Amino Acid Availability and True Metabolizable Energy Content of Corn Distillers Dried Grains with Solubles in Adult Cecectomized Roosters[1,2]

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ABSTRACT Five sources of corn distillers dried grains with solubles (DDGS), which varied in darkness of color, were collected from several processing plants in the Midwestern United States. Sources of DDGS were analyzed for their amino acid and energy contents, measured for color score, and evaluated for TME_n, apparent amino acid digestibility, and true amino acid digestibility. A precision-fed rooster assay was used, in which each DDGS sample was tube fed (25 g) to adult cecectomized roosters, and the excreta were collected for 48 h. The experiment was conducted as a randomized complete block design with 8 replicates. Seven adult roosters (averaging 75 wk of age) were used in each period, with 5 fed the DDGS sources and 2 fasted to estimate basal endogenous amino acid losses. One source (no. 5) was the darkest, 2 sources (no. 2 and 4) were light, whereas 2 other sources (no. 1 and 3) were intermediate in color as measured by a colorimeter. Total lysine content of the DDGS sources ranged from 0.48 to 0.76%, with the lowest lysine content in the darkest DDGS source. Apparent and true lysine digestibility was approximately 30 and 15 percentage units lower ($P < 0.05$), respectively, in the dark-colored source (no. 5) than in the other 4 sources. Average apparent and true digestibility of the essential amino acids were 10 and 8 percentage units lower ($P < 0.05$), respectively, in source 5 than the other 4 sources. The TME_n content of the 5 DDGS sources was also lower ($P < 0.05$) in the darkest DDGS (no. 5). Our results suggest that when the color score of a DDGS source, as measured by a colorimeter, reached a certain threshold (lightness between 28 and 34), amino acid availability and true metabolizable energy content may be reduced. This reduction was particularly evident for lysine, which had the lowest digestibility in the darkest DDGS source. These results suggest that dark-colored DDGS may have been overheated during drying, causing Maillard reactions to be more extensive and resulting in a lowered total lysine content, lysine digestibility, and TME_n content.

Key words: distillers dried grain with solubles, amino acid availability, lysine, rooster, metabolizable energy

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INTRODUCTION

Production of ethanol from 100 kg of corn using the dry-milling method produces approximately 34.4 kg of ethanol, 34.0 kg of carbon dioxide, and 31.6 kg of distillers dried grains with solubles (**DDGS**; Renewable Fuels Association, 2005). The ethanol industry in the United States is increasing by approximately 30% annually (DiPardo, 2000; Renewable Fuels Association, 2005), resulting in large increases in corn DDGS supplies for the animal industry. A majority of DDGS is fed to ruminant animals because of their ability to use the high fiber content. However, recent research has demonstrated that corn DDGS can be incorporated into poultry diets at levels up to 6% in starter diets and 12 to 15% in grower and finisher diets (Lumpkins et al., 2004). However, variable DDGS composition and poor protein quality may ultimately limit its use in poultry diets.

Differences in processing procedures may lead to large variations in the nutritional value of DDGS (Cromwell et al., 1993) and as reported for soybean meal (Parsons et al., 1992). The objective of this study was to evaluate the amino acid and energy digestibility of 5 sources of DDGS that varied in the degree of color (light to dark) by using the precision-fed cecectomized rooster assay.

MATERIALS AND METHODS

Samples of DDGS were collected at one time point from 5 dry-grind corn ethanol plants located in the Midwestern United States. Although differences in processing conditions were suggested by marked variations in the colors of the 5 samples, plant personnel requested that informa-

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tion on drying temperatures and other DDGS processing conditions not be publicly revealed. All DDGS samples were ground using a Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ) equipped with a 2-mm screen, before being fed to the roosters. The experiment was conducted as a randomized complete block in 8 replicates, using 7 adult cecectomized Single Comb White Leghorn roosters (75 wk of age). All animal handling protocols were approved by The Ohio State University Animal Care and Use Committee. Roosters were cecectomized according to the procedures outlined by Parsons (1985) at 45 wk of age. The roosters were individually housed in raised wire cages (46 cm long × 41 cm wide × 61 cm high). During each replicate, 5 birds were deprived of feed for 24 h and then tube fed 25 g of 1 of the 5 DDGS sources, and 2 roosters were fasted throughout to allow for the determination of endogenous amino acid and energy losses. Excreta were collected quantitatively from each rooster for 48 h and freeze-dried prior to analysis. The birds were returned to a conventional corn-soybean meal diet for approximately 7 to 14 d and then rerandomized to treatments for a total of 8 replicates. No attempt was made to ensure all birds received each treatment. Excreta were collected from 2 fasted roosters per replicate to estimate basal endogenous amino acid losses.

Analyses

Color score [degree of lightness (**L***), redness (**a***), and yellowness (**b***)] of the DDGS samples was measured using a colorimeter (model CR-410, Konica Minolta Photo Imaging USA Inc., Mahwah, NJ). Samples of each DDGS source were placed in aluminum pans (23 × 23 × 2.5 cm), leveled with a ruler, and measured by lightly setting the probe on the surface of the DDGS. Reported color score was the mean of 10 measurements, with the sample being mixed and leveled between each determination. Low values for L* indicated a dark color, whereas higher scores indicated a light color (0 = black; 100 = white). Higher values of a* and b* indicated greater degrees of redness and yellowness, respectively. Neutral and acid detergent fiber contents of the DDGS were determined using an Ankom fiber analyzer (model A200, Ankom Technology, Macedon, NY). Crude protein content of DDGS and excreta were calculated from the N content measured using a N analyzer (model 2410, series II, Perkin-Elmer, Norwalk, CT). Amino acids were determined using an amino acid analyzer (model 6300, Beckman Coulter, Inc., Fullerton, CA) by the method outlined by AOAC methods 994.12 (sulfur and regular) and 988.15 (tryptophan) (AOAC, 1995). Gross energy was analyzed in the DDGS, and freeze-dried excreta was analyzed using an adiabatic oxygen bomb calorimeter (model 1241, Parr Instruments, Moline, IL).

Apparent and true digestibilities of amino acids and TME_n were calculated according to the procedure outlined by Sibbald (1986). Endogenous excretion of amino acids and energy were averaged from the 2 feed-deprived birds within each replicate.

The data were analyzed using the Mixed procedure of SAS Institute (2003) following a randomized complete block design according to the method of Steel and Torrie (1980). The individual rooster was the experimental unit.

The statistical model was

$$ND_{ijk} = \mu + r_i + D_j + a_{k:i} + e_{ijk}$$

where i = 1, 2, 3, 4, 5, 6, 7, 8; j,k = 1, 2, 3, 4, 5; $r_i \sim$ iidN(0, σ^2_r); $a_{k:i} \sim$ iidN(0, σ^2_a); $e_{ijkl} \sim$ iidN(0, σ^2_e); ND = nutrient digestibility; r = random effect of replicate; D = fixed effect of the 5 corn DDGS sources; and a = random effect of rooster nested within replicate. Mean comparisons were calculated using the PDIFF option of the LSMEANS statement and the Tukey-Kramer adjustment for multiple contrasts for all pairwise comparisons. Differences were considered significant when $P < 0.05$. Coefficients of determination were calculated from the Pearson product-moment correlations between color score and both DDGS composition and amino acid digestibilities to determine any possible relationships.

RESULTS

Color scores of the 5 DDGS sources (Table 1) demonstrated that source 5 was the darkest (lowest L*), sources 2 and 4 were the lightest (the highest L*), and sources 1 and 3 were intermediate. There was little variation in the degree of a* among sources, whereas b* followed a pattern similar to the L* value.

The analyzed amino acid, crude protein, neutral detergent fiber, acid detergent fiber, and gross energy contents of the 5 DDGS sources are presented in Table 2. Total lysine contents varied from 0.48 to 0.76%, whereas the range of crude protein was narrower (27.0 to 29.8%). Neutral detergent fiber and acid detergent fiber contents ranged from 29.7 to 34.2 and 10.3 to 13.2%, respectively. Gross energy content ranged from 4,848 to 4,969 kcal/kg in the 5 DDGS samples. Coefficient of determination values between color score and analyzed lysine content were 0.86, suggesting a high correlation between color score and lysine content.

Apparent lysine digestibility of the 5 DDGS sources (Table 3) was the lowest ($P < 0.05$) in source 5 (38.6%), highest ($P < 0.05$) in source 4 (69.5%), and intermediate in sources 1 (64.1%), 2 (64.2%), and 3 (63.0%). The latter did not differ statistically (63.0 to 64.2%). Digestibilities of all amino acids in source 5, the darkest source, were the lowest ($P < 0.05$) of all DDGS sources except for leucine and glutamate. Apparent amino acid digestibilities (Table 3) were similar among sources 1 to 4, with the exception of histidine, leucine, lysine, alanine, aspartate, glutamate, and proline. Average apparent digestibility of the essential and nonessential amino acids was lower ($P < 0.05$) in source 5 than the other sources, whereas sources 1 to 4 did not differ statistically. Coefficient of determination values between color score and apparent amino acid digestibilities ranged from 0.43 to 0.72, suggesting a moderate to high relationship.

Table 1. Color characteristics of the 5 sources of distillers dried grains with solubles (DDGS)[1]

	DDGS source				
Item	1	2	3	4	5
Color scores[2]					
L*	34.1 ± 0.83	55.1 ± 0.79	39.8 ± 0.69	52.3 ± 0.88	28.0 ± 0.19
a*	7.1 ± 0.36	9.0 ± 0.06	8.6 ± 0.14	7.9 ± 0.03	6.7 ± 0.07
b*	20.1 ± 0.47	41.9 ± 0.33	28.1 ± 0.51	33.8 ± 0.66	15.8 ± 0.08

[1]Ten observations per mean ± SD.

[2]L* = lightness of sample; where 0 = black to 100 = white; higher values for a* and b* indicate greater degrees of redness and yellowness, respectively.

When adjusted for endogenous losses, the true digestibility (Table 4) of lysine was lower ($P < 0.05$) in source 5 (65.3%) than sources 1 to 4, which ranged from 78.3 to 82.4%. True digestibility of a majority of amino acids was lower ($P < 0.05$) for the darkest source of DDGS (no. 5) than the other 4 sources. However, digestibility of leucine, tryptophan, cysteine, glutamate, serine, and tyrosine was not significantly different between sources 3 and 5. True digestibilities of most amino acids were similar among sources 1 to 4. True digestibilities of histidine, leucine, tryptophan, alanine, cysteine, glutamate, proline, serine, and tyrosine were different among some of the DDGS sources. Average true digestibility of essential and nonessential amino acids was lower ($P < 0.05$) for source 5 than for the other 4 sources. Coefficient of determination values between color score and true amino acid digestibilities ranged from 0.35 (tyrosine) to 0.88 (tryptophan), suggesting a relationship ranging from low to high correlation.

True metabolizable energy was the lowest ($P < 0.05$) in source 5; the highest ($P < 0.05$) in sources 1, 2, and 4; and intermediate in source 3. Source 5, the darkest DDGS source, contained nearly 600 kcal/kg less TME_n than source 4.

DISCUSSION

This experiment demonstrated that as the degree of lightness and yellowness of the DDGS sources reached a certain threshold (L* between 28 to 34 and b* between 15 to 20), the apparent and true digestibility of amino acids declined. This reduction seemed to be particularly exacer-

Table 2. Amino acid, crude protein, gross energy, neutral detergent fiber, and acid detergent fiber contents of the 5 sources of dried grains with solubles (DDGS; as-fed basis)[1,2]

	DDGS source					
Item	1	2	3	4	5	R^2
Essential amino acids (%)						
Arg	0.96	1.06	1.08	1.06	0.86	0.65
His	0.61	0.66	0.70	0.65	0.63	0.15
Ile	0.84	1.03	1.09	0.99	0.96	0.19
Leu	2.86	3.05	3.26	3.05	3.13	0.00
Lys	0.51	0.75	0.70	0.76	0.48	0.86
Met	0.48	0.48	0.50	0.51	0.45	0.41
Phe	1.19	1.36	1.44	1.34	1.36	0.05
Thr	0.89	0.98	1.03	1.01	0.84	0.56
Trp	0.25	0.26	0.28	0.25	0.20	0.31
Val	1.21	1.32	1.42	1.29	1.26	0.10
Nonessential amino acid (%)						
Ala	1.80	1.85	1.99	1.85	1.84	0.01
Asp	1.66	1.72	1.77	1.71	1.46	0.47
Cys	0.46	0.48	0.50	0.50	0.45	0.48
Glu	4.39	3.89	3.94	3.68	4.01	0.42
Gly	0.96	1.01	1.06	0.98	0.91	0.27
Pro	1.94	2.09	2.17	1.95	1.84	0.24
Ser	1.13	1.01	1.08	1.06	0.91	0.05
Tyr	0.86	0.98	1.01	1.03	0.96	0.29
CP (%)	28.2	28.3	29.8	27.3	27.0	0.01
GE[3] (kcal/kg)	4,969	4,895	4,898	4,888	4,848	0.00
NDF[4] (%)	34.2	29.7	32.8	32.9	31.5	0.17
ADF[5] (%)	12.0	10.3	13.0	11.0	13.2	0.79

[1]Values represent a single analysis of each DDGS sample.

[2]R^2 indicates correlation between lightness value and nutrient composition.

[3]GE = gross energy.

[4]NDF = neutral detergent fiber.

[5]ADF = acid detergent fiber.

Table 3. Apparent amino acid digestibility of 5 different sources of distillers dried grains with solubles (DDGS)[1,2]

	DDGS source						
Item	1	2	3	4	5	SEM	R^2
Essential amino acids (%)							
Arg	76.5[b]	77.9[b]	77.6[b]	78.0[b]	64.1[a]	1.36	0.53
His	76.2[b]	78.4[bc]	78.0[bc]	80.8[c]	66.4[a]	1.22	0.65
Ile	75.9[b]	77.7[b]	76.1[b]	77.7[b]	68.3[a]	1.09	0.66
Leu	86.4[b]	87.1[b]	82.7[a]	87.3[b]	81.3[a]	0.64	0.57
Lys	64.1[b]	64.2[b]	63.0[b]	69.5[c]	38.6[a]	2.07	0.53
Met	83.0[b]	83.6[b]	83.3[b]	83.1[b]	77.5[a]	1.27	0.50
Phe	81.0[b]	82.4[b]	81.4[b]	82.1[b]	75.8[a]	0.84	0.65
Thr	64.1[b]	65.1[b]	64.4[b]	66.1[b]	55.3[a]	1.95	0.58
Typ	79.1[b]	81.7[b]	79.1[b]	80.2[b]	71.5[a]	1.15	0.67
Val	71.8[b]	73.3[b]	70.3[b]	73.6[b]	61.9[a]	1.40	0.64
Average	75.8[b]	77.1[b]	75.6[b]	77.8[b]	66.0[a]	1.12	0.61
Nonessential amino acids (%)							
Ala	79.6[bc]	80.4[c]	77.7[b]	81.8[c]	74.3[a]	0.86	0.68
Asp	66.5[bc]	68.0[bc]	64.9[b]	69.2[c]	56.3[a]	1.56	0.66
Cys	63.5[b]	65.9[b]	65.6[b]	67.8[b]	55.8[a]	2.57	0.68
Glu	78.5[b]	79.8[b]	74.1[a]	80.6[b]	71.9[a]	0.92	0.63
Pro	79.0[bc]	80.2[bc]	77.4[b]	80.8[c]	73.9[a]	1.16	0.72
Ser	71.3[b]	72.9[b]	70.7[b]	72.9[b]	65.0[a]	1.76	0.70
Tyr	81.5[b]	81.0[b]	79.3[b]	80.8[b]	75.1[a]	1.31	0.43
Average	74.3[b]	75.4[b]	72.8[b]	76.3[b]	67.5[a]	1.33	0.68

[a–c]Observations in the same row with different superscripts differ, $P < 0.05$.

[1]Eight observations per mean.

[2]R^2 indicates correlation between lightness and apparent nutrient digestibility.

bated for the essential amino acid lysine, which had the greatest variability among the 5 DDGS sources evaluated. These results imply that colorimetric measurements, such as L* and b*, may provide a rapid method for identifying DDGS sources with good to poor amino acid digestibility.

It is clear from previous studies (Parsons et al., 1992) that excessive heat applied during the drying process may cause Maillard reactions to affect the lysine residues and carbohydrate moieties, subsequently darkening the color of a by-product. Depending upon the extent of the Maillard reaction, some lysine may be converted to other compounds or irreversibly bound to a carbohydrate moiety, thus reducing the total lysine content of the DDGS. The bound and converted lysine is apparently not avail-

Table 4. True amino acid digestibility and true metabolizable energy content of 5 different sources of distillers dried grains with solubles (DDGS)[1,2]

	DDGS source						
Item	1	2	3	4	5	SEM	R^2
Essential amino acids (%)							
Arg	89.1[b]	89.9[b]	89.7[b]	90.1[b]	82.8[a]	1.43	0.55
His	85.4[b]	87.3[bc]	86.8[bc]	89.4[c]	78.0[a]	1.47	0.67
Ile	84.9[b]	86.3[b]	84.4[b]	86.3[b]	78.5[a]	1.12	0.67
Leu	91.7[b]	92.2[b]	87.7[b]	92.5[b]	86.9[a]	0.67	0.52
Lys	78.4[b]	78.4[b]	78.3[b]	82.4[b]	65.3[a]	2.44	0.54
Met	89.3[b]	89.8[b]	89.6[b]	89.5[b]	84.6[a]	1.13	0.50
Phe	88.5[b]	89.5[b]	88.5[b]	89.5[b]	84.0[a]	0.88	0.65
Thr	78.4[b]	78.8[b]	78.3[b]	80.1[b]	71.7[a]	1.93	0.57
Typ	87.9[ab]	89.8[b]	88.2[ab]	88.9[ab]	86.1[a]	1.25	0.88
Val	83.0[b]	84.0[b]	80.9[b]	84.5[b]	74.7[a]	1.34	0.63
Average	85.7[b]	86.6[b]	85.3[b]	87.3[b]	79.3[a]	1.08	0.63
Nonessential amino acids (%)							
Ala	86.6[bc]	87.4[c]	84.6[b]	88.6[c]	81.9[a]	0.89	0.68
Asp	78.8[b]	80.0[b]	77.2[b]	81.3[b]	70.7[a]	1.57	0.67
Cys	81.6[ab]	83.6[b]	82.7[b]	85.2[b]	75.1[a]	2.56	0.71
Glu	87.4[b]	88.5[b]	82.9[a]	89.4[b]	81.2[a]	0.96	0.60
Pro	87.6[bc]	89.0[bc]	86.1[ab]	89.6[c]	82.9[a]	1.16	0.75
Ser	84.9[b]	86.0[b]	84.0[ab]	86.4[b]	80.3[a]	1.61	0.72
Tyr	89.9[b]	88.8[b]	87.5[ab]	89.2[b]	84.3[a]	1.40	0.35
Average	85.2[b]	86.2[b]	83.6[b]	87.1[b]	79.5[a]	1.34	0.69
TME_n, kcal/kg	3,014[c]	2,994[c]	2,815[b]	3,047[c]	2,484[a]	53	0.52

[a–c]Observations in the same row with different superscripts differ, $P < 0.05$.

[1]Eight observations per mean.

[2]R^2 indicates correlation between lightness and apparent nutrient digestibility.

able for use in the animal, nor does all of it appear to be released under the acid hydrolysis conditions used in amino acid analysis (Hurrell, 1983). However, a portion of the bound lysine that is released during acid hydrolysis may be analyzed as lysine but is not available to the animal, therefore affecting digestibility values (Moughan and Rutherfurd, 1996). Therefore, the darkest colored DDGS (no. 5) may have had a greater degree of Maillard reaction occur during processing. The resulting Maillard reactions may also reduce the analyzed content of lysine as well as its digestibility.

This experiment demonstrated a reduction in the digestibility of other essential amino acids and energy in the darkest DDGS (no. 5), most likely due to excessive heating. Research by Evans and Butts (1948) suggests that excessive heating can bind amino acids and protein to compounds such as fiber, effectively reducing their digestibility. However, unlike the lysine that is altered in Maillard reactions, these bound amino acids and proteins are most likely freed during acid hydrolysis used in amino acid analysis procedure. This may explain why there was no marked reduction in the amino acid content, other than lysine, in the darker DDGS sources (Table 2). The TME_n of the high-quality DDGS sources in the current experiment were similar to the values reported by the National Research Council (1994) and by Parsons (1985) of 3,097 and 3,163 kcal/kg, respectively.

The reduction in lysine digestibility in darker colored DDGS has also been reported by Ergul et al. (2003), who demonstrated a reduction in true lysine digestibility of approximately 20% from their lightest to darkest DDGS sources. However, their average true lysine digestibility values (71.0 %) were somewhat lower than that reported in our experiment (76.5%). Additionally, the DDGS samples evaluated by Ergul et al. (2003) had L* values that ranged from 41 to 54. These samples would generally be categorized as light colored, typical of high quality DDGS. Our study evaluated a wider range of colors in the 5 DDGS sources collected, and found less difference in lysine digestibilities among the lightest colored sources.

The results of the current experiment indicate that DDGS has a higher lysine digestibility (overall mean of 76.5% for the 5 samples) than the 65 and 56.4% reported by the National Research Council (1994) and by Parsons (1985), respectively.

Overall, apparent and true amino acid digestibilities had a moderate relationship with DDGS color score. This experiment suggested that measuring the color score of a DDGS sample may provide a rapid method for identifying DDGS sources with poor amino acid digestibility.

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