JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of the Reactive Lysine Procedure To Estimate Lysine Digestibility in Distillers Dried Grains with Solubles Fed to Growing Pigs

AMEER A. PAHM,[†] CARSTEN PEDERSEN,[§] AND HANS H. STEIN*,[†]

Department of Animal Sciences, University of Illinois, 1207 West Gregory Drive, Urbana, Illinois 61801, and Danisco Animal Nutrition, Wiltshire, United Kingdom

Two experiments were conducted to measure the reactive Lys concentration in corn distillers dried grains with solubles (DDGS). In expt 1, reactive Lys was measured in 33 sources of DDGS using two procedures: the homoarginine procedure and the furosine procedure. The concentration of reactive Lys in DDGS was then correlated with the concentration of standardized ileal digestible (SID) Lys in DDGS fed to growing pigs. In expt 2, a factorial experiment was conducted using four 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, and four subsamples from each combination were freeze-dried or oven-dried at 50, 75, or 100 °C. The dried samples were designated DDG, $DDGS_{20}$, $DDGS_{40}$, and CDS, respectively. All subsamples were analyzed for total Lys and for reactive Lys using the homoarginine procedure. Results of expt 1 showed that only 74.1% of total Lys was reactive if measured by the 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 measured by the homoarginine procedure ($r^2 = 0.70$, $P < 0.05$) and by the furosine procedure ($r^2 = 0.66$, $P < 0.05$). In expt 2, the concentrations of total Lys and reactive Lys were 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, 75.7% of total Lys was reactive in DDG, but only 27.6 and 10.2% were 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 coproducts 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 (*1*). Maillard reaction during drying can bind the free NH_2 group of Lys to the reducing sugars, which may cause variability in ileal Lys digestibility in DDGS (*2, 3*). 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 utilized (*4*).

Reactive Lys may be measured using the homoarginine procedure that transforms the unbound Lys to homoarginine through a guanidination reaction (*5*). The unreactive Lys may be calculated using the furosine procedure (*4*), and the concentration of reactive Lys can be subsequently calculated by subtracting the unreactive Lys from the total Lys concentration in the sample.

It is hypothesized that the concentration of reactive Lys is correlated with the concentration of standardized ileal digestible (SID) Lys in pigs fed DDGS and that both CDS addition and heat-drying can reduce the concentration of reactive Lys in DDGS. The objectives of these experiments were 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 further objective was to measure the effect of the addition of CDS and of drying temperature on the concentration of reactive Lys and on the CV of the concentration of AA in DDGS under laboratory conditions.

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 composi-

* Author to whom correspondence should be addressed [telephone (217) 333-0013; fax (217) 333-7088; e-mail hstein@uiuc.edu].
[†] University of Illinois.
[§] Danisco Animal Nutrition.

10.1021/jf801618g CCC: $40.75
Published on Web 09/26/2008

Table 1. Mean CP and AA Composition and Standardized Ileal Digestibility (SID) of AA in 33 Sources of Corn Distillers Dried Grains with Solubles (DDGS), Experiment 1 (As-Fed Basis)[a]

	total AA, %			SID AA, %			SID AA concn, %		
	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
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

[a] Stein et al. (*3, 37*), Pahm (*7*), and Urriola (*38*).

tion, and the SID of AA had been measured using cannulated growing pigs (**Table 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 each source of DDGS 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 (*4*). During this step, the Amadori product is hydrolyzed to three AA, namely, regenerated Lys, furosine, and pyridosine (*6*), 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 (*6*). 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 between reactive Lys and unreactive Lys, leading to a potential overestimation of the Lys concentration in heated proteins. To overcome this limitation, several methods have been developed to estimate the concentration of reactive Lys in heated feed ingredients. Among these methods are the homoarginine procedure and the furosine procedure.

Homoarginine Procedure. This method chemically transforms the reactive Lys, but not the sugar-bound Lys, to homoarginine through a guanidination reaction using *O*-methylisourea before the protein sample is acid-hydrolyzed (*5*). The guanidination step 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 expt 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 days at a pH of 11.4 (*7*)]. The guanidinating solution was prepared using a procedure adopted from Rutherfurd and Moughan (*8*). To prepare 100 mL of the *O*-methylisourea reagent, 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*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 1.0 M HCl. The supernatant was adjusted to a final volume of 100 mL using degassed water. Six milliliters 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 at 20 °C 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 [method 994.12 (*9*)]. The concentration of homoarginine and other AA was determined using an HPLC system (Pickerings Laboratories, Mountain 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 on the basis of the amount of homoarginine in the samples. Homoarginine was transformed to Lys on a molar basis using the following equation:

$$\text{reactive Lys (\%)} = \text{homoarginine (\%)/MW homoarginine} \times \text{MW Lys}$$

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

$$\text{Lys conversion rate (\%)} = 100 \times [\text{mmol of homoarginine}/(\text{mmol of homoarginine} + \text{mmol Lys})]$$

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

$$\text{Lys recovery (\%)} = 100 \times (\text{reactive Lys} + \text{unreactive Lys})/\text{total Lys in unguanidinated sample}$$

Furosine Procedure. This procedure is used to calculate the concentration of reactive Lys on the basis of the concentration of furosine in heated proteins. Furosine is one of the AA that is produced from Amadori products during the acid hydrolysis step in AA analysis (*6*). Amadori products in milk yield 32% furosine, 40% regenerated Lys, and 28% pyridosine (*10*). Because of this constant proportion in the concentration of the three hydrolysis products, 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 of refluxing [method 994.12; (*9*)]. Each 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 quantify the furosine peak in the chromatogram at 570 nm. The conversion factors used to calculate for the Amadori product, unreactive Lys, and, subsequently, reactive Lys in DDGS was based on previous studies in milk products (*11*). Thus, the Amadori product was calculated as follows:

$$\text{Amadori product (\%)} = \text{furosine, \%}/(32/100)$$

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

$$\text{regenerated Lys (\%)} = \text{Amadori product (\%)} \times (40/100)$$

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

$$\text{reactive Lys (\%)} = \text{total Lys} - \text{regenerated Lys}$$

Statistical Analysis. The experimental unit was the source of DDGS. The Proc REG procedure of SAS (version 9.1, SAS Institute Inc., Cary,

Table 2. Reactive Lys and Total Lys Concentration in 33 Sources of Corn Distillers Dried Grains with Solubles, Experiment 1 (DM Basis)

	mean[a]	low	high	CV
reactive Lys				
homoarginine procedure %[b]	0.63b	0.31	0.82	17.34
furosine procedure, %	0.71c	0.35	0.97	6.67
total Lys, %	0.85d	0.56	1.03	12.25
SEM[c]	0.02	ND[d]	ND	ND

[a] Values that do not contain a common letter are different, $P < 0.05$. [b] The mean conversion of Lys to homoarginine was 78.85%, and the mean recovery of Lys after guanidination was 93.32 ± 6.5. [c] Standard error of the mean. [d] ND, not determined.

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 the homoarginine procedure, respectively.

Experiment 2. *Samples and Experimental Design.* Ten kilograms of CDS and WDG was obtained from a dry-grind ethanol plant in South Dakota. Two additional samples (1 kg each) were prepared by mixing CDS and WDG using ratios (wt/wt, as-is basis) of CDS to WDG of 20:80 and 40:60. Four 25 g 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 °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 × 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 °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 evenly spread in 11.25 × 11.25 cm aluminum pans and oven-dried using a Thelco 130DM oven dryer. A commercial freeze-dryer (Dura Dry MP, FTS Systems, Inc., Stone Ridge, NY) was used to dry the samples that were not oven-dried. Samples were ground in a coffee grinder and were stored at −20 °C until analyzed. Samples were analyzed for CP [method 990.02 (*9*)] and for total Lys using the conventional procedure [method 994.12 (*9*)] 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 × 4 factorial in completely randomized design, with a total of 16 treatment combinations of CDS addition and drying temperature. The experimental unit was the dried sample. The effect of CDS addition, drying temperature, and CDS × 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 33 DDGS samples was lower ($P < 0.05$) than the concentration of total Lys (mean = 0.85%) regardless of the procedure that was used to measure reactive Lys (**Table 2**). The concentration of reactive Lys in DDGS as a percentage of total Lys was 83.5% (0.71 vs 0.85%) if calculated from the data on the basis of the furosine procedure and 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 reactive Lys measured by the homoarginine procedure was correlated with the concentration of SID Lys, with an $r^2 = 0.70$ ($P < 0.05$), but the r^2 was 0.66 ($P < 0.05$) if measured by the furosine procedure (**Table 3**). 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 3. Coefficient of Determination (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, Experiment 1

measured variable	regression eq	r^2	RMSE[a]
CP	−0.1284 + 0.0217 (CP)	0.22	0.082NS
total Lys	−0.051 + 0.682 (total Lys)	0.60	0.059**
	reactive Lys procedure		
homoarginine	0.093 + 0.694 (reactive Lys)	0.70	0.051**
furosine	0.023 + 0.637 (reactive Lys)	0.66	0.054**

[a] Root mean square error. NS, not significant; **, $P < 0.01$.

Table 4. Initial CP and AA Concentration in Samples before Oven-Drying, Experiment 2 (DM Basis)

item	DDG[a]	$DDGS_{20}$[a]	$DDGS_{40}$[a]	CDS[a]
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[b]	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
Pro	2.98	2.58	2.27	0.61
Ser	1.97	1.72	1.54	0.51
Tyr	1.49	1.30	1.14	0.30

[a] 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. [b] Reactive Lys measured by the homoarginine procedure.

Experiment 2. *Composition of Unheated Samples.* Before oven-drying, the concentration of CP and AA was greater in DDG than in CDS (**Table 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 addition was increased (**Table 5**). Drying temperature, however, did not affect the concentration of CP in the samples.

The concentration of total Lys as percent of the initial concentration of Lys 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

Table 5. Effect of Drying and Addition of Condensed Distillers Solubles (CDS) on CP, Total Lys, and Reactive Lys in Samples, Experiment 2 (DM Basis)[a]

		total Lys		reactive Lys		
item	CP, %	%	% of initial	%	% of initial	% of total Lys
freeze-drying						
DDG	34.6f	1.1p	100.0j	0.89i	100.0i	80.9f
$DDGS_{20}$	32.2e	1.0kp	100.0j	0.80gh	100.0i	79.2f
$DDGS_{40}$	28.0d	0.84ij	100.0j	0.70f	100.0i	83.3f
CDS	14.6c	0.42e	100.0j	0.22c	100.0i	51.9d
50 °C						
DDG	34.5f	1.11p	101.3jk	0.84hi	94.7hi	75.7e
$DDGS_{20}$	32.3e	1.03o	103.0k	0.76g	95.7hi	73.9e
$DDGS_{40}$	28.3d	0.84j	100.5j	0.63e	89.8gh	74.6e
CDS	13.5b	0.36d	86.1g	0.20c	92.7h	54.3d
75 °C						
DDG	35.2f	1.05o	95.6i	0.81gh	91.1h	77.2f
$DDGS_{20}$	32.1e	0.78h	77.8e	0.45d	56.5e	57.8d
$DDGS_{40}$	28.6d	0.53g	63.2d	0.21c	29.9d	39.5c
CDS	12.9b	0.34d	81.1f	0.18c	83.4g	52.7d
100 °C						
DDG	35.2f	1.01p	91.4h	0.67ef	75.7f	67.1e
$DDGS_{20}$	32.2e	0.51f	51.0c	0.22c	27.6c	43.0c
$DDGS_{40}$	28.4d	0.28c	33.6b	0.08b	10.2b	25.2b
CDS	12.9b	0.22b	52.4c	0.07b	35.3d	34.3c
SEM[b]	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 × CDS	0.06	<0.01	<0.01	<0.01	<0.01	<0.01

[a] 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. Values within a column that do not contain a common letter are different, $P < 0.05$. [b] Mean square error.

total Lys as percent of initial concentration of Lys differed only slightly 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 recovered after drying at 100 °C, but 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 was reduced ($P < 0.05$) when drying temperature or CDS addition was increased. Interaction ($P < 0.05$) was observed 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 present after drying at 100 °C, but in $DDGS_{40}$, only 10.2% of the initial reactive Lys was present after the sample had 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 were 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 $DDGS_{20}$ were 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.

Table 6. Coefficient of Variation of CP and AA Concentrations in Samples That Were Freeze-Dried or Oven-Dried at 50, 75, or 100 °C, Experiment 2[a]

item	DDG[b]	$DDGS_{20}$[b]	$DDGS_{40}$[b]	CDS[b]
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[c]	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

[a] Samples were not analyzed for Met, Trp, and His. [b] 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. Each value within a column was calculated by obtaining the CV of mean values of AA in samples that were dried using the four drying conditions. [c] Reactive Lys measured by homoarginine procedure.

Coefficient of Variability of AA. The CV of all AA except Tyr increased with increasing amount of CDS in DDGS. The greatest CV for most AA was observed in CDS, but 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 concentrations 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 (*12*). 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 the fermentation stage (*12*). In the dehydration of thin stillage to CDS, a temperature of 100 °C or more 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 concentrations of reactive Lys in total Lys in freeze-dried samples of DDG (80.9%) and CDS (50.0%) suggest that Lys−sugar binding can occur before DDGS is dried. The cause of Lys deterioration in DDGS is often attributed to addition of solubles to WDG (*13, 14*). This is likely because WDG contains most of the total Lys, whereas CDS contains most of the low molecular weight sugars (*15*).

The reactive Lys procedure has previously been used to measure heat damage in protein sources (*16, 17*). The furosine method has been used to measure heat damage in several milk and cereal products that were moderately heated (*18*), whereas the homoarginine procedure has been used to evaluate the

reactive Lys in feedstuffs that have undergone mild to severe heating (*19*). However, these procedures have not been used to measure the extent of heat damage in DDGS. Results of these experiments concur with previous observations showing that the measured concentration of reactive Lys can be affected by the procedure used to measure the reactive Lys (*21, 22*).

The furosine method is suited for proteins that have undergone moderate heating and when Amadori products are the sole source of unreactive Lys (*18, 23*). The homoarginine procedure is effective in measuring reactive Lys in proteins that have undergone moderate or severe heating (*24*). An important observation in expt 1 is that only 74.1−83.5% of the total Lys in DDGS is reactive, whereas 16.5−25.9% is unreactive. The presence of variable amounts of unreactive Lys in DDGS may explain the reported variability in the ileal digestibility of Lys in pigs (*2*) and in the bioavailability of Lys (*25, 26*). The better correlation of SID Lys with reactive Lys than with total Lys or CP indicates that in DDGS, the concentration of reactive Lys is a better indicator of the concentration of digestible Lys than total Lys or CP. The unreactive Lys is partly digested in the small intestine (*4, 27*) and may also reduce the absorption of reactive Lys, possibly via blockage of the absorption sites (*28, 29*). Maillard products may also reduce the action of pancreatic and intestinal enzymes (*30*−*32*) through increased steric hindrance of the cleavage sites in the protein (*33*). Therefore, greater concentration of reactive Lys in DDGS is correlated with greater concentration of SID Lys in DDGS.

Results of expt 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 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 two samples were dried separately.

In a study involving heated soy protein concentrate and 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 (*34*). 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 studies using other protein sources (*35, 36*). The increase in the 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.

ABBREVIATIONS USED

AA, amino acids; CDS, condensed distillers solubles; CP, crude protein; DDG, distillers dried grains without solubles; DDGS, distillers dried grains with solubles; $DDGS_{20}$, distillers dried grains with 20% solubles; $DDGS_{40}$, distillers dried grains with 40% solubles; SID, standardized ileal digestible.

LITERATURE CITED

(1) Association of American Feed Control Officials. *AFFCO Official Publication*; 2006; p 273.

(2) Fastinger, N. D.; Mahan, D. C. Determination of the ileal amino acid and energy digestibilities of corn distillers dried grains with solubles using grower-finisher pigs. *J. Anim. Sci.* **2006**, *84*, 1722–1728.

(3) Stein, H. H.; Gibson, M. L.; Pedersen, C.; Boersma, M. G. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. *J. Anim. Sci.* **2006**, *84*, 853–860.

(4) Finot, P. A.; Magnenat, E. Metabolic transit of early and advanced Maillard products. *Prog. Food Nutr. Sci* **1981**, *5*, 193–207.

(5) Kimmel, J. R. Guanidination of proteins. *Methods Enzymol.* **1967**, *11*, 584–589.

(6) Finot, P. A.; Bricout, J.; Viani, R.; Mauron, J. Identification of a new lysine derivative obtained from acid hydrolysis of heated milk. *Experientia* **1968**, *24*, 1097–1099.

(7) Pahm, A. A. Utilization of Amino Acids in Distillers Dried Grains with Solubles (DDGS) by Pigs and Poultry and the Use of Reactive Lysine Procedures To Evaluate DDGS Quality. Ph.D. Dissertation, University of Illinois, Urbana-Champaign, IL, April 2008.

(8) Rutherfurd, S. M.; Moughan, P. J. Guanidination of lysine in selected dietary proteins. *J. Agric. Food Chem.* **1990**, *38*, 209–211.

(9) Association of Official Analytical Chemists *Official Methods of Analysis of AOAC International*, 17th ed.; Arlington, VA, 2000.

(10) Bujard, E.; Finot, P. A. Mesure de la disponibilité et du blocage de la lysine dans les laits industriels. *Ann. Nutr. Alim.* **1978**, *32*, 291–305.

(11) Finot, P. A.; Deutsch, R.; Bujard, E. The extent of Maillard reaction during processing of milk. *Prog. Food Nutr. Sci* **1981**, *5*, 345–355.

(12) Bothast, R. J.; Schlicher, M. A. Biotechnological process for the conversion of corn to ethanol. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 19–25.

(13) Cromwell, G. L.; Herkelman, K. L.; Stahly, T. S. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *J. Anim. Sci* **1993**, *71*, 679–686.

(14) Martinez Amezcua, C.; Parsons, C. M. Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. *Poult. Sci.* **2007**, *86*, 331–337.

(15) Wu, V. Determination of neutral sugars in corn distillers grains, corn distiller's solubles, and corn distiller's dried grains with solubles. *J. Agric. Food Chem.* **1994**, *42*, 723–726.

(16) Carpenter, K. J.; Booth, V. H. Damage to lysine in food processing: its measurement and significance. *Nutr. Abstr. Rev.* **1973**, *43*, 23–451.

(17) Hurrell, R. F.; Carpenter, K. J. Mechanisms of heat damage in proteins. 4. The reactive lysine content of heat-damaged material as measured in different ways. *Br. J. Nutr.* **1974**, *32*, 589–604.

(18) Erbersdobler, H. F.; Somoza, V. Forty years of furosine, forty years of using early Maillard products as indicators of the nutritional quality of foods. *Mol. Nutr. Food Res.* **2007**, *51*, 423–430.

(19) Rutherfurd, S. M.; Moughan, P. J. Application of new method for determining digestible reactive lysine to variably heated protein source. *J. Agric. Food Chem.* **1997**, *45*, 1582–1586.

(20) Möller, A. B.; Andrews, A. T., III. Methods for the estimation of lysine and sugar-lysine derivatives formed by Maillard reaction. *J. Dairy Res.* **1977**, *44*, 277–281.

(21) Campos Giménez, E.; Bénet, T.; Spack, L. The uncertainty in the calculation of the amount of blocked and reactive lysine in

milk products, as determined by the furosine method. *Accred. Qual. Assur.* **2004**, *9*, 605–614.
(22) Krause, R.; Henle, T. Studies on the formation of furosine and pyridosine during acid hydrolysis of different Amadori products of lysine. *Eur. Food Res. Technol.* **2003**, *216*, 277–283.
(23) Chiang, G. A simple and rapid high-performance liquid chromatographic procedure for determination of furosine, a lysine-reducing sugar derivative. *J. Agric. Food Chem.* **1983**, *48*, 1373–1374.
(24) Hurrell, R. F.; Carpenter, K. J. The estimation of available lysine in foodstuffs after Maillard reactions. *Prog. Food Nutr. Sci.* **1981**, *5*, 159–176.
(25) Combs, G. F.; Bossard, E. H. Available lysine content of corn distillers grains with solubles based on chick bioassay. *Proceedings of the 23rd Conference of Distillers Feed Research Council*, Cincinnati, OH, 1968; pp 58−63.
(26) Combs, G. F.; Bossard, E. H. Further studies on available amino acid content of corn distillers dried grains with solubles. *Proceedings of the 24th Conference of Distillers Feed Research Council*, Cincinnati, OH, 1969; pp 53−58.
(27) Erbersdobler, H. F.; Faist, V. Metabolic transit of Amadori products. *Nahrung* **2001**, *45*, 177–181.
(28) Lee, C. M.; Lee, T.; Chichester, C. O. Effect of Maillard reaction products on the absorption of tryptophan. *Comp. Biochem. Physiol.* **1977**, *56A*, 473–476.
(29) Sherr, B.; Lee, C. M.; Jelesciewicz, C. Absorption and metabolism of lysine Maillard products in relation to the utilization of L-lysine. *J. Agric. Food Chem.* **1989**, *37*, 119–122.
(30) Hansen, L. P.; Millington, R. J. Blockage of protein enzymatic digestion (carboxypeptidase-B) by heat-induced sugar-lysine reactions. *J. Food Sci.* **1979**, *44*, 1173–1177.
(31) Öste, R. E.; Bjorck, I.; Dahlqvist, A.; Sjöström, H.; Norén, O.; Miller, R. Effect of Maillard reaction products on protein digestion. In vitro studies. *J. Agric. Food Chem.* **1986**, *34*, 355–358.
(32) Öste, R. E.; Miller, R.; Sjöström, H.; Norén, O. Effect of Maillard reaction products on protein digestion. Studies on pure compounds. *J. Agric. Food Chem.* **1987**, *35*, 938–942.
(33) Kilara, A.; Sharkasi, T. W. Effects of temperature on food proteins and its implications on functional properties. *CRC Crit. Rev. Food Sci. Nutr.* **1986**, *23*, 323–395.
(34) Wolf, J. C.; Thompson, D. R.; Reineccius, G. A. Initial losses of available lysine in model systems. *J. Food Sci.* **1977**, *42*, 1540–1544.
(35) Yoong, F. S.; Walters, R. J.; Tester, R. F.; Gomes, M. R. A.; Ledward, D. A. The role of glutamic acid/ glutamine and lysine during non-enzymatic browning in heated gluten. *Food Chem.* **1994**, *51*, 271–274.
(36) Evangelisti, F.; Calcagno, C.; Nardi, S.; Zunin, P. Deterioration of protein fraction by Maillard reaction in dietetic milk. *J. Dairy Res.* **1999**, *66*, 237–243.
(37) Stein, H. H.; Pedersen, C.; Boersma, M. G. Energy and nutrient digestibility in dried distillers grain with solubles. *J. Anim. Sci.* **2005**, *83* (Suppl. 2), 79.
(38) Urriola, P. E. Digestibility of distillers dried grains with solubles, in vivo estimation and in vitro prediction. M.S. Thesis, University of Minnesota, St. Paul, MN, Jan 2007.

Received for review May 24, 2008. Revised manuscript received August 6, 2008. Accepted August 29, 2008.

JF801618G