The effect of cottonseed condensed tannins on the ileal digestibility of amino acids in casein and cottonseed kernel

BY FENG YU, P. J. MOUGHAN* AND T. N. BARRY

Department of Animal Science, Massey University, Palmerston North, New Zealand

(*Received 5 April 1995 – Revised 10 July 1995 – Accepted 16 August 1995*)

The effect of adding cottonseed hulls to casein- and cottonseed-kernel-based diets on the apparent and true ileal digestibility of N and amino acids, and the proportion of this effect accounted for by condensed tannin (CT), were determined using the growing rat. Sixty rats were allocated randomly to ten semi-purified diets, containing either casein (four diets) or purified unheated solvent-extracted cottonseed kernel (six diets) as the sole protein source, with Cr_2O_3 added as an indigestible marker. Two of the casein diets contained no hulls whilst the remaining two diets contained 70 g cottonseed hulls/kg. Two of the cottonseed-kernel-based diets contained no hulls, with two containing 23 g hulls/kg and the remaining two containing 46 g hulls/kg. For each pair of diets, PEG was either included or excluded. The effect of CT was quantified by comparing control rats (−PEG; CT acting) with PEG-supplemented rats (+PEG; CT inactivated) at each level of dietary hulls. The rats were given their respective experimental diets for 14 d. Each rat was given the food *ad libitum* for 10 min hourly from 08.00 to 18.00 hours. On day 14, samples of digesta were collected at death from the terminal 150 mm of ileum at 7 h from the first meal. Apparent and true ileal digestibilities were calculated for DM, N and the individual amino acids. The principal finding was that the inclusion of hulls depressed the apparent and true ileal digestibilities of N and amino acids, but with the response differing between diets. With the casein-based diet the mean apparent and true ileal amino acid digestibilities were significantly depressed from 0·89 and 0·96 to 0·85 and 0·92 respectively, by the inclusion of 70 g hulls/kg in the diet, and addition of PEG then restored these to 0·89 and 0·95. All of the depression could be explained by the CT content of the hulls. However, with the cottonseed-kernel-based diet the responses fell into three categories. The apparent and true ileal digestibilities of the essential amino acids cystine and methionine were not affected by hull addition, ileal digestibilities of leucine, isoleucine, lysine, threonine and valine were markedly depressed by hull addition with approximately 50% of the depression being explained by CT, whilst the ileal digestibilities of histidine, arginine and phenylalanine were depressed by hull addition but little or none of this effect could be explained by CT. Thus the effect of hulls on protein digestion clearly differed with source of protein. With the cottonseed-kernel-based diet it seems that components of the hulls other than CT also depressed the apparent and true ileal digestibilities of N and amino acids. The identity of these components is unknown.

Cottonseed: Condensed tannin: Ileal digestibility

Cottonseed meal is an important source of protein for single-stomached farm animals (Lusas & Jividen, 1987), but is regarded as being of variable quality and generally of low amino acid availability (Batterham *et al.* 1990; Batterham, 1992). The nutritional value of cottonseed meal is influenced by the processing conditions applied to cottonseed during oil extraction, and by the presence of antinutritional factors and NSP (Frank, 1987; Huisman *et al.* 1990; Yu *et al.* 1993). Gossypol, a naturally-occurring polyphenolic substance in cottonseed, reacts with the ϵ-amino group of lysine during heating of the seed to form insoluble, indigestible complexes (Lyman *et al.* 1959; Damaty & Hudson, 1979; Berardi &

* For reprints.

Goldblatt, 1980; Ikurior & Fetuga, 1988). Other components of the seed, such as asparagine and glutamine (Varnish & Carpenter, 1975), raffinose and phospholipids (Martinez *et al.* 1967), have been found to interact during seed processing, leading to inter- and intramolecular cross-linkages, which reduce protein digestibility by obstructing enzymic attachment (Varnish & Carpenter, 1975).

Recent studies have found that condensed tannins (CT) are present in commercially produced cottonseed meal in significant concentrations (8–16 g/kg DM; Balogun *et al.* 1990; Terrill *et al.* 1992; Yu *et al.* 1993). CT occur in cottonseed hulls (32–65 g/kg DM) mainly bound to protein and fibre, but are absent from cottonseed kernel (Yu *et al.* 1993). CT are polyphenolic compounds, capable of precipitating proteins from aqueous solutions, and hence have been shown to have antinutritional effects in non-ruminant animals (Huisman *et al.* 1990; Helsper *et al.* 1993). Specifically, they are known to increase faecal excretion of nutrients, particularly amino acids, thus reducing apparent nutrient digestibility (Mangan, 1988; Salunkhe *et al.* 1990). Moreover, *in vitro* and *in vivo* studies have demonstrated that CT can inhibit the activity of digestive enzymes (Longstaff & McNab, 1991; Jansman, 1993*b*) due to the formation of tannin–enzyme complexes which are biologically inactive (Griffiths, 1979; Griffiths & Moseley, 1980). In the rat, sorghum and faba bean CT are known to cause hypertrophy of the parotid glands, accompanied by an increased secretion of proline-rich proteins (Mehansho *et al.* 1983, 1992; Jansman, 1993*b*). CT may cause damage to the gut mucosa (Mitjavila *et al.* 1977; van Leeuwen *et al.* 1993), and may also cause an increased loss of mucin in the faeces (Sell *et al.* 1985).

There is no information on the effects of bound CT in cottonseed hulls on nutrient digestibility in single-stomached animals. The objective of the present study was to determine the effect of CT in cottonseed hulls on the apparent and true ileal digestibilities of amino acids in casein and in unheated solvent-extracted cottonseed kernel fed to the growing rat. Dietary PEG addition was used in the present work to allow an effect of the CT, consequent on an increase in the level of dietary inclusion of cottonseed hulls, to be distinguished from an effect of the increased fibre. PEG binds strongly to CT and can be used to displace protein completely from the CT–protein complexes (Jones & Mangan, 1977; Barry & Manley, 1986). Work was also conducted to demonstrate that adding PEG to the diet had no effect on protein digestion in the absence of CT.

MATERIALS AND METHODS

Preparation of cottonseed kernel and hulls

Delinted whole cottonseed (var. Siokra L22) supplied by Cotton Seed Distributors Ltd, Wee Waa, NSW, Australia was cracked using a crushing-mill (AB Thorell and Persson, Uppsala, Sweden), and separated into kernels and hulls using air-flow, at the Seed Technology Centre, Massey University, with final manual separation. The separated kernels were freeze-dried for 48 h, ground to pass a 2 mm diameter sieve, and the oil and gossypol were extracted using hexane and then acetone–water (70:30, w/w) using a modification of the Pons & Eaves (1967) procedure, as described by Yu *et al.* (1995*a*). Finally, extracted cottonseed kernel and hulls were then re-ground to pass through a 1 mm diameter sieve and were stored at −20°. The chemical compositions of the unheated solvent-extracted cottonseed kernel and hulls are shown in Table 1.

Animals and diets

Male and female Sprague-Dawley rats, which had been weaned at 4 weeks of age, were reared on a high quality diet at the Small Animal Production Unit, Massey University. The

Table 1. *Chemical compositions (g/kg DM) of the unheated solvent-extracted cottonseed kernel and hulls*

(Mean values from duplicate determinations)

	Cottonseed kernel	Cottonseed hulls
Dry matter (g/kg)	909	911
Crude protein	537	35
Oil	138	10
Neutral-detergent fibre	89	886
Acid-detergent fibre	37	624
Lignin	27	209
Free gossypol	0·8	0·2
Condensed tannin:		
Extractable	0	13
Protein-bound	0	29
Fibre-bound	0	10
Total (calculated)	0	52
Essential amino acids		
Arginine	64	1·0
Histidine	16	0·5
Isoleucine	22	0·9
Leucine	43	1·4
Lysine	30	1·1
Phenylalanine	39	0·9
Threonine	21	0·9
Valine	30	1·1
Total (calculated)	265	7·8
Non-essential amino acids		
Alanine	26	1·3
Aspartic acid	7	6·9
Glutamic acid	101	2·9
Glycine	24	1·0
Proline	23	1·2
Serine	24	1·6
Tyrosine	19	1·1
Total (calculated)	224	16·0

animals were kept individually in raised stainless steel cages with wire mesh floors, at $20 \pm 2°$ and with a 12 h light–dark cycle.

Ten semi-purified diets were formulated based on maize starch, and containing either casein or purified unheated solvent-extracted cottonseed kernel as the sole protein source (Table 2). The diets contained graded levels of cottonseed hulls, and Cr_2O_3 was added as an indigestible marker compound to all diets. At each level of dietary hulls, PEG (molecular weight 3500, Union Carbide, Danbury, CT, USA) was either included or excluded. Thus, the effect of CT could be quantified by comparing control rats (−PEG; CT acting) with PEG-supplemented rats (CT inactivated) at each level of dietary hulls. The PEG was added at a minimum level of 2 mg/mg total CT to maximize the displacement of protein from the CT–protein complexes (Yu *et al.* 1995*b*).

Experimental procedure

Sixty rats (body weight 176 (SE 4·5) g) were assigned randomly to the ten experimental diets (Table 2), such that there were three males and three females per diet. The animals were initially fed on a casein-based diet (approximately 100 g crude protein/kg) for 2 d and were

Table 2. *Ingredient (g/kg air-dry weight) and chemical compositions of the casein and unheated solvent-extracted cottonseed-kernel-based diets*

Diet...	Casein				Cottonseed kernel					
Cottonseed hulls...	0 g/kg		70 g/kg		0 g/kg		23 g/kg		46 g/kg	
PEG*...	−	+	−	+	−	+	−	+	−	+
Ingredient										
Casein	160	160	160	160	—	—	—	—	—	—
Cottonseed kernel	—	—	—	—	290	290	290	290	290	290
Cottonseed hulls	—	—	70	70	—	—	23	23	46	46
PEG*	—	8	—	8	—	5	—	2·5	—	5
Maize starch	599	591	529	521	469	464	446	443·5	423	418
Sucrose	100	100	100	100	100	100	100	100	100	100
Maize oil	50	50	50	50	50	50	50	50	50	50
Cellulose†	35	35	35	35	35	35	35	35	35	35
Mineral/vitamin premix‡	15	15	15	15	15	15	15	15	15	15
Sodium chloride	5	5	5	5	5	5	5	5	5	5
Magnesium sulphate	2	2	2	2	2	2	2	2	2	2
Potassium carbonate	4	4	4	4	4	4	4	4	4	4
Dicalcium phosphate	24	24	24	24	24	24	24	24	24	24
Chromic oxide	6	6	6	6	6	6	6	6	6	6
Nutrient content (DM basis)§										
DM	983	982	977	984	973	971	971	975	966	975
OM	951	945	950	949	924	927	926	922	927	931
Crude protein	150	156	162	156	160	160	163	161	169	165
Oil	17	20	20	15	94	94	91	94	93	91
NDF	—	—	61	61	26	26	46	46	67	67
ADF	—	—	43	43	11	11	25	25	39	39
Lignin	—	—	14	14	8	8	13	13	18	18
Gross energy (MJ/kg)	18	18	18	18	18	19	19	19	19	19
Free gossypol (mg/kg)	—	—	—	—	174	182	175	189	184	186
Condensed tannin (g/kg)										
Total	0	0	3·6	3·6	0	0	1·2	1·2	2·4	2·4
Free	0	0	0·91	0·91	0	0	0·30	0·30	0·60	0·60

OM, organic matter; NDF, neutral-detergent fibre; ADF, acid-detergent fibre.

* MW 3500.

† Avicel, Asahi Chemical Industry Company Ltd, Tokyo, Japan.

‡ Rat Pellet Premix 9327, Technik Products, Auckland, New Zealand. Supplied the following per kg diet: 3 mg retinol, 0·04 mg cholecalciferol, 30 mg α-tocopherol, 1 mg menadione, 1 mg thiamin, 4 mg riboflavin, 3 mg pyridoxine, 0·02 mg cyanocobalamin, 15 mg pantothenic acid, 1 mg pteroylmonoglutamic acid, 25 mg niacin, 125 mg antioxidant, 250 mg choline, 100 mg manganese, 35 mg iron, 10 mg copper, 60 mg zinc, 1 mg cobalt, 0·15 mg selenium.

§ Means of duplicate determinations.

then given the experimental diets for a further 14 d. The diets were offered in stainless-steel feeders with anti-spill devices similar to those described by Thomsen (1981). The rats were trained to consume their experimental diet between 08.00 and 18.00 hours with the feeder being placed in the cage for 10 min at hourly intervals. The training was achieved within 7 d, and feed intakes were recorded after each 10 min feeding. Fresh water was freely available.

On day 14 the rats were asphyxiated in CO_2 gas and decapitated (immediately ceasing all neural stimulation to the gut) at 7 h from the start of feeding. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature were folded back to expose the viscera. The final 150 mm of the ileum was immediately dissected from the body,

and the intestinal surface cleaned using absorbent tissue paper, taking care not to apply pressure to the intestine. The digesta were slowly flushed out into plastic bags with distilled water from a plastic syringe. The digesta from each animal were kept separate and packed in ice immediately after collection.

Ileal digesta and samples of the test diets were subsequently freeze-dried, finely ground and stored at −20° for the determination of N, Cr, and total amino acids. The stomach contents were inspected for signs of faecal contamination resulting from coprophagy.

Chemical analysis

The diets and ileal digesta were analysed in duplicate for total N using the Kjeldahl procedure, and crude protein was calculated as total N × 6·25. The Cr contents of duplicate 15 mg samples of ileal digesta and each diet were determined by the method of Costigan & Ellis (1987). The CT contents of the diets were determined using the method of Terrill *et al.* (1992). Free gossypol in the diets was estimated by method Ba 7-58 of the American Oil Chemists Society (1975). The neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and lignin contents were determined by the method of Robertson & van Soest (1981). The crude ash, crude oil and gross energy contents of the feeds were analysed according to conventional methods (Association of Official Analytical Chemists, 1975). The amount of freeze-dried matter (FDM) collected from the terminal ileum of each rat was determined after freeze-drying the samples for 3 d.

Amino acid composition was determined on samples of 5–7 mg using HPLC (Waters Associates, USA), using a reverse phase column and the Pico.Tag analytical method (Cohen *et al.* 1989). Duplicate samples were hydrolysed in 500 μl 6 M-HCl with added phenol (10 g/l), for 24 h at 110±1° in glass tubes sealed under vacuum. For the determination of methionine and cystine in the samples obtained from the cottonseed-kernel-based diets, separate duplicate samples were oxidized with performic acid–H_2O_2 (90:10, v/v) before hydrolysis. Methionine and cystine in the samples obtained from the casein-based diet, and tryptophan, which was partly destroyed during acid hydrolysis, were not determined. The amino acids were detected by the fluorescence of their phenylisothiocyanate (PITC) derivatives using a programmable multi-wavelength detector (Waters 490E, USA). Free amino acid molecular weights were used to calculate the weights of amino acids.

Data analysis

Apparent and true amino acid digestibility coefficients were calculated using the following equations:

$$\text{apparent amino acid (AA) digestibility } (\mu\text{g/g FDM}) = \frac{\text{dietary AA intake} - \text{ileal AA output}}{\text{dietary AA intake}},$$

$$\text{true AA digestibility } (\mu\text{g/g FDM}) = \frac{\text{dietary AA intake} - (\text{ileal AA output} - \text{endogenous AA output})}{\text{dietary AA intake}},$$

$$\text{AA output } (\mu\text{g/g FDM}) = \left(\begin{array}{c} \text{AA concentration in} \\ \text{ileal digesta} \end{array} \right) \times \frac{\text{diet total chromium}}{\text{ileal total chromium}}.$$

Endogenous amino acid flows used for calculating true amino acid digestibility coefficients in the present study were obtained in a separate but related study (Yu *et al.* 1995*c*), in which endogenous ileal amino acid flows were determined in rats given diets containing graded levels of cottonseed hulls. The enzymically hydrolysed casein (EHC) ultrafiltration method (Moughan *et al.* 1990; Butts *et al.* 1991) was used in the latter work to determine

endogenous amino acid losses. Data from the present study were subjected to ANOVA. A linear statistical model, which included terms for hulls, PEG and hulls × PEG, was initially fitted to the digestibility data for each amino acid singly, and reduction in sums of squares was used to determine levels of significance. Relevant comparisons between treatment means were made using orthogonal contrasts (Snedecor & Cochran, 1982). Where a cause-and-effect trend in the data was expected (e.g. ileal digestibility as a function of hull addition), the data (n 18) were subjected to a simple linear regression and slopes were tested for statistical significance from zero.

RESULTS

The overall mean live weight for the rats at the end of the study was 197 (SE 8·4) g. Mean feed intakes for the rats on day 13 of the study are given in Table 3. Feed intake was within the normal range for the 200 g body weight rat (National Research Council, 1978). Daily feed intake tended to be lower with the casein-based diet in comparison with the cottonseed-kernel-based diet, and dietary PEG addition did not affect feed intake on either diet (Table 3). On the last day of the study the rats had high feed intakes over the first two hourly meals and then consumed generally even-sized meals for the remainder of the feeding period (Table 3). The latter was important to ensure an even flow of digesta at the terminal ileum. Faeces were not detected in the gastric contents at slaughter indicating that coprophagy had not occurred at least on the last day of study.

There was a significant ($P < 0·05$) hulls × PEG interaction for amino acid digestibility for several of the amino acids. Accordingly, relevant comparisons were made between treatment means within the PEG or hull factors.

With both the casein and cottonseed-kernel-based diets, inclusion of PEG in the diet without hulls did not affect ileal DM digestibility or the apparent and true ileal digestibilities of N and individual amino acids (Tables 4, 5 and 6), indicating that there was no effect of PEG *per se* on dietary DM and protein digestibilities in the absence of CT.

With the casein-based diet, in the absence of PEG, addition of dietary cottonseed hulls (70 g/kg) significantly depressed ileal DM digestibility ($P < 0·001$; Table 4) and the true ileal digestibility of total N ($P < 0·001$). In the presence of hulls, dietary supplementation with PEG significantly increased ileal DM digestibility ($P < 0·01$) and the true ileal digestibility of total N ($P < 0·01$), with the values for total N attained being similar to those for diets not containing hulls.

Apparent and true ileal amino acid digestibilities for rats fed on the casein-based diet were significantly depressed ($P < 0·05$) by addition of dietary hulls (Table 4). On average, the apparent ileal amino acid digestibility was decreased from 0·89 to 0·85 by the inclusion of 70 g hulls/kg in the diet, and addition of PEG then restored this to 0·89. The mean true ileal digestibility of amino acids decreased from 0·96 for the diet not containing hulls to 0·92 for the diet containing 70 g hulls/kg. When PEG was added to the diet containing hulls (70 g/kg), the mean true ileal amino acid digestibility was restored to 0·95. Apparent and true ileal digestibilities of all individual amino acids were similarly affected by the addition of hulls and PEG.

With the cottonseed-kernel-based diet, in the absence of PEG, inclusion of dietary hulls progressively depressed ($P < 0·05$) ileal DM digestibility and apparent ileal digestibility of total N for the rats (Table 5) in a linear manner ($P < 0·05$; Table 7). Addition of dietary PEG significantly increased the apparent ileal N digestibility ($P < 0·05$), but did not affect ileal DM digestibility. However, the increased values for total N digestibility accounted for only about 67 and 50% of the depression in the ileal digestibility caused by the inclusion of different levels of dietary hulls (23 and 46 g/kg) respectively. True ileal N digestibility

Table 3. *Mean feed intakes of growing rats fed on casein-based or cottonseed-kernel-based diets with or without cottonseed hulls and PEG on day 13 and at hourly meals on day 14 (last day of study)**

(Mean values with their standard errors for six rats)

Diet	Food intake on day 13 (g)		Hourly meal intakes on day 14† (g)							
	Mean	SE	1	2	3	4	5	6	7	Total
Casein:										
−PEG‡	10	0·3	1·4	1·2	1·2	0·6	0·7	0·6	0·8	6·5
+PEG	9	0·7	1·4	1·0	0·9	0·6	0·6	1·0	1·0	6·5
70 g/kg hulls−PEG	13	0·7	2·7	1·9	1·7	1·0	0·8	0·9	1·2	10·2
70 g/kg hulls+PEG	13	1·5	2·1	1·9	1·6	1·1	0·8	1·2	1·3	10·0
Cottonseed kernel:										
−PEG	15	0·7	3·5	2·5	0·6	0·6	1·2	2·1	1·5	12·0
+PEG	15	0·6	3·1	2·3	1·4	1·0	0·9	1·3	1·2	11·2
23 g/kg hulls−PEG	15	1·0	3·4	2·5	1·9	1·7	0·8	1·4	1·2	12·9
23 g/kg hulls+PEG	16	0·9	3·4	2·8	1·4	1·2	0·8	1·6	1·5	12·7
46 g/kg hulls−PEG	16	0·9	3·9	2·6	2·1	0·7	1·0	1·3	1·2	12·8
46 g/kg hulls+PEG	16	1·1	3·7	3·2	2·1	0·8	0·8	1·5	1·4	13·5

* For details of diets and procedures, see Table 2 and pp. 684–686.
† Day of sampling ileal digesta.
‡ MW 3500.

was also linearly depressed by increased dietary hulls ($P < 0·001$; Table 6), and addition of PEG to the diets significantly restored this ($P < 0·05$ at the 46 g hulls/kg level), but not back to the original level.

In the absence of PEG, inclusion of hulls in the cottonseed-kernel-based diet significantly reduced ($P < 0·05$) the apparent and true ileal digestibilities of all amino acids except methionine and cystine (Tables 5 and 6). The significant linear regression relationships (Table 7) indicated that as the level of dietary hull increased, in the absence of dietary PEG, the apparent and true ileal amino acid digestibilities of all amino acids except cystine and methionine decreased in a linear manner, with the slopes of the regression lines being significantly different from zero ($P < 0·05$). The coefficients of determination ranged from 0·38 to 0·85, indicating that a considerable proportion of the variation in ileal digestibility was explained by fitting the regression model. Unlike the responses obtained with casein, adding hulls to a cottonseed-kernel-based diet depressed apparent and true ileal digestibilities of some amino acids more than others, with histidine, isoleucine, leucine, lysine, threonine and valine being the essential amino acids most depressed per unit of hulls added. Glycine was the non-essential amino acid most depressed.

In the diet with 23 g hulls/kg, supplementation with dietary PEG significantly increased the apparent and true ileal digestibilities of threonine and increased the apparent ileal digestibility of aspartic acid and proline (Tables 5 and 6). With the rate of hull addition increased to 46 g/kg, the apparent and true ileal digestibilities of cystine, leucine, isoleucine, threonine, valine, alanine and proline were significantly increased with PEG addition. However, unlike the casein-based diet, inclusion of dietary PEG in the diets containing hulls did not restore apparent and true ileal amino acid digestibilities to the same levels as found for diets not containing hulls and not all amino acids were affected similarly. Both the apparent and true ileal digestibilities of arginine, histidine, methionine,

Table 4. *Mean* (n 6) *apparent and true digestibilities of dry matter, nitrogen and amino acids at the terminal ileum of the growing rat given a casein-based diet*†

	Apparent digestibility								True digestibility							
Hulls‡...	0 g/kg		70 g/kg		Overall	Significance‖			0 g/kg		70 g/kg		Overall	Significance‖		
PEG§...	−	+	−	+	SE	PNH	HNP	PWH	−	+	−	+	SE	PNH	HNP	PWH
Dry matter	0·84	0·84	0·69	0·77	0·017	NS	***	**								
Nitrogen	0·88	0·88	0·87	0·88	0·013	NS	NS	NS	0·94	0·94	0·90	0·93	0·004	NS	***	**
Essential amino acids																
Arginine	0·93	0·93	0·89	0·92	0·006	NS	***	**	0·98	0·98	0·95	0·97	0·004	NS	***	**
Histidine	0·93	0·93	0·87	0·92	0·006	NS	***	***	0·98	0·98	0·93	0·97	0·005	NS	***	***
Isoleucine	0·84	0·86	0·79	0·84	0·012	NS	*	*	0·93	0·93	0·88	0·91	0·008	NS	***	**
Leucine	0·94	0·95	0·91	0·94	0·004	NS	***	**	0·98	0·98	0·95	0·97	0·003	NS	***	**
Lysine	0·94	0·96	0·92	0·95	0·004	*	**	**	0·98	0·99	0·95	0·98	0·004	NS	***	***
Phenylalanine	0·95	0·96	0·93	0·96	0·004	NS	***	***	0·98	0·99	0·96	0·98	0·003	NS	***	**
Threonine	0·82	0·85	0·78	0·83	0·010	*	**	**	0·91	0·93	0·89	0·92	0·008	NS	*	*
Valine	0·87	0·89	0·84	0·88	0·008	NS	**	**	0·95	0·96	0·91	0·93	0·007	NS	**	*
Non-essential amino acids																
Alanine	0·86	0·89	0·81	0·86	0·009	**	**	**	0·95	0·96	0·90	0·94	0·007	NS	***	**
Aspartic acid	0·85	0·87	0·80	0·85	0·010	NS	**	**	0·94	0·95	0·90	0·94	0·007	NS	***	**
Glutamic acid	0·88	0·89	0·82	0·88	0·007	NS	***	***	0·94	0·95	0·90	0·93	0·008	NS	**	*
Proline	0·93	0·94	0·89	0·92	0·004	NS	***	***	0·98	0·98	0·95	0·97	0·003	NS	***	***
Serine	0·72	0·74	0·67	0·74	0·015	NS	*	*	0·88	0·89	0·82	0·86	0·010	NS	***	**
Tyrosine	0·96	0·96	0·93	0·96	0·004	NS	**	***	0·99	0·99	0·97	0·98	0·005	NS	*	NS

* $P \leqslant 0{\cdot}05$, ** $P \leqslant 0{\cdot}01$, *** $P \leqslant 0{\cdot}001$.

† For details of diets and procedures, see Table 2 and pp. 684–688.

‡ Proportion of cottonseed hulls in the diet.

§ MW 3500.

‖ PNH: PEG+ *v.* PEG−, 0 g hulls/kg; HNP: 0 *v.* 70 g hulls/kg without PEG; PWH: PEG+ *v.* PEG−, 70 g hulls/kg.

Table 5. *Mean* (n 6) *apparent ileal dry matter, nitrogen and amino acid digestibilities for the growing rat given an extracted cottonseed-kernel-based diet*†

Hulls‡...	0 g/kg		23 g/kg		46 g/kg		Overall	Statistical significance: Hull effect‖		PEG effect¶		
PEG§...	−	+	−	+	−	+	SE	0v23	0v46	0H	23H	46H
Dry matter	0·76	0·76	0·73	0·74	0·73	0·71	0·008	*	*	NS	NS	NS
Nitrogen	0·88	0·89	0·86	0·88	0·85	0·87	0·008	NS	*	NS	*	*
Essential amino acids												
Arginine	0·94	0·94	0·91	0·92	0·90	0·91	0·007	**	***	NS	NS	NS
Cystine	0·76	0·76	0·84	0·85	0·77	0·81	0·013	***	NS	NS	NS	*
Histidine	0·88	0·88	0·82	0·83	0·79	0·80	0·012	***	***	NS	NS	NS
Isoleucine	0·81	0·80	0·78	0·80	0·61	0·75	0·018	NS	***	NS	NS	***
Leucine	0·82	0·80	0·79	0·81	0·73	0·77	0·013	NS	***	NS	NS	*
Lysine	0·82	0·80	0·77	0·78	0·71	0·74	0·015	*	***	NS	NS	NS
Methionine	0·84	0·84	0·85	0·87	0·84	0·86	0·013	NS	NS	NS	NS	NS
Phenylalanine	0·88	0·86	0·86	0·87	0·82	0·84	0·011	NS	**	NS	NS	NS
Threonine	0·74	0·73	0·64	0·70	0·63	0·67	0·015	***	***	NS	**	*
Valine	0·83	0·82	0·79	0·81	0·72	0·78	0·014	*	***	NS	NS	**
Non-essential amino acids												
Alanine	0·79	0·78	0·75	0·78	0·73	0·77	0·014	NS	**	NS	NS	*
Aspartic acid	0·83	0·83	0·77	0·81	0·76	0·78	0·012	**	***	NS	*	NS
Glutamic acid	0·90	0·90	0·88	0·89	0·84	0·87	0·007	*	***	NS	NS	*
Glycine	0·78	0·76	0·73	0·73	0·62	0·75	0·018	*	***	NS	NS	***
Proline	0·82	0·81	0·75	0·79	0·70	0·73	0·010	***	***	NS	*	*
Serine	0·83	0·82	0·75	0·78	0·71	0·75	0·016	**	***	NS	NS	*
Tyrosine	0·88	0·89	0·84	0·86	0·80	0·82	0·014	*	***	NS	NS	NS

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diets and procedures, see Table 2 and pp. 684–688.

‡ Proportion of cottonseed hulls in the diet.

§ MW 3500.

‖ Effect of hulls in diets without PEG. 0v23: 0 *v.* 23 g hulls/kg; 0v46: 0 *v.* 46 g hulls/kg.

¶ Effect of PEG within level of hull inclusion. 0H: 0 g hulls/kg; 23H: 23 g hulls/kg; 46H: 46 g hulls/kg.

Table 6. *Mean* (n 6) *true ileal nitrogen and amino acid digestibilities for the growing rat given an extracted cottonseed-kernel-based diet*†

								Statistical significance				
Hulls‡...	0 g/kg		23 g/kg		46 g/kg		Overall	Hull effect‖		PEG effect¶		
PEG§...	−	+	−	+	−	+	SE	0v23	0v46	0H	23H	46H
Nitrogen	0·93	0·94	0·90	0·91	0·88	0·90	0·005	***	***	NS	NS	*
Essential amino acids												
Arginine	0·95	0·96	0·94	0·94	0·93	0·94	0·007	NS	*	NS	NS	NS
Cystine	0·85	0·86	0·88	0·90	0·86	0·88	0·011	*	NS	NS	NS	*
Histidine	0·93	0·94	0·90	0·91	0·87	0·88	0·012	NS	***	NS	NS	NS
Isoleucine	0·94	0·94	0·92	0·93	0·88	0·91	0·015	NS	**	NS	NS	*
Leucine	0·88	0·88	0·86	0·87	0·82	0·85	0·010	NS	***	NS	NS	*
Lysine	0·86	0·86	0·83	0·84	0·76	0·81	0·011	*	***	NS	NS	**
Methionine	0·89	0·90	0·89	0·91	0·90	0·91	0·010	NS	NS	NS	NS	NS
Phenylalanine	0·90	0·90	0·88	0·90	0·86	0·87	0·009	NS	**	NS	NS	NS
Threonine	0·87	0·87	0·80	0·85	0·79	0·82	0·011	***	***	NS	**	*
Valine	0·92	0·93	0·90	0·92	0·87	0·89	0·011	NS	**	NS	NS	*
Non-essential amino acids												
Alanine	0·87	0·87	0·82	0·85	0·79	0·83	0·012	*	***	NS	NS	*
Aspartic acid	0·91	0·92	0·87	0·90	0·86	0·87	0·013	*	*	NS	NS	NS
Glutamic acid	0·97	0·98	0·97	0·98	0·95	0·95	0·006	NS	**	NS	NS	NS
Glycine	0·86	0·85	0·78	0·82	0·75	0·80	0·022	**	**	NS	NS	NS
Proline	0·96	0·97	0·93	0·95	0·90	0·92	0·011	NS	**	NS	NS	*
Serine	0·94	0·94	0·91	0·93	0·88	0·90	0·014	NS	*	NS	NS	NS
Tyrosine	0·94	0·95	0·91	0·92	0·88	0·90	0·012	NS	***	NS	NS	NS

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of diets and procedures, see Table 2 and pp. 684–688.
‡ Proportion of cottonseed hulls in the diet.
§ MW 3500.
‖ Effect of hulls in diets without PEG. 0v23: 0 *v.* 23 g hulls/kg; 0v46: 0 *v.* 46 g hulls/kg.
¶ Effect of PEG within level of hull inclusion. 0H: 0 g hulls/kg; 23H: 23 g hulls/kg; 46H: 46 g hulls/kg.

Table 7. *Linear regression relationships* (n *18*) *between ileal dry matter, nitrogen or amino acid digestibility* (Y) *and hull addition* (x; *g/kg*) *for growing rats fed on an unheated solvent-extracted cottonseed-kernel-based diet not including PEG*

	Apparent ileal digestibility			True ileal digestibility		
	Linear regression (Y)	R^2	Slope significance†	Linear regression (Y)	R^2	Slope significance†
Dry matter	$0{\cdot}756-0{\cdot}786x$	0·31	*			
Nitrogen	$0{\cdot}880-0{\cdot}679x$	0·41	*	$0{\cdot}929-1{\cdot}025x$	0·66	***
Essential amino acids						
Arginine	$0{\cdot}936-0{\cdot}893x$	0·63	**	$0{\cdot}952-0{\cdot}468x$	0·29	*
Cystine	$0{\cdot}789+0{\cdot}212x$	0·01	NS	$0{\cdot}861+0{\cdot}200x$	0·02	NS
Histidine	$0{\cdot}876-2{\cdot}072x$	0·77	***	$0{\cdot}935-1{\cdot}469x$	0·60	***
Isoleucine	$0{\cdot}829-4{\cdot}301x$	0·74	***	$0{\cdot}941-1{\cdot}217x$	0·41	**
Leucine	$0{\cdot}826-1{\cdot}960x$	0·58	***	$0{\cdot}882-1{\cdot}286x$	0·59	***
Lysine	$0{\cdot}817-2{\cdot}288x$	0·72	***	$0{\cdot}863-2{\cdot}055x$	0·69	***
Methionine	$0{\cdot}847-0{\cdot}108x$	0·01	NS	$0{\cdot}887-0{\cdot}318x$	0·06	NS
Phenylalanine	$0{\cdot}878-1{\cdot}186x$	0·51	**	$0{\cdot}903-0{\cdot}980x$	0·44	**
Threonine	$0{\cdot}730-2{\cdot}630x$	0·64	**	$0{\cdot}860-1{\cdot}704x$	0·57	***
Valine	$0{\cdot}838-2{\cdot}449x$	0·70	***	$0{\cdot}924-1{\cdot}100x$	0·41	**
Non-essential amino acids						
Alanine	$0{\cdot}790-1{\cdot}319x$	0·38	*	$0{\cdot}868-1{\cdot}686x$	0·43	**
Aspartic acid	$0{\cdot}822-1{\cdot}585x$	0·62	***	$0{\cdot}903-1{\cdot}014x$	0·33	*
Glutamic acid	$0{\cdot}901-1{\cdot}297x$	0·75	***	$0{\cdot}977-0{\cdot}573x$	0·40	*
Glycine	$0{\cdot}783-3{\cdot}197x$	0·74	***	$0{\cdot}851-2{\cdot}360x$	0·41	**
Proline	$0{\cdot}819-2{\cdot}688x$	0·85	***	$0{\cdot}954-1{\cdot}110x$	0·60	***
Serine	$0{\cdot}823-2{\cdot}635x$	0·85	***	$0{\cdot}939-1{\cdot}217x$	0·56	**
Tyrosine	$0{\cdot}876-1{\cdot}712x$	0·75	***	$0{\cdot}940-1{\cdot}314x$	0·52	**

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† Significance of difference of slope from zero.

phenylalanine, aspartic acid and tyrosine were not significantly increased by PEG addition to diets containing cottonseed hulls.

DISCUSSION

In the present study the effect of cottonseed hulls on the nutritional value of casein and unheated solvent-extracted cottonseed kernel as dietary protein sources was studied in the growing rat by determining apparent and true ileal N and amino acid digestibilities. The principal finding was that the inclusion of hulls depressed the apparent and true ileal digestibilities of N and amino acids, but with the response differing between diets. With the casein-based diet all amino acids were significantly affected and all of the depression in digestibility could be explained by the CT content of the hulls. However, with the cottonseed-kernel-based diet the responses fell into three categories. The apparent and true ileal digestibilities of the essential amino acids cystine and methionine were not affected by hull addition, ileal digestibilities of leucine, isoleucine, lysine, threonine and valine were markedly depressed by hull addition with approximately 50% of the depression being explained by CT, whilst ileal digestibilities of histidine, arginine and phenylalanine were depressed by hull addition but little or none of this effect could be explained by CT. Thus interactions with hulls, affecting amino acid digestion, clearly differed between sources of protein. With the cottonseed-kernel-based diet it seems that components of the hulls other than CT also depressed the apparent and true ileal digestibilities of N and amino acids. The identity of these components is unknown.

These studies have shown the presence of hulls to be one of the reasons for the generally low levels of amino acid digestibility in cottonseed meal, due to the effect of hulls in lowering the true ileal digestibility of amino acids (present study) and in increasing endogenous ileal amino acid excretion (Yu *et al.* 1995*c*). The reduction in true ileal amino acid digestibility per unit increase in hull content for the cottonseed-kernel diets differed for each amino acid, showing that inclusion of hulls will unbalance the digestible amino acids in cottonseed meal. This seems particularly important for amino acids that are most likely to be limiting, such as lysine and threonine, whose true ileal digestibility was most lowered by hull inclusion in the cottonseed-kernel diets.

PEG has been used to absorb plant phenolics during the extraction of enzymes (Jones, 1965) and preferentially binds with CT, preventing CT from reacting with proteins or carbohydrates and displaces CT from CT:protein or CT:carbohydrate complexes (Jones & Mangan, 1977; Barry & Manley, 1986). If PEG is included in a CT-containing diet, the CT will still be present, but will be rendered unreactive in the digestive tract because of the preferential nature of the binding between CT and PEG (McNabb, 1991). This provides a unique way of studying the effect of the presence or absence of reactive CT, without affecting the nutritive composition of the diet. Complete control of the levels of other dietary ingredients is afforded. In the present study there was no effect of PEG addition on feed intake or apparent and true ileal protein and amino acid digestibilities in the diets not containing cottonseed hulls, thus demonstrating that PEG addition *per se* had no intrinsic effect on feed intake and ileal protein digestibility in the absence of CT. Similar observations on endogenous ileal amino acid loss with rats fed on an enzymically hydrolysed casein-based diet were made by Yu *et al.* (1995*c*). Yu *et al.* (1995*b*) reported that 2 mg PEG/mg total CT was required to inactivate the CT in cottonseed hulls. It is thus assumed that in the present study PEG completely bound the CT released during digestion of cottonseed hulls and that for the hull-containing diets, comparisons, with or without PEG, represented effects of cottonseed CT.

Cottonseed hulls are a by-product of cottonseed processing, with the cottonseed being cleaned and partly dehulled before the kernels are crushed and subjected to oil extraction. Commercial cottonseed meal produced in Australia contains between 150 and 300 g hulls/kg and has a CT content of 8–15 g/kg DM, 92% of which is bound to protein and fibre (Yu *et al.* 1993). Extractable CT as found in fresh forages can readily react with dietary protein during ingestion and digestion (Barry & Manley, 1986; McNabb, 1991), but the effects of bound CT have not been previously studied in single-stomached animals. Yu *et al.* (1995*a*) found that bound CT were relatively unreactive at rumen pH (pH 7·0), but the responses to PEG in diets containing cottonseed hulls in the present study suggest that CT react with proteins in the single-stomach digestive system. CT are known to be solubilized in the stomach (Jones & Mangan, 1977) and the most probable explanation for the present result is that the bound CT in cottonseed hulls were solubilized and released in the stomach and were thus available to react with proteins in the small intestine.

The effects of CT in depressing apparent ileal protein digestibility may be explained either by a direct binding of CT to dietary proteins, by a reduced activity of protein-degrading enzymes (Longstaff & McNab, 1991), or by increased secretion of endogenous proteins (digestive enzymes, mucus or mucosal cells; Mangan, 1988; Marquardt, 1989). However, in recent work where ileal endogenous amino acid flow was determined in the rat, no effect of CT in cottonseed hulls was found (Yu *et al.* 1995*c*).

There were differences in the apparent ileal digestibility of amino acids between casein and cottonseed kernels. In the absence of hulls the apparent ileal amino acid digestibility, except that for three amino acids (arginine, glutamic acid and serine), was markedly higher for the casein-based diet than for the cottonseed-kernel-based diet. The difference in

apparent ileal amino acid digestibility between the two protein sources suggests a difference in the intrinsic quality of the proteins. This is confirmed by the lower true ileal amino acid digestibility in the cottonseed-kernel-based diet than in the casein-based diet (Tables 4 and 6). The relatively low digestibility of amino acids in cottonseed meal has been reported by other workers (Batterham *et al.* 1990; Batterham, 1992). Apparent ileal amino acid digestibility was depressed by an increase in dietary hulls in both the casein and cottonseed-kernel-based diets. This effect may be due to both the CT and fibre contents of the hulls. However, ileal amino acid digestibility for the casein diet was still higher than that for the cottonseed-kernel diet, even though the level of hull addition was higher in the casein-based diet (70 g hulls/kg, compared with 23 and 46 g hulls/kg in the cottonseed-based diet). With addition of PEG to the casein-based diet containing hulls, the depressed ileal amino acid digestibility was restored to almost the original level, but only about half of the decrease in digestibility was restored after PEG addition with the cottonseed-kernel-based diet. This suggests that only about half of the depression in ileal digestibility caused by hull addition to a cottonseed-kernel diet can be explained by CT, with the remainder presumably being due to unidentified reactions with other hull components.

It seems that CT may have different affinities for proteins with different amino acid profiles. Asquith & Butler (1986), in an *in vitro* study, noted that CT–protein interactions may be specific for different tannins as well as for different proteins. The high degree of interaction indicated that the differences in affinity were functionally significant. Hagerman & Butler (1981) found that *Sorghum* tannins have a high affinity for proteins that are relatively large, with an open, loose structure, and that are rich in hydrophobic amino acids, particularly proline. Cousins *et al.* (1981), in a study with sorghums containing different levels of CT, showed that the apparent ileal digestibilities of tryptophan, histidine, glycine and proline were more depressed than for other amino acids in high-tannin varieties. Differences in protein structure and composition may account for the different responses to cottonseed CT observed in the present study between casein and cottonseed kernel. In the present study the true ileal digestibilities of the essential amino acids histidine, leucine, isoleucine, lysine, threonine and valine in the cottonseed-kernel-based diets were more depressed with high levels of dietary CT compared with the other amino acids, suggesting that reactions between these amino acids and CT are not completely reversible in the small intestine.

The ileal digestibility coefficients presented here indicate that the correction of apparent ileal N and amino acid digestibilities for endogenous excretion, as determined by the EHC technique (Yu *et al.* 1995*c*), results in true ileal N and amino acid digestibilities which are markedly higher (about 11% for alanine, cystine, aspartic acid, and up to 22% for serine) than corresponding apparent estimates. The ileal endogenous excretion of N and amino acids, determined using the recently developed peptide alimentation–digesta ultrafiltration method (Moughan *et al.* 1990; Butts *et al.* 1991), was slightly increased by the inclusion of 50 g cottonseed hulls/kg in the EHC-based diet (Yu *et al.* 1995*c*). The authors concluded that this increase was caused by the cottonseed-hull-fibre component, and there did not appear to be an effect of CT on endogenous ileal amino acid loss. In the present study the lowered apparent ileal digestibilities of N and amino acids in the diets containing cottonseed hulls can be attributed to a decrease in digestion and absorption as reflected by the true coefficients of digestibility of dietary protein (Tables 4, 5 and 6) and also increased endogenous protein excretion.

The consumption of diets containing CT has been shown to increase the size of the parotid glands in the rat and the synthesis and secretion of proline-rich proteins (PRP; Mehansho *et al.* 1992). Tannin-induced PRP were shown to have a very high binding affinity for tannins (Mehansho *et al.* 1983). The mechanism by which tannins induce

hypertrophy in the parotid glands of the rat and increase the secretion of PRP is not clear (Jansman, 1993*a*). Although salivas of various species contain PRP (Mole *et al.* 1990), it is not clear whether species other than the rat are able to develop a similar response when consuming tannin-containing diets. In the hamster this response was absent and used as an explanation for the high sensitivity of this species to dietary tannin (Mehansho *et al.* 1987). If pigs and poultry lack this response, the rat may not be a completely valid model for establishing the nutritional effects of dietary tannins in these commercially important animals. Thus, further experiments studying the effects of cottonseed CT on ileal N and amino acid digestibilities in pigs and poultry are necessary.

The presence of CT in the hulls is part of the host-plant resistance mechanism in cotton for defence against attack by insects and pathogenic micro-organisms (Fitt *et al.* 1992). It seems, on this basis, unrealistic to select for low CT levels in cotton breeding programs. Heating, as employed during commercial cottonseed meal manufacturing, may have some effect on protein solubilization. The present study was done with unheated solvent-extracted cottonseed, so further work is needed to study the effects of hull inclusion in the presence and absence of heat treatment, and to compare the effect of CT between animal species, notably comparing rats with pigs and poultry. If cottonseed hulls increase endogenous ileal amino acid loss (Yu *et al.* 1995*c*) and depress ileal protein digestion in heated extracted cottonseed kernel to the same extent as found in the present study, then it seems that the presence of hulls in commercial cottonseed meal should be reduced to the lowest possible levels.

This study was supported by a grant from the Australian Cotton Research and Development Corporation. The authors thank Cotton Seed Distributors Ltd, Wee Waa, NSW, Australia for supplying cottonseed and Mr G. Pearson of the Monogastric Research Centre, and Ms F. S. Jackson and Mr J. A. Anderson of the Department of Animal Science, Massey University for their skilled technical assistance.

REFERENCES

American Oil Chemists Society (1975). *Official and Tentative Methods of American Oil Chemists Society*, 3rd ed., pp. Ba 7–58. Champaign, IL: American Oil Chemists Society.

Asquith, T. N. & Butler, L. G. (1986). Interactions of condensed tannins with selected proteins. *Phytochemistry* **25**, 1591–1593.

Association of Official Analytical Chemists (1975). *Official Methods of Analysis*, 12th ed. Washington, DC: Association of Official Analytical Chemists.

Balogun, T. F., Aduku, A. O., Dim, N. I. & Olorunji, S. A. S. (1990). Undecorticated cottonseed meal as a substitute for soya bean meal in diets for weaner and growing finishing pigs. *Animal Feed Science and Technology* **30**, 193–201.

Barry, T. N. & Manley, T. R. (1986). Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus* sp. and their possible consequences in ruminant nutrition. *Journal of the Science of Food and Agriculture* **37**, 248–254.

Batterham, E. S. (1992). Availability and utilization of amino acids for growing pigs. *Nutrition Research Reviews* **5**, 1–18.

Batterham, E. S., Anderson, L. M., Baigent, D. R., Beech, S. A. & Elliott, R. (1990). A comparison of the availability and ileal digestibility of lysine in cottonseed and soybean meals for grower/finisher pigs. *British Journal of Nutrition* **64**, 663–677.

Berardi, L. C. & Goldblatt, L. A. (1980). Gossypol. In *Toxic Constituents of Plant Foodstuffs*, pp. 183–237 [I. E. Liener, editor]. New York, London and Sydney: Academic Press.

Butts, C. A., Moughan, P. J. & Smith, W. C. (1991). Endogenous amino acid flow at the terminal ileum of the rat determined under conditions of peptide alimentation. *Journal of the Science of Food and Agriculture* **55**, 175–187.

Cohen, S. A., Meys, M. & Tarvin, T. L. (1989). *The Pico.Tag Method. A Manual of Advanced Techniques for Amino Acid Analysis*. Bedford, MA: Millipore Corporation.

Costigan, P. & Ellis, K. J. (1987). Analysis of faecal chromium derived from controlled release marker devices. *New Zealand Journal of Technology* **3**, 89–92.

Cousins, B. W., Tanksley, T. D., Knabe, D. A. & Zebrowska, T. (1981). Nutrient digestibility and performance of pigs fed sorghums varying in tannin concentration. *Journal of Animal Science* **53**, 1524–1537.

Damaty, S. M. & Hudson, B. J. F. (1979). The interaction between gossypol and cottonseed protein. *Journal of the Science of Food and Agriculture* **30**, 1050–1056.

Fitt, G. P., Mares, C. L., Wilson, L. J. & Thomson, N. J. (1992). Development of resistance to insects in Australian cotton varieties. In *Proceedings of 1992 Australian Cotton Conference*, pp. 307–322. Gold Coast, Queensland: The Australian Cotton Growers' Research Association.

Frank, A. W. (1987). Food uses of cottonseed protein. In *Developments in Food Protein-5*, pp. 31–80 [B. J. F. Hudson, editor]. London and New York: Elsevier Applied Science.

Griffiths, D. W. (1979). The inhibition of digestive enzymes by extracts of field beans. *Journal of the Science of Food and Agriculture* **30**, 458–462.

Griffiths, D. W. & Moseley, G. (1980). The effects of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *Journal of the Science of Food and Agriculture* **31**, 255–259.

Hagerman, A. E. & Butler, L. G. (1981). The specificity of proanthocyanidins – protein interactions. *Journal of Biological Chemistry* **256**, 4494–4497.

Helsper, J. P. F. G., Hoogendijk, J. M., van Norel, A. & Burger-Meyer, K. (1993). Antinutritional factors in Faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannins. *Journal of Agricultural and Food Chemistry* **41**, 1058–1061.

Huisman, J., van der Poel, A. F. B., Verstegen, M. W. A. & van Weerden, E. J. (1990). Antinutritional factors (ANF) in pig production. *World Review of Animal Production* **25**, 77–82.

Ikurior, S. A. & Fetuga, B. L. A. (1988). Chemical and biological evaluation of Nigerian cottonseed meal protein quality. *Animal Feed Science and Technology* **20**, 251–258.

Jansman, A. J. M. (1993*a*). Tannins in feedstuffs for simple-stomached animals. *Nutrition Research Reviews* **6**, 209–236.

Jansman, A. J. M. (1993*b*). Tannins in faba beans (*Vicia faba* L.) – antinutritional properties in monogastric animals, pp. 51–70. PhD Thesis, Wageningen Agricultural University, The Netherlands.

Jones, D. E. (1965). Banana tannin and its reaction with polyethylene glycols. *Nature* **206**, 299–300.

Jones, W. T. & Mangan, J. L. (1977). Complexes of the condensed tannins of sainfoin (*Onybrychis viciifolia* Scop.) with Fraction 1 Leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Longstaff, M. & McNab, J. M. (1991). The inhibitory effects of hull polysaccharides and tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *British Journal of Nutrition* **65**, 199–216.

Lusas, E. W. & Jividen, G. M. (1987). Glandless cottonseed, a review of the first 25 years of processing and utilisation research. *Journal of the American Oil Chemists' Society* **64**, 839–854.

Lyman, C. M., Baliga, B. P. & Slay, M. W. (1959). Reactions of proteins with gossypol. *Archives of Biochemistry and Biophysics* **84**, 486–497.

McNabb, W. C. (1991). Digestion and metabolism of sulphur containing amino acids in sheep fed fresh forage diets. PhD Thesis, Massey University, Palmerston North, New Zealand.

Mangan, J. L. (1988). Nutritional effects of tannins in animal feeds. *Nutrition Research Reviews* **1**, 209–231.

Marquardt, R. R. (1989). Dietary effects of tannins, vicine and convicine. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 141–155 [J. Huisman, A. F. B. van der Poel and I. E. Liener, editors]. Wageningen: Pudoc.

Martinez, W. H., Beradi, L. C., Frampton, V. L., Eilcke, H. L., Green, D. E. & Teichman, R. (1967). Importance of cellular constifuents to cottonseed meal protein quality. *Journal of Agricultural and Food Chemistry* **15**, 427–432.

Mehansho, H., Ann, D. K., Butler, L. G. & Carlson, D. M. (1987). Induction of proline-rich proteins in hamster salivary glands by isoproterenol treatment and an unusual growth inhibition by tannins. *Journal of Biological Chemistry* **260**, 4418–4423.

Mehansho, H., Asquith, T. N., Butler, L. G., Rogler, J. C. & Carlson, D. M. (1992). Tannin-mediated induction of proline-rich protein synthesis. *Journal of Agricultural and Food Chemistry* **40**, 93–97.

Mehansho, H., Hagerman, A., Clements, S., Butler, L. G., Rogler, J. C. & Carlson, D. M. (1983). Modulation of proline-rich protein biosynthesis in rat parotid glands by sorghum with high tannin levels. *Proceedings of the National Academy of Sciences USA* **80**, 3948–3952.

Mitjavila, S., Lacombe, C., Carrera, G. & Derache, R. (1977). Tannic acid and oxidised tannic acid on the functional state of rat intestinal epithelium. *Journal of Nutrition* **107**, 2113–2121.

Mole, S., Butler, L. G. & Iason, G. (1990). Defence against dietary tannin in herbivores: a survey for proline rich salivary proteins in mammals. *Biochemical Systematics and Ecology* **18**, 287–293.

Moughan, P. J., Darragh, A. J., Smith, W. C. & Butts, C. A. (1990). Perchloric and trichloroacetic acids as precipitants of protein in endogenous ileal digesta from the rat. *Journal of the Science of Food and Agriculture* **52**, 13–21.

National Research Council (1978). Nutrient requirements of the laboratory rat. In *Nutrient Requirements of Laboratory Animals*, pp. 7–32. Washington, DC: National Research Council.

Pons, W. A. & Eaves, P. H. (1967). Aqueous acetone extraction of cottonseed. *Journal of the American Oil Chemists Society* **44**, 460–464.

Robertson, J. B. & van Soest, P. J. (1981). The detergent system of analysis and its application in human food. In *The Analysis of Dietary Fibre in Food*, pp. 123–158 [W. P. T. James and O. Theander, editors]. New York and Basel: Marcel Dekker, Inc.

Salunkhe, D. K., Chavan, J. K. & Kadam, S. S. (1990). *Dietary Tannins: Consequences and Remedies*. Chapter 5. *Nutritional Consequences of Dietary Tannins*, pp. 113–146. Boca Raton, FL: CRC Press.

Sell, D. R., Reed, W. M., Chrisman, C. L. & Rogler, J. C. (1985). Mucin excretion and morphology of the intestinal tract as influenced by sorghum tannins. *Nutrition Reports International* **31**, 1369–1374.

Snedecor, G. W. & Cochran, W. G. (1982). *Statistical Methods*. Ames, IA: Iowa State University Press.

Terrill, T. H., Rowan, A. M., Douglas, G. B. & Barry, T. N. (1992). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* **58**, 321–329.

Thomsen, L. (1981). Bowl for feeding powdered diets to rats. *Laboratory Animals* **15**, 177–178.

van Leeuwen, P., Koninkx, J. F. J. L., Jansman, A. J. M. & Mouwen, J. M. V. M. (1993). Effect of condensed tannins in hulls of Faba beans (*Vicia faba* L.) on some morphological and functional parameters of small intestinal mucosa of piglets. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*, pp. 335–339 [A. F. B. van der Poel, J. Huisman and H. S. Saini, editors]. Wageningen: Wageningen Pers.

Varnish, S. A. & Carpenter, K. L. (1975) Mechanism of heat damage in proteins. 5. The nutritional values of heat-damage and propionylated proteins as sources of lysine, methionine and tryptophan. *British Journal of Nutrition* **34**, 325–337.

Yu, F., Barry, T. N., McNabb, W. C., Moughan, P. J. & Wilson, G. F. (1995*a*). Effect of bound condensed tannin from cottonseed upon *in situ* protein solubility and dry matter degradation in the rumen. *Journal of the Science of Food and Agriculture* **69**, 311–319.

Yu, F., Barry, T. N., Moughan, P. J. & Wilson, G. F. (1993). Condensed tannin and gossypol concentrations in cottonseed and in processed cottonseed meal. *Journal of the Science of Food and Agriculture* **63**, 7–15.

Yu, F., McNabb, W. C., Barry, T. N. & Waghorn, G. C. (1995*b*). Effect of condensed tannin in cottonseed hulls upon the *in vitro* degradation of cottonseed kernel proteins by rumen micro-organisms. *Journal of the Science of Food and Agriculture* **69**, 223–234.

Yu, F., Moughan, P. J. & Barry, T. N. (1995*c*). Effect of condensed tannin in cottonseed hulls on endogenous ileal amino acid loss in the growing rat. *Journal of the Science of Food and Agriculture* **68**, 451–455.

Printed in Great Britain