Additivity of apparent and standardized ileal digestibility of amino acid determined by chromic oxide and titanium dioxide in mixed diets containing wheat and multiple protein sources fed to growing pigs

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ABSTRACT: The aim of this study was to investigate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in mixed diets containing wheat, canola meal (CM), meat and bone meal (MBM), and sorghum distillers' dried grains with solubles (DDGS) fed to pigs with chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) as digestibility index markers. Four diets were prepared to contain wheat, CM, MBM, or DDGS as a sole source of nitrogen; three mixed diets were prepared to contain wheat, CM, and MBM; wheat, MBM, and DDGS; or wheat, CM, MBM, and DDGS; also, a nitrogen-free diet was prepared to estimate the basal ileal endogenous losses (BEL) of CP and AA. Both Cr_2O_3 and TiO_2 were incorporated at 5 g/kg into each diet. Sixteen barrows (initial body weight = 34.7 ± 0.6 kg) surgically fitted with T-cannulas at the distal ileum were allotted to a duplicate 8×4 Youden square design with eight experimental diets and four periods. During each 7-d period, the ileal digesta was collected for 2 d after a 5-d adaptation, and the AID and SID of CP and AA were determined using Cr_2O_3 or TiO_2 as marker. There was no interaction between diet and marker type for both AID and SID of CP and AA. Similar BEL, AID, and SID of CP and AA were

determined by Cr_2O_3 and TiO_2 . In the wheat–CM–MBM diet, the measured AID of CP and most AA determined with Cr_2O_3 or TiO_2 were not different from the predicted values, which was determined based on the measured AID values in wheat, CM, and MBM. In the wheat–MBM–DDGS diet, the measured and predicted AID of CP and seven indispensable AA differed ($P < 0.05$) using Cr_2O_3 as marker, and the measured and predicted AID of CP and four indispensable AA differed ($P < 0.05$) using TiO_2 as marker. The measured AID of CP and most indispensable AA were greater ($P < 0.05$) than predicted AID regardless of marker type for the wheat–CM–MBM–DDGS diet. The measured SID of CP and indispensable AA were consistent with the predicted values, except Lys in the wheat–CM–MBM diet using either marker and His in the wheat–MBM–DDGS diet with Cr_2O_3 as marker. In conclusion, more accurate prediction of ileal digestibility of CP and AA was achieved using SID rather than AID in mixed diets containing wheat, CM, MBM, and DDGS. The determination of endogenous loss, AID, and SID of CP and AA were not affected by marker type in this study. In addition, the additivity of AID and SID of CP and most indispensable AA in mixed diets was not affected by marker type.

Key words: additivity, chromic oxide, ileal digestibility, swine, titanium dioxide

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INTRODUCTION

The application of additive ileal digestibility of amino acids (AA) in feed ingredients is fundamental to match AA supply in diets to AA requirement for pigs (Jansman et al., 2002). The

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standardized ileal digestible AA is adopted to express both requirements for pigs and ingredient contents used in feed formulation (NRC, 2012). Compared with apparent ileal digestibility (AID) of AA, the standardized ileal digestibility (SID) accounts for basal ileal endogenous losses (BEL) of AA; therefore, SID is independent of dietary AA contents and more additive in mixed diets fed to pigs, especially for the diets containing low-protein cereal grain (Stein et al., 2007). Lack of additivity of AID has been reported in the mixed diets containing corn in pigs (Stein et al., 2005; Xue et al., 2014); however, the additivity of AID in the mixed diet containing wheat is still not clear. According to NRC (2012), the crude protein (CP) content in soft red wheat is 2.7% greater than yellow dent corn, and this difference in CP content might elicit a relatively large change of AID in ingredients (Fan et al., 1994), as well as the additivity of AID in mixed diets.

One of the general approaches to determine ileal digestibility of CP and AA is to include digestibility index marker in diets and collect representative ileal digesta from pigs fitted with simple T-cannulas (Nyachoti et al., 1997a; Gabert et al., 2001). Chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2) as two of the most common markers have been reported to affect the determination of ileal digestibility (Kavanagh et al., 2001; Wang et al., 2017), and this might relate to the variant recovery of these two markers in ileal digesta of pigs (Yin et al., 2000). Therefore, the choice of marker could affect the determination of ileal digestibility, consequently the additivity of ileal digestibility of CP and AA in mixed diets. In previous studies that investigated the assumption of additivity of AID and SID of CP and AA in mixed diets, Cr_2O_3 has been generally used as marker (Fan et al., 1993; Stein et al., 2005; Xue et al., 2014); however, there is a scarcity of data with TiO_2 as marker. Thus, the objective of this experiment was to test the null hypothesis that 1) additivity of AID of CP and AA in mixed diets containing wheat, CM, MBM, and DDGS is not different from SID; 2) the determination of AID, SID, and BEL of CP and AA as well as additivity of ileal digestibility is not affected by the choice of marker.

MATERIALS AND METHODS

All animal procedures used in this study were approved by the Purdue University Animal Care and Use Committee.

Dietary Treatments

Eight experimental diets including one nitrogen-free diet (NFD) were formulated (Table 1). Four semipurified diets consisted one of wheat, canola meal (CM), meat and bone meal (MBM), or sorghum distillers' dried grains with solubles (DDGS) as the sole source of CP and AA to measure the AID and SID of CP and AA in test ingredients, which were used to determine the predicted AID and SID of CP and AA in mixed diets. Three mixed diets including wheat–CM–MBM, wheat–MBM–DDGS, and wheat–CM–MBM–DDGS diets were formulated to contain approximately 17% CP and measure the AID and SID of CP and AA in mixed diets. Wheat and MBM contents were same across the three mixed diets, but the CM and DDGS contents varied among the three mixed diets. All diets were formulated to meet or exceed the estimated vitamin and mineral requirements suggested in NRC (2012). Both Cr_2O_3 and TiO_2 were incorporated into experimental diets as the marker at the level of 5 g/kg, respectively.

Animals and Sample Collection

Sixteen barrows (initial body weight = 34.7 ± 0.6 kg) were surgically fitted with T-cannulas at the distal ileum as described by Dilger et al. (2004). Pigs were allotted to two blocks with body weight as the blocking factor and assigned to a duplicate 8×4 Youden square design with eight dietary treatments and four experimental periods. All pigs were individually housed in stainless steel crates (1.22×1.22 m) equipped with a feeder and a nipple drinker. Daily feed allowance was set as 4% of the initial smallest body weight in each block in each period, and pigs were fed at 0800 and 1700 h in two equally divided meals. Water was available at all time during the experiment. In each period, there were 5 d of adaptation and 2 d of ileal digesta collection. On day 6 and 7 of each experimental period, Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 10 mL of 10% formic acid was attached to the T-cannula with a rubber O-ring to collect ileal digesta from 0800 to 1700 h. The attached bags were inspected every 30 min and the filled bags were changed and stored at -20°C until further processing. The ileal digesta from each pig during each period were pooled, subsampled, and lyophilized.

Table 1. Ingredient composition of experimental diets, % as-fed basis¹

Item	NFD	Wheat	CM	MBM	DDGS	Wheat–CM–MBM	Wheat–MBM–DDGS	Wheat–CM–MBM–DDGS
Wheat	—	88.90	—	—	—	58.00	58.00	58.00
Corn starch	72.40	—	47.50	64.40	30.90	9.60	3.00	6.90
DDGS	—	—	—	—	58.50	—	24.40	10.00
CM	—	—	42.50	—	—	17.80	—	10.50
MBM	—	—	—	27.00	—	6.00	6.00	6.00
Dextrose	10.00	—	—	—	—	—	—	—
Cellulose ²	5.00	—	—	—	—	—	—	—
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate	2.80	1.50	0.70	—	0.70	—	—	—
Chromic oxide premix ³	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Titanium dioxide premix ⁴	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	0.60	1.00	0.70	—	1.30	—	—	—
Salt	0.40	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Potassium carbonate	0.40	—	—	—	—	—	—	—
Vitamin premix ⁵	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix ⁶	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Magnesium oxide	0.10	—	—	—	—	—	—	—
Selenium premix ⁷	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

¹NFD = nitrogen-free diet; CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles.

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

³A 5 g chromic oxide was added to 20 g corn starch to provide 5 g chromic oxide/kg of complete diet.

⁴A 5 g titanium dioxide was added to 20 g corn starch to provide 5 g titanium dioxide/kg of complete diet.

⁵Provided 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.

⁶Provided the following quantities per kilogram of complete diet: Cu (as copper chloride), 9 mg; Fe (as ferrous carbonate), 194 mg; I (as calcium iodate), 0.36 mg; Mn (as manganese oxide), 17 mg; Zn (as zinc oxide), 149 mg.

⁷Provided 0.3 mg Se/kg of complete diet.

Chemical Analysis

Ingredients, experimental diets, and ileal digesta samples were ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany). The concentrations of dry matter (DM) in ingredients, diets, and ileal digesta samples were measured by drying in a forced-air drying oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). In ingredients, ether extract was analyzed without acid hydrolysis [method 920.39 (A); AOAC, 2006], and acid detergent fiber [method 973.18 (AD); AOAC, 2006] and neutral detergent fiber (Van Soest et al., 1991) contents were analyzed by a fiber analyzer (ANKOM A2000 Fiber Analyzer, ANKOM Technology, Macedon, NY). Nitrogen contents in ingredients, diets, and ileal digesta samples were determined by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP content was calculated by multiplying 6.25 by the content of nitrogen. Amino acid analyses [method 982.30 E (a, b, c); AOAC, 2006] for the diets, ingredients, and ileal digesta samples

were performed by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). The concentrations of Cr and Ti were analyzed as described by Saha and Gilbreath (1991) and Myers et al. (2004), respectively, with modifications as described by Wang and Adeola (2018).

Calculations and Statistical Analysis

The measured AID, BEL, and SID of CP and AA were determined using the following equations (Park et al., 2017):

$$\text{AID}(\%) = \left(1 - \left[\frac{M_i}{M_o} \right] \times \left[\frac{\text{AA}_o}{\text{AA}_i} \right] \right) \times 100;$$

$$\text{BEL (g / kg DM intake)} = \text{AA}_o \times \left(\frac{M_i}{M_o} \right);$$

$$\text{SID}(\%) = \text{AID} + \left(\frac{\text{BEL}}{\text{AA}_i} \right) \times 100,$$

where M_i and M_o are marker concentration (g/kg DM) of diet and ileal digesta, respectively; AA_i and AA_o are the CP or AA concentration (g/kg DM) of diet and ileal digesta, respectively. The average BEL of CP and AA were calculated from the eight pigs received the NFD to determined SID from AID.

The predicted AID or SID of CP and AA in mixed diets were determined by the measured AID or SID of CP and AA in ingredients using the following equations (Xue et al., 2014):

$$AID_p(\%) = \frac{[(CP_w \times AID_w) + (CP_{CM} \times AID_{CM}) + (CP_{MBM} \times AID_{MBM}) + (CP_{DDGS} \times AID_{DDGS})]}{(CP_w + CP_{CM} + CP_{MBM} + CP_{DDGS})},$$

where AID_p is the predicted AID of CP in a mixed diet; CP_w , CP_{CM} , CP_{MBM} , and CP_{DDGS} are the concentrations (%) of CP in the mixed diets originating from wheat, CM, MBM, and DDGS, respectively, which were determined by multiplying the concentration of CP (%) in respective ingredient by the proportion (%) of the ingredient in mixed diet; AID_w , AID_{CM} , AID_{MBM} , and AID_{DDGS} are the measured AID (%) of CP in wheat, CM, MBM, and DDGS, respectively. The predicted AID of each AA in mixed diets was determined using the same equation by replacing CP with AA in the above equation. The predicted SID of CP or each AA in mixed diets was determined using the same equation with AID by replacing the AID with SID (%) of CP or each AA in wheat, CM, MBM, and DDGS. The differences between measured and predicted values of AID or SID of CP and AA in the mixed diets were determined with both Cr_2O_3 and TiO_2 as markers.

The data on AID and SID of CP and AA were analyzed using the MIXED procedure of statistical analysis system (SAS; SAS Inst. Inc., Cary, NC) as a split-plot arrangement, with dietary treatment as whole-plot factor and the marker type as split-plot factor. The model included diet, marker type, and the interaction between diet and marker type as fixed effects; and pig, period, and interactions between pig and period, pig and marker, and period and marker as random effects. The BEL data were analyzed using the MIXED procedure with marker type as fixed effect; and pig, period, and interaction between pig and marker as random effects. One-sample, two-tailed *t*-test was conducted by the TTEST procedure of SAS to test the null hypothesis that the difference between measured and predicted AID or SID of CP and AA in mixed diets is equal to 0. The ileal digestibility was underestimated when the difference between measured and

predicted values was significantly greater than 0 or was overestimated when the difference was significantly less than 0. The experimental unit was pig in all the analyses, and statistical significance was declared at $P < 0.05$.

RESULTS

All pigs were in good condition throughout the four periods of the experiment. Among the four feed ingredients used in this study, the concentration of CP ranged from 11.19% in wheat to 60.31% in MBM and similarly, the Lys concentration ranged from 0.36% in wheat to 2.73% in MBM (Table 2). Except for the NFD and wheat diet, the other experimental diets contained approximately 17% CP (Table 3).

There were no differences in the BEL of CP and all AA determined with Cr_2O_3 or TiO_2 in pigs fed the NFD (Table 4). There was no interaction between experimental diet and marker type for both AID and SID of CP and AA, however, the values of AID and SID of CP and AA determined by both markers are presented in Tables 5 and 6. The AID of CP, DM, and AA, and SID of CP and AA in each experimental diet were not affected by the choice of marker.

In the wheat–CM–MBM diet, the measured AID of His, Lys, Trp, and Tyr determined with either marker and AID of Thr, Asp, and Pro determined with TiO_2 were greater ($P < 0.05$) than predicted values (Table 7). In the wheat–MBM–DDGS diet, the measured AID of CP, Arg, His, Lys, Trp, Pro, and Tyr determined with either marker and those of Ile, Leu, and Met determined with Cr_2O_3 were greater ($P < 0.05$) than predicted values. Regardless of marker type, the AID of CP and all indispensable AA except Arg and Val in the wheat–CM–MBM–DDGS diet was underestimated, where the predicted AID was lower ($P < 0.05$) than the measured AID.

The measured SID of Lys determined with either marker and SID of Gly determined with Cr_2O_3 were different ($P < 0.05$) from predicted SID in the mixed diet containing wheat, CM, and MBM (Table 8). For the wheat–MBM–DDGS diet, the measured SID of His determined with Cr_2O_3 and those of Pro and Tyr determined with either marker were greater ($P < 0.05$) than corresponding predicted SID. The measured and predicted SID of all AA except Cys and Tyr differed ($P < 0.05$) regardless of marker in the wheat–CM–MBM–DDGS diet.

DISCUSSION

The nutrients composition in wheat, CM, and MBM in this study were within the range of

Table 2. Analyzed nutrient composition in ingredients, % as-fed basis¹

Item	Wheat	Canola meal	Meat and bone meal ²	DDGS
Dry matter	88.55	90.49	92.94	86.32
Crude protein ³	11.19	40.24	60.31	29.82
Acid detergent fiber	3.41	19.12	5.43	9.38
Neutral detergent fiber	16.09	25.49	35.49	27.97
Ether extract	0.65	5.83	8.54	10.80
Indispensable amino acids				
Arg	0.51	2.14	3.75	1.10
His	0.25	1.00	0.96	0.54
Ile	0.40	1.62	1.96	1.33
Leu	0.70	2.62	3.58	3.15
Lys	0.36	2.03	2.73	0.72
Met	0.16	0.73	0.78	0.43
Phe	0.47	1.55	2.10	1.46
Thr	0.30	1.53	1.82	1.02
Trp	0.14	0.47	0.32	0.19
Val	0.49	2.05	2.79	1.57
Dispensable amino acid				
Ala	0.40	1.61	3.93	2.12
Asp	0.55	2.60	4.06	1.88
Cys	0.25	0.95	0.79	0.48
Glu	2.77	6.23	6.50	3.77
Gly	0.47	1.85	6.77	1.03
Pro	0.94	2.39	4.61	1.83
Ser	0.41	1.23	2.19	1.07
Tyr	0.26	1.02	1.47	1.05

¹DDGS = sorghum distillers' dried grains with solubles.

²Acid and neutral detergent fiber in meat and bone meal probably represent gelatin and other components dissolved in the acid and neutral detergent solutions rather than fiber.

³Crude protein = nitrogen $\times$ 6.25.

reported values for these ingredients (Olukosi and Adeola, 2009; Urriola et al., 2009; NRC, 2012; Xue et al., 2014). Except for the wheat diet, the CP contents in semipurified and mixed diets met the requirement for pigs (NRC, 2012).

The BEL of CP and AA determined with either Cr₂O₃ or TiO₂ in NFD in this study were within the range of values reported in previous studies (Zhai and Adeola, 2011; NRC, 2012; Park et al., 2013; Xue et al., 2014). Value for BEL of AA could be determined by several methods; however, feeding NFD is most widely used due to its simplicity (Adeola, 2001). Although the NFD method is straightforward, several studies have investigated the factors related with diet formulation or physiological status of pig, which might affect the determination of BEL values in pigs (Park et al., 2013; Kong et al., 2014). This study indicated that the determination of BEL of CP and AA were not affected by the choice of marker used in NFD. It is worth noting that the AID of DM was not affected by marker type, which therefore excludes the possibility for other errors that may result in a lack of additivity.

Similar to the determination of AID of DM, the choice of marker had no effect on the determination of AID and SID of CP and AA in all semipurified and mixed diets in this study, even with high dietary fiber. In corn–soybean meal-based and barley–wheat–soybean meal-based complete diets, however, greater AID of CP was determined with TiO₂ compared with Cr₂O₃ (Jagger et al., 1992; Wang and Adeola, 2018). It has been reported that starch-enriched diet emptied faster from the stomach compared with wheat bran- and sugar beet pulp-enriched diets (Guerin et al., 2001). Therefore, it is reasonable to speculate that the different effect of marker on digestibility between current and previous studies might be caused by discrepant basal components in diets, which was corn starch in this study.

One of the advantages of using SID instead of AID of CP and AA in diet formulation for pigs is that SID is relatively additive in mixtures of feed ingredients (Furuya and Kaji, 1991; Nyachoti et al., 1997b; Stein et al., 2005; Xue et al., 2014). This is because that SID has been corrected for the

Table 3. Analyzed nutrient composition in experimental diets, % as-fed basis¹

Item	NFD	Wheat	CM	MBM	DDGS	Wheat–CM–MBM	Wheat–MBM–DDGS	Wheat–CM–MBM–DDGS
Dry matter	87.88	87.72	88.18	90.82	87.52	88.02	87.92	87.85
Crude protein ²	0.68	9.79	16.95	17.72	16.86	16.85	17.20	17.44
Indispensable amino acids								
Arg	0.01	0.47	0.96	1.08	0.62	0.97	0.74	0.84
His	0.00	0.21	0.44	0.25	0.31	0.40	0.33	0.36
Ile	0.02	0.37	0.73	0.59	0.74	0.69	0.64	0.65
Leu	0.04	0.65	1.20	1.09	1.78	1.18	1.33	1.21
Lys	0.01	0.29	0.91	0.81	0.41	0.78	0.53	0.64
Met	0.01	0.14	0.32	0.22	0.25	0.28	0.24	0.25
Phe	0.02	0.45	0.70	0.64	0.82	0.72	0.72	0.71
Thr	0.01	0.27	0.70	0.53	0.58	0.59	0.50	0.53
Trp	0.02	0.11	0.20	0.10	0.12	0.19	0.15	0.17
Val	0.01	0.44	0.90	0.85	0.89	0.86	0.78	0.80
Dispensable amino acids								
Ala	0.02	0.36	0.75	1.21	1.23	0.82	0.94	0.85
Asp	0.02	0.50	1.18	1.20	1.13	1.11	1.01	1.02
Cys	0.01	0.21	0.42	0.23	0.29	0.40	0.28	0.36
Glu	0.04	2.56	2.89	1.98	2.43	3.32	2.94	3.11
Gly	0.02	0.44	0.88	2.07	0.61	1.09	0.90	1.01
Pro	0.02	0.92	1.06	1.41	1.09	1.29	1.22	1.24
Ser	0.01	0.36	0.59	0.61	0.58	0.59	0.56	0.57
Tyr	0.01	0.29	0.44	0.40	0.54	0.45	0.47	0.45

¹NFD = nitrogen-free diet; CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles.

²Crude protein = nitrogen $\times$ 6.25.

Table 4. Basal ileal endogenous losses of crude protein and amino acids in pigs fed nitrogen-free diet, mg/kg dry matter intake^{1,2}

Item	Cr ₂ O ₃	TiO ₂	SEM	P-value
Crude protein, g/kg dry matter intake ³	16.31	16.91	1.708	0.170
Indispensable amino acids				
Arg	503	520	71.2	0.203
His	181	188	13.7	0.159
Ile	368	383	28.4	0.148
Leu	587	611	40.8	0.132
Lys	741	774	75.6	0.149
Met	91	95	10.5	0.182
Phe	361	375	24.0	0.139
Thr	540	560	43.7	0.136
Trp	114	119	9.3	0.108
Val	531	551	39.3	0.134
Dispensable amino acids				
Ala	576	598	50.0	0.162
Asp	856	889	61.3	0.142
Cys	179	185	12.5	0.138
Glu	1,017	1,057	85.4	0.147
Gly	1,241	1,285	186.6	0.213
Pro	3,549	3,672	871.1	0.274
Ser	466	483	41.5	0.154
Tyr	276	286	18.0	0.145

¹Cr₂O₃ = chromic oxide; TiO₂ = titanium dioxide.

²Each least squares mean represents eight observations.

³Crude protein = nitrogen $\times$ 6.25.

Table 5. Measured apparent ileal digestibility (%) of crude protein, dry matter, and amino acids in ingredients and mixed diets^{1,2}

Item	Wheat		CM		MBM		DDGS		Wheat-CM-MBM		Wheat-MBM-DDGS		Wheat-CM-MBM-DDGS		P-value	
	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Diet	Marker
CP	72.2	70.8	67.6	67.9	59.5	58.7	52.0	52.7	69.7	70.0	66.5	65.5	69.2	69.6	<0.001	0.684
DM	79.5	78.3	69.5	69.8	81.0	80.7	62.0	62.5	73.3	73.5	72.2	71.3	72.3	72.6	<0.001	0.771
Indispensable AA																
Arg	81.7	80.7	83.9	84.0	74.7	74.2	64.7	65.2	82.3	82.4	76.6	75.9	79.8	80.0	<0.001	0.627
His	80.9	79.8	80.3	80.5	54.1	53.2	55.3	55.9	79.0	79.1	70.7	69.8	75.4	75.7	<0.001	0.579
Ile	79.0	77.9	75.8	76.0	63.9	63.2	65.0	65.5	76.8	77.0	72.3	71.5	75.5	75.8	<0.001	0.623
Leu	81.0	80.0	78.2	78.4	65.1	64.4	69.5	70.0	78.2	78.3	75.1	74.3	77.2	77.5	<0.001	0.619
Lys	48.5	45.9	70.5	70.7	48.2	47.4	17.0	17.9	65.9	66.1	47.0	45.4	58.7	59.2	<0.001	0.602
Met	81.5	80.4	84.7	84.8	61.9	61.1	67.3	67.7	81.0	81.1	74.1	73.3	78.6	78.8	<0.001	0.566
Phe	82.9	82.0	78.2	78.4	67.2	66.5	67.6	68.1	79.1	79.3	75.3	74.6	78.2	78.4	<0.001	0.650
Thr	63.7	61.8	70.0	70.3	54.7	53.7	55.8	56.4	67.7	68.0	61.3	60.1	65.4	65.8	<0.001	0.573
Trp	81.3	80.3	87.6	87.7	68.8	68.2	75.2	75.5	85.1	85.2	81.9	81.3	83.7	83.9	<0.001	0.507
Val	74.6	73.3	72.5	72.8	63.9	63.1	61.8	62.3	73.3	73.5	69.0	68.0	71.8	72.1	<0.001	0.620
Dispensable AA																
Ala	66.0	64.3	74.0	74.3	70.9	70.2	65.6	66.1	72.0	72.2	68.8	67.9	70.9	71.2	0.001	0.596
Asp	65.5	63.8	71.0	71.3	45.4	44.2	55.1	55.7	66.1	66.3	58.3	57.0	62.2	62.6	<0.001	0.525
Cys	81.3	80.4	74.1	74.3	44.0	42.8	55.6	56.2	75.1	75.3	65.6	64.6	74.2	74.5	<0.001	0.554
Glu	91.6	91.1	84.0	84.1	62.6	61.8	64.2	64.7	85.7	85.8	80.1	79.6	83.8	84.0	<0.001	0.674
Gly	67.2	65.5	68.3	68.5	71.3	70.8	40.4	41.1	70.2	70.3	63.4	62.4	68.2	68.6	<0.001	0.677
Pro	76.5	75.5	62.4	62.5	59.2	58.8	36.2	37.0	72.6	72.8	66.0	65.4	67.2	67.6	<0.001	0.921
Ser	78.9	77.8	72.1	72.4	58.7	57.7	58.6	59.2	72.4	72.6	68.8	67.9	71.2	71.6	<0.001	0.641
Tyr	81.8	80.9	77.3	77.5	60.2	59.5	68.4	68.8	77.6	77.8	75.1	74.3	77.3	77.5	<0.001	0.625
																0.473

¹CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr₂O₃ = chromic oxide; TiO₂ = titanium dioxide; CP = crude protein; DM = dry matter; AA = amino acids.

²Each least squares mean represents eight observations.

³SEM of diet.

Table 6. Measured standardized ileal digestibility (%) of crude protein and amino acids in ingredients and mixed diets^{1,2}

Item	Wheat		CM		MBM		DDGS		Wheat-CM-MBM		Wheat-MBM-DDGS		Wheat-CM-MBM-DDGS		P-value	
	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Cr ₂ O ₃	TiO ₂	Diet	Diet × marker
CP	86.8	86.0	76.1	76.7	67.8	67.4	60.5	61.5	78.3	78.8	74.8	74.2	77.5	78.1	<0.001	0.824
Indispensable AA																0.515
Arg	91.0	90.4	88.5	88.8	79.0	78.6	71.8	72.5	86.9	87.1	82.5	82.1	85.0	85.4	<0.001	0.954
His	88.4	87.7	83.9	84.2	60.7	60.0	60.4	61.2	82.9	83.2	75.5	74.8	79.8	80.2	<0.001	0.914
Ile	87.7	87.0	80.3	80.7	69.6	69.1	69.4	70.1	81.5	81.8	77.4	76.7	80.5	81.0	<0.001	0.993
Leu	88.9	88.3	82.5	82.9	70.0	69.5	72.4	73.0	82.6	82.9	79.0	78.4	81.5	81.9	<0.001	0.977
Lys	70.9	69.3	77.7	78.2	56.5	56.1	32.8	34.5	74.2	74.8	59.3	58.3	68.8	69.8	<0.001	0.897
Met	87.2	86.4	87.2	87.4	65.6	65.0	70.5	71.1	83.9	84.1	77.4	76.7	81.8	82.1	<0.001	0.814
Phe	90.0	89.3	82.7	83.1	72.3	71.9	71.5	72.1	83.5	83.8	79.7	79.2	82.7	83.1	<0.001	0.968
Thr	81.2	80.0	76.8	77.3	63.9	63.3	64.0	64.9	75.8	76.3	70.8	70.0	74.3	75.1	<0.001	0.992
Trp	90.4	89.7	92.6	92.9	79.2	79.0	83.5	84.2	90.3	90.7	88.6	88.3	89.6	90.1	<0.001	0.729
Val	85.2	84.3	77.8	78.2	69.6	69.0	67.0	67.7	78.7	79.1	74.9	74.2	77.6	78.1	<0.001	0.987
Dispensable AA																0.477
Ala	80.0	78.8	80.8	81.3	75.2	74.7	69.7	70.3	78.2	78.7	74.2	73.5	76.8	77.4	<0.001	0.956
Asp	80.5	79.3	77.4	77.9	51.8	50.9	61.7	62.6	72.9	73.3	65.8	64.7	69.6	70.3	<0.001	0.890
Cys	88.8	88.1	77.9	78.2	51.1	50.1	61.0	61.8	79.1	79.3	71.2	70.4	78.6	79.0	<0.001	0.862
Glu	95.1	94.8	87.1	87.3	67.3	66.7	67.8	68.5	88.4	88.6	83.2	82.7	86.7	86.9	<0.001	0.985
Gly	91.9	91.1	80.7	81.3	76.8	76.4	58.2	59.6	80.2	80.7	75.5	74.9	79.0	79.7	<0.001	0.713
Pro	110.4	110.6	91.9	93.1	82.0	82.4	64.7	66.5	96.8	97.8	91.5	91.8	92.3	93.6	<0.001	0.173
Ser	90.3	89.6	79.1	79.6	65.7	64.9	65.6	66.5	79.4	79.8	76.2	75.5	78.4	79.0	<0.001	0.933
Tyr	90.2	89.5	82.8	83.2	66.5	66.0	72.8	73.4	83.0	83.4	80.2	79.7	82.7	83.1	<0.001	0.934

¹CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr₂O₃ = chromic oxide; TiO₂ = titanium dioxide; CP = crude protein; AA = amino acids.²Each least squares mean represents eight observations.³SEM of diet.

Table 7. Differences between measured and predicted values for apparent ileal digestibility (AID, %) of crude protein and amino acids in mixed diets^{1,2}

Item	Wheat–CM–MBM				Wheat–MBM–DDGS				Wheat–CM–MBM–DDGS			
	Cr ₂ O ₃		TiO ₂		Cr ₂ O ₃		TiO ₂		Cr ₂ O ₃		TiO ₂	
	Diff	SE	Diff	SE	Diff	SE	Diff	SE	Diff	SE	Diff	SE
CP	1.9	1.15	2.7	1.27	5.6**	1.38	5.0**	1.25	3.5*	1.36	4.4*	1.55
Indispensable AA												
Arg	1.3	0.73	1.8	0.82	2.7*	1.00	2.4*	0.87	0.6	1.13	1.2	1.26
His	2.3*	0.86	2.9*	0.99	4.4**	1.09	3.9*	1.14	2.5*	0.84	3.2*	1.14
Ile	2.0	0.99	2.5	1.10	2.5*	0.86	1.9	0.90	2.9*	0.91	3.5*	1.09
Leu	1.4	0.92	2.0	1.02	2.7*	1.04	2.1	0.93	2.6*	0.93	3.1*	1.14
Lys	7.3**	1.35	8.4**	1.49	10.0*	2.88	9.3*	3.00	5.5*	1.97	6.8*	2.13
Met	1.3	0.84	1.9	0.89	2.3*	0.85	1.8	0.86	2.0*	0.77	2.6*	0.91
Phe	0.9	0.91	1.5	0.98	2.0	0.90	1.5	0.83	2.3*	0.79	2.8*	0.98
Thr	2.7	1.29	3.6*	1.48	3.2	1.45	2.5	1.44	3.1*	0.98	4.0*	1.30
Trp	2.2*	0.84	2.8*	0.86	4.2**	0.88	4.1**	1.02	2.6**	0.67	3.3**	0.82
Val	1.7	1.05	2.4	1.18	2.3	1.12	1.7	1.02	2.2	0.99	3.0*	1.25
Dispensable AA												
Ala	1.4	1.11	2.3	1.24	1.8	1.26	1.1	1.08	1.9	1.15	2.7	1.35
Asp	2.9	1.25	3.8*	1.50	2.3	1.79	1.5	1.99	1.5	1.15	2.5	1.54
Cys	2.2	1.17	2.8	1.34	−0.2	1.27	−0.9	1.34	3.5**	0.72	4.3**	0.93
Glu	0.5	0.55	0.8	0.65	1.0	0.85	0.5	0.79	0.9	0.65	1.3	0.80
Gly	−0.2	1.14	0.6	1.47	2.0	1.90	1.5	1.81	−1.4	2.08	−0.5	2.19
Pro	4.9	2.25	5.5*	2.25	12.6**	3.59	12.2**	3.34	−2.5	7.25	−1.7	7.26
Ser	0.4	1.10	1.2	1.22	2.5	1.46	1.9	1.41	1.3	1.03	2.1	1.28
Tyr	2.3*	0.83	2.8*	0.95	4.0**	0.91	3.5**	0.87	3.9**	0.72	4.4**	0.92

¹Difference = measured – predicted AID of CP and AA; predicted AID was determined based on the measured AID values in the feed ingredients contained in mixed diet.

²CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr₂O₃ = chromic oxide; TiO₂ = titanium dioxide; CP = crude protein; AA = amino acids; Diff = difference.

* $P < 0.05$; ** $P < 0.01$. Ileal digestibility was underestimated when the difference between measured and predicted ileal digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

contribution of BEL of CP and AA in ileal digesta, and consequently SID is not affected by dietary CP content (Jansman et al., 2002). Similarly, this conclusion is also supported by the data from the three mixed diets in this study, where the differences between measured and predicted ileal digestibility values of CP and indispensable AA differed from zero in 39 for AID and 3 for SID of 66 observations.

The AID of His, Lys, Trp, and Tyr were not additive in all the three mixed diets, and the possible reason for this might be the low concentrations of these AA in the mixed diets, which could result in greater errors in the measurement of these AA. Cereal grains, such as corn and wheat, contribute a considerable proportion of AA to swine diets (Sauer et al., 2001), although their CP and AA concentrations are quite low. As discussed by Fan et al. (1994), the relationship between dietary AA content and AID value of respective AA is quadratic with plateau, and the plateau AID could be determined by meeting the threshold level of AA

to become independent of the dietary AA content. When the direct method is used, the determination of AID of CP and AA in a cereal grain is generally underestimated because it is difficult to meet the threshold level of CP or AA in diet with a cereal grain as the sole source of CP or AA, and therefore underestimates the AID value in the mixed diet containing the cereal grain (Fan et al., 1994; Stein et al., 2005). This hypothesis was supported by Stein et al. (2005) and Xue et al. (2014), where the mixed diets containing corn resulted in lack of additivity. However, the AID of CP and most indispensable AA in the wheat–CM–MBM diet was additive regardless of the choice of marker in this study. The difference of additivity between corn and wheat might be explained by the greater CP and AA contents in wheat than corn, which can elicit a relatively large change in the determination of AID values (Fan et al., 1994). Furthermore, it has been reported that direct method did not underestimate the AID of AA in wheat compared with difference

Table 8. Differences between measured and predicted values for standardized ileal digestibility (SID, %) of crude protein and amino acids in mixed diets^{1,2}

Item	Wheat–CM–MBM				Wheat–MBM–DDGS				Wheat–CM–MBM–DDGS			
	Cr ₂ O ₃		TiO ₂		Cr ₂ O ₃		TiO ₂		Cr ₂ O ₃		TiO ₂	
	Diff	SE	Diff	SE	Diff	SE	Diff	SE	Diff	SE	Diff	SE
CP	-0.4	1.15	0.3	1.27	3.2	1.38	2.5	1.25	1.0	1.36	1.7	1.55
Indispensable AA												
Arg	-0.2	0.73	0.2	0.82	1.6	1.00	1.2	0.87	-0.7	1.13	-0.1	1.26
His	0.7	0.86	1.2	0.99	2.8*	1.09	2.2	1.14	1.0	0.84	1.6	1.14
Ile	0.4	0.99	0.9	1.10	1.5	0.86	0.9	0.90	1.7	0.91	2.2	1.09
Leu	0.0	0.92	0.5	1.02	1.9	1.04	1.3	0.93	1.6	0.93	2.1	1.14
Lys	3.9*	1.35	4.8*	1.49	6.2	2.88	5.3	3.00	2.4	1.97	3.6	2.13
Met	0.4	0.84	0.9	0.89	1.4	0.85	0.9	0.86	1.2	0.77	1.8	0.91
Phe	-0.3	0.91	0.2	0.98	1.2	0.90	0.7	0.83	1.3	0.79	1.8	0.98
Thr	0.1	1.29	0.9	1.48	1.2	1.45	0.5	1.44	1.0	0.98	1.9	1.30
Trp	0.1	0.84	0.6	0.86	1.9	0.88	1.7	1.02	0.6	0.67	1.1	0.82
Val	-0.1	1.05	0.6	1.18	1.1	1.12	0.5	1.02	0.9	0.99	1.6	1.25
Dispensable AA												
Ala	-0.6	1.11	0.1	1.24	0.7	1.26	0.0	1.08	0.5	1.15	1.2	1.35
Asp	0.6	1.25	1.4	1.50	0.5	1.79	-0.3	1.99	-0.3	1.15	0.7	1.54
Cys	0.5	1.17	1.0	1.34	-1.2	1.27	-1.9	1.34	1.9*	0.72	2.5*	0.93
Glu	-0.3	0.55	0.0	0.65	0.3	0.85	-0.1	0.79	0.2	0.65	0.6	0.80
Gly	-3.1*	1.14	-2.5	1.47	-0.3	1.90	-0.9	1.81	-4.2	2.08	-3.3	2.19
Pro	-0.9	2.25	-0.4	2.25	8.6*	3.59	8.1*	3.34	-7.2	7.25	-6.5	7.26
Ser	-1.4	1.10	-0.7	1.22	1.2	1.46	0.5	1.41	-0.2	1.03	0.5	1.28
Tyr	1.0	0.83	1.5	0.95	3.2**	0.91	2.6*	0.87	2.9**	0.72	3.4**	0.92

¹Difference = measured – predicted SID of CP and AA; predicted SID was determined based on the measured SID values in the feed ingredients contained in mixed diet.

²CM = canola meal; MBM = meat and bone meal; DDGS = sorghum distillers' dried grains with solubles; Cr₂O₃ = chromic oxide; TiO₂ = titanium dioxide; CP = crude protein; AA = amino acids; Diff = difference.

P* < 0.05; *P* < 0.01. Ileal digestibility was underestimated when the difference between measured and predicted ileal digestibility was significantly greater than 0 or was overestimated when the difference was significantly less than 0.

and regression methods (Hennig et al., 2008), which supports the use of wheat as sole source of nitrogen to determine AID of AA.

The wheat–CM–MBM and wheat–MBM–DDGS diets contained same levels of wheat and MBM to elucidate whether there was different effect between CM and DDGS on additivity of AID of CP and AA determined with the same marker. In the wheat–MBM–DDGS diet, AID of CP and four indispensable AA using TiO₂ as marker, and of CP and seven indispensable AA using Cr₂O₃ as marker were underestimated. The lack of additivity observed with TiO₂ and Cr₂O₃ in the wheat–MBM–DDGS diet might relate to the low recovery of marker in the presence of high concentration of fiber from DDGS contained in this diet. It has been reported that the recovery of TiO₂ was decreased from 99.9% to 67.8% and the recovery of Cr₂O₃ decreased from 84.9% to 60.6% with increasing dietary neutral detergent fiber concentration using pigs installed with post-valve T-caecum cannulas (Yin et al., 2000). Furthermore, a negative

correlation between neutral detergent fiber and AID value of CP and AA determined with Cr₂O₃ has been reported (Fan et al., 2001); however, further research is required to investigate whether similarly negative correlation exists when TiO₂ is chosen as marker.

Unlike in the mixed diets containing three feed ingredients, the AID of CP and indispensable AA were not additive in the wheat–CM–MBM–DDGS diet containing four feed ingredients using either Cr₂O₃ or TiO₂ as marker. Except for dietary AA content, fiber source and level can also affect the threshold AA level in determination of AID of AA in ingredients, and therefore affect the predicted AID in mixed diets (Fan et al., 1994; Xue et al., 2014). However, the acid detergent fiber and neutral detergent fiber contents in the wheat–CM–MBM–DDGS diet were between those in the other two mixed diets, which indicates fiber might not be the reason for the lack of additivity of AID of CP and AA in the wheat–CM–MBM–DDGS diet. On the other hand, four components were included in

the determination of predicted AID values in the wheat–CM–MBM–DDGS diet, and this accumulative calculation could magnify the error in mixed diet induced by the calculation of determining AID for ingredients (Sauer et al., 2001); however, this speculation was not supported by the data in the study reported by Xue et al. (2014), and further study is required.

In conclusion, the SID of CP and AA are generally more accurate than AID for predicting ileal digestibility of CP and AA in mixed diets containing wheat, canola meal, meat and bone meal, and sorghum distillers' dried grains with solubles. The results of the experiment also demonstrate that the determination of endogenous loss, AID, and SID of CP and AA was not affected by the choice of digestibility index marker (Cr_2O_3 or TiO_2). In addition, the choice of digestibility index marker has no effect on the additivity of AID and SID of CP and most indispensable AA in all the three mixed diets.

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