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PROJECT MNZIC

**THE EFFECT OF CONDENSED TANNIN CONTENT, HEAT
AND SOLVENT EXTRACTION UPON THE NUTRITIVE
VALUE OF COTTONSEED MEAL FOR RUMINANT AND
MONOGASTRIC LIVESTOCK**

***FINAL REPORT TO THE
COTTON RESEARCH
AND
DEVELOPMENT CORPORATION***

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The last two experiments have been completed and all follow-up laboratory work has been done. The PhD student (Mr Feng Yu) is currently writing up these experiments for the last two chapters of his PhD thesis. The thesis is due for submission to Massey University during November 1995, and a complementary copy will be sent to the CRDC at that time.

The thesis chapters have been prepared in the form of scientific papers and five of these have already been accepted for publication in International peer-reviewed scientific journals. A further two papers will be submitted before the end of 1995 (they are in draft form at this time). In addition, two papers were presented at industry conferences in Australia and one was presented at the First World Cotton Conference in Brisbane 1994. A list of all work published from the grant appears at the end of this final report.

The work has identified condensed tannin (CT) as occurring in cottonseed hulls but not in cottonseed kernels. Approximately 20% of the CT in cottonseed hulls is extractable and 80% is bound to protein and fibre. This contrasts with forage plants, where 75% of CT is extractable and 25% is bound. As Australian cottonseed meal (CSM) contains some hulls, this explains its content of CT (0.8-1.6% DM). Animal nutrition experiments were then conducted to study the effect of adding hulls upon the digestion and availability of kernel protein to ruminant and monogastric animals. During 1993 and 1994 all experiments were done with solvent-extracted unheated kernel.

Ruminant nutrition experiments were conducted by removing rumen fluid from fistulated sheep and conducting in vitro (i.e. laboratory) studies on the degradation of cottonseed proteins by mixed rumen micro-organisms. Monogastric nutrition experiments were done initially with laboratory rats as a model for production animals such as the pig, and then with pigs. In all cases the diets were given with and without polyethylene glycol (PEG; MW 3,350). PEG specifically binds and inactivates CT and can be used to deduce the effects of CT by comparing control diets (CT acting) with PEG diets (CT inactivated).

Proteins are made up from 20 individual amino acids, and these can be classified as either essential or non-essential in terms of animal nutrition. Essential amino acids are particularly important for the nutrition of monogastric animals. In this report the monogastric nutrition data is reported for the digestion of total protein, total essential amino acids and the four essential amino acids most likely to be limiting the performance of pigs and poultry (lysine, threonine, methionine and isoleucine). Details on the other six essential amino acids and on all non-essential amino acids can be obtained from the PhD thesis.

In the absence of heat, adding graded levels of hulls did reduce both the solubility and degradability of protein by rumen micro-organisms, giving a rumen "by-pass" effect (which is beneficial). However, the magnitude of these effects was very small and they are not regarded as being nutritionally significant under practical conditions of ruminant nutrition.

Adding hulls (4.6% of diet) significantly reduced the digestion of several essential amino acids in the small intestine of the rat. This effect (Table 1) is considered large, and is probably one of the factors responsible for the generally low levels of amino acid availability found for commercially produced CSM. Approximately half of the "hulls effect" is due to their content of CT and the cause of the other 50% is unknown. As there is no CT in cottonseed kernels, this explains the lack of response to PEG when the diet does not contain cottonseed hulls.

Table 1 Effect of adding hulls and polyethylene glycol (PEG) to unheated solvent extracted cottonseed kernels on the apparent ileal digestibility of protein and amino acids (AAs) in the growing rat.

	NO HULLS (0.0% CT)		WITH HULLS ¹ (0.24% CT)		SEM
	-	+	-	+	
PEG ²					
COTTONSEED KERNEL DIET:					
Protein	88	89	85	87	0.8
Total Essential AAs	83	82	75	79	1.3
Lysine	82	80	71	74	1.5
Threonine	74	73	63	67	1.5
Isoleucine	81	80	61	75	1.8
Methionine	84	84	84	86	1.3

¹4.6% cottonseed kernel-based diet.

²PEG binds and inactivates CT. Effect of CT can be calculated by comparing control rats (i.e. -PEG; CT acting) with +PEG rats (CT inactivated).

Work was then conducted in 1995 to test if the above conclusions held true in the presence of heat, which is extensively used during the extraction of oil from cottonseed. Cottonseed was manually separated into kernels and hulls and the kernels were extracted with hexane to remove the oil. A mixture of ground extracted kernels (85%) and hulls (15%) was then prepared. Half of this was then autoclaved at 110°C, 0.5 kg/pressure cm², 7-10% moisture content for 2 h as used in CSM manufacturing at Narrabri. The unheated and heated CSM were then incorporated into rat diets and fed with and without PEG (to define the effects of CT). The results are shown in Table 2 below. The combined action of heat and CT (as occurs in commercial CSM processing) substantially lowered the availability of some essential amino acids and hence reduced protein nutritional value (two -PEG columns).

depressed amino acid digestion in both the unheated and heated meals, with the amount of depression being greatest in the presence of heat. This shows that a combination of heat and CT is particularly bad for reducing amino acid availability in CSM for monogastric animals.

Table 2 Effect of heat and polyethylene glycol (PEG) on the apparent ileal digestibility of protein and amino acids (AA) in the growing rat.

PEG ¹	Unheated CSM (0.20% CT)		Heated CSM (0.22% CT)		SEM
	-	+	-	+	
Protein	79	86	70	74	1.4
Total Essential AAs:	80	82	70	74	0.9
Lysine	77	80	58	64	0.8
Threonine	69	69	56	62	1.3
Isoleucine	75	78	64	68	1.0
Methionine	82	84	66	74	1.0

¹PEG binds and inactivates CT. Effect of CT can be calculated by comparing control rats (i.e. -PEG; CT acting) with +PEG rats (CT inactivated).

The final monogastric experiment looked at the effect of CT on apparent ileal digestion of protein and amino acids in both rats and pigs fed a commercially produced CSM (which had therefore undergone extensive heat treatment). The results, shown below in Table 3, show that CT reduced protein nutritional value for both species, with the pig and the rat showing similar sensitivity to CT. Thus, the detrimental effects of CT on protein nutritional value observed with the rat will also occur with the pig.

Table 3 Effect of animal species and polyethylene glycol (PEG) on the apparent ileal digestibility of protein from commercially produced cottonseed meal (Narrabri plant).

PEG	Rat (0.42% CT)		Pig (0.42% CT)		SEM
	-	+	-	+	
Protein	69	71	66	70	1.0
Total Essential AAs:	66	68	67	68	1.0
Lysine	50	52	58	59	1.3
Threonine	50	53	56	58	0.9
Isoleucine	57	62	61	64	1.2
Methionine	61	65	59	59	1.1

Compared to the experimentally prepared unheated CSM reported in Tables 1 and 2, the commercially prepared CSM reported in Table 3 was of low amino acid availability to monogastric animals. This is due to the combined effects of heating (which is extensive in CSM manufacturing) and the presence of some hulls (and hence CT) in commercially produced CSM.

In addition to work with monogastric animals, some further work was done with *in vitro* rumen fermentations during 1995. This measured effects of heat and CT upon degradation rate and potential degradability of the major cottonseed proteins by rumen micro-organisms and is shown below in Table 4.

Table 4 Effects of heat and polyethylene glycol (PEG) upon degradation of the 48 kDa cottonseed protein by rumen micro-organisms.

PEG	Degradation rate (%/h)		Potential degradability (%)	
	-	+	-	+
Unheated cottonseed kernel (0.0% CT)	1.02	1.11	80	83
Commercial CSM:				
Brisbane (0.57% CT)	0.07	0.11	81	92
Narrabri (0.91% CT)	0.15	0.15	85	91

Whilst the action of CT did have some effect in reducing potential degradability (shown as +PEG minus -PEG), these effects are considered small. CT had little effect on degradation rate, but degradation rate was markedly slowed by the heat treatment used in commercial processing. Heat treatment warrants further detailed research as a method for reducing rumen degradation of CSM proteins.

Other work with sheep showed that cottonseed hulls contained approximately 90% fibre and that rumen digestion of this was very low at 33%. When the CT content was neutralised with PEG, this increased to 40%.

CONCLUSIONS

From the results generated from this project the following conclusions can be drawn:

- 1) Unheated pure cottonseed kernel protein (with no hulls present) is of high protein nutritional value to monogastric animals. Relative to unheated solvent extracted kernels, commercially produced CSM is of low protein nutritional value for monogastric animals (Table 3). This is shown in apparent ileal digestibility of limiting essential amino acids. The low amino acid availability in commercial CSM is probably due to a combination of heat applied during processing and the presence of some hulls, with approximately half the hulls effect being explained by their CT content. It seems that bound CT in cottonseed hulls are solubilised in the monogastric stomach and then reduce amino acid digestion.

It is understood that some hulls are left in commercial CSM to ensure a firmer "meats", from which the oil can be extracted more efficiently. This lowers the final protein content, and as shown in this study it also substantially lowers the efficiency with which limiting essential amino acids can be digested and absorbed from the monogastric digestive system. This study has shown that cottonseed hulls are not an inert substance for monogastric animals; rather they are anti-nutritional and reduce the efficiency of protein digestion.

The study has also shown that the large amount of heat applied during normal commercial CSM processing reduces the digestion of limiting essential amino acids by monogastric animals (Table 2). A combination of heat and CT from hulls is particularly damaging for lowering amino acid digestion in monogastric species.

Where CSM is intended for the monogastric livestock industries, it is recommended that the level of hulls be reduced to the lowest possible levels and that the amount of heat applied be the minimum amount to bind gossypol and no more. This subject has been discussed with Cargill Executives at their Melbourne Head Office (Robert Green, Marketing Manager; Geoff Barker, Meal Sales Manager). The company sees soybean meal as a main competitor to CSM, and whilst CSM produced from their Brisbane plant is of similar crude protein content to soybean meal, CSM is perceived by the monogastric feed industries in Australia as being of lower amino acid availability. The present study has identified the causes of the low amino acid availability in CSM.

- 2) As CT in forage plants substantially reduces rumen protein degradation in ruminants (and creates "by-pass protein"), an original objective of the project was to study the effect of CT in CSM upon rumen protein solubility and degradation. CT in forages are mainly in the extractable form (75%), with only 25% being bound to protein and fibre. In contrast, only 20% of the CT in CSM is extractable, with 80% being bound to protein and fibre.

Although action of CT did reduce the degradation of cottonseed proteins by rumen micro-organisms, the effect seems of small magnitude. The effect is smaller than that produced by similar concentrations of forage CT, and is probably explained by bound CT being relatively unreactive at rumen pH (6.0-7.0 units).

Whilst presence of CT will give some protection of proteins against rumen fermentation, the effects seem small and are of much lower magnitude than those produced by the effects of heat. Further research is necessary to accurately define the amount of heat (temperature and time) to give optimum rumen by-pass of CSM proteins.

- 3) From the results of the project it seems that CSM intended for the monogastric industries should be processed differently from CSM intended for the ruminant industries. The effects of CT and their continued reactivity after heating have been accurately defined in this project. Future work is necessary to accurately define the extent of heating required to produce CSM of optimum nutritive value for ruminant and monogastric livestock. It is recognised that some heating is necessary, to soften the seed and to bind gossypol, but the amount of heat required is likely to differ substantially in CSM intended for ruminant or monogastric markets.

A scientific description of the overall findings from the project can be found in the attached "General Discussion and Conclusions", which forms the final chapter of Mr Feng Yu's PhD thesis.

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Massey University appreciates the time put in by staff of Cargill Oilseeds, which has greatly increased our understanding of cottonseed processing. In particular, we have always been welcome at their Narrabri Plant and wish to thank Robert Spencer (Deputy Superintendent) and Mic Mittasch (Production Supervisor) for their help. Robert Green and Geoff Barker of Cargill Head Office, Melbourne are also thanked for their input.

We have greatly valued this opportunity to work with the Australian Cotton Industry.

INDIVIDUAL PUBLICATIONS RESULTING FROM GRANT MNZIC

List of Publications - Resulting from Grant MNZIC

- 1 YU FENG, T N Barry, P J Moughan and G F Wilson 1993 Condensed tannin and gossypol concentrations in cottonseed and in processed cottonseed meal. *Journal of the Science of Food and Agriculture* 63 7-15.
- 2 YU FENG, T N Barry, W C McNabb, P J Moughan and G F Wilson 1995 Effect of bound condensed tannin upon *in situ* protein solubility and dry matter digestion in the rumen. *Journal of the Science of Food and Agriculture* 69 (In press).
- 3 YU FENG, W C McNabb, T N Barry and G C Waghorn 1995 Effect of condensed tannin from cottonseed hulls upon the *in vitro* degradation of cottonseed kernel proteins by rumen micro-organisms. *Journal of the Science of Food and Agriculture* 69 (In press).
- 4 YU FENG, P J Moughan and T N Barry 1995a 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.
- 5 YU FENG, P J Moughan and T N Barry 1995b Effect of condensed tannin on the ileal digestibility of amino acids in casein and cottonseed kernel. *British Journal of Nutrition* (In press).
- 6 YU FENG, W C McNabb, T N Barry and P J Moughan 1995 Effect of heat treatment and cottonseed condensed tannin upon ruminal protein degradation and available lysine content of cottonseed kernel. *Journal of the Science of Food and Agriculture* (In preparation).
- 7 YU FENG, P J Moughan, T N Barry and W C McNabb 1995 Effect of heat treatment and cottonseed condensed tannin on the ileal digestibility of amino acids for the growing rat and pig. *British Journal of Nutrition* (In preparation).
- 8 YU FENG, T N Barry, P J Moughan and G F Wilson 1992 A preliminary study on the chemical composition of Australian cottonseed and the relevance of cottonseed as a feedstuff for ruminant and monogastric livestock. In "Proceedings of 1992 Australian Cotton Conference". pp 465-473. Broadbeach, Queensland, Australia.
- 9 YU FENG, T N Barry, P J Moughan and G F Wilson 1993 Condensed tannins in commercial cottonseed and in processed cottonseed products. In "Manipulating Pig Production IV". pp 230. Australasian Pig Science Association, Canberra, Australia.
- 10 YU FENG, T N Barry, P J Moughan and G F Wilson 1994 Extractable and bound condensed tannin content of cottonseed and of processed cottonseed meal. In "Proceedings of World Cotton Research Conference-1". Brisbane, Queensland, Australia.
- 11 YU FENG, P J Moughan and T N Barry 1995c Effect of condensed tannins in cottonseed hulls on true ileal amino acid digestibility in casein. In "Manipulating Pig Production V". Australasian Pig Science Association, Canberra, Australia (In press).
- 12 YU FENG, P J Moughan and T N Barry 1995d Effect of cottonseed condensed tannins on the ileal digestibilities of amino acids in cottonseed kernel. In "Proceedings of the Nutrition Society of New Zealand 1995". Nutrition Society of New Zealand, Auckland, New Zealand (In press).
- 13 YU FENG 1995 Nutritional value of solvent extracted cottonseed meal for ruminant and monogastric animals. PhD thesis. Massey University, Palmerston North, New Zealand.

GENERAL DISCUSSION AND CONCLUSIONS

Chapter 9 from the PhD thesis

**The Effect of Condensed Tannins on the Protein Nutritional Value of
Cottonseed Meal for Ruminant and Monogastric Livestock**

By Feng Yu

Submitted to Massey University in November 1995

9.1 INTRODUCTION

The nutritive value of feedstuffs depends on the contents of the various nutrients, their apparent digestibility and their utilisation after absorption. Both the digestibility and the utilisation of nutrients can be affected by the presence of antinutritional factors (ANFs), particularly for monogastric species. In the past decade research on ANFs, including condensed tannins (CT), in feedstuffs for both ruminant and monogastric animals has been stimulated by the search for alternative feedstuffs as protein sources. Cottonseed meal (CSM), as one of the alternatives, contains several ANFs, including gossypol, CT, phytate and other potential toxic factors (see Chapter 1).

The presence of ANFs, including gossypol and CT, is part of the host plant resistance mechanism in cotton for defence against attack by insects and pathogenic micro-organisms. Hence, from an agronomic point of view, their presence may be desirable. Katoh *et al.* (1989) found that the tannin content of leaves of Japanese cedar (*Cryptomeria japonica* D. Don) was reduced in areas with severe environmental pollution. This was accompanied by an increased predation of larvae of a herbivorous moth upon these leaves. This stresses the possible function of tannins in plants and their interaction with environmental factors.

CT in general may potentially exert a large number of antinutritional effects, but low concentrations (20-40 g kg⁻¹ DM) in forages have been suggested to have beneficial effects for ruminants (Barry 1989). In this thesis, attention has been given to several aspects of this concept for the effects and mode of action of CT in CSM. They were studied in different animal species, including rats, pigs and sheep. Because rats have served as a model for many other monogastric species in nutritional studies, including those aiming at the elucidation of effects of dietary CT, most fundamental studies were performed with rats. Information on the nutritional effects of cottonseed CT in pigs is scarce. Therefore, one study was done with this species and the rat was evaluated as a model for the pig. Although several studies indicated that forages containing low levels of extractable CT have high nutritional value for ruminants, the effect of bound cottonseed CT on protein digestion in the ruminants has not been defined.

9.2 CONDENSED TANNIN CONCENTRATION AND REACTIVITY

From results obtained in the studies reported in this thesis (Chapters 2, 7 and 8) it was concluded that CT are present in commercially produced CSM in significant concentrations. CT were present in the hulls (Table 9.1), but were not detected in the kernels. CT in cottonseed hulls, unlike CT in forages, were mainly in the protein- and

fibre-bound form. In general, only about 10-20% of total cottonseed CT were present in extractable forms, and hence their concentration would be greatly underestimated using conventional CT analytical methods which measure extractable CT only.

The results obtained from the studies in Chapters 7 and 8 showed that heat treating cottonseed hulls reduced the concentrations of total CT that could be extracted with acetone/water and SDS solution and determined with butanol/HCl. Application of heat treatment to the mixtures of kernel/hulls by autoclaving or in a forced-draught oven inactivated 29% and 13% of the total CT, respectively. Exposure to oxygen (including drying) can cause oxidative damage to CT (Goldstein and Swain 1965; McLeod 1974), and the above results may be explained by the oxidation of some CT to other compounds that may not be extracted and be detected by butanol/HCl.

Although the concentrations of total CT in cottonseed materials determined using the butanol/HCl method were reduced by the heat treatment, the results show that the effects of CT in reducing apparent ileal amino acid digestibility (Chapter 8) and in reducing N solubility and the rumen degradation of cottonseed proteins (Chapter 7) were not reduced by the heat applied, as judged by the responses to PEG. It seems that heat treatment did not diminish the reactivity of CT with cottonseed proteins. In fact, the increased responses to PEG in apparent ileal digestibility of threonine, methionine, tyrosine and lysine in heated compared to unheated CSM indicates that action of heat treatment may have increased the reactivity of CT with these amino acids, causing larger reductions in apparent ileal digestibility than heat or CT applied separately. These effects will be described further in Section 9. 3.

In both the monogastric and ruminant experiments, polyethylene glycol (MW 3,500) was either added to the diets (Chapters 5, 6 and 8), or the flasks used in the *in vitro* incubation (Chapters 3, 4 and 7) or continuously infused into the rumen (Chapter 3) to define the effect of CT. PEG has been shown to displace CT from CT-protein complexes (Jones and Mangan 1977) and completely binds extractable CT in forage at 1.8 mg mg⁻¹ total CT (Barry and Forss 1983), preventing CT from binding to protein and releasing protein from CT-protein complexes in the rumen. The studies reported in this thesis demonstrated that 2.0 mg PEG mg⁻¹ total CT was required to prevent or reverse the effect of CT in cottonseed hulls on N solubility and rumen degradation of the kernel proteins.

The lack of any PEG effect upon protein solubility and *in vitro* rumen degradability with pure cottonseed kernel, and upon ileal endogenous amino acid loss and ileal digestibility of N and amino acids in rats fed the diets containing no-hulls showed that PEG *per se* did not effect protein digestion and absorption for both

Table 9. 1 The concentrations of extractable and bound condensed tannin (g kg^{-1} DM) in the hulls of cottonseed cultivars, in cottonseed meal and other protein meals and in forages. All determinations used the butanol-HCl procedure.

	Condensed Tannin				Reference
	Extract- able	Protein bound	Fibre bound	Total	
<i>Cottonseed cultivars (hulls only)</i>					
Multiple host plant resistant					
MHR 10	7.0	26.8	7.0	40.8	This thesis (Chapter 2)
High gossypol					
HG 065	7.4	24.5	7.7	36.9	" "
High tannin					
HT-35-5-1	12.1	30.2	10.1	52.4	" "
HT-35-14-3	15.7	38.2	11.4	65.3	" "
Australian-bred cultivars					
Siokra L22	13.0	29.0	10.0	52.0	This thesis (Chapter 4)
Siokra 1-4	14.5	28.4	6.6	49.5	This thesis (Chapter 2)
Glandless					
DP 16	15.3	32.5	9.9	57.7	" "
<i>Cottonseed meals (CSM)</i>					
CSM	2.1	10.0	3.9	16.0	Terrill <i>et al.</i> (1992)
CSM Narrabri A	2.1	7.6	5.4	15.1	This thesis (Chapter 2)
CSM Narrabri B	1.8	5.2	2.1	9.1	This thesis (Chapter 7)
CSM Brisbane A	0.1	4.9	2.9	7.9	This thesis (Chapter 2)
CSM Brisbane B	0.6	3.1	2.0	5.7	This thesis (Chapter 7)
<i>Heated and unheated mixtures of cottonseed kernel (CSK) and hulls</i>					
Unheated CSK+					
16.7% hulls	2.4	4.6	0.9	7.9	This thesis (Chapter 8)
Heated CSK+16.7%					
heated hulls	0.8	3.9	0.9	5.6	" "
Unheated CSK+					
25% hulls	2.9	4.0	3.0	9.9	This thesis (Chapter 7)
Heated CSK+25%					
heated hulls	1.9	4.3	2.4	8.5	" "
Unheated CSK+					
50% hulls	5.0	7.0	5.3	17.3	This thesis (Chapter 7)
Heated CSK+50%					
heated hulls	6.0	6.1	3.1	15.2	" "
<i>Protein meals</i>					
Soya bean	1.0	0	0	1.0	Terrill <i>et al.</i> (1992)
Rapeseed	0.7	3.7	1.5	5.9	" "
<i>Forage legumes</i>					
Canary clover	83.0	54.0	6.0	143.0	" "
Birdsfoot trefoil	27.1	6.1	1.8	35.0	Wang <i>et al.</i> (1995)
<i>Grasses</i>					
Perennial ryegrass	1.1	0	0	1.1	Terrill <i>et al.</i> (1992)
Yorkshire fog	1.1	0.3	0.4	1.8	" "

ruminant and monogastric animals when the diet does not contain CT. Thus, the studies carried out in this thesis demonstrate that the application of PEG can be used to deduce nutritional effects of CT in cottonseed hulls, and mixtures of cottonseed kernel/hulls, as found for forages (Barry and Forss 1983; McNabb *et al.* 1993; Wang *et al.* 1995).

9.3 NUTRITIONAL EFFECTS OF COTTONSEED CT IN MONOGASTRIC ANIMALS

Endogenous ileal amino acid loss was increased by inclusion of cottonseed hulls in an enzymically hydrolysed casein (EHC) based diets fed to growing rats (Chapter 5). This was probably caused by an effect of the hull fibre component on endogenous ileal amino acid loss, as the cottonseed CT did not appear to influence this loss.

Jansman (1993) fed pigs diets containing faba bean hulls with low and high concentrations of CT, and found that either dietary fibre and, or CT in faba bean led to a decreased true ileal digestibility of dietary protein and increased excretion of endogenously secreted proteins. The increase of endogenous excretion of protein may be due to an enhanced secretion of endogenous proteins or to a reduced degradation and reabsorption of endogenously secreted proteins. The latter could be relevant since Souffrant *et al.* (1986) found that 70 and 82% of endogenous secreted proteins in the alimentary tract of pigs are reabsorbed up to the terminal ileum and the rectum, respectively. Griffiths and Moseley (1980) suggested that dietary tannins may increase pancreatic secretion of digestive enzymes. Tannins may induce pancreatic secretion in a manner analogous to that of protease inhibitors from legume seeds (Liener 1989). The consumption of diets containing tannins was shown to specifically increase the size of the parotid glands in the rat and the synthesis and secretion of proline-rich proteins (PRPs; Mehansho *et al.* 1992). Tannin-induced PRPs were shown to have a very high binding affinity for tannins. The binding of tannins to both dietary and endogenous proteins has also been used to explain the reduced apparent digestibility of protein in tannin-containing diets. However, evidence for dietary cottonseed CT increasing endogenous ileal amino acid loss was not found in the study reported in this thesis.

It has been shown in Chapters 6 and 8 that inclusion of cottonseed hulls in the diets depressed apparent and true ileal digestibility of nitrogen and amino acids in rats, and reduced apparent ileal digestibility of nitrogen and amino acids in pigs (Table 9. 2). These depressions were mainly attributed to the cottonseed CT present in the diets. However, the response was different between protein sources in the diets. With the casein diets, all of the depression could be explained by the CT content of the hulls (Chapter 6), but with the cottonseed kernel diets, only part of the depression could be

explained by CT (Chapters 6 and 8). It seems that some unknown components of the hulls other than CT also depressed the apparent and true ileal digestibility of nitrogen and amino acids in cottonseed kernel. This was also shown again for chemically determined FDNB available lysine in Chapter 7. The responses to PEG in diets containing hulls reported in this thesis indicate that bound cottonseed CT were reacting with proteins in the monogastric digestive system. CT are known to be solubilised in the stomach (Jones and Mangan 1977), so that the most probable explanation for results obtained from Chapters 6 and 8 is that the bound CT in hulls were solubilised and released at the low pH 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 and McNab 1991), or by increased secretion of endogenous proteins (digestive enzymes, mucus or mucosal cells; Mangan 1988; Marquardt 1989). Our results (Chapter 5) did not indicate that cottonseed CT increased endogenous protein. Therefore, the lowered apparent ileal digestibility of N and amino acids in the diets containing CT in cottonseed hulls may be attributed to a decrease in digestion and absorption as reflected by the true coefficients of digestibility of dietary protein.

It was also shown that cottonseed CT selectively bind to proteins *in vivo* (Chapters 6 and 8). There were differences in the effect of CT on apparent ileal digestibility of individual amino acids. The difference in apparent ileal amino acid digestibility between protein sources (casein and cottonseed kernel) suggests a difference in the intrinsic quality of the proteins. The major amino acid deficiency in CSM is that of lysine. For pigs, methionine, threonine, leucine and isoleucine become limiting after lysine (Chapter 1; Fisher and Quisenberry 1971). The ileal digestibility of lysine, threonine, isoleucine and methionine were more depressed by cottonseed CT compared with the other amino acids, particularly for heat treated or processed CSM. This may partly explain why these amino acids are deficient in commercially produced CSM. Differences in the effect of faba bean CT on ileal and faecal digestibility of individual amino acids have also been reported by Jansman (1993). It seems that CT may have a different affinity for proteins with different amino acid profiles.

The apparent ileal digestibility of lysine, methionine, threonine and isoleucine are much lower in commercially produced CSM than in unheated kernel diets that do not contain cottonseed hulls (Table 9. 2). The principle reason for this seems to be a combination of heat treatment and the presence of some cottonseed hulls in commercially produced CSM, with this combination causing major reductions in digestibility. As the

responses to PEG for these amino acids were greater in heated than in unheated CSM (Chapter 8), it seems that the action of autoclaving (110°C; 2 h) increased the reversible reactivity of cottonseed hull CT with kernel proteins. A further reason is the increase in endogenous ileal protein flow caused by the fibre component of hulls in the CSM.

Asquith and Butler (1986), in an *in vitro* study, noted that CT/protein interaction 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 and 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 studies reported in this thesis for cottonseed kernel and casein.

There appear to be major similarities between the rat and pig in digestive anatomy and physiology, requirements for nutrients and relative growth rates, especially when comparison is made at a physiologically comparable age (Donkoh *et al.*, 1994). Other studies (Taverner 1979; Picard *et al.* 1984; Smith *et al.* 1990; Donkoh *et al.* 1994) have shown similarities between rats and pigs for ileal amino acid digestibility with several protein sources. However, this may not be so for all feedstuffs and particularly for feedstuffs containing antinutritional factors (ANFs). Several studies indicate that rats and piglets may respond differently to ANFs in raw soya bean (Coms *et al.* 1967) and chickpeas (Visitpanich *et al.* 1985). Moreover, Moughan *et al.* (1984) found significant differences between rats and pigs for apparent ileal protein digestibility in peas. Huisman *et al.* (1989, 1991) compared the sensitivity of various animal species (pigs, rats, chickens and mice) to ANFs in legume seeds and demonstrated that there is a difference in sensitivity between animal species. These authors concluded that piglets were distinctly more sensitive to ANFs in beans and peas than rats and chickens and that results obtained with rats and chickens cannot be extrapolated to pigs. The findings of this thesis indicate that there are significant differences in the apparent ileal digestibility of some amino acids in CSM between rats and pigs. Therefore, studies into the effects of CT should be carried out in the target animal species.

Table 9.2 Apparent ileal digestibility of some essential amino acids in different animal species fed diets containing cottonseed hulls (CSH) or cottonseed meal (CSM)

Diets	Species\PEG ¹	Apparent ileal digestibility of amino acids								Reference
		Lysine		Methionine		Threonine		Isoleucine		
		-	+	-	+	-	+	-	+	
<i>Casein diets</i> ²										
+0% CSH	rats	0.98	0.99	-	-	0.91	0.93	0.93	0.93	This thesis (Chapter 6)
+7% CSH	rats	0.95	0.98	-	-	0.89	0.92	0.88	0.91	" "
<i>Unheated cottonseed kernel (CSK) diets</i>										
+0% CSH	rats	0.82	0.80	0.84	0.84	0.74	0.73	0.81	0.80	This thesis (Chapter 6)
+4.6% CSH	rats	0.71	0.74	0.84	0.86	0.63	0.67	0.61	0.75	" "
<i>Unheated/heated CSK diets</i> ³										
Unheated+5% CSH	rats	0.77	0.80	0.82 ⁴	0.84 ⁴	0.69	0.69	0.75	0.78	This thesis (Chapter 8)
Heated+5% CSH	rats	0.58	0.64	0.66 ⁴	0.74 ⁴	0.56	0.62	0.64	0.68	" "
<i>Commercial CSM diets</i>										
+40% CSM (Prepress solvent)	rats	0.50	0.52	0.61 ⁴	0.65 ⁴	0.50	0.53	0.57	0.62	" "
+40% CSM (Prepress solvent)	pigs	0.58	0.59	0.59 ⁴	0.59 ⁴	0.56	0.58	0.61	0.64	" "
+34% CSM (Direct solvent)	pigs	0.62	-	0.65	-	0.62	-	0.66	-	Tanksley <i>et al.</i> (1981)
+34% CSM (Screw press)	pigs	0.64	-	0.66	-	0.65	-	0.70	-	" "
+26% CSM (Prepress solvent)	pigs	0.56	-	0.72	-	0.51	-	0.63	-	Batterham <i>et al.</i> (1990)
+26% CSM (Prepress solvent)	pigs	0.67	-	0.80	-	0.64	-	0.73	-	" "

¹ Polyethylene glycol, MW 3,500; PEG-, without PEG addition; PEG+, with PEG addition.

² True ileal amino acid digestibility in rats fed casein based diets.

³ Cottonseed kernel (CSK) and hulls (CSH) were heat-treated by autoclaving at 110°C for 120 min.

⁴ Determined using conventional HCl amino acid hydrolysis. Data not included in the main body of the thesis (i.e. Chapter 8).

The value of dietary protein is influenced largely by the proportion of the protein that can be digested and absorbed. Application of heat treatment to proteins can reduce their digestion and absorption by the sugar-protein Maillard reaction and the formation of isopeptide cross-links (Hurrell and Carpenter 1977; Erbersdobler and Anderson 1983). As a number of amino acids such as lysine, threonine, methionine and tryptophan are affected by heat treatment (Batterham 1992), it is possible that chemical reactions occur between amino acids within a protein molecule, in addition to the specific Maillard reaction between lysine and carbonyl groups of reducing sugars. The results obtained from a study in Chapter 8 showed that heat treatment of cottonseed kernel/hulls by autoclaving at 110°C for 120 min significantly reduced the apparent ileal digestibility of all individual amino acids, particularly for lysine, threonine, isoleucine, and heat treatment may also cause other as yet unknown reaction involving CT that further lower apparent ileal digestibility of limiting essential amino acids. Lysine, tyrosine and cystine are more sensitive to heat treatment than are other amino acids (Ashes *et al.* 1984). A finding of relevance to this work is that of Moughan *et al.* (1995), who found that the apparent ileal digestibility of most amino acids for pigs fed a heated glucose/casein mixture was lower compared with an unheated mixture.

9. 4 NUTRITIONAL EFFECTS OF COTTONSEED CT IN RUMINANT ANIMALS

Condensed tannins in cottonseed hulls appear to reduce protein solubility in heated (Chapter 7) and unheated (Chapters 3 and 7) solvent extracted cottonseed kernels determined using both *in vitro* incubation in mineral buffer (pH 7.0) and the *in situ* polyester bag technique, and to reduce degradability of the two major seed storage proteins (52 and 48 kDa) present in heated (Chapter 7) and unheated (Chapters 4 and 7) cottonseed kernels estimated using *in vitro* incubations in rumen fluid and identification of individual proteins by SDS-PAGE. This is the result of the formation of stable complexes between CT and proteins, which do not dissociate at rumen pH (Jones and Mangan 1977).

A summary of the effects obtained upon N solubility is shown in Table 9. 3. Addition of hulls and application of heat consistently reduced N solubility, but the magnitude of the changes were small. PEG consistently increased N solubility measured *in vitro* but not *in situ*. Both the *in vitro* and *in situ* data indicate that a component in hulls other than CT also depressed solubility of kernel total nitrogen.

Table 9. 3 Effect of addition of hulls and heat treatment upon the nitrogen solubility of cottonseed kernel during *in vitro* incubation with mineral buffer¹ and *in situ* in the rumen²

PEG ³	<i>In vitro</i> incubation		<i>In situ</i> in the rumen		References
	Nitrogen		N predicted		
	Solubility (%)		Solubility (%)		
	-	+	-	+	
<i>Unheated cottonseed kernel</i>					
+0% hulls	42	42	86	87	Chapter 3
+50 hulls	33	38	82	81	" "
+100% hulls	29	36	78	80	" "
+200% hulls	23	31	75	74	" "
<i>Unheated/heated cottonseed kernel</i>					
Unheated+0% hulls	49	50	ND ⁴	ND	Chapter 7
Heated+0% hulls	45	47	ND	ND	" "
Unheated+50% hulls	37	46	ND	ND	" "
Heated+50% hulls	35	39	ND	ND	" "
<i>Commercial cottonseed meal (CSM)</i>					
CSM Narrabri	33	37	ND	ND	Chapter 7
CSM Brisbane	18	18	ND	ND	" "

¹ Samples were incubated with phosphate mineral buffer (pH 7.0) in a shaking water bath (90 rpm) at 39°C for 2 h.

² Samples suspended in the rumen of sheep using the polyester bag technique (Mehrez and Ørskov 1977).

³ Polyethylene glycol (PEG), MW 3,500; PEG-, no PEG added to incubation; PEG+, PEG added to incubation.

⁴ ND, not determined.

The data collected from *in vitro* rumen degradation studies in Chapters 4 and 7 have been used to calculate predicted rumen degradability, by correcting for rumen protein outflow rate using the equation: $P=A+[BC/(C+k)]$ (Ørskov and McDonald 1979). These data are shown in Table 9. 4, and represent predicted degradability of cottonseed proteins at a rumen outflow rate (k) of 0.046^{-1} (Ørskov and McDonald 1979), equivalent to a rumen retention time of 22 h. The values presented in Table 9. 4 are considered to be relative rather than absolute values, as degradation constants determined *in vitro* (A, B and C) may be different in magnitude to *in vivo*. Nevertheless, Table 9. 4 gives a good basis for comparing all the treatments applied in this thesis, as all the data were treated identically.

In general, predicted degradability values were higher for the cottonseed used in Chapter 4 than Chapter 7, perhaps representing differences between different sources of seed. However, in the absence of hull addition or heat, predicted degradability of

cottonseed kernel proteins was high at 76-94%. Adding hulls consistently lowered predicted degradability, with the effects being small in Chapter 4 but somewhat larger in Chapter 7. However, in Chapter 7 only part of the depression in predicted degradability could be explained by CT, as judged by the effects of PEG addition, emphasising that other components of hulls in addition to CT must also have been reducing degradability. Interestingly, as found for the monogastric data (Chapter 8), a combination of heat and CT produced the biggest reduction in rumen degradability (Chapter 7), with the responses to PEG showing that effects of CT were still reversible in heated meal. Heat-stimulated reaction with CT may change the properties of CSM proteins to decrease their solubility or the reaction sites for trypsin-like enzymes of ruminal microbes (Broderick and Craig 1980).

Table 9. 4 Effect of addition of hulls and heat treatment upon the predicted degradability¹ of cottonseed kernel proteins during *in vitro* incubation with rumen fluid

PEG ²	52 kDa protein predicted <u>degradability (%)</u>		48 kDa protein predicted <u>degradability (%)</u>		References
	-	+	-	+	
	<i>Unheated cottonseed kernel</i>				
+0% hulls	93	94	92	93	Chapter 4
+50% hulls	92	ND ³	90	ND	" "
+100% hulls	86	92	85	92	" "
+200% hulls	76	ND	75	ND	" "
<i>Unheated/heated cottonseed kernel</i>					
Unheated+0% hulls	76	76	77	80	Chapter 7
Heated+0% hulls	68	66	71	66	" "
Unheated+50% hulls	61	68	65	68	" "
Heated+50% hulls	48	56	54	63	" "
<i>Commercial cottonseed meal (CSM)</i>					
CSM Narrabri	72	74	71	76	Chapter 7
CSM Brisbane	58	61	56	70	" "

¹ Predicted rumen protein degradability (P %) was calculated from the equation (Ørskov and McDonald 1979): $P=A+[BC/(C+k)]$ where A, B and C are constants, and k is the rumen protein outflow rate assumed to be 0.046 h^{-1} (Ørskov and McDonald 1979).

² Polyethylene glycol (PEG), MW 3,500; PEG-, no PEG added to incubation; PEG+, PEG added to incubation.

³ ND, not determined.

Commercial CSM produced in Brisbane was of lower predicted rumen degradability than CSM produced at Narrabri, suggesting that greater heat had been

generated in processing at the Brisbane plant. The responses to PEG indicate that CT was contributing to reduce degradability in CSM manufactured at both locations.

Condensed tannins are able to bind simultaneously at multiple sites to protein by hydrogen bonding, and by hydrophobic and covalent interactions (Spencer *et al.* 1988b; Hagerman 1989). Presumably, CT-protein complexes are less susceptible to degradation by rumen micro-organisms. However, the hydrogen bonds are continuously broken and reformed randomly (McLeod 1974), while the degree of bonding between CT and protein is also affected by a large number of other factors, such as the chemical and molecular weight of the protein, presence of detergent, concentrations and nature of both CT and proteins (Asquith and Butler 1986; Horigome *et al.* 1988; Hagerman 1989). It is assumed that the optimum pH for the formation of insoluble CT-protein complexes is around the isoelectric point of the protein involved. The pH in the digestive tract of ruminants varies from 6-7 in the rumen to 2-3 in the abomasum. Therefore, the CT are able to complex with dietary proteins in the oral cavity during chewing and by-pass from rumen, and then may release protein in the abomasum or proximal duodenum at low pH for digestion and absorption. It also means CT, subsequently, may bind to other proteins of either exogenous (feed) or endogenous origin in the small intestine.

Rumen degradation of dietary protein is influenced by protein content, amino acid composition and protein structure (Romagnolo *et al.* 1990). The tertiary structure of protein affects the ability of the microbial population to gain access to peptide bonds. Ovalbumin, a soluble protein with a tight, convoluted tertiary structure (Cotta and Hespell 1986), is more slowly degraded in the rumen than casein (Mangan 1972), whilst proteins with numerous cross linkages are more resistant to degradation (Nugent and Mangan 1978). Mahadevan *et al.* (1980) concluded that the level of disulphide bonding within a protein was a major factor in determining its resistance to rumen degradation. As the sulphur amino acid content of cottonseed proteins is much lower than for ovalbumin, it seems that a low prevalence of disulphide bonds may be one of the factors contributing to the relatively high rumen degradability of globulin storage proteins in unheated cottonseed kernel.

The lack responses of cottonseed CT upon *in situ* N solubility may possibly be explained by CT being more effective at reducing protein degradation rather than protein solubility. Similar observation was also found by McNabb *et al.* (1995) for CT in the forage of *Lotus pedunculatus*. Spencer *et al.* (1988a) noted that the loss of N from synthetic-fibre bags suspended in the rumen measured the solubilization of plant protein, and that the rates of protein solubilization and degradation in the rumen were not necessarily similar. Positive correlation between predicted degradability of the two major kernel proteins (52 and 48 kDa) and solubility of total N *in vitro* was found

(Figure 9. 1). The data using in Figure 9. 1 were calculated from Tables 9. 3 and 9. 4. However, the correlation coefficient for 52 kDa protein was only $r = 0.52$ ($p=0.08$, $n=12$), accounting for approximately 25% of the variation. The equation is:

$$Y = 49.97 + 0.41x \quad (1)$$

For the 48 kDa protein, the correlation coefficient was only $r = 0.49$ ($p = 0.10$, $n = 12$), accounting for approximately 25% of the variation. The equation is:

$$Y = 54.33 + 0.36x \quad (2)$$

For both equations 1 and 2, Y represents the predicted rumen degradability of the kernel proteins (52 or 48 kDa) and x represents the solubility of total N in buffer solution (pH 7.0).

This suggests that total N solubility *in vitro* was not a good index for estimating cottonseed protein degradation in the rumen. For estimating relative rumen degradation of cottonseed kernel proteins, the *in vitro* incubation with rumen fluid, followed by fractionation of individual proteins using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and their quantification using imaging densitometry, which has been developed and used in the studies reported in this thesis, is recommended.

Rumen DM digestibility of cottonseed hulls was 30-40% (Chapter 3; Tuncer *et al.* 1992). The DM digestibility was increased by PEG addition, indicating that bound CT was reducing the rumen digestion of cottonseed hulls. As cottonseed hulls are almost pure fibre, it seems that bound CT reduced fibre digestion. That CT in forages depressed fibre digestion in the rumen has been widely reported (Barry and Manley 1984; Barry *et al.* 1986; McAllister *et al.* 1993; Waghorn *et al.* 1994; Wang 1995). As Barry *et al.* (1986) and Wang (1995) showed the digestion of hemicellulose is more sensitive to forage CT than cellulose, it is possible that fibre digestion in the rumen begins to decline at a relative low level of CT. Therefore, Wang (1995) concluded that the optimum concentration of extractable CT in forages appears to be around 20 g kg⁻¹ DM. At this level, CT had no effect on rumen fibre digestion, but substantially reduced protein degradation in the rumen and increased amino acid flux to the small intestine.

The overall results obtained from monogastric studies showed that cottonseed CT markedly depressed apparent ileal digestibility of amino acids. These effects occurred with the addition of low concentrations of hulls, and suggest that cottonseed hulls depressed digestion of cottonseed protein in the small intestine to a much greater extent than in the rumen, where large concentrations of hulls were required to produce depressions in degradability. Assuming the same effect occurs in the small intestine of the ruminant, bound CT in cottonseed products will probably have a small effect in reducing dietary protein degradation in the rumen, but may have a significant effect in

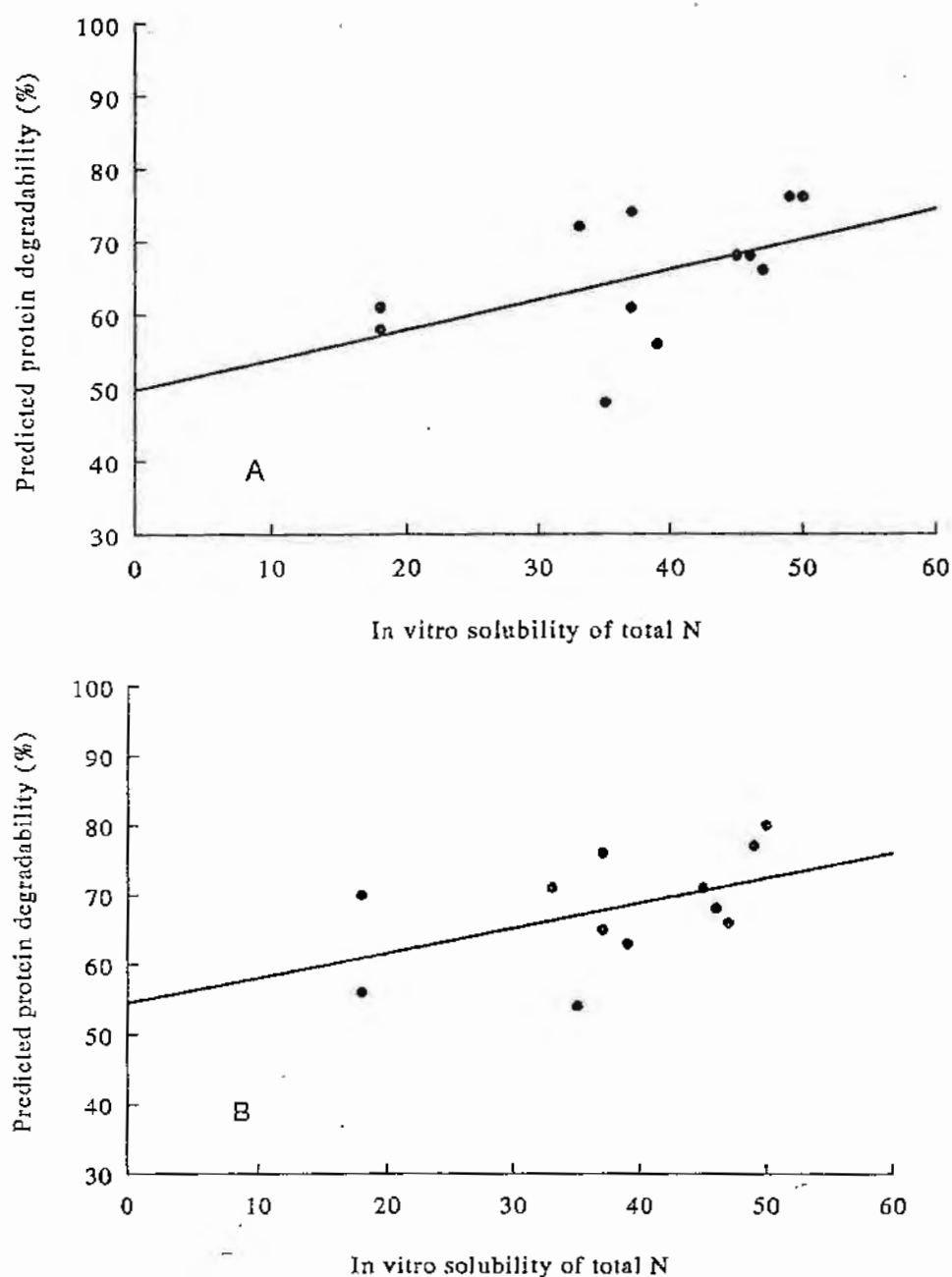


Fig 9. 1 The correlation between solubility of total N *in vitro* and predicted rumen degradability of the 52 kDa protein (A) and 48 kDa protein (B) in cottonseed kernel. For the 52-kDa protein, $r = 0.52$, $n = 12$, $p = 0.08$; For the 48 kDa protein, $r = 0.49$, $n = 12$, $p = 0.10$

reducing amino acid absorption from the small intestine. Thus, bound CT in cottonseed products, unlike extractable CT in forages, may have a greater adverse than beneficial effect upon dietary protein utilisation in ruminants. This may be due to bound CT being relatively unreactive and not moving from particle to particle at rumen pH, but being solubilised in the abomasum and being more reactive in the small intestine.

9.5 CONCLUSIONS

From the results generated in this thesis the following conclusions can be drawn:

- 1) Condensed tannins are present in commercially produced CSM in significant concentrations. CT are present in cottonseed hulls, but are not detected in the kernels. CT in cottonseed hulls, unlike CT in forages, are mainly in the protein- and fibre-bound form, with only 10-20% of total CT being extractable in acetone/water and determined with butanol/HCl. The presence of CT in commercially produced CSM is therefore due to the presence of some hulls in the CSM. During industrial processing, a component of hulls is left in the CSM to allow more efficient extraction of the oil (the most valuable commodity) during screw pressing.
- 2) Normal analytical methodology measures extractable CT only, and this grossly underestimates the total concentration of CT in CSM. For use with CSM and other processed meals, analytical methods are required which measure bound as well extractable CT.
- 3) Ileal amino acid digestibility is high in monogastric animals fed unheated solvent extracted cottonseed kernel diets, where no hull is present. Relative to unheated solvent extracted kernels, commercially produced CSM is of low protein nutritional value for monogastric animals. In particular, the ileal digestibility of lysine, methionine and threonine are low. The low ileal amino acid digestibility in CSM is probably due to a combination of heat applied during processing and the presence of some hulls in the CSM, with approximately half the hulls effect being explained by their CT content. It seems that bound CT in cottonseed hulls are solubilised in the monogastric stomach and then reduce amino acid digestion in the small intestine. Leaving some hulls in CSM substantially lowers the efficiency with which amino acids can be digested and absorbed from the small intestine in monogastric animals. The study has shown that cottonseed hulls are not an inert substance for monogastric animals; rather they are antinutritional and reduce the efficiency of protein digestion.

The study has also shown that the large amount of heat applied during normal commercial CSM processing reduces the digestion of amino acids by monogastric animals. A combination of heat and CT from hulls is damaging for lowering amino acid digestion, particularly for the dietary limiting essential amino acids lysine, methionine and threonine. Where CSM is intended to be used in the monogastric livestock industries, it is recommended that the level of hulls in CSM should be reduced to the lowest possible levels and that the amount of heat applied should be the minimum amount to bind gossypol.

- 4) In the absence of hulls or heat, rumen degradation of cottonseed kernel proteins is high (76-94%). As CT in forage plants substantially reduces rumen protein degradation in ruminants, an objective of the project was to study the effect of CT in CSM upon rumen protein solubility and degradation. Although action of bound cottonseed CT did reduce the degradation of cottonseed kernel proteins by rumen micro-organisms, the effect seems of small magnitude. The effect is smaller than that produced by similar concentrations of forage CT, and is probably explained by bound CT being relatively unreactive at rumen pH (6.0-7.0). Total N solubility measured either *in vitro* or *in situ* was not a good index for estimating cottonseed protein degradation in the rumen. For estimating rumen degradation of cottonseed proteins, the *in vitro* incubation with rumen fluid, followed by fractionation of individual proteins using SDS-PAGE and their quantification using imaging densitometry should be used.

Whilst the presence of CT will give some protection of cottonseed kernel proteins against rumen fermentation, the effects seem small in unheated CSM but tended to be somewhat greater in the presence of heat. Further research is necessary to accurately define the amount of heat (temperature, time) required to give optimum rumen bypass of CSM proteins. As the moisture and pressure used in CSM processing enhances the heating process, making it more effective than dry heat at a given temperature, moisture content and pressure should therefore be included as factors in future heating studies. Interactions among heating temperature, heating time, moisture content, hulls and CT need to be defined in future work.

As cottonseed CT lowered the protein and amino acid digestion in the small intestine of monogastric animals, it is likely that this will also happen in ruminants. The possibility therefore exists that with ruminants, negative effects of cottonseed CT in the small intestine may outweigh any beneficial effects in reducing rumen degradation of kernel proteins.

- 5) Action of cottonseed CT lowered the digestion of cottonseed hulls by rumen micro-organisms. As the hulls are almost pure fibre, it seems that bound CT may reduce rumen fibre digestion.

- 6) From the results of the thesis it seems that CSM intended for the monogastric industries should be processed differently from CSM intended for the ruminant industries. The effects of CT and their continued reactivity after heating have been accurately defined in this thesis. Future work is necessary to accurately define the extent of heating required to produce CSM of optimum nutritional value for ruminant and monogastric livestock, and the interactions with heat, CT and moisture content and their effect upon protein nutritional value also need to be defined. It is recognised that some heating is necessary, to soften the seed and to bind gossypol, but the amount of heat required is likely to differ substantially in CSM intended for ruminant and monogastric markets.

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