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**THE EFFECT OF CONDENSED TANNIN UPON THE PROTEIN
NUTRITIONAL VALUE OF SOLVENT EXTRACTED
COTTONSEED MEAL FOR RUMINANT AND
MONOGASTRIC ANIMALS**

A Thesis Presented in Partial Fulfilment of
the Requirements for the Degree of Doctor of
Philosophy in Animal Science at Massey University

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1995

DECLARATION

The studies presented in this thesis were completed by the author whilst a postgraduate student in the Department of Animal Science, Massey University, Palmerston North, New Zealand. This is all my own work and the views presented are mine alone. Any assistance received is acknowledged in the thesis. All references cited are included in the bibliography.

I certify that the substance of the thesis has not already been submitted for any degree and is not being currently submitted for any other degree. I certify that to the best of my knowledge any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



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ABSTRACT

(Feng Yu, 1995 *The Effect of Condensed Tannin upon the Protein Nutritional Value of Solvent Extracted Cottonseed Meal for Ruminant and Monogastric Animals*. Ph D thesis, Department of Animal Science, Massey University, Palmerston North, New Zealand)

A series of indoor experiments were conducted at Massey University and AgResearch Grasslands, Palmerston North, New Zealand, to study the effect of cottonseed condensed tannin (CT) upon the nutritional value of solvent extracted cottonseed meal (CSM) for ruminant and monogastric livestock. Ruminant nutrition experiments were conducted using samples suspended *in situ* in the rumen of fistulated sheep and by incubating samples with rumen fluid *in vitro*, to study effects upon solubility and degradability of cottonseed proteins. 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 half of the animals were supplemented with polyethylene glycol (PEG; MW 3500). PEG specifically binds and inactivates CT and can be used to deduce the effects of CT by comparing control animals (CT acting) with PEG supplemented animals (CT inactivated).

1. Experimental varieties of cottonseed and of industrial CSM were analysed for extractable and bound CT and free gossypol, crude protein, oil and fibre. CT was present in the hulls of all varieties, with higher concentrations recorded for high tannin and glandless selections (55 g kg⁻¹ and 58 g kg⁻¹ DM) than for the multiple host plant resistant and high gossypol selections (38 g kg⁻¹ DM). CT was present in trace amounts in the kernels of high tannin selections, but was not detected in the kernels of all other selections. On average for the hulls of all varieties, approximately 22, 60 and 18% of total CT was present in the extractable, protein-bound and fibre-bound forms, respectively. Free gossypol was mainly found in the kernels, with negligible amounts being found in the hulls of the experimental varieties. Kernels of high gossypol selections contained higher concentrations of free gossypol (18 g kg⁻¹ DM) than kernels of multiple host plant resistant, high tannin and commercial selections (10-12 g kg⁻¹ DM), with free gossypol concentration being very low (0.8 g kg⁻¹ DM) in the kernels of glandless cottonseed. A negative correlation ($r = -0.50$, $P < 0.05$) between free gossypol in the kernels and total CT in the hulls was found.

The mean crude protein, oil and fibre (neutral detergent fibre; NDF) contents of the kernels were 346, 348 and 228 g kg⁻¹ DM, respectively. The hulls contained small

amount of crude protein (37 g kg⁻¹ DM) and oil (12 g kg⁻¹ DM), and large amount of fibre (NDF; 891 g kg⁻¹ DM).

Commercially produced CSM contained 8-15 g CT kg⁻¹ DM, due to the presence of some hulls, and 0.8 g free gossypol kg⁻¹ DM. The mean contents of crude protein, oil and fibre (NDF) in CSM were 449, 70 and 253 g kg⁻¹ DM, respectively. The results are discussed in relation to plant defence mechanisms against insect attack and in relation to the nutritive value of CSM for ruminant and monogastric livestock.

2. The effect of adding cottonseed hulls upon the solubility of protein in unheated solvent extracted cottonseed kernels was studied using both *in vitro* incubation in mineral buffer and the *in situ* polyester bag technique. The latter technique was also used to study effects on rumen DM digestion. Cottonseed hulls contained 51 g CT kg⁻¹ DM, with 56 and 20% of the total CT being bound to protein and fibre, respectively; no CT was detected in kernel.

In the absence of hulls, 42% of the total nitrogen (N) in cottonseed kernel was soluble in mineral buffer *in vitro*, whilst potential *in situ* N solubility and predicted rumen N solubility (corrected for rumen outflow rate) were 99 and 86% respectively. Addition of hulls linearly reduced both *in vitro* N solubility and potential *in situ* N solubility, with 100% hulls addition (i.e. 1 g kernel + 1 g hulls) reducing potential N solubility and predicted rumen N solubility to 94 and 79% respectively. PEG addition had no effect upon the protein solubility of kernels, but increased N solubility in mixtures of hulls and kernels *in vitro* but not *in situ*. Two mg PEG mg⁻¹ total CT was shown to reverse the effect of CT in reducing *in vitro* protein solubility.

Potential *in situ* DM digestion and predicted rumen DM digestion (corrected for rumen outflow rate) were substantially lower for cottonseed hulls (41 and 33%) than for kernels (99 and 88%). Increasing the addition of hulls to kernels lowered the rumen DM digestion of mixtures in a quadratic manner, with increasing rate of hulls causing progressively smaller depressions. Addition of PEG had no effect upon the digestion of kernel DM, but increased potential DM digestibility and predicted rumen DM digestion of hulls to 47 and 40% respectively.

It was concluded that the high protein solubility of unheated solvent extracted cottonseed kernels can be linearly reduced by the addition of cottonseed hulls, with the magnitude of the reduction being small, and that the presence of bound CT in hulls substantially depressed fibre digestion by rumen micro-organisms.

3. A 24 h *in vitro* rumen incubation procedure was developed to measure the effect of adding cottonseed hulls upon degradation of the 52 and 48 kDa major seed storage proteins present in unheated solvent extracted cottonseed kernels. Proteins were

fractionated using sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the protein bands quantified using imaging densitometry. A set of *in vitro* experiments was conducted, in which degradation rate and potential degradability were measured.

In the absence of hulls, potential degradability of both kernel proteins was very high (99%), with approximately 97% of this taking place within 8 h, and the addition of PEG did not effect degradation. Increasing rates of hull addition reduced the potential degradability of both proteins in a linear manner, but did not affect degradation rate. Equal weights of hulls and kernel (i.e. 100% hulls) reduced potential degradability of the 52 and 48 kDa proteins by approximately 10% units. Addition of PEG increased degradation of both proteins in incubations involving mixtures of hulls and kernels, with 2 mg PEG mg⁻¹ CT being required to maximise this effect. However, the increase obtained accounted for only 50% of the depression in protein degradation caused by the addition of hulls. In all experiments, the 52 and 48 kDa proteins were similarly affected by the treatments applied.

It was concluded that *in vitro* rumen degradability of the 52 and 48 kDa storage kernel proteins was very high and close to 100%, and that this could be reduced by the addition of hulls in a linear relationship, with approximately half of the depression in potential degradability caused by hulls being due to effects of CT.

4. The effect of CT in cottonseed hulls on endogenous ileal amino acid flow in the growing rat was evaluated. Twenty-four rats were allocated to four semi-synthetic diets, which contained enzymically hydrolysed casein (EHC) as the sole source of dietary N and chromic oxide as an indigestible marker. Two of the diets contained no hulls while the remaining two contained 50 g kg⁻¹ hulls. At each level of hull inclusion, PEG was added to one of the diets. The rats were given their respective experimental diets *ad libitum* for 14 days. Samples of digesta were collected at slaughter from the terminal 15 cm of ileum. The digesta samples were centrifuged and the supernate ultrafiltered. The precipitate plus retentate (MW > 10,000) fraction affords an estimate of endogenous loss.

Inclusion of hulls in the EHC based diet increased ileal flow of total N (1387 vs. 1623 mg kg⁻¹ dry matter intake; $p < 0.05$), increased ileal flow of total amino acids (23%; $p < 0.01$), and significantly increased ileal flow of several individual amino acids. There was no significant effect of PEG and no PEG \times diet interaction, showing that the hull effects could not be explained by action of CT. The presence of some hulls in commercial CSM will contribute to lowering apparent ileal amino acid digestibility, due to its effect in increasing endogenous loss.

5. The effect of adding cottonseed hulls to casein and to 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 CT, was determined using the growing rat. Sixty rats were allocated randomly to 10 semi-synthetic diets, containing either casein (4 diets) or purified unheated solvent-extracted cottonseed kernel (6 diets) as the sole protein sources, with chromic oxide added as an indigestible marker. Two of the casein diets contained no hulls whilst the remaining two diets contained 70 g hulls kg⁻¹. Two of the cottonseed kernel based diets contained no hulls, with two containing 23 g hulls kg⁻¹ and the remaining two containing 46 g hulls kg⁻¹. For each pair of diets, PEG was either included or excluded. Samples of digesta were collected at death from the terminal 15 cm of ileum.

The inclusion of hulls depressed the apparent and true ileal digestibility 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 hulls 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 response 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. With the cottonseed kernel based diet it seems that unknown components of the hulls other than CT also depressed the apparent and true ileal digestibility of N and amino acids.

6. The effects of heat treatment, with or without the addition of cottonseed hulls, on the chemical composition of CSM and upon reactivity of the CT were studied. Heat treatment (100°C for 2 h, in a forced draught oven) reduced the concentrations of free gossypol and fluorodinitrobenzene (FDNB) available lysine by small amounts, reduced measurable total CT content by 13%, reduced the solubility of total N, and reduced potential degradability of the 52 and 48 kDa cottonseed storage proteins by mixed rumen micro-organisms. Addition of hulls further depressed solubility of total N and ruminal degradation of the two major storage proteins in cottonseed kernel. The action of PEG *in vitro* indicated that only part of the depression caused by hull addition could be explained by the presence of CT in the hulls, and that the effects of CT upon N solubility and potential degradability in heated CSM were similar to that

in unheated CSM. Addition of hulls also substantially reduced FDNB available lysine.

Although application of heat inactivated 13% of the total CT such that it could no longer be detected with butanol/HCl, it did not seem to change the reversible reactivity of CT with kernel proteins. Commercial CSM produced from the Brisbane mill had a lower total CT content, lower N solubility and ruminal protein degradation rate than CSM produced from the Narrabri mill, but a similar level of FDNB available lysine.

7. The effect of CT from heated and unheated cottonseed on the apparent ileal digestibility of amino acids for the growing rat and pig was determined. In Experiment 1, twenty-four rats were allocated to four semi-synthetic diets, which contained cottonseed kernel/hulls as the sole protein source. Two of the diets contained unheated solvent extracted cottonseed kernel/hulls while the remaining two diets contained similar material but which had been heat-treated by autoclaving at 110°C for 120 min. In Experiment 2, twelve rats and twelve pigs were fed four semi-synthetic diets containing commercial CSM as the sole protein source. Chromic oxide was added to all diets as an indigestible marker. For each pair of diets in both experiments, PEG was either included or excluded. Ileal contents from the terminal 15 and 45 cm of ileum were collected at slaughter for the rats and pigs, respectively.

Apparent ileal amino acid digestibility for rats fed the cottonseed kernel/hulls diet was significantly depressed by the heat treatment, particularly for lysine and threonine. On average, apparent ileal amino acid digestibility in the diets without PEG was decreased from 0.80 to 0.70 by heat treatment. Dietary cottonseed CT depressed apparent ileal protein digestibility in the pig and in the rat. The addition of PEG to the diets significantly increased the apparent ileal digestibility of N and some amino acids for the pigs and the rats. The mean increase in apparent ileal digestibility due to PEG addition for the 14 amino acids was 2% units in both species fed the commercial CSM diets, and 2 or 4% units in rats fed the unheated or the heated cottonseed kernel/hull diets, respectively. The effect of PEG was similar in the heated and unheated cottonseed kernel/hulls for most amino acids, but responses to PEG for lysine, threonine and tyrosine were greater in heated than unheated CSM.

For several of the amino acids there were significant animal species differences in apparent ileal digestibility. Studies into the effects of cottonseed CT should be carried out in the target animal species. The commercial CSM had a low apparent ileal amino acid digestibility overall, particularly for the essential amino acids lysine and threonine. It was concluded that effects of heating did not eliminate the reversible reactivity of

cottonseed CT on amino acid digestion in rats and pigs but rather appeared to increase it for threonine, tyrosine and lysine in Experiment 1, causing large reductions in apparent ileal digestibility.

It was concluded that CT in CSM are mainly in the protein- and fibre-bound forms (80%), with only 10-20% of CT being extractable. In the absence of heat, adding graded levels of hulls reduced both the solubility and degradability of protein by rumen micro-organisms, giving a rumen 'by-pass' effect. However, the magnitude of these effects was small and they are not regarded as being nutritionally significant under practical conditions of ruminant nutrition. Adding hulls significantly depressed the digestion of amino acids in the small intestine of the monogastric animals. This effect 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 'hull 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. 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 especially damaging for lowering ileal amino acid digestion in monogastric species, particularly for the limiting essential amino acids lysine and threonine.

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