein et al., 2006). When Lys is complexed with reducing sugars, it becomes unreactive Lys (Hurrell and Carpenter, 1981; Friedman, 1982). Because unreactive Lys is biologically unavailable but may be partly absorbed in the intestine (Finot and Magnenat, 1981), it is hypothesized that the

conventional digestibility measurement may over-estimate the amount of bioavailable Lys in DDGS.

While measurement of digestibility and relative bioavailability values of AA are established procedures of assessing AA quality in feed ingredients, these procedures are relatively expensive and tedious. Among the in vitro methods that may be used to estimate bioavailable Lys is the reactive Lys procedure that measures the amount of free ε-NH_2 groups of Lys in heated proteins (Hurrell and Carpenter, 1981). There is, however, no information on the correlation between the measured quantity of reactive Lys in DDGS and the quantity of standardized digestible (**SDD**) Lys and the amount of relative bioavailable Lys in DDGS fed to poultry.

It may also be possible to evaluate the quality of DDGS based on the color because darker DDGS results in lower ADG of broiler chicks (Cromwell et al., 1993). However, there is no information about the correlation between bioavailable Lys and color in DDGS.

The objective of this study, therefore, was to compare the concentration of relative bioavailable Lys and SDD Lys in DDGS fed to poultry. The second objective was to determine if the concentration of reactive Lys or the color of DDGS can be used to predict the concentration of SDD Lys and bioavailable Lys.

MATERIALS AND METHODS

Samples of DDGS

Seven sources of DDGS from dry grind ethanol plants in MN, MI, MO, IL, and SD were used in the experiment (Table 7.1). The SDD of AA in each source of DDGS

was measured using cecectomized roosters, whereas the relative bioavailability of Lys was measured by chick growth assay. The DDGS sources were analyzed for reactive Lys and the degree of lightness, yellowness, and redness was measured using the Hunterlab colorimeter. All studies were conducted at the University of Illinois in Urbana-Champaign. All experimental protocols involving use of animals were approved by the Institutional Animal Care and Use Committee at the University of IL.

Animals, Housing, Experimental Design

Ileal AA Digestibility Study. Cecectomized Single Comb White Leghorn roosters (45 wk old) were used in the experiment. Roosters were cecectomized using the procedure by Parsons (1985). The roosters were housed in an environmentally controlled room equipped with 22.5 x 36 cm individual cages with raised wire floors and a 16-h light and 8-h dark cycle was provided. Water was accessible at all times. Five roosters were allotted to each of the 7 DDGS sources in a completely randomized design. The roosters were deprived of feed for 24 h and then fed 30 g of DDGS via crop intubation. The basal endogenous losses of AA were measured from 5 additional roosters that were deprived of feed for 48 h. The excreta were collected quantitatively for 48 h starting immediately after crop intubation with collection via a plastic tray that was placed under each rooster. The SDD of AA were calculated using the method described by Sibbald (1979).

Lysine Bioavailability Study. The relative bioavailability of Lys in the 7 sources of DDGS was measured using the standard curve method (de Muelenare, 1967a, b). Crystalline L-Lys HCl was used as the reference AA. New Hampshire x Columbian Plymouth Rock male chicks (total of 220 chicks with average BW of 97.8 g) were fed a

pretest starter diet based on corn and soybean meal for 7 d. This diet was formulated to contain nutrients according to NRC (1994) requirements. Chicks were housed in an environmentally controlled room equipped with battery cages and raised wire floors. Water and artificial light was provided at all times. On d-8 post-hatch, chicks were randomly allotted to 11 diets in a completely randomized design with 5 chicks per pen, and 4 pens (replicates) allotted per diet.

To construct the growth standard curve, 3 diets were mixed by adding L-Lys HCl at 0.093, 0.1875 and 0.281%, respectively, to the basal diet at the expense of cornstarch (Table 7.2). Thus, the calculated supplemental Lys from L-Lys HCl (78% L-Lys) was 0, 0.07, 0.15%, and 0.22% for the basal diet and the 3 supplemented diets, respectively. At these levels of Lys supplementation, the growth of chicks in response to increasing Lys intake was verified to be linear in a preliminary experiment. Seven additional diets were formulated that included 20% of each source of DDGS in the basal diet at the expense of cornstarch. Chicks were fed the experimental diets from d-8 to 17 post-hatch and the total weight gain and feed consumption during this period were recorded.

Reactive Lys Analysis. The quantity of reactive Lys was analyzed in the 7 DDGS sources using the homoarginine procedure that involves guanidination of samples with O-methylisourea (Rutherfurd and Moughan, 1990). A 0.2 g of each source of DDGS was placed in a 125-mL flask and 6 mL of 0.6 *M* O-methylisourea solution (pH of 11.4) was added to the flask. The samples were stirred for 12 h using a magnetic stirrer (MultiMagnestir 1278, Lab-line Instruments, Melrose Park, IL) followed by a 60-h incubation at 20^{o}C (Pahm et al., unpublished data). The guanidinated samples were air-dried and then sent to South Dakota State University for homoarginine analysis

(procedure 982.30E, step a; AOAC International, 2006). The reactive Lys was calculated based on the amount of homoarginine in the samples.

Colorimetry. The 7 DDGS sources were analyzed for degree of lightness (**L**), redness (**a**), and yellowness (**b**) using Hunterlab Miniscan XE (Hunter Associates Laboratory, Reston, VA). Each DDGS sample was placed in a 1cm-deep clear petri-dish with transparent cap. Ten color measurements were obtained for each sample. Based on the Hunterlab scale (Hunterlab, 2001), a lower L value represents darker color (0 = black), whereas a greater L value represents lighter color, with white color having an L value of 100. Positive, negative, or 0 values of "a" indicate that the sample is predominantly red, green, or neutral, respectively. Positive, negative, or 0 values of "b" indicate that the sample is predominantly yellow, blue, or neutral, respectively.

Other Analyses. All DDGS samples and the dried excreta from the cecectomized roosters were analyzed at the University of Missouri Experiment Station Chemical Laboratories. The CP in DDGS samples was analyzed using the combustion method (procedure 990.03, AOAC International, 2006). All diets, DDGS, and dried excreta were analyzed for AA concentrations. Methionine and Cys were analyzed after oxidation with performic acid (procedure 982.30E step b, AOAC, 2006). Tryptophan was analyzed after alkali oxidation using 4.2 *M* NaOH and boiling at 110^{o}C for 24h (procedure 982.30E, step c; AOAC, 2006). All other AA were analyzed after acid hydrolysis using 6 *M* HCL and boiling at 110^{o}C for 22h (procedure 982.30E step a; AOAC International, 2006). The AA concentration of samples was quantified using HPLC. The DDGS samples were also analyzed for ADF (procedure 973.18, AOAC International, 2006), NDF (Holst, 1973), and fat (procedure 954.02; AOAC International 2006).

Calculations

The SDD of AA was calculated using the following equation (Sibbald, 1979):

SDD AA% = 100 x [AA intake, mg – (AA in excreta, mg – endogenous AA loss, mg)]/AA intake, mg

where SDD is the standardized digestibility of AA, and endogenous AA is the AA from excreta of roosters that were deprived of feed.

For Lys bioavailability in the chick assay, the standard curve for the relative bioavailability assay was constructed from the total gain of chicks fed the basal diet and the 3 diets containing increasing concentration of L-Lys HCl and the best fit regression equation was then derived. Bioavailable Lys was estimated by plotting the total weight gain of chicks (y - axis) fed each source of DDGS and estimating the corresponding concentration of bioavailable Lys (x - axis) in the linear regression equation of the standard curve (Sasse and Baker, 1973). Relative bioavailability of Lys in DDGS was then calculated as follows:

Relative bioavailability of Lys, % = calculated bioavailable Lys, %/analyzed Lys, % x 100

where the calculated bioavailable Lys is the bioavailable Lys in each of DDGS source, and analyzed Lys is the Lys measured by HPLC in each source of DDGS.

The concentration of reactive Lys in DDGS was calculated based on the concentration of homoarginine in the samples after guanidination. The concentration of homoarginine was then converted to Lys (reactive Lys) on a molar basis:

Reactive Lys,% = (homoarginine, %/MW of homoarginine) x MW of Lys (Rutherfurd et al., 1997).

Statistical Analysis

Digestibility data were analyzed as a completely randomized design using PROC MIXED of SAS (SAS Institute, Cary, NC). The experimental unit was the rooster, the fixed effect was source of DDGS, and the random effect was the replicate. Mean digestibility values were calculated as least square means and compared using the PDIFF option of SAS.

In the bioavailability study, the standard curve was constructed from the linear regression equation that was calculated from the weight gain of chicks fed diets containing increasing concentration of L-Lys HCl using the PROC REG procedure of SAS. The calculated concentration of relative bioavailable Lys in the 7 sources of DDGS was then compared using PROC MIXED of SAS. The experimental unit was the pen or replicate group of chicks, the random effect in the model was the replicate, and the fixed effect was the source of DDGS. Means were calculated as least square means and separated using the PDIFF option of SAS. The concentration of relative bioavailable Lys and SDD Lys in the 7 sources of DDGS were compared by T-test procedure of SAS (Rao, 1997) using SAS.

Concentrations of SDD Lys and relative bioavailable Lys in each source of DDGS were predicted from the concentrations of NDF, ADF, reactive Lys, and Hunterlab color scores using PROC CORR of SAS. In all comparisons, a difference of $P < 0.05$ was considered significant.

RESULTS

The average CP, ADF, NDF, and crude fat concentration in the 7 sources of DDGS were 26.4, 9.8, 37.3, and 13.0% (Table 7.1). In the digestibility study, no roosters were removed and no sign of sickness was observed during the excreta collection period. The DDGS sources differed ($P < 0.05$) in SDD for Leu, Lys, Glu, and Pro, whereas the SDD for all other AA was similar among sources (Table 3). The mean SDD for Lys was 61.4%. Source 6 (52.7%) had the lowest ($P < 0.05$) SDD for Lys, whereas source 1 (70.4%) had the greatest ($P < 0.05$) SDD for Lys.

The best fit regression equation in the bioavailability study was chick gain (g) = 77.29 + 118.88 (supplemental bioavailable Lys intake, g), $r^2 = 0.96$ (Figure 7.1). The calculated average relative bioavailability of Lys as percent of analyzed Lys in each source of DDGS was 69.0%. Differences were observed in the gain:feed of chicks fed different DDGS sources. However, differences were not observed in total weight gain of chicks and Lys bioavailability among DDGS sources.

The mean concentrations of SDD Lys and bioavailable Lys were 0.47, and 0.53% (Table 7.5). Two of DDGS sources showed greater concentration of bioavailable Lys than SDD Lys, but the remaining 5 sources of DDGS showed no difference between concentrations of bioavailable Lys and SDD Lys.

Hunterlab L, a, and b scores had average values of 52.81, 12.48, and 39.51, respectively in the 7 sources of DDGS (Table 7.6). The CV for the Hunterlab L scores was 5.16% compared with 11.37 % for Hunterlab a scores, and 7.46% for Hunterlab b scores. The concentration of reactive Lys was correlated with the concentration of SDD Lys in DDGS ($r^2 = 0.84$, $P < 0.05$; Table 7.7). However, the concentration of SDD Lys

was not correlated with the concentration of ADF and NDF. Likewise, there was no correlation between any of the color scores and SDD Lys. The concentration of reactive Lys was poorly correlated with the concentration of relative bioavailable Lys ($r^2 = 0.46$). No correlation was observed between the concentration of ADF and NDF and the concentration of relative bioavailable Lys. The concentration of bioavailable Lys was correlated with Hunterlab L score ($r^2=0.90$, $P < 0.05$), but bioavailable Lys was poorly correlated with Hunterlab b score ($r^2=0.47$) and not correlated with Hunterlab a score.

DISCUSSION

The composition of DDGS obtained in this study is similar to previously published values (NRC, 1994; Spiehs et al., 2002; Stein et al., 2006). The range of values of SDD for AA obtained in this study is close to previously reported values (NRC, 1994; Batal and Dale, 2006; Fastinger et al., 2006). The SDD for Trp, however, was unexpectedly high.

The higher CV for the SDD of Lys compared with the SDD for other indispensable AA (9% vs. less than 3%, data not shown) is in agreement with the observations in other studies that the variability in ileal digestibility of Lys is greater than in most AA in DDGS (Fiene et al., 2006; Parsons, 2006; Stein et al., 2006) and may be attributed to the negative effect of heat processing. Several steps in the starch extraction in the ethanol plant (jet cooking, liquefaction, saccharification) involves application of heat, and drying of wet distillers grains and condensed distillers solubles (Rausch and Belyea, 2005). Although drying of wet distillers grains and condensed solubles is the stage where heat application is most aggressive, some Lys in the wet distillers grains and

condensed solubles appears to have already been heat damaged before drying (Pahm et al., unpublished data).

The range of the concentration of relative bioavailable Lys (0.43 to 0.62%) agree with values (0.48 to 0.71%) reported earlier by Combs and Bossard (1969) for DDGS from beverage plants. Thus, the bioavailable Lys in recent batches of DDGS from dry grind ethanol plants appears to be similar to DDGS from beverage plants. The lack of a difference between the concentration of SDD Lys and the concentration of bioavailable Lys suggests that the digestible Lys in DDGS is fully bioavailable to chicks. This result does not support studies in pigs (Wiseman et al., 1991; Van Barneveld et al., 1994) and rats (Craig and Broderick, 1981) showing that heat application in feedstuffs lower the efficiency of utilization of digested Lys. The fact that only 75% (0.58% reactive Lys vs. 0.77% analyzed Lys) of the Lys is reactive suggests that part of the Lys in DDGS is bound to reducing sugars. This agrees with previous results showing that approximately 24% of the Lys in DDGS is unreactive (Pahm et al., unpublished data). It appears that when fed to chicks, these unreactive Lys do not cause an overestimation of the concentration of digestible Lys in relation to the concentration of bioavailable Lys. The SDD procedure appears to correct for the reduction in the efficiency of digested Lys by taking into account the unavailable Lys in DDGS.

The strong correlation between SDD Lys and reactive Lys is an indication that greater amounts of Lys are digested by roosters when the ε-NH_2 group of Lys is unbound to sugars. A similar relationship between the amount of reactive Lys in DDGS and ileal digestibility was obtained in pigs fed DDGS-containing diets (Pahm et al., 2006). Maillard products can reduce the digestibility of Lys by competing with absorption of

Lys (Sherr et al., 1989), or inhibit the release of protein-bound Lys by inhibition of carboxypeptidases (Hansen and Millington, 1979).

The low correlation between the concentration of SDD Lys and Hunterlab L scores was due to source 1 having a relatively dark color but a high concentration of SDD Lys. Excluding this sample, the r^2 between the concentration of SDD Lys and Hunterlab L score was 0.70 (data not shown). Heat processing such as roasting of corn is accompanied by brown discoloration (Costa et al., 1976) and may indicate that non-enzymatic browning (Moran and Summers, 1968). A moderate correlation (r^2=0.52) of SDD Lys and Minolta L* scores in DDGS have also been observed (Fastinger et al., 2006), whereas a high correlation (r = 0.87) between the concentration of SDD Lys and Minolta L* score was reported by Batal and Dale (2006). This suggests that the relationship of color score and SDD Lys in DDGS can vary and may be influenced by the type of colorimeter and the procedure used to measure color (Pedersen et al., 2005).

The strong correlation between Hunterlab L score and the concentration of bioavailable Lys indicate that darker colored DDGS may have undergone considerable binding of Lys with reducing sugars, which initiated a browning reaction. Results of this experiment agree with previous data (Cromwell et al., 1993) showing that a positive relationship between Hunterlab L and a, but not b scores, and chick performance (weight gain, gain:feed). A similar relationship between color and performance has been reported in diets containing heated soybean meal fed to broilers (McNaughton et al., 1981).

The guanidination procedure (Mauron and Bujard, 1964) can be used to measure reactive Lys and it appears that values obtained with this procedure correlate well with Lys utilization in vivo (e.g. Hurrell and Carpenter, 1974; Nair et al., 1978). However, the

relatively low correlation between reactive Lys and the concentration of bioavailable Lys obtained in this study may be due to the inability of some of the reactive Lys in DDGS to be absorbed in the small intestine, possibly due to some of the reactive Lys being trapped in indigestible peptides (Desrosiers et al., 1989; Moughan et al., 1996).

In conclusion, the concentration of SDD Lys in DDGS fed to poultry appears to be close to the concentration of bioavailable Lys. The concentration of reactive Lys and Hunterlab L values are alternative methods to evaluate DDGS quality, in addition to AA digestibility and chick growth assay.

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Table 7.1. Composition of 7 sources of distillers dried grains with solubles (DDGS), as fed basis

	DDGS source							
Item	1	2	3	4	5	6	7	Mean
DM, %	87.5	86.9	89.4	87.9	86.8	88.3	83.3	87.2
CP, %	26.9	25.8	23.9	28.0	24.6	26.5	28.9	26.4
ADF, %	12.5	9.2	8.1	8.7	8.3	9.2	12.8	9.8
NDF, %	41.5	33.8	33.8	36.6	36.0	38.1	41.1	37.3
Crude fat, %	13.0	9.6	9.3	10.3	10.2	11.0	9.0	13.0
Indispensable AA, %								
Arg	1.35	1.12	1.03	1.24	1.04	1.19	1.15	1.16
His	0.80	0.70	0.65	0.76	0.62	0.71	0.69	0.70
Ile	1.08	1.00	0.92	1.04	0.84	1.02	1.02	0.99
Leu	3.32	3.09	2.83	3.45	2.67	3.17	3.21	3.11
Lys	0.94	0.78	0.65	0.84	0.71	0.74	0.72	0.77
Met	0.57	0.51	0.46	0.57	0.45	0.52	0.49	0.51
Phe	1.40	1.29	1.19	1.43	1.14	1.35	1.34	1.31
Thr	1.05	0.93	0.91	1.10	0.88	1.05	1.04	0.99
Trp	0.18	0.17	0.16	0.18	0.16	0.18	0.18	0.17
Val	1.49	1.36	1.26	1.41	1.14	1.39	1.39	1.35
Dispensable AA, %								
Ala	2.01	1.84	1.69	2.00	1.61	1.86	1.91	1.85
Asp	1.81	1.63	1.52	1.77	1.45	1.70	1.73	1.66

Table 7.1. (Cont.)

Cys	0.54	0.49	0.45	0.55	0.43	0.49	0.48	0.49
Glu	4.20	3.79	3.36	3.91	3.22	3.49	3.79	3.68
Gly	1.19	1.02	0.96	1.09	0.93	1.06	1.04	1.04
Pro	1.99	1.81	1.68	2.00	1.61	1.82	0.19	1.59
Ser	1.23	1.05	1.05	1.33	1.06	1.25	1.23	1.17
Tyr	1.03	0.98	0.92	1.16	0.90	1.07	1.06	1.02

Table 7.2. Composition of the Lys-deficient basal diet and the diets containing distillers dried grains with solubles (DDGS) used in the Lys bioavailability study, as fed basis

Item	Diets	
	Basal	DDGS
Cornstarch	20.00	-
Ground corn	40.00	40.00
Corn gluten meal	25.00	25.00
Soybean meal	8.00	8.00
DDGS	-	20.00
Soybean oil	2.00	2.00
Dicalcium phosphate	2.00	2.00
Limestone	1.40	1.40
Salt	0.40	0.40
Vitamin premix[1]	0.20	0.20
Trace mineral premix[2]	0.15	0.15
Choline chloride (60%)	0.13	0.13
L-Trp	0.10	0.10
L-Thr	0.12	0.12
L-Lys	-	-
L-Arg	0.45	0.45
DL-Met	0.05	0.05

Table 7.2. (Cont.)

Bacitracin premix[3]	0.025	0.025
Total	100.0	100.0
Calculated analysis:		
Crude protein, min. %	22.78	28.38
TMEn, kcal/kg	3386	3326
Digestible Lys, %	0.52	0.64

[1]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2]Provided per kg of diet: Fe, 75 mg ($FeSO_4.H_2O$); Zn, 75 mg (ZnO); Mn, 75 mg (MnO); Cu, 5mg ($CuSO_4.5H_2O$); I, 0.75 mg (ethylenediamine dihydroiodide); Se, 0.1 mg (Na_2SeO_3).

[3]Contributed 25 mg bacitracin per kilogram of diet as bacitracin methylene disalicylate (Alpharma, Inc., Fort Lee, NJ).

Table 7.3. Standardized amino acid digestibility of 7 sources of distillers dried grains with solubles (DDGS)[1,2]

	DDGS source									
	1	2	3	4	5	6	7	Mean	SEM	*P*-value
Indispensable AA, %										
Arg	89.3	88.9	88.2	87.3	87.6	87.9	90.0	88.5	1.18	0.649
His	88.0	87.6	86.3	87.6	86.7	84.7	85.8	86.7	1.28	0.555
Ile	84.1	85.0	83.2	82.8	82.6	82.0	85.2	83.5	1.41	0.593
Leu	91.0^{y}	92.6^{y}	91.1^{y}	91.8^{y}	91.2^{y}	91.0^{y}	88.2^{x}	91.0	0.79	0.024
Lys	70.4^{z}	63.1^{yz}	57.7^{xy}	63.5^{yz}	62.3^{yz}	52.7^{x}	59.8^{xy}	61.4	3.16	0.021
Met	87.9	88.7	87.2	87.4	84.9	83.6	88.5	86.9	1.24	0.054
Phe	87.9	88.7	87.2	87.9	87.3	86.8	88.8	87.8	0.98	0.743
Thr	78.8	78.5	75.6	78.7	77.5	76.1	77.8	77.6	1.74	0.781
Trp	101.2	104.8	103.7	101.1	102.9	100.7	103.2	102.5	1.53	0.426
Val	84.2	85.9	83.6	83.4	82.5	82.4	84.4	83.8	1.50	0.691
Dispensable AA, %										

Table 7.3. (Cont.)

Ala	86.1	87.4	84.7	86.5	85.8	84.7	84.8	85.7	1.14	0.551
Asp	79.0	78.7	76.2	77.4	76.8	75.6	76.3	77.1	1.64	0.709
Cys	85.4	85.1	84.6	83.8	81.7	80.4	81.9	83.3	1.88	0.409
Glu	89.3^{z}	89.9^{z}	87.2^{xyz}	88.2^{yz}	87.7^{yz}	85.7^{xy}	84.4^{x}	87.5	1.01	0.008
Pro	89.8^{y}	89.9^{y}	87.7^{y}	89.6^{y}	88.7^{y}	87.4^{y}	-29.6^{x}	72.0	5.54	<0.010
Ser	85.6	85.4	83.0	85.6	84.2	84.0	83.5	84.5	1.51	0.788
Tyr	87.8	90.1	88.6	89.2	88.3	89.3	89.1	88.9	1.07	0.801

[x,y,z]Digestibility values within a row with different superscript letters differ, $P < 0.05$.

[1]Each digestibility value is the mean of 5 observations.

[2]Standardized digestibilities of AA were calculated by correcting the apparent AA digestibilities for the basal endogenous AA losses. Basal ileal endogenous losses (mg) were: Arg, 35.6; His, 25.8; Ile, 23.1; Leu, 41.4; Lys, 37.4; Met, 8.84; Phe, 24.5; Thr, 38.1; Trp, 12.9; Val, 34.0; Ala, 32.0; Asp, 56.4; Cys, 27.2; Glu, 98.6; Gly, 202.6; Pro, 45.7; Ser, 41.5; and Tyr, 24.5.

Table 7.4. Relative bioavailability of Lys in 7 sources of distillers dried grains with solubles (DDGS) fed to chicks, as fed basis[1]

Source of DDGS	Chick total gain, g	Gain:feed ratio	Relative bioavailability of Lys, %[2]
1	102.9	0.53^{yz}	58.1
2	107.6	0.54^{z}	79.0
3	99.6	0.49^{x}	70.2
4	105.3	0.52^{y}	68.2
5	106.1	0.52^{yz}	82.6
6	100.1	0.52^{y}	65.0
7	96.8	0.52^{yz}	59.9
Mean	102.6	0.52	69.0
SEM	3.27	0.007	7.72
P-value	0.238	0.010	0.264

[x,y,z]Values within a column with different superscript letters differ, $P < 0.05$.

[1]Values are least square means of 4 replicate pens per diet.

[2]Calculated from the best fit regression equation: chick gain (g) = 77.29 + 118.88 x supplemental Lys intake, g, r^2=0.96.

Table 7.5. Comparison of rooster standardized digestible (SDD) Lys (%) and relative chick bioavailable Lys (%) in 7 sources of distillers dried grains with solubles (DDGS), as fed basis

DDGS source	SDD Lys[1]	Relative bioavailable Lys[2]	SED[3]	*P*-value
1	0.66	0.55	0.05	0.054
2	0.49	0.62	0.02	< 0.010
3	0.38	0.46	0.06	0.220
4	0.53	0.57	0.03	0.286
5	0.44	0.59	0.06	0.038
6	0.39	0.48	0.08	0.303
7	0.43	0.43	0.07	0.990
Mean	0.47	0.53	-	-

[1]Measured by the cecectomized rooster assay.

[2]Measured by the chick growth assay.

[3]Standard error of difference.

Table 7.6. Color scores of 7 sources of distillers dried grains with solubles (DDGS)[1]

DDGS source	Color score[2]		
	L	a	b
1	53.38	10.37	36.81
2	56.43	13.38	42.02
3	50.00	11.78	36.70
4	55.66	13.20	43.93
5	53.57	12.95	41.49
6	51.34	11.20	36.95
7	49.28	14.47	38.68
Mean	52.81	12.48	39.51
CV	5.16	11.37	7.46

[1]Measured by using the Hunterlab color scale. Hunterlab L, a, or b is a measure of degree of lightness, redness, and yellowness, respectively.

[2]Each value is a mean of 10 measurements.

Table 7.7. Correlation of chemical composition and color score with concentration (% of sample) of standardized ileal digestible (SDD) Lys and relative bioavailable Lys in 7 sources of distillers dried grains with solubles

	SDD Lys [1]		relative bioavailable Lys[2]	
Predictor	r^2	*P*-value	r^2	*P*-value
Chemical composition				
Reactive Lys[3]	0.84	0.003	0.46	0.093
NDF	0.23	0.272	0.11	0.463
ADF	0.21	0.301	0.14	0.403
Color score[4]				
L	0.29	0.215	0.90	0.001
a	0.10	0.500	0.00	0.973
b	0.02	0.749	0.47	0.088

[1]Measured the using adult cecectomized roosters.

[2]Measured by the chick growth assay.

[3]Measured by the homoarginine procedure (Rutherfurd and Moughan, 1990). The concentration of reactive Lys in DDGS sources 1,2,3,4,5,6, and 7 were 0.72, 0.59, 0.43, 0.69, 0.59, 0.51, and 0.50%, respectively (mean = 0.58%).

[4]Measured by the Hunterlab color scale. Hunterlab L, a, or b scores is a measure of degree of lightness, redness, and yellowness, respectively.

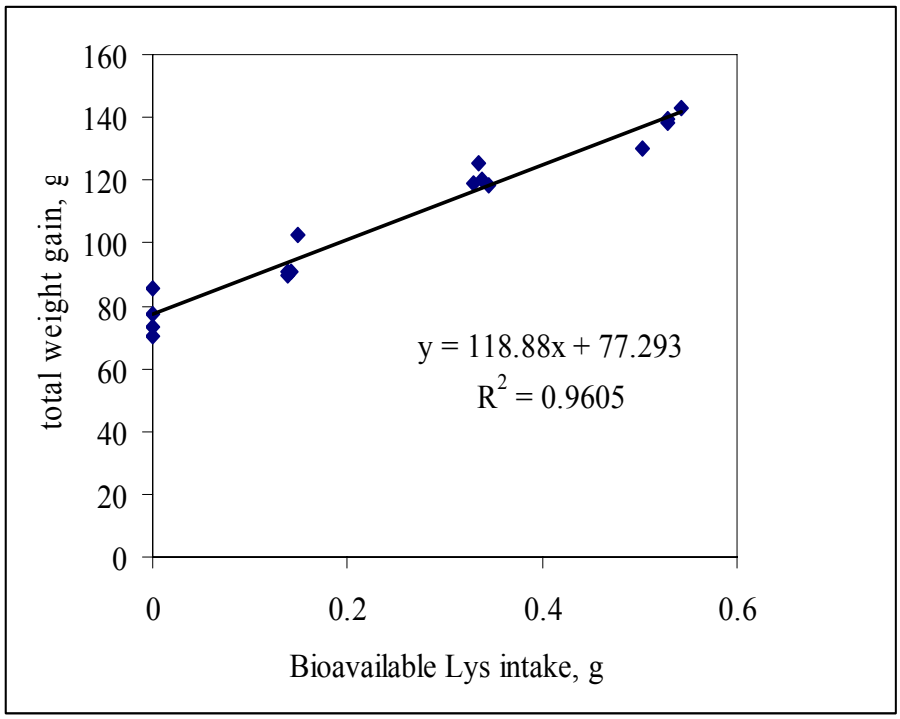

Figure 7. 1. Total weight gain of chicks in response to increasing intake of supplemental bioavailable Lys from L-Lys HCl.

GENERAL CONCLUSION

The ileal AA digestibility of distillers dried grains with solubles (DDGS) fed to pigs is influenced by drying temperature, and plant process such as the amount of condensed distillers solubles (CDS) added to the wet distillers grain. Differences in ileal AA digestibility due to the type of facility producing DDGS (dry-grind ethanol plant vs. beverage plant) appears minimal. Increased addition of CDS exacerbates the effect of drying on Lys deterioration in DDGS. Reactive Lys by guanidination may be used to evaluate the extent of heat damage to DDGS. The optimal condition for guanidination of DDGS during reactive Lys measurement is 3 d incubation with 0.6 M O-methylisourea at pH 11 to 12. The concentration of ileal digestible reactive Lys may be a better measure of bioavailable Lys than the concentration of SID Lys in DDGS fed to pigs. However, the 3 d guanidination of DDGS and the need to guanidinate the ileal digesta of pigs fed DDGS makes this a tedious procedure. Alternatively, the concentration of reactive Lys in DDGS *per se* can be used to predict the amount of ileal digestible Lys in pigs and poultry. Other effective methods is by measuring the degree of lightless in color (Hunterlab L score) of DDGS. Lighter-colored DDGS is associated with greater concentration of relative bioavailable Lys in chicks. The use of ileal digestibility procedure using cecectomized roosters appears to be an effective method to estimate the concentration of bioavailable Lys in DDGS fed to poultry.

AUTHOR'S BIOGRAPHY

Ameer A. Pahm was born on the 3rd of December, 1966 in the province of Tacurong, Republic of the Philippines. He obtained his high school diploma at the Notre Dame of Cotabato Boys Department in Cotabato City. He pursued his bachelor's degree in Veterinary Medicine at the University of the Philippines in Los Baños (UPLB), Laguna from 1983 to 1988. He then worked as research associate in the same university from 1989 to 1990. He earned his Master's degree in Monogastric Nutrition from UPLB in 1994 and his thesis focused on the effect of feeding processed grains and addition of full-fat soybean on the growth of pre-weaned piglets. In 1992, he started working as Animal Nutritionist for San Miguel Corporation, a private multi-national company in the Philippines and had brief international assignments. He came to the United States in the summer of 2004 to pursue his doctorate degree in swine nutrition under Dr. Hans H. Stein. From 2004 to 2006, he attended school at the South Dakota State University in Brookings, where he received the Britzman scholarship award. He eventually moved to University of Illinois in Urbana-Champaign, where he consummated the remaining 2 years of his doctorate program. He is married to Sarah Flordeliz Cervantes-Pahm in 2000 and they have a son.