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Original Research Article

Basal diet and indigestible marker influence apparent digestibilities of nitrogen and amino acids of cottonseed meal and soybean meal in pigs

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This study was to determine the apparent ileal nitrogen (N) and amino acid digestibilities of cottonseed meal (CSM) and soybean meal (SBM) in simple carbohydrate-based and more complex wheat-based diets. Twenty five Large White × Landrace boars (40.9 kg) were randomly allocated to one of 5 dietary treatments: a wheat diet, 40% CSM in either a sugar:starch (1:1) or wheat-based diet, or 40% SBM in either a sugar:starch or wheat-based diet for 14 d. All diets contained vitamins, minerals, and contained acid-insoluble ash (AIA) and Cr_2O_3 as indigestible markers. Rations were offered (1,800 g/pig per d) in 3 meals/d on d 1 − 11 and 8 meals/d on d 12 − 13. On d 14, the pigs were fed hourly for 8 h. After the 8th meal, pigs were anaesthetised with isoflurothane, and ileal and faecal digesta sampled from the terminal ileum and rectum before lethal injection of barbiturate. Apparent ileal digestibility (AID) of N was greater (12.1%) when CSM was fed in the wheat-based diet as compared to the simple carbohydrate-based diet, whereas apparent ileal N digestibility of SBM was slightly lower (−4.5%) in the wheat-based diet as compared to the sugar:starch-based diet. Apparent ileal amino acid digestibility generally respondeds similarly to N. Therefore, while there was a wide difference in apparent N and amino acid digestibilities of SBM and CSM when they were fed in the sugar:starch-based diets, these differences were less apparent when they were fed the wheat-based diet. There was an apparent net release of N into the hindgut of pigs fed CSM in both base diets. Conversely, there was quite substantial apparent digestion of N in the hindgut of pigs fed SBM in both base diets. The use of Cr_2O_3 as an indigestible marker resulted in lower apparent ileal and faecal digestibilities than using AIA, particularly for diets containing CSM. These data demonstrate that the basal diet and choice of indigestible marker can substantially influence the estimate of apparent N digestibility, this response can differ for different protein meals.

1. Introduction

Cotton is grown in many parts of the world with global production of cotton seeds being 44 million tonnes per year which, after removal of oil, hulls and lint, provides approximately 15 million tonnes of cottonseed meal available for livestock (Soy and Oilseed Bluebook, 2015). However, cottonseed meal (CSM) can be of variable quality and in general has a poorer amino acid digestibility and availability than other oilseed meals (Prawirodigdo et al., 1998; Gonzalez-Vega and Stein, 2012; Ma et al., 2018), although the use of CSM in pig diets has been little researched (Stein et al., 2016).

Digestibility of dietary protein may be affected by several factors including the amino acid profile of the protein source, the concentration of the protein source and the nutrient profile of other feeds consumed with the protein source. However, data are equivocal with respect to the absolute effect of these factors, or any interactions, on the digestibility of dietary protein sources. For instance, protein digestibility in piglets was more dependent upon

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the amino acid profile of the protein source rather than on other components in the diet including carbohydrates (Turlington et al., 1989). Moreover, wide variations in dietary protein content may influence the apparent ileal digestibility (AID) or total tract (ATTD) of nitrogen (N) or amino acids in growing pigs (Fan et al., 1994; Ma et al., 2018). Variation in processing technique can also contribute to variation in AID. For instance, Ma et al. (2018) found that cottonseed co-products treated using a range of different processing technologies also resulted in widely varying AID. In addition, using a meta-analysis approach, Messad et al. (2016) found that the AID of amino acids in oilseeds was inversely related to their neutral detergent fibre content and that this is partially related to increased endogenous losses of amino acids (Stein et al., 2007). Leterme et al. (1994) observed substantial differences in AID of N in pigs fed different carbohydrate fractions, and suggested that this was due to an effect of specific carbohydrate fractions and the rate of endogenous protein loss. Furthermore, Wigan et al. (1995) found that digestible energy of lupins was higher when they were fed in a wheat-based diet compared to a sugar-based diet; it was suggested that the higher digestibility when pigs were fed the wheat-based diet may have been due to more of the complex carbohydrates passing to the hindgut and providing increased substrate for hindgut fermentation. Thus, carbohydrates and fibre present in the basal diet may substantially influence the AID of amino acids (Messad et al., 2016). Therefore, the present study was conducted to determine AID of N and amino acids in pigs fed cottonseed meal (CSM) or soya bean meal (SBM) in a simple (sugar + starch) and a complex (wheat) carbohydrate-based diet. Soybean and cottonseed meals were chosen as test oilseed protein meals to provide additional comparative information on CSM and because the oilseeds meals differ widely in their apparent amino acid digestibilities. A further objective was to compare chromic oxide (Cr_2O_3) and acid-insoluble ash (AIA) as indigestible markers.

2. Materials and methods

2.1. Diets, animals and experimental design

The protocol used in this experiment conformed to all Animal Experimentation Ethics Committee regulations concerning the health and care of experimental animals and was approved by the Victorian Institute of Animal Science Animal Ethics Committee.

The measured nutrient contents of SBM, CSM and wheat and estimated energy content are presented in Table 1. The present study used 5 formulated diets (Table 2). The basal control diet contained 926 g wheat/kg with wheat providing the sole source of dietary carbohydrate and protein. The other 4 diets contained 400 g/kg CSM in a sugar + starch (1:1 wt/wt) based or a wheat-based and 400 g/kg SBM in both basal diets. Iron sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) was added (2 g/kg) to the CSM diets to inactivate the gossypol present in CSM (Knabe et al., 1979). Chromic oxide and acid insoluble ash (Celite, a diatomaceous earth produced by Celite Cooperation Lompoc, California, USA) were included in the diets (2 g/kg) as indigestible markers.

Twenty-five Large White × Landrace entire male pigs (40.9 ± 2.6 kg, mean ± standard deviation) were individually penned and randomly assigned to one of the 5 experimental diets (Table 2) in a completely randomised design. The experimental diets were offered to pigs in a mash form for 14 d. Water was constantly available via nipple drinkers during the experimental period. The diets were introduced to the pigs over a 3-d period. Rations were offered (1,800 g/pig per day) in 3 meals/d (at 06:00, 12:00 and 18:00) from d 1 to 11 and in

Table 1
Protein and amino acids profile of the cottonseed meal (CSM), soybean meal (SBM) and wheat used in the present experiment (g/kg, air-dry basis).

Item	CSM	SBM	Wheat
Dry matter	911	887	892
Organic matter	849	828	881
Crude protein (N × 6.25)	371	443	133
Indispensable amino acids			
Arginine	38.1	31.5	6.9
Histidine	11.4	13.8	3.0
Isoleucine	12.4	22.0	5.0
Leucine	20.6	32.9	9.1
Lysine	15.9	27.2	3.6
Phenylalanine	18.9	24.0	7.1
Threonine	11.8	20.2	3.9
Tyrosine	8.9	16.6	4.6
Valine	17.5	22.6	6.3
Dispensable amino acids			
Alanine	13.8	20.1	4.7
Aspartic acid	30.9	48.5	6.6
Glutamic acid	67.0	74.0	37.8
Glycine	14.6	18.4	5.4
Proline	13.4	23.4	13.6
Serine	13.4	24.9	13.6

Table 2
Composition of the experimental diets (g/kg, air-dry basis).

Item	Diet				
	Wheat	Cottonseed meal		Soybean meal	
		Simple	Wheat	Simple	Wheat
Ingredient					
Cottonseed meal	–	400	400		
Soybean meal	–	–	–	400	400
Wheat	926	–	526	–	526
Cane sugar (sucrose)	–	263	–	263	–
Starch (wheat starch)	–	263	–	263	–
$FeSO_4.7H_2O$	–	2.0	2.0	–	–
K_2SO_4	1.5	–	–	1.5	1.5
Minerals + Vitamins premix[1]	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	30.0	30.0	30.0	30.0	30.0
Cr_2O_3	2.0	2.0	2.0	2.0	2.0
Acid-insoluble ash (Celite)	2.0	2.0	2.0	2.0	2.0
Tallow	33.5	33.5	33.5	33.5	33.5
Determined analysis					
Dry matter	894	917	896	908	888
Organic matter	854	871	828	860	820
Crude protein (N × 6.25)	123	149	219	177	246
Indispensable amino acids					
Arginine	5.5	13.8	16.6	12.4	14.5
Histidine	2.8	3.4	5.6	4.5	6.5
Isoleucine	4.8	4.5	7.2	8.6	11.2
Leucine	8.8	7.8	1.3	13.6	18.0
Lysine	7.0	6.0	7.9	10.9	12.7
Phenylalanine	6.2	7.6	10.5	9.3	12.1
Threonine	5.4	4.0	6.8	7.0	10.7
Tyrosine	4.1	4.0	5.8	6.2	8.7
Valine	6.0	6.3	9.8	9.0	12.3
Dispensable amino acids					
Alanine	4.7	5.1	7.8	7.8	10.5
Aspartic acid	6.8	11.3	15.8	19.5	22.8
Glutamic acid	37.3	24.1	44.4	31.7	49.8
Glycine	5.4	5.4	8.4	7.4	10.1
Proline	3.4	4.6	12.5	8.8	16.4
Serine	6.9	5.6	9.7	9.3	13.5
Calculated analysis					
Digestible energy, MJ/kg[2]	14.4	14.3	14.1	14.9	14.7

[1] The minerals and vitamins premix was added to contribute (mg/kg air dry diet): retinol, 6.4; cholecalciferol, 0.083; α-tocopherol, 22; menadione, 0.60; riboflavin, 3.3; nicotinic acid, 16.5; pantothenic acid, 5.5; pyridoxine, 1.1; biotin, 0.056; choline, 1,100; cyanocobalamin, 0.017; Fe, 88; Zn, 55; Mn, 22; Cu, 6.6; I, 0.22; Se, 0.1.

[2] Estimated from ingredients using AUS pig (Black et al., 1986).

8 meals/d (every 3 h) from d 12 to 13. There were no feed refusals at this level of feed offered.

Commencing at 01:00 on d 14, the pigs received their daily allocation of feed in 8 portions that were fed hourly. After the 8th meal, each pig was sedated using 5 mL of Stresnil (Janssen Pharmaceutical, Beerse, Belgium) injected intramuscularly and left undisturbed in their pens for 15 min. The sedated pigs were then anaesthetised by inhalation of isoflurane (Rhone Merieux, Australia). After each pig was anaesthetized, a ventral abdominal midline incision was made, the caecum was located and then a 150 cm portion of the terminal ileum and the rectum were excised to enable simultaneous collection of the ileal and faecal digesta. The ileal digesta was gently expelled, collected and stored at −20 °C until analysed. Faecal digesta was also expelled, collected and stored frozen until analysis. Finally, the anaesthetised pigs were killed with a lethal injection (15 mL) of pentobarbitone sodium (Valabarb 300 mg/mL; Boehringer Ingelheim Pty. Ltd, NSW, Australia) administered directly into the vena cava. Ileal digesta from all pigs were freeze-dried, finely ground and analysed for amino acids, N, organic matter (OM), Cr_2O_3 and AIA contents. Faecal digesta were freeze-dried and ground for total N, OM, Cr_2O_3 and AIA determination.

2.2. Chemical analyses

Dry matter content of wheat, CSM, SBM and experimental diets was determined by drying for 16 hr in a forced-air oven at 100 °C. Organic matter was measured in the wheat, CSM and SBM, experimental diets, and ileal and faecal digesta by combustion of the samples in a furnace chamber at 650 °C for 4 h. Total N content of the samples was determined using a Micro Kjeldahl method (AOAC, 1990). The amino acid contents of the diet and ileal digesta were determined following acid hydrolysis in 6 mol/L hydrochloric acid. The mixture of amino acids was separated on an HPLC ion exchange (strong cation exchange) column (Waters Australia Pty. Ltd., Box Hill, Victoria, Australia) using post column derivatization with ninhydrin (Rayner, 1985).

Concentrations of chromium in the diets, ileal and faecal digesta were determined using a modification of the atomic absorption spectrophotometric method of Williams et al. (1962) and the chromium contents used to calculate AID and ATTD as described by Saha and Gilbreath (1993). The concentration of AIA in the diets and digesta was determined using a gravitation technique, modified from the technique described by McCarthy et al. (1974). Since Cr_2O_3 is insoluble in 4 mol/L hydrochloric acid (McCarthy et al., 1974), when AIA is simultaneously used as an inert marker with Cr_2O_3 in a diet, the non-Cr_2O_3 AIA values should be obtained by difference (McCarthy et al., 1974). Therefore, non-Cr_2O_3 AIA was calculated as total AIA concentration minus Cr_2O_3 concentration.

2.3. Statistical analyses

For the analyses of the AID of individual amino acids, the data were analysed by analysis of variance (ANOVA) with the model being a (2 base diets × 2 protein sources) + wheat diet treatment structure split for marker within each pig. The respective factors were protein source (CSM and SBM), basal diets (sugar + starch and wheat) and markers (Cr_2O_3 and AIA). In studying the effects of basal diets on the AID and ATTD of N, OM and DM, the statistical analyses were performed so that the differences between site of sampling (ileum versus rectum) and their interactions could be determined. Thus, site of sampling (ileal and faecal) was used as an additional factor. All statistical analyses were performed using GENSTAT version 18 (VSN International Ltd, Hertfordshire, UK).

3. Results

3.1. Apparent ileal digestibility of indispensable amino acids

The AID of indispensable amino acids (IAA) are presented in Table 3. The AID of IAA was significantly ($P < 0.05$) higher in SBM diets compared to CSM diets, respectively, for arginine (81% vs. 73%), histidine (73% vs. 59%), isoleucine (83% vs. 57%), leucine (77% vs. 55%), lysine (82% vs. 48%), phenylalanine (74% vs. 70%), threonine (77% vs. 46%), tyrosine (76% vs. 59%) and valine (79% vs. 60%). The AID was significantly ($P < 0.05$) higher in the sugar and starch base diet compared to the wheat-based diet, respectively, for arginine, isoleucine, phenylalanine, tyrosine, but was lower for threonine. There was no effect ($P > 0.05$) of base diet on the AID for histidine, leucine, lysine and valine. The AID was significantly ($P < 0.05$) lower when determined using Cr_2O_3 compared to AIA, respectively, for arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine and valine (Table 4).

There were also several interactions between protein sources, basal diets and indigestibility marker. For example, AID determined using Cr_2O_3 was decreased more significantly ($P < 0.01$) than that determined using AIA in diets containing CSM compared to SBM for arginine, histidine, leucine, lysine, phenylalanine, threonine, tyrosine and valine. For arginine, the AID was decreased ($P < 0.01$) to a greater extent in a sugar and starch diet compared with a wheat-based diet when calculated using Cr_2O_3 and AIA as digestibility markers, respectively. The AID was significantly higher for SBM in a simple carbohydrate-based diet compared to a complex carbohydrate-based diet, respectively, for all IAA, but variable for CSM when comparing the base diets.

3.2. Apparent ileal digestibility of total nitrogen and dispensable amino acid

Apparent ileal digestibility of dispensable amino acids (DAA) was significantly ($P < 0.001$) higher in SBM diets compared to CSM diets, respectively, for alanine (71% vs. 50%), aspartic acid (75% vs. 63%), glutamic acid (73% vs. 69%), glycine (58% vs. 42%), proline (74% vs. 9%) and serine (79% vs. 60%). Digestibility was significantly higher in the simple base diet compared to the complex base diet for alanine (63 vs. 58%) and glutamic acid (76% vs. 58%), but was lower for proline (16% vs. 68%) and serine (67% vs. 72%). There was no effect ($P > 0.10$) of base diet on AID for aspartic acid and glycine. The AID was significantly ($P < 0.001$) lower when digestibility was determined using Cr_2O_3 compared to AIA, respectively, for alanine (57% vs. 68%), aspartic acid (65% vs. 73%), glycine (45% vs. 58%), proline (43% vs. 57%) and serine (67% vs. 75%).

There were also several interactions between protein sources, base diets and indigestibility marker. For example, AID was significantly ($P < 0.001$) higher for SBM in a simple base diet compared to a complex base diet, respectively, for all DAA, except glycine and proline, whereas for CSM digestibility was lower in a simple base diet compared to the complex base diet for all DAA except glutamate. For proline, AID was substantially less and indeed negative. The AID determined using Cr_2O_3 was decreased significantly ($P < 0.001$) more than for digestibility determined using AIA in diets containing CSM compared to SBM. There was also an interaction such that AID was decreased to greater extent in a simple base diet compared with a complex base diet for glycine and proline when calculated using Cr_2O_3 and AIA digestibility markers, respectively.

Table 3
Effect of marker, protein source and basal diets and on the apparent ileal digestibility of indispensable amino acids of the experimental diets (%).[1]

Marker	Chromium					Acid insoluble ash					SED[2]	SED[3]	Significance[4]
Protein source	Wheat	Cottonseed meal		Soybean meal		Wheat	Cottonseed meal		Soybean meal				
Basal diet		Simple	Wheat	Simple	Wheat		Simple	Wheat	Simple	Wheat			
Arginine	65.1	73.9	67.8	84.0	74.6	76.0	80.8	71.0	87.5	75.9	1.52	0.95	D^{***}; P^{***}; B^{***}; M^{***}; $P\times M^{**}$; $B\times M^{**}$
Histidine	66.2	49.0	55.8	77.4	65.3	76.7	62.3	67.4	82.2	69.0	3.43	1.63	D^{*}; P^{***}; M^{***}; $P\times B^{***}$; $P\times M^{**}$
Isoleucine	75.2	54.8	49.3	82.2	75.4	82.7	66.9	57.8	86.1	88.2	2.74	1.48	D^{**}; P^{***}; B^{***}; $P\times B^{**}$; M^{***}; $P\times B\times M^{***}$
Leucine	73.0	46.5	49.8	78.3	68.9	83.1	60.8	64.3	83.3	78.9	2.80	1.71	D^{***}; P^{***}; M^{***}; $P\times B^{**}$; PxM^{***}
Lysine	66.2	36.7	41.4	82.0	77.0	76.7	54.9	57.2	86.0	81.0	2.89	1.83	D^{**}; P^{***}; M^{***}; $P\times B^{*}$; $P\times M^{***}$
Phenylalanine	83.0	70.2	63.7	81.7	63.6	88.4	78.1	69.3	83.3	67.8	2.07	1.16	D^{***}; P^{*}; B^{***}; M^{***}; $P\times B^{**}$; $P\times M^{**}$; $P\times B\times M^{*}$
Threonine	71.3	32.2	46.1	72.5	75.8	80.2	50.1	57.2	78.5	81.3	3.59	1.96	D^{***}, P^{***}; B^{**}; M^{***}; $P\times M^{***}$
Tyrosine	69.3	55.7	51.7	76.9	69.8	78.9	67.6	59.5	81.9	73.7	3.85	1.67	D^{*}; P^{***}; B^{*}; M^{***}; $P\times M^{**}$
Valine	73.6	52.0	55.4	79.4	74.5	81.8	64.7	68.2	83.9	78.7	2.45	1.70	D^{***}; P^{***}; M^{***}; $P\times B^{*}$; $P\times M^{***}$

SED = standard error of difference.
[1] Basal diets were either a 1:1 sugar and starch mix (simple) or pure wheat (wheat); protein sources were either 400 g/kg cottonseed meal or soybean meal mixed with the basal diet or diet containing 926 g/kg wheat.
[2] When comparing different basal diet means.
[3] When comparing same basal diet means.
[4] Significance of effects of pure (926 g/kg) wheat vs. other diets (D), protein source (P), basal diet (B) or indigestible marker (M) and interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4
Effect of marker, protein source and basal diets and on the apparent ileal digestibility of dispensable amino acids of the experimental diets (%).[1]

Marker	Chromium					Acid insoluble ash					SED[2]	SED[3]	Significance[4]
Protein source	Wheat	Cottonseed meal		Soybean meal		Wheat	Cottonseed meal		Soybean meal				
Basal diet		Simple	Wheat	Simple	Wheat		Simple	Wheat	Simple	Wheat			
Alanine	62.3	39.3	46.3	76.6	59.1	74.0	55.5	60.6	81.7	66.2	3.50	2.07	D^{**}; P^{***}; B^{*}; M^{***}; $P\times B^{***}$; $P\times M^{***}$
Aspartic acid	63.6	54.1	62.0	77.2	67.5	74.9	66.3	69.5	82.2	73.2	2.73	1.48	P^{***}; M^{***}; $P\times B^{***}$; $P\times M^{*}$
Glutamic acid	88.6	68.0	62.8	78.0	61.9	92.2	76.4	68.4	82.8	68.8	2.56	1.18	D^{***}; P^{*}; B^{***}; $P\times B^{*}$
Glycine	49.3	22.1	48.3	58.9	47.4	65.1	42.6	56.7	67.8	55.8	5.43	2.62	P^{***}; $P\times B^{***}$; M^{***}; $P\times M^{*}$; $B\times M^{*}$; $P\times B\times M^{*}$
Proline	78.9	−63.3	55.2	69.5	74.4	85.4	−19.6	63.1	76.0	77.6	15.0	4.94	D^{**}; P^{***}; B^{***}; M^{***}; $P\times B^{***}$; $P\times M^{***}$; $B\times M^{***}$; $P\times B\times M^{**}$
Serine	74.4	46.8	61.1	78.2	75.7	82.4	60.9	69.4	82.9	80.5	2.99	1.61	D^{***}; P^{***}; B^{*}; M^{***}; $P\times B^{**}$; $P\times M^{***}$

SED = standard error of difference.
[1] Basal diets were either a 1:1 sugar and starch mix (simple) or pure wheat (wheat); protein sources were either 400 g/kg cottonseed meal or soybean meal mixed with the basal diet or diet containing 926 g/kg wheat.
[2] When comparing different basal diet means.
[3] When comparing same basal diet means.
[4] Significance of effects of pure (926 g/kg) wheat vs. other diets (D), protein source (P), basal diet (B) or indigestible marker (M) and interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

3.3. Apparent ileal and faecal digestibilities of nitrogen, organic matter and dry matter

The AID and ATTD of N, OM and dry matter are given in Table 5. The apparent digestibility of N was higher in SBM than in CSM (81% vs. 68%), was lower when digestibility was determined with Cr_2O_3 as compared to AIA (71% vs. 79%) and was lower when measured in the terminal ileum compared to the rectum (74% vs. 76%) (Table 5). However, there was an interaction ($P < 0.001$) such that the AID of N in CSM was higher than the ATTD (69% vs. 67%) whereas the converse was true for SBM (79% vs. 83%). The apparent digestibility of OM was higher in SBM than in CSM (84% vs. 74%), lower when digestibility was determined with Cr_2O_3 as compared to AIA (75% vs. 83%) and when measured in the terminal ileum compared to the rectum (74% vs. 76%) and higher when measured using a simple basal diet (84% vs. 74%). However, there was an interaction ($P < 0.001$) such that digestibility of organic matter in CSM increased to a lesser degree between the ileum and rectum (71% vs. 77%) than it did in SBM (78% vs. 87%). While there were a number of other interactions, these were mainly related to different magnitudes of response rather than any differences in direction of response. Responses in apparent digestibility of dry matter were similar to organic matter (Table 5).

4. Discussion

These data demonstrate that the AID of amino acids and N of both CSM and SBM diets differ when these protein sources are offered to the pigs in different basal diets. The trends in the AID changes were consistent regardless of whether Cr_2O_3 and AIA were used as indigestible markers, although the latter yielded consistently higher values. In addition, the responses of the pigs to the different basal diets were variable as indicated by significant interactions between dietary protein sources, basal diets and markers. These interactions have important implications for how digestibility of N and amino acids may differ from experimental values and may be influenced by the complex diets that are used commercially.

These data clearly show that the choice of indigestible marker can have a profound effect upon the estimates of the AID of amino

Table 5
Effect of marker, protein source, basal diets and site of sampling on the apparent digestibility (%) of nitrogen (N), dry matter and organic matter of the experimental diets.[1]

Marker	Chromium					Acid insoluble ash					SED[2]	SED[3]	Significance[4]
Protein source	Wheat	Cottonseed meal		Soybean meal		Wheat	Cottonseed meal		Soybean meal				
Basal diet		Simple	Wheat	Simple	Wheat		Simple	Wheat	Simple	Wheat			
N													
Ileal	73.4	57.1	73.0	79.0	71.4	81.6	67.3	75.9	82.7	78.8	1.20	1.08	D*; P***; M***; S*; P×S***
Faecal	77.2	56.5	63.9	81.5	78.0	82.4	67.7	76.2	86.3	85.7			
Dry matter													
Ileal	65.8	64.4	53.7	77.4	60.0	77.2	75.0	64.5	84.5	68.5	1.37	1.21	D*; P***; B***; M***; S***; B×M**;
Faecal	78.9	73.7	61.1	89.2	77.1	83.3	80.5	74.4	92.0	84.9			P×M**; B×S***; M×S**; P×S***; B×M×S**
Organic matter													
Ileal	69.4	69.2	61.8	81.1	67.0	82.6	80.3	70.4	87.9	74.8	1.29	1.15	P***; B***; M***; S***; B×M***; M×S*;
Faecal	82.0	77.3	64.8	92.4	81.3	86.7	84.6	79.2	94.9	88.7			P×S***; B×M×S***

SED = standard error of difference.
[1] Basal diets were either a 1:1 sugar and starch mix (Simple) or pure wheat (Wheat); protein sources were either 400 g/kg cottonseed meal or soybean meal mixed with the basal diet or diet containing 926 g/kg wheat.
[2] When comparing different basal diet means.
[3] When comparing same basal diet means.
[4] Significance of effects of pure (926 g/kg) wheat vs other diets (D), protein source (P), basal diet (B), indigestible marker (M) or site (S) and interactions: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

acids. The use of Cr_2O_3 resulted in lower estimates of the AID of amino acids than AIA, particularly for most of the amino acids in CSM. The AID of amino acids and N of SBM in a simple basal diet determined using Cr_2O_3 as a marker in the present study were more similar to those determined in our laboratory in an earlier study (Prawirodigdo et al., 1998), again using Cr_2O_3 as a marker, than estimates obtained using AIA. However, the AID of amino acids and N of CSM in a simple basal diet determined using AIA as an indigestible marker were very similar to those determined previously (Prawirodigdo et al., 1998). The concentration of Cr_2O_3 in the diets and digesta samples was measured by X-ray spectrometry in the previous study and by atomic absorption spectrophotometry in the present study and this may have contributed to differences. A drawback of the atomic absorption spectrophotometry technique to measure Cr_2O_3 content is that it appears to not always provide an accurate determination for Cr_2O_3 content (Jagger et al., 1992; Kozloski et al., 1998), possibly because other minerals such as Ca, P or Mg can interfere with Cr analyses (Saha and Gilbreath, 1991a,b). It is also likely that at least some of the Cr is soluble in the alkaline or acidic fluids in the gastrointestinal tract and that these become volatile during sample digestion prior to atomic absorption analyses (Saha and Gilbreath, 1991a,b). Hence, AIA was also included as an indigestible marker in the present study.

A number of researchers have shown that estimation of AID and endogenous losses of amino acids could be impacted by the choice of indigestible marker. For example, Favero et al. (2014) found that both AID and endogenous losses of amino acids were lower when determined using Cr_2O_3 as an indigestible marker as compared to both AIA or titanium oxide (TiO_2) which is consistent with the present findings. On the other hand, Fan and Sauer (1995) found that AID was higher in high fibre diets when determined using Cr_2O_3 as compared to AIA which they attributed to possible separation of the markers with Cr_2O_3 being associated with the liquid phase. Yin et al. (2000a,b) compared the use of total collection via the post-valve "T" caecal (PVTC) cannula and the use of Cr_2O_3 and TiO_2 as indigestible markers and found that Cr_2O_3 underestimated the ATTD of crude protein and DM. On the other hand, the ATTD assessed using TiO_2 as a marker agreed well with the total collection method using PVTC. Furthermore, Yin et al. (2000a,b) found that both markers, but in particular Cr_2O_3, were poorly recovered from ileal digesta collected using PVTC, from pigs consuming high fibre diets. Finally, Moughan et al. (1991) reported quantitative recovery of both endogenous and exogenous AIA but relatively poor recovery of Cr_2O_3 in pigs fed barley-based diets. However, separation of markers into solid and liquid phases shouldn't be an issue when collecting total digesta as in the present study so there may be other reasons for the discrepancies between the methods.

To further investigate the effect of marker on the ATTD of N, OM and DM, Prawirodigdo (1999) compared Cr_2O_3 and AIA with total faecal collection in pigs fed similar diets to those used in the present study. Prawirodigdo (1999) found that the ATTD of N determined using AIA as a marker was highly correlated ($R^2 = 0.95$) to that determined by total faecal collection and that the slope (0.99) and intercept (0.01) were not significantly different from 1 to 0, respectively. On the other hand, the relationship between ATTD of N determined using Cr_2O_3 was less well correlated ($R^2 = 0.87$) with total faecal collection and both the slope (0.75) and intercept (0.21) were significantly different than 1 and 0, respectively. Prawirodigdo (1999) reported that for diets with a high digestibility the three methods gave similar estimates of digestibility, whereas for diets with lower digestibilities, Cr_2O_3 seriously underestimated N digestibility determined using total faecal collection.

Since, for reasons outlined above, we have greater confidence in digestibilities determined using AIA as a marker, the following discussion about the effect of basal diet on digestibilities will use the AIA marker method as the standard measurement. The present data show a generally higher AID of N and the majority of amino acids in CSM when fed in a complex wheat-based diet compared to when CSM is provided in a simple base diet, possibly due to the different protein and/or carbohydrate profiles of the diets. However, the general response with diets containing SBM was for either unchanged or a small decrease in apparent ileal digestibility of most amino acids when a complex rather than simple base diet was used. In this context, the alloxan-induced diabetic neuromast (AIDN) of whole commercial diets containing 25% CSM and SBM in a predominantly sorghum base were similar suggesting some synergy between cereal and CSM with respect to the AID of N (Power, 2000). Also, other workers who have determined the AID of amino acids in CSM using complex cereal basal diets have found most values near the upper end of the range of normally encountered values (Batterham et al., 1990; Li et al., 2000). Conversely, the AID of amino acids obtained using simple carbohydrate basal diets have been towards the lower end of the range (Knabe et al., 1989; Prawirodigdo et al., 1998).

Most recent ileal digestibility studies of protein meals or protein sources in Australia have used a cereal base diet to simulate the conditions under which they are fed commercially (King et al., 2000; Collins et al., 2005, 2006). A possible reason for the increase in the AID of amino acids when CSM is mixed with a wheat diet may be related to the increase in protein content of the whole diet. Some workers have found that AID of amino acids and N increased as the dietary protein content increased (Fan et al., 1994; Fan and Sauer, 1995; Langer and Fuller, 1996), possibly because of a dilution of endogenous protein losses (Power, 2000). In the present experiment the increase in the AID of proline, threonine, glycine and serine in CSM in a wheat-based diet appeared to be the major amino acids contributing to the significant improvement in the AID of N compared to when CSM was offered in a simple base diet. It is possible that the pigs given CSM in a sugar + starch-base diet secreted a much greater proportion of endogenous protein relative to dietary protein into the small intestine, than the pigs consuming CSM protein in a wheat-based diet. Other investigators (Holmes et al., 1974; Sauer et al., 1977) have also found high levels of glycine and threonine in the ileal digesta of growing pigs given a protein-free diet. In addition, glycine is a major component of bile salt conjugates, and accounts for more than 90% of the amino acids secreted in bile juice of pigs (Souffrant, 1991; Fan and Sauer, 1995). Moreover, the endogenous secretions of the small intestine contain mucins that are rich in threonine, serine and proline (Neutra and Forstner, 1987). An increased mucin secretion may explain why the AID of these amino acids in CSM in a simple basal diet were relatively low compared to the AID of other amino acids in the present experiment. In this context, if there was any free gossypol present in the CSM diets this could also increase mucin secretion as was the case in the rat proximal colon (Kuhn et al., 2002).

There was a very low AID for proline (−40%) in pigs fed CSM in a simple base diet, as was the case in our earlier study (Prawirodigdo et al., 1998). It is possible that the secretion of endogenous proteins high in proline caused a negative balance of proline input (dietary origin): proline output ratio (endogenous origin). Furthermore, when CSM was fed to pigs as the sole dietary protein source in a simple base diet, the AID of N of CSM was markedly lower than that in a pure wheat diet. It is possible that interaction between the protein in CSM and that in wheat may have increased the AID of N for CSM in the wheat-based diet. Finally, the sugar and starch in the simple diet would have been rapidly digested and absorbed in the proximal small intestine (Beech et al., 1991; Noah et al., 1999) resulting in the digesta being comprised of relatively undigested and fibrous CSM in much of the small intestine. This could also increase the endogenous losses of amino acids and decrease the AID of amino acids.

Data from the present study confirm those of previous studies (Batterham et al., 1990; Prawirodigdo et al., 1998) where there was no further digestion of N in the hindgut of pigs fed CSM diets. There are two possible reasons for this. Firstly, the bound N of the CSM may have been resistant to microbial attack due to Maillard reaction during processing of CSM. A second alternative is that while hindgut microbes may have broken down the bound protein, gossypol released at the same time may have been toxic to hind gut microbes and possibly mucosal cells (Berardi and Goldblatt, 1980) thereby increasing N recovery in the faecal digesta. This free gossypol may also have stimulated the pigs to protect their body tissue by secretion of endogenous N from goblet cells of hindgut. Low and Zebrowska (1989) stated that the goblet cells secrete a protective sulphated carbohydrate–protein complex. In the present experiment the secretion may have contributed N to the total N content of the faecal digesta of the pigs. Thus, the AFDN value of CSM was similar to the AIDN.

There was further N digestion in the hind gut of pigs fed SBM in both basal diets, which is again consistent with the observations of Prawirodigdo et al. (1998). In addition, the further degradation of N in the hindgut of pigs given pure wheat diets suggests that there was no anti-nutritive effect of wheat on digestibility of N in pig. Consistent with the AFDOM values found in our previous experiment (Prawirodigdo et al. (1998), the AFDOM of CSM and SBM diets in the present experiment was also higher than the IDOM. These data also show that there was further OM digestion in the hindgut of pigs given the pure wheat diet. Similarly, the AFDDM of CSM, SBM and wheat was higher than AIDDM.

Cottonseed meal contains gossypol which is a polyphenolic compound that is toxic to monogastric animals, and this has lead producers to limit the presence of CSM in feeds for both poultry and pigs (Berardi and Goldblatt, 1980; Tanksley, 1990; Swiatkiewicz et al., 2016; Duodu et al., 2018). Even though the acute oral toxicity of gossypol is relatively low, if even small amounts of free gossypol are ingested over a prolonged period, it can be fatal (Berardi and Goldblatt, 1980). Also, gossypol can reduce protein digestibility by inhibition of pepsin and trypsin activity in the intestine and binding dietary iron. Consequently, to increase the utilization of CSM as a dietary protein source for pigs, any free gossypol must be inactivated. Although heat treatment can inactivate gossypol, the use of these thermal processes may produce inert and indigestible complexes between gossypol and protein (Berardi and Goldblatt, 1980). Gossypol toxicity can also be prevented by the addition of iron salts to the diet, such as ferrous sulfate, iron forms an insoluble and irreversible complex with gossypol in the intestinal tract, avoiding its absorption (Knabe et al., 1979) and this was the approach taken in the present study. While it is possible that the addition of iron sulphate may have an effect on amino acid digestibility, there is little data to support this. Indeed, there was a very small increase (ca. <1%) in the amino acid digestibility in rats receiving ferrous sulphate compared to ferrous lactate although there was no control diet without the addition of iron with which to compare (Perez-Llamas et al., 2001).

5. Conclusions

In conclusion, the present study suggests that the basal diet used can influence the AID of amino acids and N and the response can differ for different protein sources. Thus, the AID of amino acids in CSM appears to be higher in a complex basal diet than when fed in a simple basal diet. Given that CSM is generally fed to pigs in a complex diet under conventional production systems, the AID of amino acids may be higher than sometimes assumed. Also, AIA appears to be a more reliable digestibility indicator than Cr_2O_3.

Conflicts of interest

The authors declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

AOAC. In: Helrich K, editor. Official methods of analysis. 15 ed. Arlington, Virginia, USA: Association of Official Analytical Chemists; 1990.

Batterham ES, Andersen LM, Baigent DR, Darnell RE, Taverner MR. A comparison of the availability and ileal digestibility of lysine in cottonseed and soya-bean meals for grower/finisher pigs. Br J Nutr 1990;64(3):663–77.

Beech SA, Batterham ES, Elliot R. Utilization of ileal digestible amino acids by growing pigs: threonine. Br J Nutr 1991;65:381–90.

Berardi LC, Goldblatt LA. Gossypol. In: Liener IE, editor. Toxic constituents of plant foodstuffs. New York: Academic Press; 1980. p. 183–225.

Black JL, Campbell RG, Williams IH, James KJ, Davies GT. Simulation of energy and amino acid utilisation in the pig. Res Dev Agric 1986;3:121–45.

Collins CL, Dunshea FR, Henman DJ, McCauley I, King RH. The apparent ileal digestibility of amino acids in common vetch (Vicia sativa cv. Morava). Aust J Exp Agric 2005;45(6):705–9.

Collins CL, Eason PJ, Dunshea FR, Higgins TJV, King RH. Starch but not protein digestibility is altered in pigs fed transgenic peas containing alpha-amylase inhibitor. J Sci Food Agric 2006;86:1894–9.

Duodu CP, Adjei-Boateng D, Edziyie RE, Agbo NW, Owusu-Boateng G, Larsen BK, Skov PV. Processing techniques of selected oilseed by-products of potential use in animal feed: effects on proximate nutrient composition, amino acid profile and antinutrients. Anim Nutr 2018;4:442–51.

Fan MZ, Sauer WC, Hardin RT, Lien KA. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J Anim Sci 1994;72:2851–9.

Fan MZ, Sauer WC. Determination of apparent ileal amino acid digestibility in peas for pigs with direct, difference and regression method. Livest Prod Sci 1995;44:61–72.

Favero A, Ragland D, Vieira S, Owusu-Asieda A, Adeola O. Digestibility marker and ileal amino acid digestibility in phytase-supplemented soybean or canola meals for growing pigs. J Anim Sci 2014;92:5583–92.

Gonzalez-Vega JC, Stein HH. Amino acid digestibility in canola, cottonseed, and sunflower products fed to finishing pigs. J Anim Sci 2012;90:4391–400.

Holmes JHG, Bayley HS, Leadbeater PA, Horney FD. Digestion of protein in the small intestine and large intestine of the pig. Br J Nutr 1974;32:479–89.

Jagger S, Wiseman J, Cole DJA, Craigon J. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. Br J Nutr 1992;68:729–39.

King RH, Dunshea FR, Morrish L, Eason PJ, van Barneveld RJ, Mullan BP, Campbell RG. The energy value of *Lupinus angustifolius* and *Lupinus albus* for growing pigs. Anim Feed Sci Tech 2000;83:17–30.

Knabe DA, Tanksley TD, Hebby JH. Effect of lysine, crude fibre and free gossypol in cottonseed meal on the performance of growing pigs. J Anim Sci 1979;49:134–42.

Knabe DA, LaRue DC, Gregg EJ, Martinez GM, Tanksley TD. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J Anim Sci 1989;67:441–58.

Kozloski GV, de Moraes Flores EM, Martins AF. Use of chromium oxide in digestibility studies: variations of the results as a function of the measurement method. J Sci Food Agric 1998;76:373–6.

Kuhn G, Cermak R, Minck K, Vujicic Z, Scharrer E. Gossypol induces chloride secretion in rat proximal colon. Eur J Pharmacol 2002;457:187–94.

Langer S, Fuller MF. The effects of excessive amounts of protein on lysine utilization in growing pigs. Br J Nutr 1996;76:743–56.

Leterme P, van Leeuwen P, Thewis A, Huisman J. Effect of the carbohydrate fraction of pea seeds on the endogenous amino acid flow at the ileum of the pig. In: Verstegen MWA, Huisman J, den Hartog J, editors. Proceedings of the Vth International symposium on digestive physiology in pigs. Waegeningen: Pudoc; 1994. p. 67–70.

Li D, Xu XX, Qiao SY, Zheng CT, Chen Y, Piao XS, et al. Growth performance of growing-finishing pigs fed diets supplemented with Chinese cottonseed meal based on amino acid digestibilities. Asian-Australas J Anim Sci 2000;13:521–7.

Low AG, Zebrowska T. Digestion in pigs. In: Bock HD, Eggum BO, Low AG, Sim O, Zebrowska T, editors. Protein metabolism in farm animals: evaluation, digestion, absorption, and metabolism. Deutcher Landwirtschaftsverslag. Berlin: Oxford Science Publications; 1989. p. 53.

McCarthy JF, Aherne FX, O DB. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can J Anim Sci 1974;54:107–9.

Ma DL, Ma XK, Liu L, Zhang S. Chemical composition, energy, and amino acid digestibility in 7 cottonseed co-products fed to growing pigs. J Anim Sci 2018;96:1338–49.

Messad F, Letourneau-Montminy MP, Charbonneau E, Sauvant D, Guay F. Meta-analysis of the amino acid digestibility of oilseed meals in growing pigs. Animal 2016;10:1635–44.

Moughan PJ, Smith WC, Schrama J, Smits C. Chromic oxide and acid-insoluble ash as faecal markers in digestibility studies with young pigs. N Z J Agric Res 1991;34:85–8.

Neutra MR, Forstner JF. Gastrointestinal mucus:synthesis, secretion and function. In: Johnson LR, editor. Physiology of the gastrointestinal tract. New York: Raven Press; 1987. p. 975–1009.

Noah L, Lecannu G, David A, Kozlowski F, Champ M. Digestion of starch and glycaemic response to mixed meals in pigs. Reprod Nutr Dev 1999;39:245–54.

Perez-Llamas F, Garaulet M, Martinez JA, Marin JF, Larque E, Zamora S. Influence of dietary protein type and iron source on the absorption of amino acids and minerals. J Physiol Biochem 2001;57:321–8.

Power GN. Dietary influences on the total and individual components of endogenous protein in ileal digesta of pigs. Australia: La Trobe University; 2000.

Prawirodigdo S. Digestion, retention and excretion of nitrogen and amino acids in pigs fed cottonseed and soybean meals. Australia: The University of Melbourne; 1999.

Prawirodigdo S, Gannon NJ, van Barneveld RJ, Kerton DJ, Leury BJ, Dunshea FR. Assessment of apparent ileal digestibility of amino acids and nitrogen in cottonseed and soyabean meals fed to pigs determined using ileal dissection under halothane anaesthesia or following carbon dioxide- stunning. Br J Nutr 1998;80(2):183–91.

Rayner C. Protein hydrolysis of amino feeds for amino acid content. J Agric Food Chem 1985;33(722):725.

Saha DC, Gilbreath RL. Analytical recovery of chromium from diet and faeces determined by colorimetry and atomic absorption spectrophotometry. J Sci Food Agric 1991a;56:433–46.

Saha DC, Gilbreath RL. A modified chromic oxide indicator ratio technique for accurate determination of nutrient digestibility. Can J Anim Sci 1993;73:1001–4.

Saha DC, Gilbreath RL. Faecal recovery and diurnal variation in excretion of dietary chromium by mature swine. J Sci Food Agric 1991b;56:407–18.

Sauer WC, Stothers SC, Parker RJ. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can J Anim Sci 1977;57:775–84.

Souffrant WB. Endogenous nitrogen losses during digestion in pigs. In: Verstegen MWA, Huisman J, den Hartog J, editors. Proceedings of the Vth international symposium on digestive physiology in pigs. Waegeningen: Pudoc; 1991. p. 147.

Soy, Oilseed Blue Book. Annual directory of the World oilseed Industry. Southwest Harbor, Maine USA: Soyatech LLC; 2015. 2015.

Stein HH, Seve B, Fuller MF, Moughan PJ, de Lange CF. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci 2007;85(1):172–80.

Stein HH, Lagos LV, Casas GA. Nutritional value of feed ingredients of plant origin fed to pigs. Anim Feed Sci Technol 2016;218:33–69.

Swiatkiewicz S, Arczewska-Wlosek A, Jozefiak D. The use of cottonseed meal as a protein source for poultry: an updated review. World Poultry Sci J 2016;72:473–83.

Tanksley TD. Cottonseed meal. In: Thacker PA, Kirkwood RN, editors. Nontraditional feed sources for use in swine production. Boston: Butterworths; 1990. p. 139–51.

Turlington WH, Allee GL, Nelssen JL. Effect of protein and carbohydrate sources on digestibility and digesta flow rate in weaned pigs fed a high-fat, dry diet. J Anim Sci 1989;67:2333–40.

Wigan GC, Batterham ES, Farrell DJ. Need title. In: Hennessy DP, Cranwell PD, editors. Manipulating pig production V. Werribee, Victoria, Australia: Australasian Pig Science Association; 1995. p. 33.

Williams CH, David DJ, Iismaa O. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J Agric Sci 1962;59:381–5.

Yin YL, McEvoy JDG, Schulze H, Hennig U, S WB, McCracken KJ. Apparent digestibility (ileal and overall) of nutrients as evaluated with PVTC-cannulated or ileo-rectal anastomised pigs fed diets containing two indigestible markers. Livest Prod Sci 2000a;62:133–41.

Yin YL, McEvoy JDG, Schulze H, Hennig U, Souffrant WB, McCracken KJ. Apparent digestibility (ileal and overall) of nutrients and endogenous nitrogen losses in growing pigs fed wheat (var. Soissons) or its by-products without or with xylanase supplementation. Livest Prod Sci 2000b;62(2):119–32.