Oven drying of ileal digesta from growing pigs reduces the concentration of AA compared with freeze drying and results in reduced calculated values for endogenous losses and elevated estimates for ileal digestibility of AA

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ABSTRACT: Two experiments were conducted to evaluate the influence of drying method on the composition of ileal digesta and the standardized ileal digestibility (**SID**) of CP and AA in feed ingredients fed to pigs. The hypothesis was that oven drying of ileal digesta samples results in loss of N and AA, and therefore, in greater calculated values for SID of CP and AA compared with lyophilized samples. In Exp. 1, eight barrows (13.8 ± 0.5 kg BW) were equipped with a T-cannula in the distal ileum and randomly allotted to a replicated 4 × 4 Latin square design with four diets and four periods, for a total of eight replicate pigs per diet. Three diets containing three different sources of soybean meal as the sole source of AA and an N-free diet were used. In Exp. 2, 18 cannulated growing barrows (72.5 ± 9.2 kg BW) were allotted to a completely randomized design with three diets and six replicate pigs per diet. The three diets included two diets based on two sources of distillers dried grains with solubles and an N-free diet. In both experiments, ileal digesta samples were collected for 8 h on days 6 and 7 of the 7-d feeding period. At the conclusion, two representative sub-samples were collected from each ileal digesta sample. One sub-sample was lyophilized and the other was oven dried at 60 °C using a forced air oven. Results indicated that in both experiments, DM was greater ($P < 0.05$) in lyophilized samples than in oven dried samples. There was no difference in the SID of CP between the two drying methods. However, except for Ala and Val in Exp. 1, the concentration of AA (88% DM-basis) was greater ($P < 0.05$) in lyophilized samples than in oven dried samples, which resulted in reduced calculated values for basal endogenous losses of AA. Therefore, values for the SID of AA (except for Ala in Exp. 1 and Trp and Gly in Exp. 2) were greater ($P < 0.05$) when calculated from oven dried samples than from lyophilized samples. In conclusion, regardless of the diet, oven drying of ileal digesta samples does not result in loss of N, but appears to damage or convert AA to other N-containing compounds, which results in reduced estimates for basal endogenous losses and greater calculated values for SID of AA. Thus, freeze drying of ileal digesta samples is recommended when data for the SID of AA are calculated for feed ingredients fed to pigs.

Key words: AA digestibility, drying method, freeze drying, ileal digesta, oven drying, pigs

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INTRODUCTION

Ileal digestibility of AA is calculated by subtracting the AA output in ileal digesta from the quantity of AA ingested by the animal (Stein et al., 2007). An accurate estimation of the concentration of AA in the diet and in ileal digesta is, therefore, required in experiments that aim at estimating ileal digestibility of AA. Although it is easier and less expensive to oven-dry samples, freeze drying has usually been used as the drying

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method because of the assumption that some N compounds may evaporate during oven drying (Manoukas et al., 1964; Sibbald, 1979). Indeed, for poultry, an average of 5.5% and 8.8% N losses was reported if excreta were oven dried at 65 and 55 °C, respectively, compared with fresh samples (Manoukas et al., 1964; Ribeiro et al., 2001). However, there appears to be no differences in N losses between lyophilized and oven dried (60 °C) excreta from laying hens (Shannon and Brown, 1969). Concentrations of most AA in lyophilized excreta samples from broiler chickens were greater than in samples that were oven dried at 60 °C for 24 h (Wallis and Balnave, 1983), which concurs with data indicating that the true digestibility of Arg, Met, Lys, and Tyr determined in roosters was greater if samples were oven dried (60 °C) instead of lyophilized (Dale et al., 1985).

Apparent total tract digestibility (**ATTD**) of CP in pigs was not influenced by drying method of feces (Jørgensen et al., 1984), and there was no difference in the concentration of N in fresh, oven dried, or lyophilized feces from pigs (Jacobs et al., 2011). By contrast, N losses were observed in pig slurry in oven dried samples, but not in lyophilized samples (Mahimairaja et al., 1990). To our knowledge, there is, however, no information about the influence of drying method on nutrient composition of ileal digesta or the ileal digestibility of AA in diets fed to pigs. Therefore, the objective of this work was to test the hypothesis that oven drying of ileal digesta results in loss of N and AA, and therefore, in greater calculated values for the apparent ileal digestibility (**AID**) and standardized ileal digestibility (**SID**) of CP and AA compared with freeze drying of samples.

MATERIALS AND METHODS

Two experiments were conducted following protocols reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in both experiments were the offspring of Line 359 boars mated to Camborough sows (PIC, Hendersonville, TN).

Experiment 1: AA Digestibility in Soybean Meal

Eight growing barrows (initial BW: 13.82 ± 0.52 kg) were equipped with a T-cannula in the distal ileum and allotted to a replicated 4 × 4 Latin square design with four diets and four periods in each square. There were, therefore, eight replicate pigs per treatment. Pigs were housed in individual pens equipped with a feeder and a nipple drinker in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors.

Four diets were prepared (Table 1). Three sources of soybean meal (**SBM**) were included as the only source of AA in three diets, and an N-free diet that was used to measure basal endogenous losses of CP and AA was also used. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.4% chromic oxide as an indigestible marker and all diets were provided in a meal form.

Pigs were fed their assigned diets on an ad libitum basis (Chastanet et al., 2007) and water was available at all times. Each period lasted 7 d. The initial 5 d of each period were considered an adaptation period to the diet, but ileal digesta were collected for 8 h on days 6 and 7 by attaching a plastic bag to the barrel of the opened cannula. Bags were removed whenever they were filled with digesta or every 30 min, and stored at –20 °C.

Experiment 2: AA Digestibility in Distillers Dried Grains with Solubles

Eighteen growing barrows with an approximate initial BW of 72.5 ± 9.2 kg were equipped with a T-cannula in the distal ileum and allotted to a completely randomized design with three diets and six replicate pigs per diet. Pigs were housed as described for Exp. 1.

Two sources of distillers dried grains with solubles (**DDGS**) were procured, and each source was used in one diet as the sole source of AA and CP (Table 2). An N-free diet was also formulated. Pigs were fed at a level of three times the maintenance energy requirement (i.e., 197 kcal ME per kg$^{0.60}$; NRC, 2012). The daily allotment of feed was divided into two equal meals and provided at 0800 and 1600 hours, and water was available at all times. Ileal digesta collection followed procedures described for Exp. 1.

Chemical Analysis and Data Processing

At the conclusion of Exp. 1, ileal digesta samples were thawed and mixed within pig and period, and for each sample, two representative sub-samples were collected for analysis. One of the sub-samples was lyophilized in a freeze dryer (Gamma 1-16 LSCplus, IMA Life, Tonawanda, NY) for 8 d and the other was oven dried at 60 °C for 6 d in a forced-air oven (METALAB,

Table 1. Ingredient composition and nutrient analysis of experimental diets containing three sources of soybean meal (SBM), as-fed basis, Exp. 1

	Diet			
Item	SBM-1	SBM-2	SBM-3	N-free
SBM-1, %	38.00	—	—	—
SBM-2, %	—	38.00	—	—
SBM-3, %	—	—	46.00	—
Soybean oil, %	3.00	3.00	3.00	4.00
Ground limestone, %	0.75	0.75	0.75	0.45
Dicalcium phosphate, %	1.10	1.10	1.10	2.15
Lactose, %	20.00	20.00	20.00	20.00
Cornstarch, %	36.05	36.05	28.05	67.80
Solca flok[1], %	—	—	—	4.00
Magnesium oxide, %	—	—	—	0.10
Potassium carbonate, %	—	—	—	0.40
Sodium chloride, %	0.40	0.40	0.40	0.40
Chromic oxide, %	0.40	0.40	0.40	0.40
Vitamin–mineral premix[2], %	0.30	0.30	0.30	0.30
Total, %	100.00	100.00	100.00	100.00
Analyzed composition				
DM, %	94.83	95.51	92.40	95.24
CP, %	21.32	21.59	21.47	0.19
Indispensable AA, %				
Arg	1.24	1.37	1.56	0.01
His	0.56	0.53	0.58	0.00
Ile	0.97	0.97	1.02	0.02
Leu	1.56	1.60	1.71	0.02
Lys	1.23	1.08	1.43	0.02
Met	0.28	0.28	0.31	0.01
Phe	1.00	1.05	1.11	0.01
Thr	0.74	0.80	0.85	0.01
Trp	0.30	0.31	0.33	0.02
Val	1.01	1.03	1.09	0.01
Dispensable AA, %				
Ala	0.88	0.91	0.96	0.01
Asp	2.20	2.30	2.46	0.02
Cys	0.26	0.27	0.30	0.01
Glu	3.50	3.69	3.91	0.02
Gly	0.87	0.88	0.93	0.01
Pro	0.91	1.05	1.11	0.03
Ser	0.74	0.89	0.95	0.01
Tyr	0.53	0.65	0.70	0.01
Total AA, %	18.78	19.66	21.31	0.25

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

[2]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 2. Ingredient composition and nutrient analysis of experimental diets containing two sources of distillers dried grains with solubles (**DDGS**), as-fed basis, Exp. 2

	Diet		
Item	DDGS-1	DDGS-2	N-free
DDGS-1, %	37.00	—	—
DDGS-2, %	—	50.00	—
Soybean oil, %	2.00	2.00	4.00
Ground limestone, %	1.00	1.25	0.50
Monocalcium phosphate, %	0.80	0.15	1.75
Sucrose, %	—	—	20.00
Cornstarch, %	58.10	47.50	68.15
Solca flok[1], %	—	—	4.00
Magnesium oxide, %	—	—	0.10
Potassium carbonate, %	—	—	0.40
Sodium chloride, %	0.40	0.40	0.40
Chromic oxide, %	0.40	0.40	0.40
Vitamin–mineral premix[2], %	0.30	0.30	0.30
Total	100.00	100.00	100.00
Analyzed composition			
DM, %	91.85	91.85	94.97
CP, %	14.08	14.14	0.27
Indispensable AA, %			
Arg	0.65	0.71	0.01
His	0.38	0.43	0.00
Ile	0.63	0.66	0.02
Leu	1.72	1.87	0.03
Lys	0.58	0.58	0.02
Met	0.27	0.29	0.00
Phe	0.77	0.85	0.02
Thr	0.56	0.61	0.01
Trp	0.13	0.13	0.02
Val	0.81	0.87	0.02
Dispensable AA, %			
Ala	1.05	1.14	0.02
Asp	1.02	1.06	0.02
Cys	0.28	0.31	0.02
Glu	2.35	2.41	0.03
Gly	0.61	0.67	0.01
Ser	0.59	0.63	0.01
Tyr	0.53	0.57	0.01
Total AA, %	14.10	15.11	0.40

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

[2]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Equipment Corp., Hicksville, NY). All dried samples were ground through a 1 mm screen (Wiley Mill Model 4; Thomas Scientific; Swedesboro, NJ). Diets, ingredients, and ileal digesta samples were analyzed in duplicate for DM by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2007). CP was analyzed by combustion (Method 999.03; AOAC Int., 2007) using a Rapid N cube apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and for AA [Method 982.30 E (a, b, c); AOAC Int., 2007]. Diets and ileal digesta samples were also analyzed for chromium (Method 990.08; AOAC Int., 2007).

Data for the composition of ileal digesta were adjusted to 88% DM. Basal endogenous losses were calculated from pigs fed the N-free diet and values for AID and SID of CP and AA were calculated for the three diets containing SBM for both oven dried and lyophilized ileal digesta samples (Stein et al., 2007). Because SBM was the only AA-containing ingredient in the diets, values calculated for each diet also were representative for the three sources of SBM. Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Data for AA composition of ileal digesta as well as data for the SID of CP and AA were analyzed using the PROC MIXED of SAS with pig as the experimental unit. The statistical model only included the fixed effect of diet and drying method because the interaction between diet and drying method was not significant. The model also included the random effects of period and pig. Treatment means were calculated using the LSMEANS statement and LSmeans were separated using the PDIFF option of SAS. Statistical significance and tendency were considered at $P \leq 0.05$ and $0.05 \leq P < 0.10$, respectively.

At the conclusion of Exp. 2, ileal digesta were thawed, mixed, and two sub-samples were collected. One sub-sample was lyophilized, and the other sub-sample was oven dried as described for Exp. 1. After grinding, diets, ingredients, and ileal digesta samples were analyzed in duplicate for DM, CP, AA, and chromium.

Table 3. Effect of diet and drying method on the concentration of DM, Cr, CP, and AA in ileal digesta from pigs fed diets containing three sources of soybean meal (**SBM**) or an N-free diet, Exp. 1[1]

	Diet						Drying method			
Item	SBM-1	SBM-2	SBM-3	N-free	SEM	*P*-value	Freeze dried	Oven dried	SEM	*P*-value
DM, %	87.78[b]	87.06[b]	87.70[b]	90.58[a]	0.63	<0.001	90.12	86.44	0.53	<0.001
Cr, %	1.13[a]	1.04[a]	1.04[a]	1.82[b]	0.08	<0.001	1.21	1.30	0.06	0.234
CP, %	21.64[b]	25.01[a]	19.50[bc]	18.66[c]	1.35	<0.001	20.80	21.60	1.27	0.244
Indispensable AA, %										
Arg	0.42[b]	0.55[a]	0.41[b]	0.45[ab]	0.05	0.010	0.64	0.28	0.05	<0.001
His	0.29[b]	0.37[a]	0.24[bc]	0.20[c]	0.02	<0.001	0.32	0.22	0.01	<0.001
Ile	0.66[ab]	0.70[a]	0.59[b]	0.41[c]	0.03	<0.001	0.61	0.57	0.03	0.031
Leu	1.04[ab]	1.12[a]	0.96[b]	0.64[c]	0.06	<0.001	1.01	0.88	0.05	0.002
Lys	1.02[b]	1.43[a]	0.72[c]	0.39[d]	0.08	<0.001	1.07	0.71	0.07	<0.001
Met	0.15[a]	0.15[a]	0.13[a]	0.10[b]	0.01	<0.001	0.15	0.11	0.01	<0.001
Phe	0.62[ab]	0.69[a]	0.60[b]	0.40[c]	0.03	<0.001	0.63	0.52	0.03	<0.001
Thr	0.82	0.80	0.77	0.82	0.06	0.751	0.84	0.76	0.05	0.050
Trp	0.13	0.13	0.13	0.12	0.01	0.935	0.18	0.08	0.01	<0.001
Val	0.80[a]	0.85[a]	0.73[a]	0.59[b]	0.05	<0.001	0.75	0.73	0.04	0.635
Total	5.94[b]	6.80[a]	5.27[b]	4.13[c]	0.35	<0.001	6.19	4.87	0.32	<0.001
Dispensable AA, %										
Ala	1.04[a]	1.01[a]	0.88[b]	0.67[c]	0.06	<0.001	0.82	0.98	0.06	<0.001
Asp	1.76[a]	1.93[a]	1.46[b]	0.87[c]	0.10	<0.001	1.74	1.27	0.09	<0.001
Cys	0.37[ab]	0.39[a]	0.33[ab]	0.29[b]	0.03	0.018	0.40	0.29	0.02	<0.001
Glu	2.40[a]	2.89[a]	1.71[b]	0.94[c]	0.19	<0.001	2.37	1.60	0.16	<0.001
Gly	1.44[a]	1.44[a]	1.17[b]	1.65[a]	0.16	<0.001	1.50	1.36	0.15	0.054
Ser	0.64	0.63	0.58	0.59	0.05	0.623	0.69	0.53	0.04	<0.001
Tyr	0.35[a]	0.35[a]	0.38[a]	0.26[b]	0.02	<0.001	0.42	0.25	0.02	<0.001
Total	7.99[a]	8.65[a]	6.51[b]	5.27[c]	0.51	<0.001	7.93	6.28	0.46	<0.001
All AA	13.93[a]	15.44[a]	11.77[b]	9.40[c]	0.84	<0.001	14.12	11.15	0.77	<0.001

[a–c]Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

[1]Values for Cr, CP, and AA were adjusted to 88% (DM-basis).

Values for AID, endogenous losses, and SID of CP and AA were calculated for the oven dried as well as for the lyophilized samples in the two diets containing DDGS (Stein et al., 2007). Data were analyzed as described for Exp. 1, with the exception that the model only included the random effect of pig.

RESULTS

Experiment 1: AA Digestibility in Soybean Meal

The concentration of DM, Cr, CP, and most AA in ileal digesta samples was different ($P < 0.05$) between the three diets containing SBM and the N-free diet (Table 3). The concentration of DM was greater ($P < 0.05$) in lyophilized samples than in oven dried samples, but there was no difference in the concentration of CP or Cr (88% DM basis) between the two drying methods. Likewise, the concentration of Val (88% DM basis) in ileal digesta samples was not different between drying methods. The concentration of Ala was less ($P < 0.05$) in lyophilized samples compared with oven dried samples. However, for all other AA (88% DM basis), the concentration of AA was greater ($P \le 0.05$) in lyophilized samples than in oven dried samples.

The SID of CP and most AA was different ($P \le 0.05$) among the three experimental diets containing SBM (Table 4). The SID of CP and Ala was not different between drying methods, but the SID of CP and Ala was not different between drying methods. The SID of all other AA was greater ($P < 0.05$) in oven dried samples than in lyophilized samples.

Experiment 2: AA Digestibility in Distillers Dried Grains with Solubles

The concentration of DM and most AA (88% DM basis) in ileal digesta samples was greater ($P \le 0.05$) in the samples from the two sources of DDGS than in samples from pigs fed the N-free diet (Table 5). There was no difference in the concentration of CP among diets, but the

Table 4. Effect of diet and drying method on the standardized ileal digestibility (%) of CP and AA in three sources of soybean meal (SBM) fed to growing pigs, Exp. 1[1]

	Diet					Drying method			
Item	SBM-1	SBM-2	SBM-3	SEM	*P*-value	Freeze dried	Oven dried	SEM	*P*-value
CP, %	89.5[a]	82.7[b]	87.9[a]	1.88	<0.001	85.7	87.7	1.80	0.063
Indispensable AA, %									
Arg	97.7[a]	94.5[b]	97.2[a]	1.03	0.007	93.7	99.2	0.94	<0.001
His	93.8[a]	88.1[b]	94.1[a]	0.87	<0.001	90.0	94.0	0.81	<0.001
Ile	91.3[a]	88.7[b]	90.8[a]	0.86	0.002	89.4	91.1	0.81	0.007
Leu	91.3[a]	88.9[b]	90.9[a]	0.89	0.004	89.1	91.6	0.85	<0.001
Lys	87.3[b]	74.6[c]	91.1[a]	1.96	<0.001	80.6	88.1	1.86	<0.001
Met	93.4[ab]	92.3[b]	93.8[a]	0.78	0.047	91.8	94.7	0.74	<0.001
Phe	92.1[a]	89.7[b]	91.3[a]	0.80	0.002	89.3	92.7	0.76	<0.001
Thr	89.3	88.4	88.6	1.34	0.754	86.9	90.7	1.24	0.001
Trp	96.0	95.2	95.1	0.82	0.517	92.2	98.7	0.75	<0.001
Val	90.5[a]	87.1[b]	89.9[a]	1.07	0.001	88.1	90.2	1.01	0.004
Mean	91.8[a]	87.6[b]	92.0[a]	1.02	<0.001	88.5	92.5	0.97	<0.001
Dispensable AA, %									
Ala	84.9[ab]	82.4[b]	86.0[a]	1.85	0.042	84.6	84.3	1.76	0.811
Asp	88.6[a]	84.9[b]	90.1[a]	1.22	<0.001	84.4	91.3	1.12	<0.001
Cys	82.7[ab]	78.2[b]	83.4[a]	1.68	0.051	77.8	85.0	1.42	<0.001
Glu	89.4[a]	85.5[b]	92.3[a]	1.56	0.001	85.4	92.8	1.41	<0.001
Gly	89.5	85.9	90.6	4.20	0.191	85.5	91.9	4.06	0.005
Ser	91.3	91.5	92.0	1.14	0.786	89.4	93.8	1.06	<0.001
Tyr	92.3	92.0	91.6	0.82	0.768	88.5	95.4	0.73	<0.001
Mean	88.9[ab]	86.3[b]	90.7[a]	1.54	0.008	85.6	91.7	1.44	<0.001
All AA	90.4[a]	86.4[b]	91.4[a]	1.27	<0.001	86.8	91.9	1.20	<0.001

[a–c]Means within a row lacking a common superscript letter are different ($P \le 0.05$).

[1]Values for SID were calculated by correcting values for AID for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DMI) as CP, 21.85; Arg, 0.72; His, 0.29; Ile, 0.53; Leu, 0.83; Lys, 0.61; Met, 0.13; Phe, 0.52; Thr, 0.96; Trp, 0.19; Val, 0.68; Ala, 0.71; Asp, 1.16; Cys, 0.38; Glu, 1.28; Gly, 2.15; Ser, 0.75; and Tyr, 0.37, for lyophilized ileal digesta samples; and as CP, 22.65; Arg, 0.49; His, 0.21; Ile, 0.46; Leu, 0.73; Lys, 0.44; Met, 0.10; Phe, 0.46; Thr, 0.86; Trp, 0.12; Val, 0.69; Ala, 0.86; Asp, 1.11; Cys, 0.24; Glu, 1.32; Gly, 2.09; Ser, 0.61; and Tyr, 0.30 for oven dried ileal digesta samples.

Table 5. Effect of diet and drying method on the concentration of DM, Cr, CP, and AA in ileal digesta from pigs fed diets containing two sources of distillers dried grains with solubles (DDGS) or an N-free diet, Exp. 2[1]

	Diet					Drying method			
Item	DDGS-1	DDGS-2	N-free	SEM	*P*-value	Freeze dried	Oven dried	SEM	*P*-value
DM, %	93.54[a]	93.12[a]	89.90[b]	0.96	0.039	94.26	90.12	0.61	<0.001
Cr, %	1.07[b]	0.83[b]	2.43[a]	0.11	<0.001	1.36	1.52	0.07	0.018
CP, %	18.38	16.99	14.28	2.32	0.472	17.26	15.84	1.40	0.092
Indispensable AA, %									
Arg	0.46	0.38	0.60	0.08	0.201	0.72	0.24	0.05	<0.001
His	0.29[a]	0.29[a]	0.14[b]	0.02	<0.001	0.33	0.14	0.01	<0.001
Ile	0.57[a]	0.61[a]	0.29[b]	0.04	<0.001	0.55	0.43	0.03	<0.001
Leu	0.92[a]	1.16[a]	0.49[b]	0.09	<0.001	0.98	0.73	0.05	<0.001
Lys	0.64[a]	0.65[a]	0.32[b]	0.05	0.001	0.67	0.41	0.04	<0.001
Met	0.16[a]	0.19[a]	0.06[b]	0.01	<0.001	0.16	0.12	0.01	<0.001
Phe	0.53[a]	0.57[a]	0.28[b]	0.04	0.001	0.57	0.35	0.03	<0.001
Thr	0.72[a]	0.68[a]	0.40[b]	0.04	<0.001	0.71	0.49	0.03	<0.001
Trp	0.15[a]	0.13[a]	0.09[b]	0.01	<0.001	0.13	0.11	0.01	0.012
Val	0.80[a]	0.86[a]	0.41[b]	0.06	<0.001	0.74	0.64	0.04	0.005
Total	5.23[a]	5.51[a]	3.10[b]	0.39	0.001	5.56	3.66	0.25	<0.001
Dispensable AA, %									
Ala	0.92[ab]	0.99[a]	0.63[b]	0.10	0.046	0.92	0.77	0.06	0.010
Asp	1.30[a]	1.14[a]	0.62[b]	0.08	<0.001	1.27	0.77	0.06	<0.001
Cys	0.32[a]	0.32[a]	0.14[b]	0.02	<0.001	0.32	0.20	0.01	<0.001
Glu	1.50[a]	1.68[a]	0.86[b]	0.15	0.006	1.56	1.14	0.10	<0.001
Gly	1.35	1.11	1.75	0.21	0.140	1.64	1.17	0.14	0.001
Ser	0.56[a]	0.50[a]	0.36[b]	0.03	0.002	0.59	0.35	0.02	<0.001
Tyr	0.30[a]	0.32[a]	0.19[b]	0.03	0.028	0.38	0.17	0.02	<0.001
Total	6.26	6.0	4.56[a]	0.56	0.108	6.68	4.57	0.36	<0.001
All AA	11.48[a]	11.58[a]	7.66[b]	0.94	0.017	12.24	8.24	0.60	<0.001

[a–e]Means within a row lacking a common superscript letter are different ($P \leq 0.05$).

[1]Values for Cr, CP, and AA were adjusted to 88% DM.

concentration of Cr was greater ($P < 0.05$) in the ileal digesta from pigs fed the N-free diet than from pigs fed the DDGS diets. The concentration of DM and all AA (88% DM basis) was greater ($P < 0.05$) in lyophilized samples compared with oven dried samples, and there was a tendency ($P = 0.092$) for the concentration of CP to be greater in the lyophilized samples compared with oven dried samples. However, the concentration of Cr (88% DM basis) was greater ($P < 0.05$) in oven dried samples than in lyophilized samples.

With the exception of Leu and Met, no differences in the SID of CP and AA between the two sources of DDGS were observed (Table 6). The SID of CP was not different between drying methods, but the SID of all AA except Trp and Gly was greater ($P < 0.05$) in samples that were oven dried compared with samples that were lyophilized.

Effect of Drying Method on Calculated Values for Endogenous Losses of CP and AA

In Exp. 1, there was no difference in the concentration of endogenous losses of CP and AA between the two drying methods with the exception that the endogenous loss of Arg was greater ($P < 0.05$) if calculated based on lyophilized samples than from oven dried samples (Table 7). In Exp. 2, there was no difference in the concentration of endogenous losses of CP between the two drying methods, but for most AA, greater ($P < 0.05$) endogenous losses were calculated from lyophilized ileal digesta samples than from oven dried samples.

DISCUSSION

Soybean meal and DDGS are the two ingredients used in the United States that provides most AA to diets for pigs. To test the hypothesis that drying method influences AID and SID of AA, we therefore focused on these two ingredients. We included lactose in the diets for the weanling pigs and not in the diets for the finishing pigs and thereby provided differences in the carbohydrate sources in the diets.

Freeze drying has been used as the preferred method to dry ileal digesta from pigs for at least three decades (Moughan et al., 1987) because data

Table 6. Effect of diet and drying method on the standardized ileal digestibility of CP and AA in two sources of distillers dried grains with solubles (DDGS) fed to growing pigs[1]

	Diet				Drying method			
Item	DDGS-1	DDGS-2	SEM	*P*-value	Freeze dried	Oven dried	SEM	*P*-value
CP, %	81.5	79.8	2.83	0.685	80.3	81.0	2.08	0.593
Indispensable AA, %								
Arg	90.9	92.3	1.93	0.621	88.4	94.8	1.50	<0.001
His	86.8	85.7	1.14	0.506	80.9	91.5	0.87	<0.001
Ile	84.5	80.9	1.66	0.163	80.2	85.2	1.28	0.001
Leu	90.8	86.8	1.16	0.033	86.9	90.6	0.88	<0.001
Lys	80.7	76.4	2.14	0.185	72.9	84.3	1.69	<0.001
Met	89.4	85.3	1.26	0.045	85.3	89.5	0.95	<0.001
Phe	88.3	86.0	1.32	0.230	83.6	90.7	1.04	<0.001
Thr	78.3	77.8	1.65	0.834	74.3	81.8	1.34	<0.001
Trp	80.8	80.7	1.29	0.975	79.8	81.6	1.18	0.258
Val	82.9	79.4	1.61	0.158	79.3	83.0	1.24	0.004
Mean	86.5	83.9	1.34	0.206	82.2	88.1	1.01	<0.001
Dispensable AA, %								
Ala	85.6	82.6	1.93	0.290	83.1	85.1	1.44	0.059
Asp	77.6	78.0	1.99	0.901	72.3	83.2	1.62	<0.001
Cys	79.7	78.6	1.24	0.568	74.1	84.2	1.04	<0.001
Glu	89.2	85.5	1.59	0.127	85.3	89.5	1.22	0.001
Gly	82.0	77.7	5.26	0.580	78.8	80.8	3.83	0.310
Ser	84.5	85.0	1.21	0.809	81.4	88.2	0.94	<0.001
Tyr	90.5	88.4	1.45	0.341	85.2	93.7	1.14	<0.001
Mean	84.5	82.9	2.05	0.605	81.0	86.5	1.53	<0.001
All AA	85.5	83.4	1.66	0.402	81.6	87.3	1.24	<0.001

[1]Values for SID were calculated by correcting values for AID for basal ileal endogenous losses. Basal ileal endogenous losses were determined (g/kg of DMI) as CP, 16.69; Arg, 0.76; His, 0.18; Ile, 0.29; Leu, 0.49; Lys, 0.34; Met, 0.06; Phe, 0.30; Thr, 0.43; Trp, 0.09; Val, 0.39; Ala, 0.61; Asp, 0.70; Cys, 0.16; Glu, 0.88; Gly, 1.81; Ser, 0.42; and Tyr, 0.23, for lyophilized ileal digesta samples; and as CP, 10.81; Arg, 0.11; His, 0.05; Ile, 0.18; Leu, 0.28; Lys, 0.17; Met, 0.04; Phe, 0.17; Thr, 0.22; Trp, 0.05; Val, 0.25; Ala, 0.32; Asp, 0.31; Cys, 0.08; Glu, 0.48; Gly, 0.87; Ser, 0.16; and Tyr, 0.08, for oven dried ileal digesta samples.

from poultry demonstrated that oven drying of excreta results in loss of N (Manoukas et al., 1964; Shannon and Brown, 1969). The observation that the concentration of DM is greater in lyophilized ileal digesta samples than in oven dried samples indicates that the freeze dryer under the conditions of these experiments removed more water than the drying oven. However, AA concentration of ileal digesta as presented in this manuscript is not influenced by differences in DM because all values were adjusted to 88% DM.

The concentration of N in feces and the ATTD of CP is not affected by drying method (Jørgensen et al., 1984; Jacobs et al., 2011), which raises the question if freeze drying is needed for ileal digesta from pigs. To our knowledge, there are no data demonstrating that it is necessary to freeze dry ileal digesta samples from pigs and, if there is no loss of AA or N from ileal digesta during oven drying, it was speculated that it may be possible to use oven drying instead of freeze drying.

The observation that there is no difference in the concentration of CP or the SID of CP between lyophilized and oven dried ileal digesta samples concurs with the data for ATTD of N in feces and indicates that there is no loss of N in ileal digesta from pigs as a result of the drying method. However, the present data clearly indicated that the concentration of AA decreased if ileal digesta samples were oven dried, indicating that oven drying may damage AA in the ileal digesta or convert AA to non-AA compounds. This may be a consequence of the greater temperature used during oven drying compared with freeze drying, which may result in Maillard reactions, which bind the free NH_2 group of proteins or AA to the carbonyl group of a reducing sugar (Pahm et al., 2008). In this work, the source of reducing sugars may be the lactose supplied in the diets in Exp. 1, and the resistant starch from DDGS, which may be up to 18% (Li et al., 2014), in the DDGS diets in Exp. 2. The AA that is most susceptible to Maillard reaction is Lys because of the

Table 7. Effect of drying method on basal ileal endogenous losses (g/kg DMI) of CP and AA calculated from pigs fed N-free diets

	Exp. 1				Exp. 2			
Item	Freeze dried	Oven dried	SEM	*P*-value	Freeze dried	Oven dried	SEM	*P*-value
CP	21.85	22.36	2.64	0.833	16.69	10.98	2.26	0.149
Indispensable AA								
Arg	0.72	0.46	0.10	0.037	0.76	0.12	0.12	0.006
His	0.29	0.21	0.04	0.171	0.18	0.05	0.02	0.003
Ile	0.54	0.48	0.08	0.494	0.29	0.19	0.04	0.111
Leu	0.83	0.75	0.13	0.582	0.49	0.29	0.08	0.087
Lys	0.61	0.45	0.10	0.236	0.34	0.20	0.05	0.085
Met	0.13	0.11	0.02	0.348	0.06	0.04	0.01	0.031
Phe	0.52	0.48	0.09	0.674	0.30	0.17	0.04	0.060
Thr	0.96	0.95	0.17	0.947	0.43	0.22	0.04	0.004
Trp	0.19	0.13	0.04	0.211	0.09	0.05	0.01	0.012
Val	0.68	0.70	0.12	0.889	0.39	0.26	0.06	0.167
Total	5.46	4.74	0.83	0.419	3.35	1.51	0.40	0.013
Dispensable AA								
Ala	0.71	0.86	0.12	0.333	0.61	0.33	0.10	0.130
Asp	1.16	1.14	0.19	0.934	0.70	0.31	0.08	0.024
Cys	0.38	0.31	0.06	0.145	0.16	0.08	0.02	0.009
Glu	1.28	1.33	0.21	0.853	0.88	0.51	0.14	0.079
Gly	2.15	2.03	0.46	0.811	1.81	0.87	0.19	0.009
Ser	0.75	0.66	0.11	0.442	0.42	0.16	0.05	0.006
Tyr	0.37	0.31	0.06	0.311	0.23	0.08	0.03	0.020
Total	6.80	6.62	1.09	0.883	4.81	2.31	0.55	0.012
All AA	12.26	11.34	1.89	0.665	8.15	3.82	0.94	0.012

exposed NH_2 group (González-Vega et al., 2011). Therefore, the reduction in Lys concentration in oven dried ileal digesta samples observed in these experiments supports the hypothesis that Maillard reaction took place. However, because oven drying resulted in reduction in the concentration of almost all AA, it is likely that other forms of AA damage also occurred.

The observation that oven drying reduced the concentration of most AA in ileal digesta from pigs fed diets containing SBM, DDGS, or an N-free diet indicate that the effect of drying method on the composition of ileal digesta is not related to a specific type of diet. Values for the ileal digestibility of AA are calculated by subtracting the AA concentration in ileal digesta from the amount ingested by the pig (Stein et al., 2007). Therefore, the reduced concentration of AA in oven dried samples resulted in greater calculated values for SID of AA in samples that were oven dried compared with samples that were lyophilized, which has the consequence that oven drying will result in an overestimation of the SID of AA.

The increase in the concentration of Cr in oven dried ileal digesta samples in Exp. 2 compared with lyophilized samples indicates that volatile compounds disappeared from the samples during oven drying. It is, therefore, possible that the digestibility of energy or other nutrients also are affected by drying method, but additional research is needed to address this hypothesis.

The observation that in Exp. 2 there was reduced endogenous losses of AA from oven dried ileal digesta samples compared with lyophilized samples further indicates that AA disappeared or were changed during oven drying. As a result, the increased SID values that were calculated for oven dried samples in both experiments is a result of the combined effects of fewer AA in the ileal digesta from pigs fed the protein-containing ingredients and reduced endogenous losses calculated for oven dried samples.

CONCLUSION

Oven drying of ileal digesta samples from pigs does not result in losses of N, but reduces the concentration of AA, which results in reduced estimates for basal endogenous losses and greater calculated values for the SID of AA. This effect is not diet specific, and therefore, for AA digestibility experiments, ileal digesta samples from pigs need to be lyophilized prior to analysis rather than oven dried.

Conflict of interest statement. None declared.

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