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# Standardized Ileal Digestibility of Reactive Lysine in Distillers Dried Grains with Solubles Fed to Growing Pigs

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The Maillard reaction can occur during the production of distillers dried grains with solubles (DDGS) as a result of the addition of condensed solubles to the wet distillers cake during drying. The Maillard reaction can lead to the formation of unavailable or unreactive lysine as a result of binding of reducing sugars to the $\varepsilon$-$NH_2$ group of Lys. The Lys that remains unbound is called reactive Lys. The conventional procedure to measure the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of total Lys in DDGS may overestimate the amount of digestible Lys in DDGS because this procedure does not discount the unreactive Lys in DDGS, although only the reactive Lys is available for use by animals. By measuring the ileal digestibility of only the reactive Lys, it is expected that estimation of the amount of bioavailable Lys in DDGS will be more accurate. The objective of this experiment, therefore, was to test the hypothesis that the concentrations of AID and SID of reactive Lys are lower than the concentrations of AID and SID of total Lys in DDGS. Twelve DDGS sources, diets containing each of these 12 DDGS sources, and ileal digesta from pigs fed these diets were obtained from two previous experiments. Samples were guanidinated with *O*-methylisourea and analyzed for the concentration of homoarginine. It was assumed that only the reactive Lys, but not the unreactive Lys, would be transformed to homoarginine. This procedure, therefore, allows the separate measurement of reactive and unreactive Lys in DDGS-containing diets and ileal digesta. Subsequently, the ileal digestibility of reactive Lys can be calculated. The AID and SID of reactive Lys (percent) and the concentration of apparent ileal and standardized ileal digestible reactive Lys (g/kg) were then calculated and compared with the previously calculated values for total Lys. Results showed that only 76% of the total Lys in DDGS is reactive. The AID and SID of reactive Lys in DDGS (average = 60.1 and 66.9%, respectively) were similar to the AID and SID of total Lys (60.9 and 66.5%, respectively). When calculated as grams per kilogram, the concentration of standardized ileal digestible reactive Lys (3.9 g/kg) was lower ($P < 0.05$) than the concentration of standardized ileal digestible total Lys (5.1 g/kg). Thus, 24% of the concentration of standardized ileal digestible total Lys that was calculated using the conventional ileal AA digestibility procedure was unreactive Lys. The implication of these results is that the conventional AA digestibility procedure overestimates the concentration of digestible Lys in DDGS, and measurement of the concentration of digestible reactive Lys may more accurately estimate the amount of Lys in DDGS that is bioavailable to the pig.

**KEYWORDS: Amino acids; digestible reactive Lys; distillers dried grains with solubles; pigs**

## INTRODUCTION

Distillers dried grains with solubles (DDGS) is a coproduct of the dry-grind ethanol industry that is produced by blending the wet distillers cake and the condensed solubles, followed by drying using rotary drum driers. During drying of DDGS, the heat-sensitive amino acids (AA), such as Lys, can bind with reducing sugars via the Maillard reaction. The Lys in feedstuffs may become unavailable to animals when the $\varepsilon$-$NH_2$ group of Lys reacts with reducing sugars, polyphenols, and fats to form unreactive Lys (*1, 2*). In contrast, the Lys with a free $\varepsilon$-$NH_2$ group, called reactive Lys, is considered to be bioavailable (*3*). To optimally use DDGS and other byproducts with low AA digestibility, AA in feedstuffs are better expressed on an ileal digestible AA basis because such values give a close estimate of the amount of bioavailable AA, particularly for unheated proteins (*4, 5*). For heated proteins, however, conventional ileal AA digestibility may overestimate the amount of digestible Lys

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because of the presence of unreactive Lys that may be partly digested (*6*). 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 and unreactive Lys), the amount of bioavailable Lys in heated feedstuffs may be more closely estimated (*7*). Only 73% of the total Lys in 33 samples of DDGS is reactive (*8*), which indicates that considerable heat damage can occur 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 experiment was to test the hypothesis that the amount of standardized ileal digestible reactive Lys is lower than the amount of standardized ileal digestible total Lys in DDGS fed to pigs.

## MATERIALS AND METHODS

**Samples.** The DDGS, experimental diets, and ileal digesta were obtained from two experiments that were conducted to measure the ileal AA digestibility in five (expt 1) and seven (expt 2) sources of DDGS using 20 cannulated growing pigs (*9*). The DDGS was obtained from commercial dry grind ethanol plants located in the Midwest. Thus, 12 DDGS sources, 12 diets that each contained 1 of the 12 DDGS sources (66.7%), and the ileal digesta from pigs fed each diet were used. Ileal digesta were used to measure apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AA in DDGS. Two N-free diets were included in the experiment to measure basal ileal endogenous losses of AA. All diets contained cornstarch (27.1%), sugar (9%), and chromic oxide (0.30%), and vitamins and minerals were included to meet or exceed current estimates for requirements for pigs (*10*). A detailed description of animals, diets, collection procedures, sample analyses, and calculations has been published (*9*).

**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 the free $\varepsilon$-$NH_2$ group into homoarginine through a guanidination reaction with *O*-methylisourea (*11*). The homoarginine is then measured, and the amount of reactive Lys is obtained by calculating the equivalent amount of Lys from homoarginine on a molar basis. The amount of unreactive Lys, which is the Lys that was not transformed to homoarginine after guanidination, was obtained by measuring the amount of remaining Lys in the guanidinated samples.

Guanidination conditions for DDGS and ileal digesta were based on results from a previous experiment (*8*) in which it was determined that DDGS and ileal digesta from pigs fed DDGS-containing diets should be guanidinated in 0.6 M *O*-methylisourea at a pH of 11.4 and incubated for 3 days at 20 °C. The optimum pH of *O*-methylisourea for guanidination was determined by guanidinating DDGS and ileal digesta in 0.6 M *O*-methylisourea reagent at a pH of 9, 10, 11, 12, or 13 for 3 days. The optimum duration of guanidination was determined by using 0.6 M *O*-methylisourea reagent and incubating DDGS for 1, 3, 6, or 9 days and incubating ileal digesta from pigs fed diets containing DDGS for 1, 3, or 6 days.

In the present experiment, 6 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 analyzed for homoarginine after acid hydrolysis with 30 mL of 6 N HCl followed by refluxing for 24 h at 110 °C (*12*). The concentrations of homoarginine and other AA were measured using an HPLC system (Pickerings Laboratories, Moutain View, CA). The reactive Lys concentration was calculated on the basis of the molar weight ratio of Lys to homoarginine of 0.7767.

**Digestibility Calculations, Data Collection.** The AID and SID of reactive Lys were calculated on the basis of the conventional procedure to calculate the AID and SID of AA (*13*). 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 concentrations of apparent digestible and standardized digestible reactive Lys (g/kg) in DDGS were subsequently calculated by multiplying the AID and SID values for reactive Lys by the concentration (g/kg) of reactive Lys in the DDGS sample (*13*).

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

**Statistical Analysis.** Comparison between ileal digestibility values of total Lys and reactive Lys and between concentrations of ileal digestible total Lys and reactive Lys in each DDGS source was performed using a *t* test in SAS. Comparisons between the concentrations of other AA in unguanidinated and guanidinated DDGS sources, diets, and ileal digesta were also performed using a *t* test. In all analyses, a probability of $P < 0.05$ was considered to be significant.

**Table 1.** Concentrations of Total Lys and Reactive Lys in 12 Sources of Distillers Dried Grains with Solubles (DDGS), DM Basis[a]

| DDGS source | total Lys (g/kg) | reactive Lys (g/kg) |
|---|---|---|
| 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 |
| Avg | 7.6 | 5.8 |
| SD | 0.9 | 1.2 |
| CV | 11.7 | 20.0 |

[a] Reactive Lys is the amount of Lys that does not have an $\varepsilon$ amino group that is blocked by a reducing sugar. This Lys is, therefore, available for protein synthesis in the animal.

## RESULTS

The concentrations of total Lys in the 12 DDGS samples (**Table 1**) ranged from 5.6 to 8.8 g/kg (mean = 7.6 g/kg), whereas the concentrations of reactive Lys ranged from 3.1 to 7.0 g/kg (mean = 5.8 g/kg). The coefficient of variation (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 2**). However, DDGS sources 11 and 12 had a greater ($P < 0.05$) AID for reactive Lys than for total Lys, whereas DDGS sources 2−4 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 apparent ileal digestible reactive Lys (g/kg DDGS) than of apparent ileal digestible total Lys. The concentrations of apparent ileal digestible reactive Lys ranged from 1.2 to 4.2 g/kg (mean = 3.5 g/kg), whereas the concentrations of apparent ileal digestible total Lys ranged from 2.3 to 5.7 g/kg (mean = 4.7 g/kg).

Most of the DDGS sources had similar SID values for reactive Lys and total Lys (**Table 3**), but DDGS sources 1 and 11 had a greater ($P < 0.05$) SID for reactive Lys than for total Lys. In

**Table 2.** Comparison of Apparent Ileal Digestibility (AID) of Total Lys and AID of Reactive Lys in 12 Sources of Distillers Dried Grains with Solubles (DDGS), DM Basis[a–c]

| DDGS | AID Lys (%) | | | | AID Lys concn (g/kg) | | | |
|---|---|---|---|---|---|---|---|---|
| source | 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 |
| Avg | 60.9 | 60.1 | | | 4.7 | 3.5 | | |

[a] $N = 8$ for DDGS sources 1–5 and $N = 7$ for DDGS sources 6–12. [b] Reactive Lys is the amount of Lys that does not have an $\varepsilon$ amino group that is blocked by a reducing sugar. This Lys is, therefore, available for protein synthesis in the animal. [c] The average concentration of AID unreactive Lys in DDGS is 1.04 g/kg.

**Table 3.** Comparison of Standardized Ileal Digestibility (SID) of Total Lys and SID of Reactive Lys in 12 Sources of Distillers Dried Grains with Solubles (DDGS), DM Basis[a–c]

| DDGS | SID Lys (%) | | | | SID Lys concn (g/kg) | | | |
|---|---|---|---|---|---|---|---|---|
| source | 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 |
| Avg | 66.5 | 66.9 | | | 5.1 | 3.9 | | |

[a] $N = 8$ for DDGS sources 1–5 and $N = 7$ for DDGS sources 6–12. [b] Reactive Lys is the amount of Lys that does not have an $\varepsilon$ amino group that is blocked by a reducing sugar. This Lys is, therefore, available for protein synthesis in the animal. [c] The average concentration of SID unreactive Lys in DDGS is 1.10 g/kg.



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%), and the SID for total Lys ranged from 48.6 to 74.4% (mean = 66.5%). In all sources of DDGS, the concentration of standardized ileal digestible reactive Lys (g/kg) was lower ($P < 0.05$) than the concentration of standardized ileal digestible total Lys. The concentrations of standardized ileal digestible reactive Lys in DDGS ranged from 1.5 to 4.5 g/kg (mean = 3.9 g/kg), whereas the concentrations of standardized ileal digestible 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, which had lower ($P < 0.05$) concentrations in guanidinated DDGS compared with nonguanidinated DDGS (**Table 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 nonguanidinated DDGS.

The average recoveries of Lys (i.e., the 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 containing DDGS, and 93.7 ± 3.7% for ileal digesta from pigs fed diets containing DDGS (**Table 5**).

## DISCUSSION

Reduction in the nutritive value in feedstuffs after heat processing can be caused by binding of reducing sugars and other compounds to Lys (*14*) and other AA (*15*), leading to a reduced Lys utilization by animals (*16*). The extent of Lys binding in heated proteins can be measured through the determination of reactive Lys (*3, 17*). Data from this experiment show that only 76% of the total Lys in DDGS is reactive Lys. This suggests that some of the Lys in DDGS is unreactive. The bound, unreactive Lys may have been produced during the drying procedure of DDGS in the ethanol plant. During drying, the wet distillers grains and the solubles are blended and dried together in rotary drum dryers at a temperature between 300 and 600 °C. Deterioration of DDGS during drying has been demonstrated to be exacerbated by the amount of solubles added to the wet distillers grains and by drying temperature as a result of the increased formation of unreactive Lys (*8*).

The amount of analyzed reactive Lys in proteins is believed to represent the amount of potentially bioavailable Lys (*18*) and, therefore, is expected to be highly absorbable in the small intestine. However, the results of this experiment show that this is not the case for the Lys in DDGS because only 67% of the reactive Lys is digested. Therefore, the amount of reactive Lys in DDGS is not equal to the quantity of Lys that is absorbed by the animal. This result is in agreement with data showing that not all reactive Lys in other heated proteins is absorbed (*19, 20*). The reason some of the reactive Lys in DDGS is not digested may be that DDGS contains approximately 30% NDF. Fiber can increase the specific endogenous N loss through increased mucosal cell sloughing and mucus production (*21*). 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. Addition of purified NDF leads to a decreased N digestibility due to increased ileal losses of both endogenous and exogenous proteins (*22*). It is also possible that some of the reactive Lys in DDGS is associated with the structural fibers and, therefore, is less accessible for digestive enzymes than Lys bound in other proteins.

Another reason for the relatively low digestibility of reactive Lys in DDGS is the extent of heat application during the drying of DDGS. The CV of 20% for the 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 (*23*) or by blocking the cleavage of protein-bound Lys by inhibition of carboxypeptidases (*24*). During severe heating, protein aggregation and cross-linking may occur in heated feedstuffs, which also may limit the digestion of reactive Lys via increased hindrance at the site of cleavage in the protein chain, thereby trapping the reactive Lys in indigestible peptides (*19, 25–27*).

The SID of reactive Lys in milk products, field peas, and cottonseed meal is greater than the SID of total Lys, but blood meal, meat and bone meal, and soybean meal tend to have similar SID values for reactive and total Lys (*28, 29*). The present data show that the SID for reactive Lys in DDGS is

**Table 4.** Amino Acid Concentration and Apparent (AID) and Standardized (SID) Ileal Digestibility of AA in 12 Sources of Distillers Dried Grains with Solubles (DDGS) That Were either Guanidinated or Not Guanidinated, DM Basis[a]

| | AA concn in DDGS (%) | | | | AID (%) | | | | SID (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA | guanidinated | unguanidinated | SE | *P* value | guanidinated | unguanidinated | SE | *P* value | guanidinated | unguanidinated | SE | *P* value |
| Arg | 1.4 | 1.3 | 0.02 | 0.01 | 74.1 | 75.4 | 0.04 | 0.55 | 83.6 | 84.3 | 0.68 | 0.30 |
| Ile | 1.1 | 1.1 | 0.01 | 1.00 | 73.8 | 74.6 | 0.19 | 0.56 | 81.1 | 78.5 | 0.42 | <0.01 |
| Leu | 3.2 | 3.4 | 0.14 | 0.27 | 81.9 | 82.5 | 0.69 | 0.58 | 84.0 | 84.6 | 1.44 | 0.68 |
| Phe | 1.4 | 1.4 | 0.04 | 0.73 | 80.0 | 78.7 | 0.34 | 0.28 | 82.9 | 83.1 | 1.09 | 0.87 |
| Thr | 1.0 | 1.1 | 0.02 | <0.01 | 65.9 | 65.1 | 0.20 | 0.58 | 73.9 | 73.2 | 0.43 | 0.16 |
| Val | 1.5 | 1.5 | 0.05 | 0.21 | 73.9 | 73.3 | 0.27 | 0.47 | 77.9 | 77.8 | 0.40 | 0.78 |
| Ala | 2.1 | 2.1 | 0.10 | 0.63 | 77.0 | 75.8 | 0.06 | 0.61 | 81.5 | 80.5 | 0.58 | 0.10 |
| Asp | 2.1 | 1.9 | 0.06 | 0.03 | 64.6 | 64.5 | 0.82 | 0.82 | 73.0 | 71.1 | 0.64 | 0.01 |
| Glu | 5.0 | 5.0 | 0.29 | 0.91 | 78.9 | 80.0 | 0.59 | 1.89 | 82.1 | 83.0 | 1.68 | 0.57 |
| Gly | 1.1 | 1.2 | 0.04 | 0.29 | 49.4 | 44.0 | <0.01 | 0.75 | 74.6 | 72.4 | 0.93 | 0.03 |
| Ser | 1.5 | 1.4 | 0.04 | <0.01 | 71.8 | 71.4 | 0.61 | 0.74 | 79.9 | 78.9 | 0.40 | 0.03 |

[a] Each value represents the average of 12 observations.

**Table 5.** Recovery of total Lys in Guanidinated and Unguanidinated Samples of Distillers Dried Grains with Solubles (DDGS), Diets Containing DDGS, and Ileal Digesta of Pigs Fed DDGS-Containing Diets, DM Basis[a,b]

| | total Lys in DDGS (g/kg) | | | total Lys in diet (g/kg) | | | total Lys in digesta (g/kg) | | |
|---|---|---|---|---|---|---|---|---|---|
| DDGS source | guanidinated | nonguanidinated | recovery (%) | guanidinated | nonguanidinated | recovery (%) | guanidinated | nonguanidinated | 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 |
| 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 |
| Avg | 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 |

[a] $N = 8$ for DDGS sources 1−5 and $N = 7$ for DDGS sources 6−12. [b] Total AA in guanidinated samples = sum of reactive and unreactive Lys.



also similar to the SID for total Lys, which has also been shown for other proteins (*30*). This is most likely a consequence of the relatively high digestibility of unreactive Lys in DDGS (**Tables 2** and **3**). The greater the digestibility of unreactive Lys is, the more similar are the SID values for reactive and total Lys. It has been reported that as much as 70% of early Maillard products may be absorbed, but, because these products cannot be used in protein synthesis, they are eliminated through the urine (*6, 31*). However, in this experiment, we did not measure the concentration of early Maillard products in the urine.

The low concentration of standardized ileal digestible reactive Lys (g/kg DDGS) compared with the concentration of standardized ileal digestible total Lys shows that the amount of standardized ileal digestible total Lys in DDGS may be greater than the amount of Lys that is actually available for utilization by pigs. This implies that the amount of bioavailable Lys will be overestimated by the conventional SID procedure for Lys. It is usually assumed that all AA that are absorbed by the animal are bioavailable (*13*) but the absorption of unreactive Lys by pigs fed DDGS may lead to a reduced efficiency of utilization of digested total Lys because the absorbed unreactive Lys is not bioavailable (*32, 33*). This hypothesis is in agreement with data showing that for heat-processed feedstuffs, Lys availability may be lower than Lys digestibility (*34, 35*).

The use of ileal AA digestibility is a well-accepted method in feedstuff evaluation (*13*), but, if heat-treated feed ingredients are used, diets formulated on the basis of digestible total AA do not support performance in pigs as well as diets formulated with unheated feedstuffs (*36*). This implies that when DDGS is used as a substitute for a high-quality protein source such as soybean meal, the efficiency of utilization of SID Lys may be lower due to the absorption of unreactive Lys from DDGS. Formulating diets on the basis of SID of reactive Lys can minimize the overestimation of bioavailability of Lys.

The homoarginine procedure was used in this experiment because it allows for measuring reactive Lys in both early and advanced stages of Maillard reaction (*16*). There are, however, drawbacks to this procedure. Guanidination may not lead to a full recovery of total Lys (*3*), but a high recovery of total Lys was obtained in this experiment for DDGS, for diets containing DDGS, and for ileal digesta from pigs fed DDGS-containing 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 affected only the Lys.

In conclusion, the hypothesis that the amount of standardized ileal digestible total Lys in DDGS is greater than the amount of standardized ileal digestible reactive Lys is confirmed by the results of this experiment. The reason for this observation is that some of the unreactive Lys in DDGS is absorbed, which results in an overestimation of Lys availability if data for SID of total Lys are used. This overestimation of available Lys can be avoided if diets containing DDGS are formulated on the basis of digestible reactive Lys rather than on the basis of digestible total Lys.

## ABBREVIATIONS USED

AA, amino acids; AID, apparent ileal digestibility; DDGS, distillers dried grains with solubles; SID, standardized ileal digestibility; DM, dry matter; Avg, average; SD, standard deviation; CV, coefficient of variation; SE, standard error.

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