Amino acid digestibility of corn distillers' dried grains with solubles with the addition of casein in pigs¹

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ABSTRACT: This experiment was conducted to determine the ileal digestibility of CP and AA in casein by regression analysis and to investigate the effects of casein in experimental diets on the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA in corn distillers' dried grains with solubles (DDGS) fed to pigs. Twenty barrows (initial BW = 50.5 ± 4.46 kg) surgically fitted with T-cannulas at the distal ileum were used. Eighteen pigs were assigned to a triplicate 6 × 3 incomplete Latin Square design with 6 diets and 3 periods. Two pigs were used as replacements. Three diets were prepared to contain 60, 100, or 140 g/kg casein to determine the ileal digestibility of CP and AA in casein by regression analysis, and 2 diets were prepared to contain either 480 g/kg DDGS or 308 g/kg DDGS and 60 g/kg casein. Casein and DDGS were the sources of nitrogen (N) in experimental diets. A N-free diet was prepared based on cornstarch and dextrose. Experimental periods consisted of 5 d of adaptation and 2 d of ileal digesta collection periods. The AID of CP and AA in casein linearly increased ($P < 0.01$) with increasing concentration of casein in the diets. The SID of indispensable AA in casein

ranged from 94.7% (SEM = 1.16) for Ile in pigs fed the diet containing 60 g/kg casein to 103.3% (SEM = 2.21) for Arg in pigs fed the diet containing 100 g/kg casein. Except for Arg, pigs fed the diet containing DDGS and casein had greater ($P < 0.001$) SID of CP and indispensable AA than those fed the diet containing DDGS without casein. The ileal digestibility of indispensable AA in casein determined by regression analysis ranged from 96.8% (SE = 3.14) for Thr to 103.1% (SE = 4.40) for Arg. The AID and SID of CP and AA for DDGS in the diet containing casein were calculated by difference method using the ileal digestibility of CP and AA in casein determined by regression analysis. The AID of Lys for DDGS in the diet containing casein was greater ($P = 0.035$) than those without casein; however, the AID of CP and the other indispensable AA, except for Arg, Phe, and Trp, for DDGS in the diet containing casein was less ($P < 0.05$) than those without casein. The SID of Lys and Phe for DDGS in the diet containing casein was greater ($P < 0.05$) than those without casein. In conclusion, improved AA composition in semi-purified experimental diets may affect the SID of AA in low-quality protein ingredients.

Key words: amino acid, casein, digestibility, distillers' dried grains with solubles, protein, swine

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INTRODUCTION

Most of previous studies to determine the digestibility of CP and AA in feed ingredients have been conducted using pigs fed semi-purified diets containing feed ingredients of interest as the sole source of nitrogen (N). However, there may

be AA deficiency in semi-purified diets because the concentration of AA in most of feed ingredients, especially for cereal grains and their byproducts, is not sufficient or balanced to maintain the normal physiological condition of pigs. Although this methodology is straightforward, impaired condition of pigs caused by feeding diets containing poor quality of AA composition may cause variation in the determined digestibility of CP and AA.

Corn distillers' dried grains with solubles (DDGS) has been widely used in swine diets, and many studies have been conducted to determine the standardized ileal digestibility (SID) of CP and AA in DDGS (Urriola et al., 2009; Kim et al., 2012; Adeola and Ragland, 2016). However, most of previous studies used semi-purified diets containing DDGS as the sole source of N, which could not provide adequate amount of AA for normal physiological function.

Casein extracted from bovine milk has been considered as a high-quality protein because of its greater concentration of digestible CP and AA (Eklund et al., 2008; Cervantes-Pahm and Stein, 2010). Therefore, the addition of casein into experimental diets containing poor-quality protein can increase the quality of CP and AA in diets and reduce malnutrition of pigs. However, to the best of our knowledge, the effects of the addition of casein into semi-purified experimental diets on digestibility of CP and AA in feed ingredient have not been addressed. Therefore, the objective of this experiment was to determine the ileal digestibility of CP and AA in casein by regression analysis and to investigate the effects of addition of casein in experimental diets on the apparent ileal digestibility (AID) and SID of CP and AA in DDGS fed to growing pigs.

MATERIALS AND METHODS

Experimental protocols with animals were reviewed and approved by the Purdue University Animal Care and Use Committee.

Animals, Housing, and Experimental Design

Twenty crossbred barrows with an initial BW of 50.5 ± 4.46 kg were used. Pigs were surgically fitted with T-cannulas at the distal ileum described by Dilger et al. (2004) when the mean BW of pigs was approximately 20 kg and used for two digestibility trials before the current experiment. Following 2 digestion studies, pigs were fed a normal corn-soybean meal (SBM)-based diet for 13 d before

the initiation of the current experiment. Pigs were individually housed in metabolism crates located in an environmentally controlled room. At the beginning of the experiment, 18 pigs were divided into 3 blocks based on BW and assigned to a triplicate 6×3 incomplete Latin Square design with 6 dietary treatments and 3 periods using a spreadsheet program (Kim and Kim, 2010). Two pigs, the heaviest and lightest among 20 pigs, were used as replacements and fed randomly assigned dietary treatments to achieve 10 observations per dietary treatment.

Experimental Diets, Feeding, and Sample Collection

Casein, obtained from a local supplier, and DDGS, purchased from a local ethanol plant (Iroquois Bio-energy Company, Rensselaer, IN; Table 1), were used as the sources of N in experimental diets. Three cornstarch-dextrose-based purified diets were formulated to contain 60, 100, or 140 g/kg casein (Table 2). To determine the effects of casein on the AID and SID of CP and AA in DDGS, two isonitrogenous semi-purified diets were prepared to contain either 480 g/kg DDGS or 308 g/kg DDGS and 60 g/kg casein. A N-free diet was prepared based on cornstarch and dextrose to determine the basal ileal endogenous losses of CP and AA in pigs. Diets were formulated to meet or exceed the vitamin and mineral requirement estimates of pigs with the BW ranged from 50 to 75 kg (National Research Council [NRC], 2012). A 5 g/kg chromic oxide was added in all the diets as an indigestible marker.

Feeding, collection of ileal digesta, and sample processing were as previously described in Park et al. (2017).

Chemical Analysis

Casein, DDGS, experimental diets, and lyophilized ileal digesta samples were finely ground using a coffee grinder. Ground ingredients, diets, and ileal digesta samples were dried at 105°C for 24 h in a forced-air drying oven (Precision Scientific Co., Chicago, IL) to measure the DM concentration (method 934.01; Association of Official Analytical Chemists [AOAC], 2006). Ingredients, diets, and ileal digesta samples were analyzed for N by the combustion method (TruMac N, LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP concentration was calculated by multiplying 6.25 by the concentration of N. The analysis of AA was conducted by the University

Table 1. Analyzed chemical composition of casein and corn distillers' dried grains with solubles (DDGS), g/kg as-fed basis^{1,2}

Item	Ingredient	
	Casein	DDGS
DM	923	874
GE, kcal/kg	5,419	4,089
CP (N × 6.25)	880	311
Ether extract	3.6	25.2
Crude fiber	–	35.6
Ash	12.6	39.6
NDF	–	122
ADF	–	74.5
Indispensable AA		
Arg	26.9	19.7
His	25.3	8.1
Ile	50.2	14.3
Leu	87.4	28.2
Lys	69.2	16.2
Met	27.7	5.0
Phe	46.1	16.0
Thr	39.2	11.8
Trp	14.0	3.7
Val	60.4	15.4
Dispensable AA		
Ala	26.1	16.0
Asp	60.3	30.4
Cys	3.3	5.0
Glu	180.9	52.3
Gly	15.9	13.3
Pro	107.0	18.4
Ser	40.0	13.1
Tyr	50.1	11.2

¹Approximately 1 kg subsamples were collected for analyses.

²Mean values of duplicate analyses except for ether extract ($n = 6$) in DDGS and AA ($n = 1$) in casein.

of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Before the analysis for AA concentration, ingredients, diets, and ileal digesta samples were hydrolyzed in 6 M HCl (or BaOH for the analysis of Trp) at 110°C for 24 h under N atmosphere. For the analysis of Met and Cys, performic acid oxidation was conducted before acid hydrolysis. The concentration of AA in hydrolyzed samples was measured by HPLC after postcolumn derivatization (AOAC, 2006; method 982.30 E (a, b, c)). The concentration of GE in casein and DDGS was analyzed by an isoperibol bomb calorimeter (Model 6200, Parr Instrument Co., Moline, IL). Casein and DDGS were analyzed for ether extract (method 920.39 (A); AOAC, 2006) and ash (method 942.05; AOAC, 2006). The concentration of crude fiber (method 978.10; AOAC, 2006), NDF (Van Soest et al.,

1991), and ADF (method 973.18 (AD); AOAC, 2006) was analyzed by a fiber analyzer (Ankom 200 Fiber Analyzer, Ankom Technology, Macedon, NY). The concentration of Cr in experimental diets and ileal digesta samples was measured by a spectrophotometer at 450 nm (Spectronic 21D; Milton Roy Co., Rochester, NY) after the wet digestion in nitric acid and 70% perchloric acid (Fenton and Fenton, 1979).

Calculation

The AID and SID (%) of CP and AA in casein, DDGS, and diet containing DDGS and casein were calculated by the concentration of Cr and CP or AA in experimental diets and ileal digesta samples as described by Park et al. (2017). The concentration of apparent ileal digestible CP and AA (g/kg DMI) was regressed against the concentration of CP and AA (g/kg DMI) in diets to determine the ileal digestibility (%) of CP and AA in casein using the model suggested in Akinmusire and Adeola (2009):

$$AA_D = (ID \times AA_I) - EL,$$

where AA_D represents the concentration of apparent ileal digestible CP or AA, which was calculated by multiplying AID values and the concentration of CP or AA in diets; ID represents the coefficient of ileal digestibility, which is the slope of the regression model; AA_I represents the concentration of CP or AA in diets; EL represents the endogenous losses of CP or AA as the y-intercept of the regression model.

The AID and SID of CP and AA for DDGS in the diet containing casein were calculated using the difference method described by Kong and Adeola (2014) using the following equation:

$$AID_{DG} = ID_{CAS} + [(AID_{Diet} - ID_{CAS}) / P_{DG}],$$

where AID_{DG} and AID_{Diet} represent the AID of CP or AA (%) in DDGS and the diet containing DDGS and casein, respectively; ID_{CAS} represents the ileal digestibility (%) of CP or AA in casein determined by regression analysis; P_{DG} represents the proportion of CP or AA in diet contributed from DDGS, which was calculated as the concentration of CP or AA contributed from DDGS divided by the concentration of CP or AA in the diet containing DDGS and casein. The SID of CP and AA for DDGS in the diet containing casein was calculated using the same equation by replacing AID with SID.

Table 2. Ingredient composition of experimental diets, g/kg as-fed basis

Item	Casein, g/kg: DDGS, g/kg:	Diet ¹					NFD
		60	100	140	–	60	
Cornstarch		710.1	670.1	630.1	340.6	452.6	768.6
Casein		60.0	100.0	140.0	0.0	60.0	0.0
DDGS		0.0	0.0	0.0	480.0	308.0	0.0
Dextrose		100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil		30.0	30.0	30.0	30.0	30.0	30.0
Cellulose ²		40.0	40.0	40.0	0.0	0.0	40.0
Limestone		10.5	10.5	10.5	13.5	13.5	9.5
Monocalcium phosphate		12.5	12.5	12.5	4.0	4.0	15.0
Salt		4.0	4.0	4.0	4.0	4.0	4.0
Potassium carbonate		4.0	4.0	4.0	0.0	0.0	4.0
Magnesium oxide		1.0	1.0	1.0	0.0	0.0	1.0
Vitamin premix ³		1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix ⁴		0.9	0.9	0.9	0.9	0.9	0.9
Selenium premix ⁵		0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide premix ⁶		25.0	25.0	25.0	25.0	25.0	25.0
Total		1,000	1,000	1,000	1,000	1,000	1,000

¹DDGS = corn distillers' dried grains with solubles; NFD = nitrogen-free diet.

²Solka-Floc 40 FCC (International Fiber Corporation, Urbana, OH).

³Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D₃, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; D-pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B₁₂, 0.02 mg.

⁴Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

⁵Provided 0.3 mg Se/kg of complete diet.

⁶Provided 5 g chromic oxide/kg of complete diet.

Statistical Analysis

Data for the AID and SID of CP and AA in experimental diets were analyzed by MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included dietary treatment as a fixed variable and block, period, and animal within block as random variables. Two pigs for replacement were considered as another block and included in data analysis. Orthogonal polynomial contrasts were conducted to test the linear and quadratic effects of casein and to compare the AID and SID values for DDGS and diet containing DDGS and casein. The REG procedure of SAS was used for the regression analysis to estimate the ileal digestibility of CP and AA in casein. In the model, the concentration of CP or AA in the diets (g/kg DMI) was used as the independent variable, and the concentration of apparent ileal digestible CP or AA (g/kg DMI) was used as the dependent variable. The ileal digestibility (%) of CP and AA was calculated from the estimated parameters for the slopes multiplied by 100. Data for the AID and SID of CP and AA in DDGS from pigs fed diet containing DDGS with or without the addition of casein were analyzed by two-sample, two-tailed *t*-test by TTEST procedure of SAS. In all statistical analyses, experimental unit was the pig, and the significance was declared at an α level of 0.05.

RESULTS

All pigs were in good condition during the experimental periods. Due to the limited amount of the diet containing 100 g/kg casein, one pig was excluded from the last experimental period. Therefore, there were 9 observations for the diet containing 100 g/kg of casein.

Chemical Analysis

The analyzed CP concentration was 880 g/kg for casein and 311 g/kg for DDGS on an as-fed basis (Table 1). The analyzed concentration of ether extract in DDGS was 25.2 g/kg as-fed basis. The analyzed CP and AA concentrations in experimental diets were comparable with the values calculated from the concentrations of CP and AA in casein and DDGS (Table 3).

Digestibility of CP and AA in Experimental Diets

The AID of CP and AA in casein linearly increased ($P < 0.01$) with increasing concentration of casein in experimental diets (Table 4). However, there was no quadratic effect of graded concentration of casein on the AID of CP and AA. Pigs fed the diet containing DDGS and casein had greater

Table 3. Analyzed concentration of CP and AA in experimental diets, g/kg as-fed basis

Item	Casein, g/kg: DDGS, g/kg:	Diet ¹					NFD
		60	100	140	–	60	
CP (N × 6.25)		55.9	86.8	123.5	133.0	136.5	7.52
Indispensable AA							
Arg		1.60	2.30	3.30	5.40	5.00	0.10
His		1.60	2.40	3.50	3.40	3.60	0.00
Ile		3.40	5.10	7.60	5.35	6.65	0.10
Leu		5.80	8.70	12.40	14.80	14.85	0.20
Lys		4.60	6.80	9.80	3.95	6.65	0.10
Met		1.70	2.50	3.60	2.35	3.15	0.10
Phe		3.10	4.50	6.40	6.25	6.85	0.10
Thr		2.60	3.90	5.50	4.90	5.55	0.10
Trp		0.80	1.20	1.60	1.00	1.25	0.00
Val		4.00	5.90	8.50	6.55	7.80	0.20
Dispensable AA							
Ala		1.80	2.70	3.80	9.10	7.55	0.20
Asp		4.00	6.00	8.50	8.70	9.30	0.20
Cys		0.20	0.30	0.50	2.50	1.90	0.10
Glu		12.70	19.10	26.90	19.80	24.10	0.70
Gly		1.10	1.60	2.30	5.55	4.60	0.10
Pro		6.70	10.30	14.80	9.85	12.50	0.10
Ser		2.90	4.30	6.00	5.35	5.95	0.10
Tyr		1.70	2.40	3.30	3.85	4.80	0.10

¹DDGS = corn distillers' dried grains with solubles; NFD = nitrogen-free diet.

($P < 0.001$) AID of CP and indispensable AA, except for Arg, compared with those fed the diet containing DDGS without casein.

The SID of CP and AA in casein was not affected by the concentration of casein in experimental diets (Table 5). The SID of indispensable AA in casein ranged from 94.7% for Ile in pigs fed the diet containing 60 g/kg casein to 103.3% for Arg in pigs fed the diet containing 100 g/kg casein. Similar to the AID, the SID of CP and indispensable AA, except for Arg, in the diet containing DDGS and casein was greater ($P < 0.001$) than those in the diet containing DDGS without casein.

Ileal Digestibility of CP and AA in Casein Determined by Regression Analysis

In the regression analyses for CP and indispensable AA, the r^2 values ranged from 0.951 for Arg to 0.996 for Met (Table 6). In the model for CP, the ileal digestibility was 100.8%, and the endogenous loss was 15.5 g/kg DMI with an r^2 value of 0.975. The ileal digestibility of indispensable AA ranged from 96.8% for Thr to 103.1% for Arg. The endogenous losses of indispensable AA ranged from 0.088 g/kg DMI for Met to 0.718 g/kg DMI for Lys. The ileal digestibility of dispensable AA ranged from 95.5% for Cys to 117.4% for Gly.

Effects of Casein on Digestibility of CP and AA in DDGS

Pigs fed the DDGS diet containing casein had greater ($P = 0.035$) AID of Lys than those without casein (Table 7). However, the AID of CP, His, Ile, Leu, Met, Thr, and Val in DDGS without casein was greater ($P < 0.05$) than in DDGS diet containing casein. The AID of Ala, Glu, and Ser for DDGS diet containing casein was less ($P < 0.05$) than those without casein. The SID of Lys and Phe for DDGS diet containing casein was greater ($P < 0.05$) than those without casein. The SID of Asp, Gly, and Pro for DDGS diet containing casein was greater ($P < 0.05$) than those without casein.

DISCUSSION

The analyzed concentration of CP and AA in casein agreed with the values in previous reports (Cervantes-Pahm and Stein, 2010; NRC, 2012; Brestenský et al., 2017). The analyzed concentrations of CP and AA in DDGS were also within the range of values suggested in previous studies (Urriola et al., 2009; Kim et al., 2012; Kiarie et al., 2013; Xue et al., 2014), except for Arg and Lys, which were greater than the previously reported values. In addition, the concentrations of GE and

Table 4. Apparent ileal digestibility (%) of CP and AA in experimental diets fed to pigs¹

Item	Casein, g/kg:	Diet ²					SEM	P-value ³		
	DDGS, g/kg:	60	100	140	–	60		Linear	Quadratic	D vs. D + C
CP		75.2	85.5	89.1	64.3	74.9	1.83	<0.001	0.155	<0.001
Indispensable AA										
Arg		71.5	82.5	87.9	75.9	80.0	2.21	<0.001	0.314	0.179
His		88.4	93.3	94.5	72.7	80.5	1.08	<0.001	0.179	<0.001
Ile		86.6	92.3	93.7	71.9	81.3	1.16	<0.001	0.099	<0.001
Leu		90.6	93.8	95.1	82.1	87.0	0.92	<0.001	0.364	<0.001
Lys		85.4	90.6	92.6	33.6	68.4	1.75	0.002	0.439	<0.001
Met		94.3	96.6	96.8	76.3	86.9	0.70	0.002	0.137	<0.001
Phe		89.9	93.1	94.5	75.0	83.6	1.08	0.002	0.452	<0.001
Thr		77.9	84.4	87.7	56.6	69.7	1.74	<0.001	0.472	<0.001
Trp		84.9	90.5	91.5	68.3	80.5	1.68	0.006	0.282	<0.001
Val		85.2	90.5	92.2	66.7	77.4	1.16	<0.001	0.197	<0.001
Dispensable AA										
Ala		69.6	81.0	85.6	75.3	78.1	2.45	<0.001	0.256	0.399
Asp		81.3	88.7	90.3	59.3	71.5	1.82	<0.001	0.193	<0.001
Cys		24.7	47.8	67.0	63.7	65.9	6.44	<0.001	0.803	0.802
Glu		89.6	93.9	94.9	77.7	85.3	0.89	<0.001	0.141	<0.001
Gly		6.0	52.0	65.0	55.1	60.5	8.57	<0.001	0.106	0.627
Pro		53.9	77.4	84.1	63.3	79.9	4.94	<0.001	0.135	0.009
Ser		78.6	85.7	89.1	65.9	73.6	1.33	<0.001	0.286	<0.001
Tyr		86.8	90.9	92.4	70.7	83.3	1.42	0.003	0.436	<0.001

¹Each least squares mean represents 10 observations except for the diet containing 100 g/kg casein (9 observations).

²DDGS = corn distillers' dried grains with solubles.

³Linear = linear effect of casein; Quadratic = quadratic effect of casein; D vs. D + C = contrast between diet containing DDGS and that containing DDGS plus casein.

ether extract in DDGS were less than the previously reported values. This difference may be due to the differences in the sources of corn used to produce DDGS, the ratio between distillers' dried grains to added solubles, or the time for the fermentation of starch (Spiehs et al., 2002).

The AID of CP and AA in casein linearly, but not quadratically, increased with increasing concentration of casein in experimental diets. Mariscal-Landín and Reis de Souza (2006) reported that the AID of Arg, His, Phe, Thr, Cys, and Pro linearly increased, but the AID of remaining AA, except for Gly, quadratically increased with increasing concentration of casein in 4 experimental diets from 55.3 to 221.3 g/kg. Fan et al. (1994) reported that the AID of CP and AA in SBM quadratically increased and reached at the plateau as the concentration of CP in 6 experimental diets increases from 40 to 240 g/kg. Therefore, it may be concluded that the range of CP and AA in experimental diets used in the current experiment was not enough to observe the quadratic effects on the AID of CP and AA; however, it was appropriate to conduct the regression analysis between dietary and digestible CP and AA concentrations (Eklund et al., 2008).

The SID of CP and AA in casein was similar with the values reported in Chung and Baker (1992) and Cervantes-Pahm and Stein (2010).

Regression analysis has been used to evaluate the ileal digestibility of CP and AA (Mariscal-Landín and Reis de Souza, 2006; Eklund et al., 2008; Reis de Souza et al., 2013) as well as to evaluate the digestibility of other nutrients (Johnston et al., 2013; Kim et al., 2013; Zhai and Adeola, 2013). However, the descriptive term for the digestibility (i.e., standardized or true) was not consistent although digestibility of nutrients was determined by the same regression analysis. It has been reported that the basal ileal endogenous losses of CP and AA in pigs can be estimated by regression analysis and that true ileal digestibility can be determined by correcting the total ileal endogenous losses of CP and AA, which can be determined by homoarginine method or isotope tracer dilution method, from the AID of CP and AA (Stein et al., 2007; Adeola et al., 2016). Therefore, the digestibility determined by regression analysis seems to be considered as the standardized digestibility; however, it may also be considered as the true ileal digestibility because the endogenous losses determined

Table 5. Standardized ileal digestibility (%) of CP and AA in experimental diets fed to pigs^{1,2}

Item	Casein, g/kg: DDGS, g/kg:	Diet ³					SEM	P-value ⁴		
		60	100	140	–	60		Linear	Quadratic	D vs. D + C
CP		98.0	100.2	99.4	73.8	84.2	1.83	0.591	0.518	<0.001
Indispensable AA										
Arg		101.3	103.3	102.3	84.6	89.9	2.21	0.737	0.598	0.085
His		97.7	99.5	98.7	76.8	84.7	1.08	0.479	0.345	<0.001
Ile		94.7	97.8	97.3	76.7	85.4	1.16	0.072	0.184	<0.001
Leu		98.1	98.8	98.6	84.9	89.9	0.92	0.667	0.637	<0.001
Lys		96.6	98.1	97.8	46.2	76.3	1.75	0.574	0.639	<0.001
Met		98.6	99.6	98.9	79.4	89.2	0.70	0.711	0.239	<0.001
Phe		98.8	99.3	98.8	79.3	87.7	1.08	0.973	0.691	<0.001
Thr		96.4	96.7	96.4	66.3	78.5	1.74	0.989	0.879	<0.001
Trp		95.8	97.8	96.9	76.9	87.2	1.68	0.616	0.505	<0.001
Val		96.8	98.4	97.6	73.5	83.4	1.16	0.562	0.397	<0.001
Dispensable AA										
Ala		97.3	99.5	98.7	80.6	84.7	2.45	0.678	0.611	0.213
Asp		97.2	99.3	97.7	66.5	78.5	1.82	0.815	0.400	<0.001
Cys		92.0	92.8	93.8	69.3	73.4	6.44	0.832	0.990	0.638
Glu		95.6	97.9	97.7	81.5	88.6	0.89	0.076	0.269	<0.001
Gly		104.5	120.0	112.1	73.8	84.1	8.57	0.503	0.249	0.365
Pro		107.4	112.3	108.3	97.7	108.3	4.94	0.890	0.421	0.086
Ser		92.9	95.3	96.0	73.4	80.7	1.33	0.091	0.601	<0.001
Tyr		98.3	99.1	98.3	76.2	87.8	1.42	0.996	0.647	<0.001

¹Each least squares mean represents 10 observations except for the diet containing 100 g/kg casein (9 observations).

²Values for the standardized ileal digestibility were calculated by correcting basal ileal endogenous losses of CP and AA (g/kg DMI) in pigs fed nitrogen-free diet from values for the apparent ileal digestibility: CP, 14.1; Arg, 0.529; His, 0.165; Ile, 0.306; Leu, 0.481; Lys, 0.568; Met, 0.082; Phe, 0.308; Thr, 0.533; Trp, 0.096; Val, 0.514; Ala, 0.553; Asp, 0.704; Cys, 0.150; Glu, 0.846; Gly, 1.204; Pro, 3.984; Ser, 0.460; Tyr, 0.218.

³DDGS = corn distillers' dried grains with solubles.

⁴Linear = linear effect of casein; Quadratic = quadratic effect of casein; D vs. D + C = contrast between diet containing DDGS and that containing DDGS plus casein.

by regression analysis depend on feed ingredients as well as the concentration of a feed ingredient in experimental diets, which may be considered as the feed ingredient-specific endogenous losses. Therefore, to prevent the confusion with previous studies and with the various results in the current experiment, neither “standardized” nor “true” was used to describe the digestibility values determined by regression analysis in the current experiment. Likewise, neither “basal” nor “total” was used to describe the endogenous losses determined by the extrapolation to y-intercept.

Eklund et al. (2008) reported that if the range of CP and AA in experimental diets is above the plateau responses of AID values, the ileal digestibility determined by regression analysis represents the AID, and therefore, the range of CP and AA needs to be close to the origin of the coordinate. In the current experiment, although it was not clear whether the range of CP and AA was close enough to the origin of the coordinate, the range of CP and AA was below the plateau responses of

AID values. The ileal digestibility of CP and AA in casein determined by regression analysis was comparable with the values reported in previous studies using growing pigs (Jørgensen and Gabert, 2001; Mariscal-Landín and Reis de Souza, 2006). The endogenous losses of CP and AA determined by y-intercept were also comparable with the previous reports (Jørgensen and Gabert, 2001; Mariscal-Landín and Reis de Souza, 2006) except for Lys, which was greater in the current experiment. The reason for this discrepancy remains unclear; however, it may be due to the differences in the ingredient composition of experimental diets or in the sources of casein used.

Deficiency or imbalance of CP and AA in semi-purified diets has been concerned in previous studies. Liu and Adeola (2016), however, reported that the total tract digestibility of P in SBM determined by regression analysis was not affected by the addition of casein into semi-purified diets containing SBM as the sole source of P. Pedersen et al. (2007) also reported that the DE and ME contents

Table 6. Ileal digestibility (%) and endogenous losses (g/kg DMI) of CP and AA in pigs fed diets containing graded concentration of casein determined by regression analysis¹

Item	Regression analysis parameters ²				Statistical parameters		
	Slope		Intercept		RMSE ³	r ²	P-value
	Digestibility	SE	Endogenous loss	SE			
CP	100.8	3.07	15.5	3.18	5.197	0.975	<0.001
Indispensable AA							
Arg	103.1	4.40	0.548	0.1226	0.188	0.951	<0.001
His	99.9	1.77	0.196	0.0516	0.084	0.991	<0.001
Ile	99.5	1.79	0.464	0.1125	0.189	0.991	<0.001
Leu	99.1	1.61	0.539	0.1682	0.266	0.993	<0.001
Lys	99.7	2.06	0.718	0.1693	0.268	0.988	<0.001
Met	99.2	1.13	0.088	0.0343	0.054	0.996	<0.001
Phe	98.9	1.89	0.304	0.1021	0.156	0.990	<0.001
Thr	96.8	3.14	0.546	0.1460	0.228	0.971	<0.001
Trp	98.6	3.17	0.117	0.0439	0.063	0.972	<0.001
Val	98.9	2.08	0.589	0.1482	0.234	0.988	<0.001
Dispensable AA							
Ala	99.7	4.82	0.590	0.1550	0.241	0.938	<0.001
Asp	98.6	3.17	0.737	0.2272	0.357	0.972	<0.001
Cys	95.5	9.10	0.158	0.0362	0.069	0.796	<0.001
Glu	99.7	1.51	1.369	0.3435	0.536	0.994	<0.001
Gly	117.4	15.04	1.293	0.2912	0.453	0.681	<0.001
Pro	112.4	7.70	4.265	0.9526	1.559	0.884	<0.001
Ser	99.3	2.59	0.661	0.1320	0.201	0.981	<0.001
Tyr	98.5	2.76	0.218	0.0784	0.111	0.979	<0.001

¹Regression analysis was conducted with the concentration of CP or AA (g/kg DMI) and the concentration of apparent ileal digestible CP or AA (g/kg DMI) in diets containing 60, 100, and 140 g/kg casein (29 observations).

²Ileal digestibility was estimated by multiplying the slope by 100, and the endogenous loss was estimated by the absolute value of y-intercept.

³RMSE = root mean square error.

in 2 sources of corn were not affected by the addition of crystalline AA into diets containing corn as the sole source of energy. In the study to determine the ileal digestibility of CP and AA, a mixture of crystalline AA was provided to pigs during the adaptation period before collecting ileal digesta samples (Pedersen et al., 2007; Strang et al., 2017). Similar approach has been used in determination of basal ileal endogenous losses of CP and AA in pigs by adding low concentration of casein into N-free diet to prevent the abnormal physiological condition caused by AA deficiency (Adeola et al., 2016).

The AID and SID of CP and AA in DDGS from pigs fed the diet containing DDGS without casein agreed with the previously reported values (Urriola et al., 2009; Kim et al., 2012; Kiarie et al., 2013; Xue et al., 2014) except for Lys, which was lower in the current experiment. It has been reported that the variation in the digestibility of Lys among sources of DDGS is greater than the other AA due to the Maillard reaction caused by heat damage (Stein and Shurson, 2009; Kim et al., 2012; Almeida et al., 2013). Therefore, it may be concluded that DDGS used in the current experiment may be treated at

high temperatures during the process, and thus bio-availability of Lys decreased.

Digestibility of CP and AA in pigs may increase from an upsurge in digestive enzyme secretion or increased expression of peptide or AA transporters in the small intestine or both. Morales et al. (2017) reported that the activity of trypsin and chymotrypsin increased in the duodenum and jejunum of pigs fed the diet containing 281 g/kg CP compared with pigs fed the diet containing 192 g/kg CP with the supplementation of crystalline AA. However, in the current experiment, two diets containing DDGS with or without casein were formulated to contain the same concentration of CP. Moreover, Valette et al. (1992) reported that the activity of chymotrypsin in pigs fed the diet containing casein was less than that in pigs fed the diet containing rapeseed-concentrate protein, although the diets were prepared to contain the same concentration of CP as 120 g/kg from casein or rapeseed-concentrate protein as the sole source of N. Therefore, it may be concluded that the pancreatic enzyme activity in pigs used in the current experiment was not affected by the diets. Nevertheless, the SID of Lys

Table 7. Apparent and standardized ileal digestibility (%) of CP and AA in corn distillers' dried grains with solubles (DDGS) with or without the addition of casein fed to pigs^{1,2}

Item	Apparent ileal digestibility				Standardized ileal digestibility			
	DDGS		SE	P-value	DDGS		SE	P-value
	– Casein	+ Casein			– Casein	+ Casein		
CP	64.3	61.2	1.28	0.024	73.8	75.6	1.28	0.171
Indispensable AA								
Arg	75.9	74.5	1.21	0.278	84.6	87.1	1.21	0.050
His	72.8	68.9	1.18	0.004	76.9	75.5	1.18	0.267
Ile	71.9	69.0	1.21	0.026	76.8	76.0	1.21	0.516
Leu	82.1	79.7	0.76	0.005	84.9	84.4	0.76	0.520
Lys	33.6	41.6	3.53	0.035	46.2	56.5	3.53	0.009
Met	76.4	73.6	0.78	0.002	79.4	78.4	0.78	0.222
Phe	75.0	75.2	1.03	0.853	79.3	81.6	1.03	0.044
Thr	56.6	51.7	1.88	0.019	66.3	66.5	1.88	0.884
Trp	68.4	67.6	2.06	0.722	76.9	79.2	2.06	0.287
Val	66.7	61.4	1.45	0.002	73.4	72.0	1.45	0.332
Dispensable AA								
Ala	75.3	71.0	0.92	<0.001	80.6	79.8	0.92	0.403
Asp	59.4	61.3	1.84	0.328	66.6	70.9	1.84	0.030
Cys	63.8	61.9	1.64	0.259	69.3	70.4	1.64	0.524
Glu	77.8	75.5	0.89	0.022	81.5	81.0	0.89	0.583
Gly	55.1	51.3	2.53	0.157	73.8	80.3	2.53	0.019
Pro	62.9	56.8	4.68	0.212	97.3	117.1	4.68	<0.001
Ser	65.8	58.0	1.83	<0.001	73.3	69.5	1.83	0.050
Tyr	70.8	69.9	1.40	0.547	76.3	78.5	1.40	0.127

¹Each mean represents 10 observations.

²Digestibility of CP and AA in the diet containing DDGS with the addition of casein at 60 g/kg was calculated using the difference method with the ileal digestibility of CP and AA in casein determined by regression analysis.

and Phe increased when casein was added in the experimental diet. Therefore, it may be speculated that the expression or activity of transporters of peptide or AA in the small intestine increased due to the increased protein quality of the diet containing DDGS and casein. However, the evidence to explain the increased expression or activity of peptide or AA transporters is limited. Gilbert et al. (2008) reported that the mRNA expression of transporters in broiler chickens was affected by the quality of protein sources in diets with the results showing that the mRNA expression of intestinal peptide transporter 1 (PepT1) and Na⁺-independent cationic and zwitterionic amino acid transporter (b⁰,+AT) in the small intestine of broiler chickens fed the diet containing 467 g/kg SBM was greater than those fed the diet containing 342 g/kg corn gluten meal. Because Lys is one of the main substrates for b⁰,+AT (Bröer, 2008), it may partially explain the increased digestibility of Lys in the current experiment. However, because the substrates for PepT1 are di- and tripeptide (Herrera-Ruiz and Knipp, 2003), it remains unclear why the digestibility of Phe, but not all AA, increased. Further research is needed to verify the relationship between dietary

protein quality and the expression or activity of peptide or AA transporters.

The standardized ileal digestible AA concentration in all experimental diets in the current study except for the diet containing 140 g/kg casein was less than the suggested standardized ileal digestible AA requirement in NRC (2012). However, the SID of AA in diets containing 60 or 100 g/kg casein was not underestimated based on the results that there were no linear or quadratic effects of graded concentration of casein on the SID of AA in casein. Thus, the digestibility of AA from readily digestible protein was not affected by deficiency of AA.

In the current experiment, two diets containing DDGS with or without casein were formulated to contain the same concentration of CP to prevent the potential effect of dietary CP concentration on digestibility of CP and AA. However, when the digestibility of CP and AA in DDGS was calculated from pigs fed the diet containing DDGS and casein, the CP and AA contributed from casein were deducted by their digestibility values, which eventually decreased the concentration of CP and AA contributed from DDGS in the diet. Due to the decreased CP contributed from DDGS, the

AID of CP and several AA for DDGS in the diet containing casein decreased compared with those without casein. This result was consistent with previous reports which suggested that the AID of CP and AA increased with increasing concentration of dietary CP concentration (Fan et al., 1994) due to the decreased proportional contribution of endogenous losses of CP and AA in ileal digesta (Kong and Adeola, 2014). However, it remains unclear whether increased SID of Lys and Phe for DDGS in the diet containing casein was due to the addition of casein or decreased contribution of CP from DDGS. Although Adebiyi et al. (2015) reported that the dietary concentration of CP as 150 or 190 g/kg did not affect the AID and SID of CP and AA in pigs fed the diets containing corn and DDGS, further research is needed to clarify whether the supplementation of casein increases the digestibility of AA in DDGS regardless of dietary CP concentration.

In conclusion, the ileal digestibility of CP and AA determined by regression analysis was close to 100%, which indicated that the CP and AA in casein were highly digestible in the small intestine of pigs. The addition of casein in DDGS diet increased the SID of Lys and Phe compared with values for DDGS diet without casein. Therefore, improved AA composition in the semi-purified experimental diet may increase the SID of several AA in test ingredients with low quality of AA composition.

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