

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

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Two experiments were conducted to determine apparent ileal digestibility of amino acids (AIDAA) and nitrogen (AIDN) in cottonseed meal (CSM) and soyabean meal (SBM) fed to growing pigs. In the first experiment, twenty-four male pigs (37.3 (SE 2.7) kg) were individually penned and randomized to either CSM or SBM diets. The diets contained 40% of the protein meal (either CSM or SBM) in a wheat starch–sucrose (1:1, w/w) base containing vitamins and minerals, and Cr₂O₃ as an indigestible marker. Pigs were acclimated to the experimental diets over a 3 d period and on day 4 through to day 14 were offered 1800 g/d of the diet. Diets were offered in three meals/d from day 4 to day 11 and in eight meals/d from day 12 to day 13. After the eighth hourly-meal on day 14, twelve pigs were anaesthetized with halothane while the remaining twelve pigs were CO₂-stunned and processed using commercial slaughter procedures. Ileal digesta were collected from a 1500 mm portion of the terminal ileum of each pig and subsequently analysed for amino acids, N, organic matter and Cr. Results indicated that AIDAA of CSM and SBM were lower when digesta were collected following CO₂-stunning than when digesta were obtained under halothane anaesthesia. Consistently, AIDN in CSM (0.51 v. 0.56) and SBM (0.55 v. 0.71) were lower ($P < 0.05$) in CO₂-stunned pigs than in halothane-anaesthetized pigs. Furthermore, when digesta collection was conducted under halothane anaesthesia, AIDN of CSM was lower ($P < 0.001$) than that of SBM. In the second experiment, six male pigs (45 (SE 2.6) kg) were fitted with T-piece cannulas implanted in the terminal ileum, housed individually in metabolism cages, and randomly allocated to either CSM or SBM diets in a single reversal arrangement. Ileal digesta were collected for AIDAA and AIDN determination. Although statistical comparisons could not be made between the two experiments, the AIDAA and AIDN data obtained via cannulated pigs were similar to those values obtained using the halothane-anaesthesia method. Overall, the CO₂-stunning method is not recommended for studies of amino acid or nitrogen ileal digestibilities, but may be useful for the study of other dietary constituents.

Ileal digestibility: Cottonseed meal: Soyabean meal: CO₂-stunning

Typically, ileal digestibility of nutrients in pig diets have been determined following the surgical preparation of animals to facilitate digesta collection from the terminal ileum. Recently, a technique involving collection of ileal digesta under halothane anaesthesia before exsanguination or barbiturate injection has been described as an alternative to the cannulation technique (Moughan & Smith, 1987; Moughan *et al.* 1991). An advantage of the anaesthesia approach is that surgical intervention is minimal and labour

required to maintain the cannulas is obviated. A disadvantage of the anaesthesia technique, however, is that the presence of anaesthetic and barbiturate in the pig makes the carcass unsuitable for human consumption.

Traditional electrical stunning of the pig at a commercial abattoir with collection of ileal digesta during evisceration is not an alternative to the use of anaesthetics and barbiturates for studying ileal digestibility. The stress of transport and lairage and, more importantly, the sloughing

Abbreviations: AIDAA, apparent ileal digestibility of amino acids; AIDN, apparent ileal digestibility of nitrogen; CSM, cottonseed meal; OM, organic matter; SBM, soyabean meal.

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of intestinal cells following electrocution may significantly increase the protein content of the ileal digesta resulting in an underestimation of digestibility. For example, Badawy *et al.* (1957) demonstrated that collection of digesta following slaughter of sheep was not suitable for measuring digestibility. One possible alternative is to use CO₂-stunning combined with the commercial slaughter procedures and the careful removal of the ileal digesta during evisceration. If the CO₂-stunning technique provides similar estimates of amino acid digestibility as removal under halothane anaesthesia, then exploitation of CO₂-stunning will allow ileal digestibility of feedstuffs to be analysed without forfeiting the cost of the animal. Therefore, the aim of the present study was to determine whether CO₂-stunning and halothane anaesthesia before exsanguination give similar estimates for the apparent ileal digestibility of amino acids (AIDAA) from diets with two feedstuffs (cottonseed meal and soyabean meal) differing widely in their apparent ileal digestibility in growing pigs using the slaughter technique. A confirmatory experiment was conducted with pigs surgically prepared with T-piece cannulas.

Materials and methods

Protein sources and diets

The experiment used prepress solvent-processed cottonseed meal (CSM) and prepress solvent-processed soyabean meal (SBM) as the only sources of protein in the experimental diets. The chemical compositions of the two protein sources are presented in Table 1. The experimental diets were formulated to contain 400 g/kg of either CSM or SBM in a wheat starch–sucrose (1:1, w/w) base. Sucrose (cane sugar) and wheat starch were chosen as they provide N-free sources of energy. Cr₂O₃ was added (2.5 g/kg diet, LaRue *et al.* 1985) as an indigestible marker to enable calculation of the apparent ileal and faecal digestibilities. FeSO₄.7H₂O

Table 1. Composition of the cottonseed meal and soyabean meal used in experimental diets (g/kg, air-dry basis)

Constituent	CSM	SBM
Dry matter	911.0	900.0
Crude protein (N×6.25)	381.6	492.6
Organic matter	849.1	832.5
Indispensable amino acids		
Arginine	38.1	34.5
Histidine	11.4	14.0
Isoleucine	12.4	24.3
Leucine	20.6	35.8
Lysine	15.9	29.8
Phenylalanine	18.9	25.3
Threonine	11.8	19.4
Tyrosine	8.9	16.5
Valine	3.5	4.3
Dispensable amino acids		
Alanine	13.8	22.5
Aspartic acid	30.9	51.3
Glutamic acid	67.0	82.9
Glycine	14.6	21.3
Proline	13.4	24.0
Serine	15.7	25.3

CSM, cottonseed meal; SBM, soyabean meal.

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

Diets ...	CSM-based	SBM-based
Component		
Cottonseed meal	400.0	—
Soyabean meal	—	400.0
Cane sugar (sucrose)	263.5	263.5
Wheat starch	263.5	263.5
FeSO ₄ .7H ₂ O	2.0	—
K ₂ SO ₄	—	1.5
MgSO ₄	—	0.5
Minerals and vitamins premix*	5.0	5.0
Ca ₂ PO ₄	30.0	30.0
Cr ₂ O ₃	2.5	2.5
Tallow	33.5	33.5
Analysed		
Dry matter	954.7	945.3
Organic matter	897.8	884.9
Crude protein (nitrogen×6.25)	156.1	196.8
Lysine	6.8	12.7
Estimated		
Digestible energy (MJ/kg)	15.6	15.0

CSM, cottonseed meal; SBM, soyabean meal.

*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, 1100; cyanocobalamin, 0.017; Fe, 88; Zn, 55; Mn, 22; Cu, 6.6; I, 0.22; Se, 0.1.

was included (2 g/kg) in the CSM diet to inactivate free gossypol present in CSM (Tanksley *et al.* 1981; Batterham *et al.* 1990a). MgSO₄ and NaSO₄ were added (1.5 and 0.5 g/kg, respectively) to the SBM diet to balance sulfate inclusion in the experimental diets (Table 2). The diets were offered to the pigs in a mash form.

Experiment 1: animals and management

The first experiment was conducted at Victorian Institute of Animal Science, Werribee, Victoria. Twenty-four Large White×Landrace male pigs (37.3 (SE 2.7) kg), supplied by the Institute's piggery, were penned individually and randomized to either the CSM or SBM diets. Water was supplied *ad libitum* throughout the experimental period via nipple drinkers. The pigs were acclimated to their diets over a 3 d period and on day 4 through to day 14 were given 1800 g/d of the experimental diet. Diets were offered in 3 meals/d (08.00, 13.00 and 18.00 hours) from day 4 to 11. From day 12 to 13, pigs were offered 225 g of feed every 3 h (8 meals/d). On day 14, diets were offered hourly and in the morning after their fifth feed the pigs were individually transported 1 km to a lairage area which serviced a pilot abattoir and experimental surgery. Pigs were placed in individual pens for 3 h and received hourly feeds before CO₂-stunning or halothane anaesthesia.

Twelve pigs were sedated using 5 ml of Stresnil (Janssen Pharmaceutical, Beerse, Belgium) injected intramuscularly and left undisturbed in a peaceful environment for 15 min. The sedated pigs were then anaesthetized by inhalation of Fluothane (Rhone Merieux, Australia). After each pig was anaesthetized a ventral abdominal midline incision was made, the caecum located and then a 1500 mm portion of terminal ileum and the rectum were excised to enable

simultaneous collection of the ileal and faecal digesta. The ileal digesta were gently expelled, collected and stored at -20° until analysed. Faecal digesta were also expelled, collected and stored at -20° until analysis. Finally, the anaesthetized 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.

The remaining twelve pigs were gently and quietly driven individually into the stunning crate, lowered down to the stunning pit (Dip-lift stunner Butina, Denmark) and stunned with the CO_2 gas (concentration 85%) for 120 s (Troeger & Woltersdorf, 1991). The unconscious pig was raised from the pit and slaughtered in accordance with standard commercial slaughter procedures. This involved exsanguination, followed by scalding and dehairing after the pig was placed in a water bath at 65° for 5 min. The pig was then hosed and eviscerated. On average, it took 20 min to process the slaughtered pig before evisceration. During evisceration, the whole intestine and the rectum was collected in a tray, the caecum identified and then the digesta from the 1500 mm of terminal ileum were carefully collected and stored at -20° before freeze-drying and subsequent analyses. Simultaneously, after the rectum of each pig was excised, faecal digesta were removed, collected and stored at -20° before being freeze-dried.

Ileal digesta from all pigs were freeze-dried, finely ground and analysed for amino acids, total N, organic matter (OM) and Cr. Faeces from both groups of pigs were freeze-dried and ground for total N, OM and Cr determination.

Experiment 2: animals and management

The second experiment was conducted at The South Australian Research and Development Institute, Pig and Poultry Production Institute, Roseworthy, Australia using six Large White \times Landrace male pigs (35–40 kg) provided by the Institute's piggery. Each pig was surgically fitted with a simple T-piece cannula about 150 mm anterior to the ileo-caecal valve as described by Van Barneveld *et al.* (1994), housed individually in solid-sided pens (1.5 \times 2.2 m) and fed on a commercial grower diet (0.7 g available lysine/MJ digestible energy, 14.0 MJ digestible energy/kg) for a recovery period of 7 d. In this experiment the skin barriers for use around stoma in human ileostomy patients (Stomahesive® System 2 with 70 mm flange; Bristol-Myers Squibb, Princetown, NJ, USA) were incorporated between the flange of the cannula and the skin to promote healing of the wound and to prevent any leakage around the cannula.

After the 7 d recovery period the pigs (45 (SE 2.6) kg) were moved to a metabolism room, and housed individually in the metabolism cages. Pigs were blocked on weight and position in the experimental facilities, and randomly assigned to either CSM or SBM diets. The experimental diets were the same diets described in Experiment 1. Water was provided *ad libitum* via nipple drinkers. A single reversal experimental design was employed with each pig receiving both diets.

In the first period of collection of ileal digesta and faeces samples, experimental diets were introduced over a 3 d

period then fed for a further 5 d. Each pig received 1800 g/d experimental diet and the diets were offered to the pigs in 3 meals/d. Ileal digesta samples were collected on days 6 and 7 via the T-piece cannula and stored at -21° . However, since Cr was used as an inert marker for digestibility determination, only a part of the ileal digesta was sampled to allow collection of faeces. Faeces were collected daily using a metal tray placed underneath the rear part of each metabolism cage, bulked and stored at -21° .

In the second period of sample collection, on day 8, the diets were exchanged. Pigs were acclimated to their new diets for a 3 d period and conditioned to the same schedule as employed in the first collection period.

At the end of the experimental period, ileal digesta and faeces samples were thawed, mixed, subsampled, and stored at -20° before freeze-drying. The freeze-dried ileal digesta samples were finely ground and analysed for OM, N, amino acid and Cr as described in Experiment 1. The freeze-dried faeces samples were ground and analysed using the same procedures for OM and N content.

Chemical analyses

The DM content of the feedstuffs and experimental diets was determined by drying in a forced-air oven at 95° for 24 h. OM was measured in the feed, experimental diets, ileal digesta and faeces by ashing at 550° for 6 h (Association of Official Analytical Chemists, 1990). Total N content of the samples was assayed using a macro-Kjeldahl method (Association of Official Analytical Chemists, 1990) in all experiments of this study. The samples were digested in H_2SO_4 and a Se-catalyst followed by steam distillation, and analysed by an automatic N-analyzer (Tecator Kjeltec Auto Sampler System 1035 Analyzer; Radiometer Pacific-Copenhagen, Blackburn, Vic., Australia). Amino acids were determined following acid hydrolysis in 6 M-HCl to hydrolyse the peptide bonds of the protein in the ileal digesta and diet samples as recommended by Rayner (1985). The mixture of amino acids was separated on an HPLC ion exchange (strong cation exchange) column (Waters Australia Pty. Ltd., Box Hill, Vic., Australia) using post-column derivatization with ninhydrin.

Concentrations of Cr in the diets, ileal digesta and faeces were determined by X-ray spectrometry (Philips PW 1404/10 X-ray spectrometer; Mulgrave, Vic., Australia) based on the method of Jenkins & De Vries (1967). Each sample was finely ground using the LM 1 Laboratory mill (Labtechnics Australia, Ridleyton, SA., Australia), and approximately 0.5 g of sample was pressed using a hydraulic press to produce a circular tablet 5 mm thick and 38 mm in diameter before being placed in the X-ray spectrometer.

Cr concentrations in the diets, ileal digesta and faeces were used for calculating the apparent ileal digestibilities of amino acids, N and OM, and faecal digestibility of N and OM, as described by Saha & Gilbreath (1993). The general formula used for calculating apparent ileal digestibilities of amino acids, N and OM is presented below using lysine as an example:

$$\text{AIDL (\%)} = 100 - ((\text{Cr}_d/\text{Cr}_{id}) \times (\text{L}_{id}/\text{L}_d) \times 100),$$

where AIDL is apparent ileal digestibility of lysine, Cr_d and

Cr_{id} are the concentrations of chromium (%) in the experimental diet and ileal digesta respectively, L_{id} represents the lysine content (%) of the ileal digesta, and L_d is the lysine content (%) of the diet.

Statistical analyses

Experiment 1. A completely randomized design with a factorial 2×2 arrangement was employed in Experiment 1. For the analyses of individual amino acid digestibilities, the data were analysed by two-way ANOVA with the respective factors being protein source (CSM *v.* SBM), and method (halothane *v.* CO₂-stunning). For digestibilities of N and OM, site of sampling (ileum and rectum) was used as an additional factor ($2 \times 2 \times 2$) with pig used as a blocking factor.

Experiment 2. The experimental design of Experiment 2 was a single reversal design and data were analysed by ANOVA with protein source (SBM *v.* CSM) as the main effect.

There was no statistical comparison made between data of Experiment 1 and Experiment 2 because these two experiments were conducted in different environments and with different experimental animals.

All statistical analyses were performed using the GENSTAT 5 program as described by Payne *et al.* (1993).

Results

Experiment 1

The CO₂-stunning technique consistently provided lower AIDAA (except proline, Table 3) and AIDN ($P < 0.05$, Table 4) estimates than the halothane-anaesthesia technique. In contrast to the AIDAA and AIDN data, the apparent ileal digestibility of OM of CSM or SBM diets was not affected by method of sampling (Table 4). When ileal digesta were collected under halothane anaesthesia (an accepted standard of measurement) the AIDAA, AIDN

Table 3. Apparent ileal digestibilities of amino acids in pigs fed on cottonseed meal-based (n 8) and soyabean meal-based (n 8) diets measured under different slaughter techniques (Experiment 1)†

Diet...	Digestibility (%)				SED	Significance‡
	CO ₂ -stunned		Halothane-anaesthetized			
	CSM-based	SBM-based	CSM-based	SBM-based		
Indispensable amino acids						
Arginine	71.1	70.3	81.3	81.4	3.95	M***
Histidine	48.5	55.6	66.5	73.8	6.93	M**
Isoleucine	37.0	60.1	60.2	72.1	7.94	D**; M**
Leucine	36.8	59.4	59.9	71.2	8.19	D**; M**
Lysine	27.5	59.7	55.4	75.3	8.01	D***; M***
Phenylalanine	60.6	62.5	74.5	72.7	5.84	M**
Threonine	30.4	50.7	50.8	64.5	9.22	D*; M*
Tyrosine	34.8	56.2	56.1	69.0	9.49	D**; M***
Valine	42.8	52.2	62.6	69.6	8.56	M**
Dispensable amino acids						
Alanine	32.5	53.3	56.4	68.5	7.32	D**; M***
Aspartic acid	52.0	59.1	66.2	69.9	6.53	M*
Glutamic acid	66.4	63.9	78.4	76.1	4.96	M**
Glycine	24.0	35.0	52.0	56.4	10.56	M**
Proline	-9.9	50.9	16.5	65.9	15.55	D***
Serine	47.4	63.7	61.3	70.9	6.49	D**; M*

CSM, cottonseed meal; D, diet; M, method; SBM, soyabean meal; SED, standard error of the difference for method \times site of collection.

† For details of diets and procedures, see Tables 1 and 2 and pp. 184–186.

‡ Statistical analysis was undertaken using two-way ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Influence of collection method and site of digesta collection on apparent nitrogen and organic matter digestibilities for pigs fed on cottonseed meal-based (n 8) and soyabean meal-based (n 8) diets (Experiment 1)†

Collection site...		Digestibility (%)				SED	Significance‡
		CO ₂ -stunned		Halothane-anaesthetized			
		Ileal	Faecal	Ileal	Faecal		
N	CSM-based	51.0	56.4	64.5	56.1	5.37	D***; M*; S***;
	SBM-based	54.9	81.1	70.5	80.4		D \times S***; M \times S***.
OM	CSM-based	69.7	74.3	71.8	73.8	4.07	D***; S***;
	SBM-based	72.0	92.7	77.2	91.5		D \times S***.

CSM, cottonseed meal; OM, organic matter; SBM, soyabean meal; SED, standard error of the difference for method \times site of collection.

† For details of diets and procedures, see Tables 1 and 2 and pp. 184–186.

‡ Statistical analysis was undertaken using two-way ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and the apparent ileal digestibility of OM in the CSM diet were significantly lower ($P < 0.001$) than in the SBM diets (Tables 3 and 4). Similarly, faecal digestibilities of N and OM in the CSM diet were lower ($P < 0.001$) than in the SBM diet.

Faecal digestibilities of N and OM were higher ($P < 0.01$) than the ileal digestibilities in pigs consuming the SBM diet. However, there was no significant difference ($P > 0.05$) between ileal and faecal digestibilities of N and OM in pigs consuming the CSM diet. There were significant interactions affecting digestibility of N between dietary protein source and site of collection ($P < 0.001$) and between method of collection and site of sampling ($P < 0.01$). This resulted in a significant ($P < 0.001$) interaction between dietary protein source and site of collection in the digestibility of OM (Table 4). Mean daily body weight gain was lower (508 v. 708 g/d; $P < 0.001$) and feed:body weight gain ratio was higher (3.31 v. 2.42, $P < 0.001$) in pigs fed on the CSM diet compared with pigs fed on the SBM diet.

Experiment 2

In Experiment 2 the AIDAA and AIDN of CSM were significantly lower than that of SBM. The results of the present experiment were in accordance with the data obtained in Experiment 1 (Table 5). Similarly, the data suggested that both apparent ileal digestibilities of indispensable and dispensable amino acids were lower (from $P < 0.05$ to $P < 0.001$) when CSM was compared with SBM. For instance, the apparent ileal digestibilities of lysine and threonine of CSM were much lower ($P < 0.001$ and $P < 0.01$ respectively) than those of SBM.

Furthermore, it was found that within the indispensable amino acids the difference between the apparent ileal digestibilities of amino acids of CSM and SBM was greatest for lysine (32.7%; see Table 5), and also most pronounced for tyrosine (30.8%) followed by threonine (27.8%), isoleucine (26.8%) and leucine (23.6%).

In addition, there had been further digestion of N and OM in the hindgut of pigs consuming the SBM diet. The cannulated pigs fed on the CSM diet exhibited similar differences between apparent ileal and faecal digestibilities of N compared with the halothane-anaesthetized pigs. The apparent faecal digestibility of OM in pigs fed on SBM was significantly higher ($P < 0.001$) than the apparent ileal digestibility of OM. However, while there was no significant difference between ileal and faecal digestibilities of N, more OM disappeared (9.8%; $P < 0.001$) in the hindgut than in the small intestine of cannulated pigs consuming the CSM diet. Table 6 displays the differences between apparent ileal and faecal digestibilities of N and OM in the cannulated pigs consuming the CSM and SBM diets.

There were also significant interactions between dietary protein source and site of collection ($P < 0.001$) for the digestibility of N. Nevertheless, there was no significant interaction between dietary protein source and site of collection for the digestibility of OM. The faecal digestibility of OM in pigs fed on the CSM diet was lower ($P < 0.05$) than in pigs fed on the SBM diet.

Discussion

Effect of sampling techniques on the apparent ileal digestibilities of amino acids, nitrogen and organic matter

The halothane technique is considered to be an accepted approach for estimating ileal digestibility (Moughan & Smith, 1987; Moughan *et al.* 1991). The alternative approach described in this paper for collecting ileal digesta by using CO₂-stunning was a modification of the slaughter technique using halothane anaesthesia developed by Moughan & Smith (1987). However, collection of ileal digesta following CO₂-stunning and using commercial slaughter conditions to reduce the cost of routine evaluation of protein meals was not satisfactory, since

Table 5. Apparent ileal digestibilities of amino acids of cottonseed meal and soyabean meal in pigs (n 6) fitted with T-piece cannulas (Experiment 2)†

Amino acid	Digestibility (%)		SED	Difference (%)	Significance (P)‡
	CSM	SBM			
Indispensable amino acids					
Arginine	79.2	85.1	2.25	5.9	*
Histidine	65.8	81.9	2.34	16.1	***
Isoleucine	50.4	77.2	4.73	26.8	**
Leucine	53.4	77.0	4.45	23.6	**
Lysine	51.1	83.8	3.62	32.7	***
Phenylalanine	71.1	80.1	3.15	9.0	*
Threonine	41.7	69.5	5.06	27.8	**
Tyrosine	63.2	94.0	3.63	30.8	***
Valine	60.2	78.2	3.87	18.0	**
Dispensable amino acids					
Alanine	49.8	71.4	5.44	21.6	*
Aspartic acid	61.7	75.1	4.00	13.4	*
Glutamic acid	77.3	82.3	1.90	4.95	*
Glycine	43.4	61.2	6.63	17.8	*
Proline	27.7	72.8	7.96	45.1	**
Serine	56.4	77.4	3.66	21.0	**

CSM, cottonseed meal; SBM, soyabean meal.

† For details of diets and procedures, see Tables 1 and 2 and pp. 184–186.

‡ Statistical analysis was undertaken using ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 6. Apparent ileal and faecal digestibilities of nitrogen and organic matter of cottonseed meal and soyabean meal in pigs (*n* 6) fitted with T-piece cannulas (Experiment 2)†

		Digestibility (%)		SED	Significance‡
		Site of collection			
		Ileal	Faecal		
N	CSM	61.5	62.0	2.52	D × S*
	SBM	74.6	85.6		
OM	CSM	68.3	78.1	2.67	D*, S***
	SBM	79.3	92.7		

CSM, cottonseed meal; D, diet; OM, organic matter; S, site; SED, standard error of the difference for diet × site; SBM, soyabean meal.

† For details of diets and procedures, see Tables 1 and 2 and pp. 184–186.

‡ Statistical analysis was undertaken using ANOVA: * $P < 0.05$, *** $P < 0.0001$.

AIDAA and AIDN were consistently underestimated. In contrast, OM digestibility was unaffected by the method of obtaining ileal digesta. One explanation for these findings may be sloughing of intestinal mucosa (Badawy *et al.* 1957) during the slaughter process post-CO₂-stunning. Intestinal mucosal cells contributing significant amounts of protein would increase the N content of ileal digesta, thus reducing the apparent amino acid and N digestibilities.

While no information is available for the pig, Badawy *et al.* (1957) demonstrated remarkable histological changes of the duodenum when sheep were stunned with a captive bolt and then exsanguinated. Although the duodenal mucosa remained intact when the specimen was taken under pentobarbitone anaesthesia, there was much shedding of the epithelium and a loss of nitrogenous material from Brunner's glands when the specimen was taken after the sheep was slaughtered (Badawy, 1964).

A contributing factor to the sloughing of the intestinal cells may have been the considerable agitation of the small intestine during the commercial slaughter process, particularly during the dehairing process. On the other hand the relatively small contribution of OM by sloughed cells to the OM content of the ileal digesta would not significantly alter OM digestibility.

While it is possible that CO₂-stunning and exsanguination combined with immediate sampling of ileal digesta may rectify the apparent problem of increased endogenous secretion, the CO₂-stunning technique is probably not a satisfactory method for routine measurement of amino acid digestibility. However, since OM digestibility was not significantly affected by the method of sampling, the CO₂-stunning and commercial processing technique may be suitable for studying the digestion of dietary components that are not found in appreciable amounts in intestinal epithelial cells or other ileal sections (such as starch, oligosaccharides or NSP; Govers *et al.* 1997).

Comparison of apparent ileal digestibilities of cottonseed meal and soyabean meal

When comparing the ileal digestibilities of CSM and SBM the halothane technique will be used as the standard throughout this discussion. Although no statistical

comparisons could be made between the two methods, the halothane and T-piece cannulation techniques provided similar estimates of ileal digestibility. In general, when the samples were obtained through the T-piece cannula there was a slight underestimation of ileal digestibility of CSM (–3%) and overestimation of AIDAA of SBM (+4.1%) compared with when samples were obtained under halothane anaesthesia (Table 7). These differences may be due to the differences in weight and genotype of pigs, feeding regimen or pattern of digesta flow between Experiment 1 and Experiment 2. Furthermore, the variation (SED) of ileal digestibility of amino acids in Experiment 1 (Table 3) was consistently higher than in Experiment 2 (Table 5). Since ileal digestibilities of amino acids for CSM and SBM were measured in the same pigs in a single reversal design, this may have contributed to lower SED in Experiment 2.

The data obtained from the present experiments provide further evidence that CSM has lower AIDAA than SBM. Other researchers (Tanksley *et al.* 1981; LaRue *et al.* 1985; Furuya & Kaji, 1989; Batterham *et al.* 1990b; Lekule *et al.* 1990; Leibholz, 1992; Yin *et al.* 1994) have also reported that the AIDAA of SBM was greater than that of CSM (Table 7). Erbersdobler (1976) and Sauer & Ozimek (1986) suggested that incomplete digestion of amino acids in some feeds may be caused by inaccessibility of the protein due to a higher indigestible fibre content, inhibition of the enzyme binding-site in the protein or the presence of protease inhibitors.

The presence of high levels of indigestible fibre in the diet has been shown to decrease the digestibilities of protein and amino acids in pigs (Mitaru *et al.* 1984; Jondreville *et al.* 1994), presumably due to the ability of fibre to adsorb amino acids and peptides, partly preventing them from being absorbed by the gastrointestinal tract (Mitaru *et al.* 1984). It is also well documented that CSM contains higher levels of indigestible cell wall components than SBM (Tanksley *et al.* 1981; Batterham *et al.* 1990b; Yin *et al.* 1994), and this could possibly account for part of the lower amino acid and N digestibilities in CSM. Interestingly, Schneeman *et al.* (1982) demonstrated that inclusion of fibre in the diets of rats increased the amount of sloughing of small intestine mucosal cells causing an increase in the endogenous N loss, which in turn resulted in underestimation of AIDAA and AIDN measurements. Close (1993) and Low (1993) suggested that increasing the concentration of the fibre in the diet generally causes reduction in the apparent digestibility of particular nutrients. Certainly, the ileal OM digestibility of CSM is lower than that of SBM. Furthermore, there is little further digestibility of OM in the hindgut in pigs fed on CSM.

The Maillard reaction which occurs during heat processing is one of the factors that decreases the digestibility of proteins and amino acids of protein sources. The prepress solvent-processed CSM used as a source of dietary protein in the present experiment was subjected to heating before mechanical breakdown and subsequent solvent extraction (Godin & Spensley, 1971).

It is also possible that during the heating and extraction process of cottonseed oil, the ϵ -amino acids are bound with carbohydrate and/or gossypol (Berardi & Goldblatt,

Table 7. Various reported values of apparent ileal digestibilities of lysine, threonine and nitrogen in cottonseed and soyabean meals

	Chemical analysis (g/kg)			Apparent ileal digestibility (%)			Source of carbohydrate	Technique	Authors
	Crude protein	Lysine	Threonine	Crude fibre	Nitrogen	Lysine			
CSM	431.0	16.0	12.0	118.0	74.6	64.0	Corn starch	TPC	Tanksley <i>et al.</i> (1981)
CSM	ND	ND	ND	ND	74.2	54.0-68.0	Corn starch	TPC	Leibholz & Mollah (1988)
CSM; PE	361.0	14.0	12.0	129.0	71.0-73.0	56.0-67.0	Wheat + starch	TPC	Batterham <i>et al.</i> (1990b)
CSM; EE	445.0	18.0	13.0	84.0	78.0	71.0	Wheat + starch	TPC	
CSM	379.0	13.0	12.0	60.0*	73.0	43.0	Corn starch	TPC	Yin <i>et al.</i> (1994)
CSM	348.0	16.0	12.0	150.0	64.5	55.4	Wheat starch and sucrose	H	Present study
					61.5	51.1	Wheat starch and sucrose	TPC	
					51.0	27.5	Wheat starch and sucrose	CDS	
SBM	505.0	28.0	17.0	60.0	81.6	88.5	Corn starch	TPC	Tanksley <i>et al.</i> (1981)
SBM	ND	ND	ND	ND	85.8	83.0-92.0	Corn starch	TPC	Leibholz & Mollah (1988)
SBM	425.0	23.0	17.0	59.0	83.0	87.0	Wheat + starch	TPC	Batterham <i>et al.</i> (1990b)
SBM	477.0	22.0	16.0	33.0*	82.4	85.4	Corn starch	TPC	Yin <i>et al.</i> (1994)
SBM	444.0	29.8	19.0	ND	70.5	75.3	Wheat starch and sucrose	H	Present study
					74.6	83.8	Wheat starch and sucrose	TPC	
					54.9	59.7	Wheat starch and sucrose	CDS	

CDS, CO₂-stunning; CSM, cottonseed meal; EE, expeller extracted; ND, not detected; H, halothane anaesthesia; PE, prepress solvent-extracted; SBM, soyabean meal; TPC, T-piece cannula.
* Neutral-detergent fibre.

1980). Furthermore, Öste *et al.* (1987) found some competitive inhibitors of amino-peptidase N when they examined the effect of Maillard reaction products on the digestion of protein. It is possible that the mechanical process for producing CSM may produce compounds that damage the activity of secreted proteases in pigs fed on CSM. A further possibility is that CSM contains some anti-nutritive factors such as condensed tannins (Yu *et al.* 1996) which may result in a very low AIDAA and AIDN. Huisman (1989) explained that tannins can inhibit trypsin activity.

The ileal digestibilities of lysine (0.55) and threonine (0.51) of CSM in the present study (Table 3) was low, which is in close agreement with Tanksley & Knabe (1993) and Yu *et al.* (1996). The AIDAA and AIDN of CSM and SBM in the present experiment were lower than determined in earlier investigations (Leibholz & Mollah, 1988; Batterham *et al.* 1990b). While this could represent variabilities in the quality of CSM and SBM, it could also reflect an interaction between carbohydrate source and N digestibility. In the experiment of Leibholz & Mollah (1988) and Batterham *et al.* (1990b) corn starch and wheat were used in the basal diet, while in the present experiment a sucrose and starch-based diet was used. Various values of AIDAA and AIDN of CSM and SBM from previous experiments (Leibholz & Mollah, 1988; Batterham *et al.* 1990b; Yin *et al.* 1994) and the data from the present study are given in Table 7. Recently, we have confirmed that the basal diet can have marked effects upon N digestibility of protein sources (Prawirodigdo *et al.* 1997).

Table 3 also shows that ileal digestibility of proline (0.17) of CSM was very low. Rérat (1978) reviewed the literature and suggested that very large amounts of proline were recovered in ileal digesta, presumably because proline was poorly absorbed in the small intestine of pigs. Sauer *et al.* (1977) demonstrated that proline made up 42% of the endogenous amino acid levels in the ileal digesta of pigs fed on a protein-free diet. Thus, the higher level of proline recovered in the ileal digesta of pigs consuming the CSM diet in the present study is probably due to endogenous protein secretion and poor absorption of proline.

Comparison of apparent faecal digestibilities of cottonseed meal and soyabean meal

In the pigs (Experiment 1) fed on SBM there were further increases in digestibility of both N and OM in the hindgut, whereas for the CSM there were no further changes in digestibility in the hindgut, confirming that there is no apparent net effect of microbial synthesis of amino acids in the hindgut in pigs fed on CSM (Batterham *et al.* 1990a). This is possibly due to either inhibitory effects of gossypol on microflora (Batterham *et al.* 1990a) or to protein-gossypol complexes formed during processing (Tanksley *et al.* 1981; Sauer & Ozimek, 1986). However, in cannulated pigs fed on the CSM diet there was a significant increase in digestion of OM between the ileum and rectum (Experiment 2) which was not apparent in intact pigs (Experiment 1). In contrast, Huisman *et al.* (1984) reported that apparent faecal digestibilities of nutrients in pigs were not influenced by the insertion of ileocaecal re-entrant cannulas.

Overall, the results of the present experiments demonstrate that in pigs, apparent ileal digestibilities of amino acids, N and OM for CSM are lower than for SBM. Although the CO₂-stunning technique is not recommended for collection of ileal digesta in the study of ileal digestibility of amino acids and N, this method may be suitable to measure the digestibility of OM or other dietary constituents of feedstuffs and diets.

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References

- Association of Official Analytical Chemists (1990) *Official Methods of Analysis*. Washington, DC: Association of Official Analytical Chemists.
- Badawy AM (1964) Changes in the protein and non-protein nitrogen in the digesta of the sheep. In *The Role of the Gastro-intestinal Tract in Protein Metabolism*, pp. 175–185 [HN Munro, editor]. Oxford: Blackwell.
- Badawy AM, Campbell RM, Cuthbertson DP & Fell BF (1957) Changes in the intestinal mucosa of the sheep following death by humane killer. *Nature* **180**, 756–757.
- Batterham ES, Andersen LM, Baigent DR, Beech SA & Elliott R (1990a) Utilization of ileal digestible amino acids by pigs: lysine. *British Journal of Nutrition* **64**, 679–690.
- Batterham ES, Andersen LM, Baigent DR, Darnell RE & Taverner MR (1990b) A comparison of availability and ileal digestibility of lysine in cottonseed and soya-bean meals for grower/finisher pigs. *British Journal of Nutrition* **64**, 663–677.
- Berardi LC & Goldblatt LA (1980) Gossypol. In *Toxic Constituents of Plant Foodstuffs*, 2nd edition, pp. 183–225 [IE Liener, editor]. New York: Academic Press.
- Close WH (1993) Fibrous diet for pigs. *Animal Production Journal in Developing Countries, Occasional Publication* **16**, 107–117.
- Erbersdobler H (1976) Amino acid availability. In *Protein Metabolism and Nutrition*, pp. 139–158 [DJA Cole, KN Boorman, PJ Lewis, RJ Neale and H Swan, editors]. London: Butterworths.
- Furuya S & Kaji Y (1989) Estimation of true ileal digestibility of amino acids and nitrogen from their apparent values for growing pigs. *Animal Feed Science and Technology* **26**, 271–285.
- Godin VJ & Spensley PC (1971) *Oil and Oilseeds*. London: The Tropical Products Institute, Foreign and Commonwealth Office.
- Govers MJAP, Gannon NJ, Dunshea FR, Fielding M, Kiliass D, Gibson PR & Muir JG (1997) Altering the site of fermentation in the pig: Implications for colon cancer risk in humans. In *Manipulating Pig Production VI*, p. 181 [PD Cranwell, editor]. Werribee, Victoria, Australia: Australasian Pig Science Association.
- Huisman J (1989) Antinutritional factors in the nutrition of monogastric farm animals. In *Nutrition and Digestive Physiology in Monogastric Farm Animals*, pp. 17–35 [EJ van Weerden and J Huisman, editors]. Wageningen, Holland: Pudoc.
- Huisman J, van Weerden EJ & van Leeuwen P (1984) Effect of the insertion of cannulas on rate passage and nutrient digestibilities in pigs. *Journal of Animal Science* **59**, Suppl. 1, 271.
- Jenkins R & de Vries JL (1967) *Practical X-ray Spectrometry*. New York: Springer-Verlag New York Inc.
- Jondreville C, van Den Broecke J, Delpech A, Gatel F, Bertin JM, Beaux MF & Grosjean F (1994) Facteurs de variation de la digestibilité ileale des acides amines des cereales chez le porc charcutier (Factors influencing the ileal digestibility of amino acids from cereals in the pig). *Journées de la Recherche Porcine en France* **26**, 251–258.
- LaRue DC, Knabe DA & Tanksley TD Jr (1985) Commercially processed glandless cottonseed meal for starter, grower and finisher swine. *Journal of Animal Science* **60**, 495–502.
- Leibholz J (1992) The availability of lysine in diets for pigs: comparative methodology. *British Journal of Nutrition* **67**, 401–410.
- Leibholz J & Mollah Y (1988) Digestibility of threonine from protein sources for growing pigs. II: The digestibility of threonine to the terminal ileum in pigs given six protein sources. *Australian Journal of Agriculture Research* **39**, 721–728.
- Lekule FP, Jørgensen H, Fernandez JA & Just A (1990) Nutritive value of some tropical feedstuffs for pigs. Chemical composition, digestibility and metabolizable energy content. *Animal Feed Science and Technology* **28**, 91–101.
- Low AG (1993) Role of dietary fibre in pig diets. In *Recent Development in Pig Nutrition 2*, pp. 137–162 [DJA Cole, W Haresign and PC Garnsworthy, editors]. Nottingham: Nottingham University Press.
- Mitaru BN, Blair R, Reichert RD & Roe WE (1984) Dark and yellow rapeseed hulls, soybean hulls and a purified fiber source: their effect on dry matter, energy, protein and amino acid digestibilities in cannulated pigs. *Journal of Animal Science* **59**, 1510–1519.
- Moughan PJ & Smith WC (1987) A note on the effect of cannulation of terminal ileum of the growing pig on the apparent ileal digestibility of amino acids in ground barley. *Animal Production* **4**, 319–321.
- Moughan PJ, Smith WC, Pearson G & James KAC (1991) Assessment of apparent ileal lysine digestibility for use in diet formulation for the growing pig. *Animal Feed Science and Technology* **34**, 95–109.
- Öste RE, Miller R, Sjöström H & Norén O (1987) Effect of Maillard reaction products on protein digestion. Studies on pure compounds. *Journal of Agriculture Food Chemistry* **35**, 938–942.
- Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Wilson GT, Welham SJ & White RP (1993) *Genstat™ 5 Release 3 Reference Manual*. Oxford: Clarendon Press.
- Prawirodigdo S, Gannon NJ, Kerton DJ, Leury BJ & Dunshea FR (1997) Basal diet can influence apparent ileal and faecal digestibility of nitrogen in protein meals. In *Manipulating Pig Production VI*, pp. 246 [PD Cranwell, editor]. Werribee, Victoria, Australia: Australasian Pig Science Association.
- Rayner C (1985) Protein hydrolysis of animal feeds for amino acid content. *Journal of Agricultural and Food Chemistry* **33**, 722–725.
- Rérat A (1978) Digestion and absorption of carbohydrates and nitrogenous matters in the hindgut of the omnivorous nonruminant animal. *Journal of Animal Science* **46**, 1808–1837.
- Saha DC & Gilbreath RL (1993) A modified chromic oxide indicator ratio technique for accurate determination of nutrient digestibility. *Canadian Journal of Animal Science* **73**, 1001–1004.
- Sauer WC & Ozimek L (1986) Digestibility of amino acids in swine: Results and their practical applications. A review. *Live-stock Production Science* **15**, 367–388.
- Sauer WC, Stothers SC & Parker RJ (1977) Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. *Canadian Journal of Animal Science* **57**, 775–784.
- Schneeman BO, Richter DB & Jacobs LR (1982) Response to

- dietary wheat bran in the exocrine pancreas and intestine of rats. *Journal of Nutrition* **112**, 283–286.
- Tanksley TD Jr & Knabe DA (1993) Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In *Recent Developments in Pig Nutrition 2*, pp. 85–105 [DJA Cole, W Haresign and PC Garnsworthy, editors]. Nottingham: Nottingham University Press.
- Tanksley TD Jr, Knabe DA, Purser K, Zebrowska T & Corley JR (1981) Apparent digestibility of amino acids and nitrogen in three cottonseed meals and one soybean meal. *Journal of Animal Science* **52**, 769–777.
- Troeger K & Woltersdorf W (1991) Gas anaesthesia of slaughter pigs: 1. Stunning experiments under laboratory conditions with fat pigs of known halothane reaction type: meat quality, animal protection. *Fleischwirtsch* **71**, 1063–1068.
- Van Barneveld RJ, Batterham ES & Norton BW (1994) The effect of heat on amino acids for growing pigs. 1. A comparison of ileal and faecal digestibilities of amino acids in raw and heat-treated field peas (*Pisum sativum* cultivar Dundale). *British Journal of Nutrition* **34**, 221–241.
- Yin YL, Chen CM, Zhong HY, Huang RL & Chen XS (1994) Apparent digestibilities of energy, cell wall constituents, crude protein and amino acids of Chinese oil seed meals for growing pigs. *Animal Feed Science and Technology* **45**, 283–298.
- Yu F, Moughan PJ, Barry TN & McNabb WC (1996) The effect of condensed tannins from heated and unheated cottonseed on the ileal digestibility of amino acids for the growing rat and pig. *British Journal of Nutrition* **76**, 359–371.