

Forage tannins in ruminant nutrition

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ABSTRACT

Chemically distinct, hydrolysable and more stable condensed type of tannins have been identified in forages. The values depend on the analytical method used. Factors affecting tannin content of foliage were: climatic factors (higher in tropical species and under drier conditions), soil fertility, species (shrubs and tree species having higher levels than pasture types), stage of growth (less mature having higher levels) and plant part of significance was the accessibility to browsing and the act itself resulting in higher tannin levels, recognized as a defense mechanism mediated from affected plants to others through ethylene produced due to browsing to increase tannin levels in unaffected plants to discourage browsing. Younger parts and those accessible for browsing having higher levels are other defense mechanisms. Proline in saliva of deer binds with tannin in shrubs and tree species offering a counter mechanism by browsers while the saliva of grazers (cattle and sheep) is proline-free. Depending on the level of tannins in a diet, the effect may vary from beneficial (2-4%) through antinutritional (5-9%) to toxic and lethal (>9%). Effects are mainly due to complexing with enzymes/protein thereby reducing rumen NH₃, microbial activity, nitrogen and dietary dry matter utilization, resulting in decreased intake and animal performance. Polyethylene glycol (PEG) in feed preferentially binding condensed tannins improved the utilization of protein more than that of the dry matter with the effect being greater at higher tannin levels. Split compared to single dose PEG seemed more effective. Limited evidence indicated that PEG promotes selection and intake of high tannin forages. Attempts to improve silage quality using tannins have also been reported.

Key words: condensed tannins, hydrolysable tannins, browse plants, Proline, Polyethylene glycol

INTRODUCTION

Fodder from trees and shrubs (top feed) have been an integral component of feed for ruminants in arid and semi arid regions in many countries of the world. In general, top feeds are a good source of protein for grazing animals. The intake of these materials by ruminants is reported to vary widely from none or low to high levels. However, in some cases not only has their crude protein digestibility been observed to be low, but also several episodes of loss of livestock have been associated with the presence of undesirable secondary plant compounds in the foliage of some fodder trees such as *Prosopis cineraria*. In particular, tannins have been implicated in the failure of ruminants to consume some forage legumes (e.g. *Desmodium*). The most important property of tannins is its strong affinity for enzymes and feed protein. Tannins are known to occur in oilseeds, oak (*Quercus* sp.) trees, and legumes and in other fodder species (Kumar and Singh, 1984; Leiner, 1990). The digestibility of protein by micro-organisms is reduced by tannin binding (Mangan, 1988). But in some circumstances it may be advantageous due to, increase protein availability in the lower gut (Barry *et al*, 1986).

Tannins produce adverse effects that cannot be accounted for by digestion inhibition, primarily due to rapid and drastic decreases in food intake (Provenza *et al*, 1994). They are best accounted for by lesions of gut mucosa and toxicity (Kumar and Singh, 1984). At high levels, tannins may have detrimental effects on the nutritive value of forages by reducing their palatability, intake and digestibility (Palmer and Schlink, 1992). Tannins can adversely affect the microbial and enzyme activities, nutritional absorption and utilization by animals, and may cause toxicity and death in severe cases (Barry and Duncan, 1984). Moderate quantities of tannins may, however, prevent bloat and enhance the supply of by-pass proteins (undegraded dietary proteins) for the digestion in the small intestine. (Kumar and D'Mello, 1995) and improved the utilization of dietary essential amino acids (McNabb *et al*, 1993).

Tannins appear to be quite mobile chemicals in plants. Work from South Africa (Van Hooven, 1984) has demonstrated that a number of trees normally browsed by wild animals of the deer group responded to grazing by releasing ethylene. Ethylene apparently transmitted a message to the other trees causing them to raise their tannin content. In other trees it has been shown that foliage out of reach of

grazers has no tannin whilst those in the grazing zone contain considerable tannins. This has considerable importance for the use of tree forages and research must be established to examine tannin content, effect of harvesting foliage, height of cutting and interval of harvesting of appropriate fodder trees that grow locally. Tannins at 2-4 % in the diet may be highly beneficial as they protect the leaf protein against degradation in the rumen provided that the protein remains digestible. As tannin levels increase, digestibility and feed intake are reduced possibly through an effect on the microbial ecosystem of the rumen. The use of polyethylene glycol to specifically detoxify tannins is also a potential strategy to make use of tanniniferous plants (Makkar *et al.*, 1995).

The chemistry of tannins

Tannins are a special type of phenolic compounds of plant origin, traditionally distinguished by their ability to tan animal hides. Tannins are involved in the formation of insoluble tannin protein complexes, a characteristic property of tannins, which can readily be demonstrated *in vitro* with protein solutions.

There are two chemically distinct types of tannins (Fig 1)

They are:

1. Hydrolysable tannins (Gallo tannins and ellagitannins). These are polyesters of gallic acid, and other phenolic acids derived from it, with a sugar (normally glucose), which are readily hydrolysed by acid.
2. Condensed tannins (flavolans). These are polymers (M.W ~ 1,000 to >20,000) of catechins, which are flavonoid phenols. The linkage between monomers, typically a carbon condensation, is relatively stable under the conditions, which cleave ester linkages in hydrolysable tannins.

Investigations of tannins in plant foliage

Condensed tannins (CT) are present in only some plant species. In general shrub and tree foliage is likely to be higher in tannins than pasture plants while leguminous forages from the tropics are higher in tannin than those from the temperate countries.

Crude protein and CT contents together with digestibilities of different tropical forage legumes and tree fodder species are presented in Tables 1 & 2 respectively. Most of the tested species had CT, varying from 3 to 194 g/kg DM in forage legumes (Table 1) and from 0 to 262 g/kg DM in tree fodder

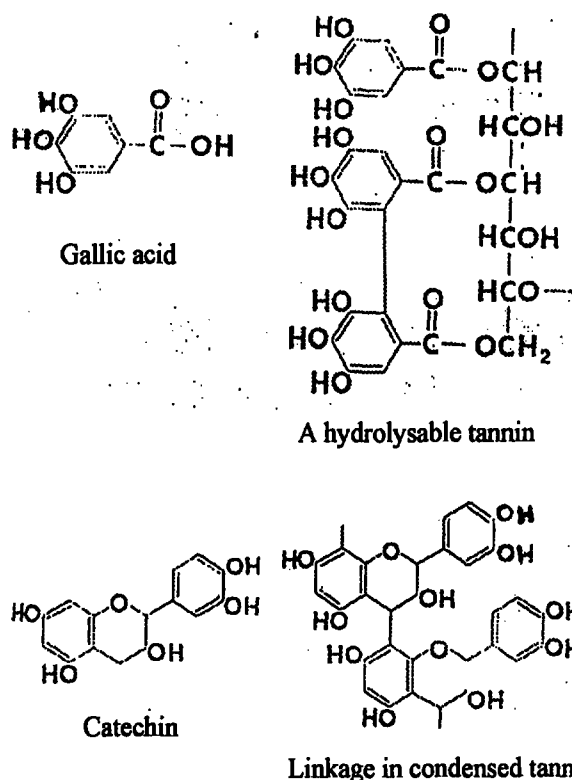


Fig. 1. Structures of hydrolysable and condensed tannins and constituent units

Table 1. Some values for the concentrations (g/kg dry matter) of crude protein, and tannin content and *in vitro* digestibility of leaf from tropical forage legumes (from Lowry *et al.* 1992).

Species	Crude protein	Condensed tannins ¹	In vitro DMD% ²
Contains tannins			
<i>Aeschynomene Americana</i>	210	8	70,64 ⁴
<i>Clitoria laurifolia</i>	150-180	20-60	-
<i>Desmodium heterophyllum</i>	130-140	17-26	-
<i>D. intortum</i>	110-245	32-34 ³	36-45,64
<i>D. ovalifolium</i>	153-230	83-14	51
<i>Indigofera spicata</i>	170-210	6-10	-
<i>Mimosa pigra</i>	210-230	80	40
<i>Peuraria phaseoloides</i>	160-190	3	-
<i>Vigna hosei</i>	190-240	4	-
No tannins			
<i>Calopogonium mucinoides</i>	150-210	nd	63
<i>Centrosema pubescens</i>	120-30	nd	54
<i>Chamaerista rotundifolia</i>	80-140	nd	56-44
<i>Desmodium triflorus</i>	150-180	nd	-

nd= not detected. ¹tannins measured by pepsin precipitation using tannic acid standards (Hagerman and Butler 1978). ²DMD=dry matter digestibility.

species (Table 2). Both types had species with no detectable tannins. Values for fodder tree species in fact depend on the method of analysis while pepsin precipitation method is common for both tables. Vanillin HCl method detected higher CT levels

Table 2. Mean values and ranges for the concentrations of crude protein, condensed tannins and *in vivo* (and *in vitro* in parenthesis) digestibilities of dry matter from a selection of tannin-containing and tannin-free fodder tree legumes. sources: Lowry *et al.* 1992 and those referenced in table footnote.

Species	Crude protein (N× 6.25)	Condensed tannins			In vivo DMD%
		Pepsin Peptn ¹	Vanillin HCL ²	Butanol HCL ³	
<i>Contains tannins</i>					
<i>Acacia aneura</i>	92-203	-	31-4496	11-14	4-63
<i>A. angustissima</i>	210-230	-	59-66	nd ⁴	-
<i>A. auriculiformis</i>	110-170	11-83	-	-	40
<i>A. cyanophylla</i>	112-212	40-70	-	-	51-53
<i>A. nilotica</i>	112-167	9-0	-	-	69
<i>A. senegal</i>	141-336	4	-	-	nm ⁶
<i>A. seyal</i>	111-293	2-4	-	-	nm
<i>A. sieberiana</i>	123-158	37	-	-	54
<i>A. tortilis</i>	103-210	40-61	-	-	54
<i>A. villosa</i>	220-280	6	-	-	nm
<i>Albizia chinensis</i>	151-263	10-22	24-33	12-15	35-48
<i>A. falcataria</i>	230	22	-	-	nm
<i>Calliandra calothyrsus</i>	173-212	40-90	79-111	15-21	35-48
<i>Codariocalyx gyroides</i>	128-198	-	42-71	26-28	3-44
<i>Flemingia macrophylla</i>	175	130-190	155	-	9-36
<i>Gliricidia sepium</i>	200-280	0	0-30 ⁵	0-17 ⁵	68-74
<i>Leucaena spp</i>	174-380	7-40	37-43	1-262	63-68
<i>Prosopis juliflora</i>	142-222	-	-	-	nm
<i>P. cineraria</i>	119-154	-	-	105	39
<i>P. tamarugo</i>	90-357	105	-	-	32
<i>Ziziphus nummularia</i>	141	-	-	130	41-46
<i>No tannins</i>					
<i>Albizia lebbek</i>	181-240	0	nd	nd	43-64
<i>Enterolobium</i>					
<i>Cyclocarpum</i> ⁵	168-250	0	nd	nd	69
<i>Samanea saman</i>	240	0	nd	nd	65
<i>Sesbania grandiflora</i>	206-348	0	-	-	36,67
<i>S. sesban</i>	152-263	-	nd	nd	65,68
<i>Tipuana tipu</i>	200-260	-	0-42	nd	62,64

¹Hagerman and Butler 1978 tannic acid standards. ²Broadhurst and Jones 1978 vanillin-HCl-catechin standards. ³Bate-Smith 1981- Butanol-HCL- tannic acid standard, tannic acid equivalents (g)/kg DM. ⁴ nd = not detected. ⁵ No tannins detected in dried samples. ⁶ nm = not measured.

compared to the two methods (Table 2). Irrespective of the CT content, all species had higher CP contents compared with standards for tropical grasses

The level of tannins, particularly in leguminous forages has been found to vary considerably depending on a number of factors. For example in New Zealand, *Lotus pendunculatus* grown on fertile, high moisture soils had about one third the condensed tannin content of *Lotus* grown on hill country under water stress (Barry, 1983).

The literature in this area is often confusing as reported levels of tannins often seem to be higher than can be explained and these values are often rejected by reviewers as possibly due to errors in analysis. For example, *Prosopis* leaves have been

reported to contain tannin levels of 2.2% DM (Sehgal, 1984) but in 15 individual trees Joshi *et al* (1983) reported levels ranging from 10.6 to 25.3% which suggested that there may have been major analytical differences between the two sets of results from the Indian scientists.

It is almost certain that the level of tannins in the foliage of trees such as those from the *prosopis* family are highly variable, depending on environmental stress (fertiliser use, soil water relationships, insect attack etc.). Also it was reported that new leaves often have higher tannin content than older leaves (Vaithyanathan and Singh, 1989).

Tannins at 14-16% are present in the bark of *Prosopis* (Dalziel, 1948) indicating a very large pool

in the tree that can possibly be mobilised. In general it could be expected that the green bark of new growth would contain less tannin than the brown bark, and the leaves and petals less than the bark.

Mupangava *et al.*, (2000) investigated the soluble, total-bound and fibre bound condensed tannins (CT) in *Cassia rotundifolia* (Cassia), *Lablab purpureus* (Lablab) and *Macroptilium atropurpureum* (Siratro) grown in tropical and sub-tropical regions by the butanol-HCl method. Cassia had the highest total CT content of 29.5g/kg DM, while that of Lablab at 16.9 g/kg DM was intermediate and that of Siratro, 12.4 g/kg DM, the least. Even with the relatively low CT content of Lablab and Siratro, they still high enough not to confer beneficial effects on legume nitrogen utilization in ruminants as reported in other studies. Infact, high CT content in Cassia may even cause astringency leading to low intake. The presence of condensed tannins in these legumes may influence their utilization as protein supplements for low quality roughages fed to ruminants during the dry season in tropical regions.

Twelve tropical shrubs (10 legumes and 2 non legumes) varying in tannin content was measured by Balogun *et al.* 1998. Five terminal and fully expanded leaves were harvested by hand plucking, chopping fresh to about 3 mm lengths (either as fresh or after oven drying at 65C for 24 h) for 48 h. Extractable condensed tannin levels ranged from 0.5 to 11.2% for vanillin/HCl and from 0 to 9.4% for butanol/HCl extraction (Table 3). Bound tannins also varied in the protein bound component of both *Gliricidia sepium* and *Bauhinia rufescens* exceeding 2% of the dry matter. *B. rufescens* also had a high fibre-bound tannin level of 0,9%.

The shrubs used in this study can be classified, on the

basis of their contents of ECTvan, thus,

Traces of CT (<1%): *A. lebbek*, *G. sepium* and *C. erythrophloem*.

Low level CT (1-3%): *A. richardiana*.

Medium level CT (4-6%): *A. currassavica* and *L. leucocephala*

High-level CT (>6%) *B. Rufescens*, *C. acapulcensis*, *C. calothyrsus*, *C. apiculatum*, *U. umbellatum* and *L. watsonii*.

A similar classification is also obtained by using the ECTbut values

Tannin mobilisation

Several studies have indicated that the tannin content of foliage may be controlled in some way and can be elevated at times of high risk due to defoliation. (i.e. by insect attack, cutting and harvesting, or grazing).

An investigations in south Africa (Van Hoooven, 1984) of the death of a number of Kudu (a member of the deer family) on a wild life farm has led to a highly significant advancement of knowledge that has major implication for management of fodder trees (Table 4).

A number of Kudu died after grazing on a small woodland of *Acacia* trees. Subsequent studies have shown that in the wild these Kudu would approach such woodlands and after grazing on the trees on the periphery move relatively quickly to a distant group of trees. However, because of the enclosure on the wild life farm, these animal were forced to consume more of the foliage from one woodland. Subsequent studies implicated that tannin caused the death of the animals and this led to a study of tannin in tree foliage. Tannin levels rose sharply over a 15 minute - 1 hour period, not only in trees grazed by deer, but also in the trees adjacent to those that were being

Table 3. The composition (%) of extractable condensed tannin by vanillin (ectvan) or butanol-hcl (ectbut), protein bound (pct), fibre bound (fct) and total condensed tannin (tct) of shrub legumes.

Species	ECTvan	ECTbut	PCT	FCT	TCT
<i>Leucaena leucocephala</i>	5.58	5.70	0.89	0.23	6.8
<i>Combretum apiculatum</i>	10.29	5.87	0.25	0.07	6.19
<i>Combretum erythrophloem</i>	0.35	nd	0.03	0.04	0.07
<i>Bauhinia rufescens</i>	8.40	9.34	2.27	0.92	12.53
<i>Calliandra acapulcensis</i>	8.98	5.00	0.97	0.35	6.32
<i>Calliandra calothyrsus</i>	8.91	5.29	0.30	0.45	6.04
<i>Cathormium umbellatum</i>	9.58	7.60	1.23	0.49	9.32
<i>Acacia currassavica</i>	5.81	3.98	0.27	0.02	4.27
<i>Gliricidia sepium</i>	0.75	0.89	2.41	0.34	3.64
<i>albizia lebeck</i>	0.37	nd	0.06	0.03	0.09
<i>Lysiloma watsonii</i>	11.62	7.07	0.46	0.33	7.86
<i>Albizia richardiana</i>	1.60	2.16	0.34	0.20	3.00

nd: condensed tannin not detected.

Source :Balogun *et al.*, 1998

damaged. It was subsequently shown that ethylene released by trees being harvested increased leaf tannin levels to such an extent that it caused a cessation of leaf consumption by animals. Trees communicate with each other in order to protect themselves against graziers, presumably insects and man (with his machete) as well as animals.

The implications are that there is a pool of tannins (presumably in the bark, pith perhaps as precursors in the leaf), which can be readily mobilised by activation of specific enzymes sensitive to air borne materials (ethylene in this case) released from damaged foliage. This obviously has been an important survival mechanism.

The effect of simulated grazing on tannin content of a number of tree leaves is shown in Table 4.

Table 4. Tannin content in three tree species in response to physical stimulus.

Species	% Rise in 15 mins	% rise in 60 mins
<i>Peltophorum africanum</i> (Weeping wattle)	44	256
<i>Rhus leptodictya</i> (Mountain sumac)	76	275
<i>Acacia caffra</i> (Hookthorn acacia)	94	282

Source: Prof W. Van Hooven, Centre of Wildlife Studies, University of Pretoria.

Above findings are supported by Teague (1989) in a study to investigate the patterns of browse selection by Boer goats in a representative *Acacia karroo* community in the eastern zone of South Africa (Tables 5 & 6). His results showed that goats do select for the highest density of leaves, thereby maximising intake for the least effort, when eating *A. karroo*. Generally, following browsing tannin levels increased significantly and *in vitro* digestibility decreased significantly. Increase in tannin content

Table 5. *In vitro* digestibility (%) of hand harvested leaves and shoots from *acasia karroo* trees 1.5 m high during a period of utilization by goats

Day	Leaves		Shoots	
	Young	Mature	Young	Mature
0	51.41	51.30	7-48	42.87
3	52.78	51.62	47.81	48.86
7	46.32	493.61	48.92	40.00
15	47.29	40.46	40.46	44.77
LSD	0.01=3.92; 0.05=2.83; 0.10=2.38			

Source: Teague (1989)

Table 6. Tannin content (mg/g) of hand harvested leaves and shoots from *acasia karroo* trees 1.5 m high, during a period of utilization by goats.

Day	Leaves			Shoots		
	Young	Mature	Mean	Young	Mature	Mean
0	211	203	207	192	201	197
3	282	204	243	230	171	201
7	218	144	181	217	181	148
15	207	223	215	267	200	234
LSD	0.01=13.9; 0.05=10.1					

Source: Teague (1989)

of *A. karroo* was most remarkable soon after grazing which tended to decline with time in young plant material compared to mature plant material indicating a greater defensive mechanism in young plant material preferred by grazing livestock.

Therefore, the factors affecting plant tannin content are numerous including the species, plant part (leaf, stem, inflorescence, seed), stage of growth, environmental factors and most interesting by the defoliation by cutting or grazing herbivores, including insects as a means of defence.

Effects of tannins on rumen function and gastro intestinal tract utilization of nitrogen

McLeod (1974) reported that tannins above 5% can become a serious anti-nutritional factor in plant materials fed to ruminants. Barry (1983) and his colleagues demonstrated that the ideal concentration of condensed tannins in plants was between 2-4% of the diet dry matter, at which level they bind with the diet proteins during mastication by the animal and protect the protein from microbial attack in the rumen. The protein tannin complex dissociates and the protein can be digested in the lower gut. Tannins at higher levels (5-9%) become detrimental (Barry, 1983), as they reduce digestibility of fibre in the rumen (Reed *et al*, 1985) by inhibiting the activity of bacteria (Chesson *et al*, 1982) and anaerobic fungi (Akin & Rigsby, 1985) and above 9% tannins their effects can become lethal. Tannin-protein interactions in nutrition have long been recognized while the detailed knowledge of the chemistry of it has been relatively more have only recent. Large proteins tend to bind tannins more tightly, although proline-rich proteins (PRP) bind stronger due to high proline in proteins resulting in open structure containing sites for H-bonding with tannins (Hagermann, 1989). Mole *et al*, (1990) observed that post transactional modifications such as glycosylation may enhance the affinity for tannins

through a more open protein conformation tannins (Hagerman, 1989).

Tannin-protein interactions also depend on the pH, which is of significance due to varying pH in different regions of the gastrointestinal tract. Fate of plant protein due to condensed tannins in the diet in different regions of the gastrointestinal tract of a ruminant is illustrated in fig. 2.

Variation in the anti nutritional effects of tannins due to animal species as a result of the ability to

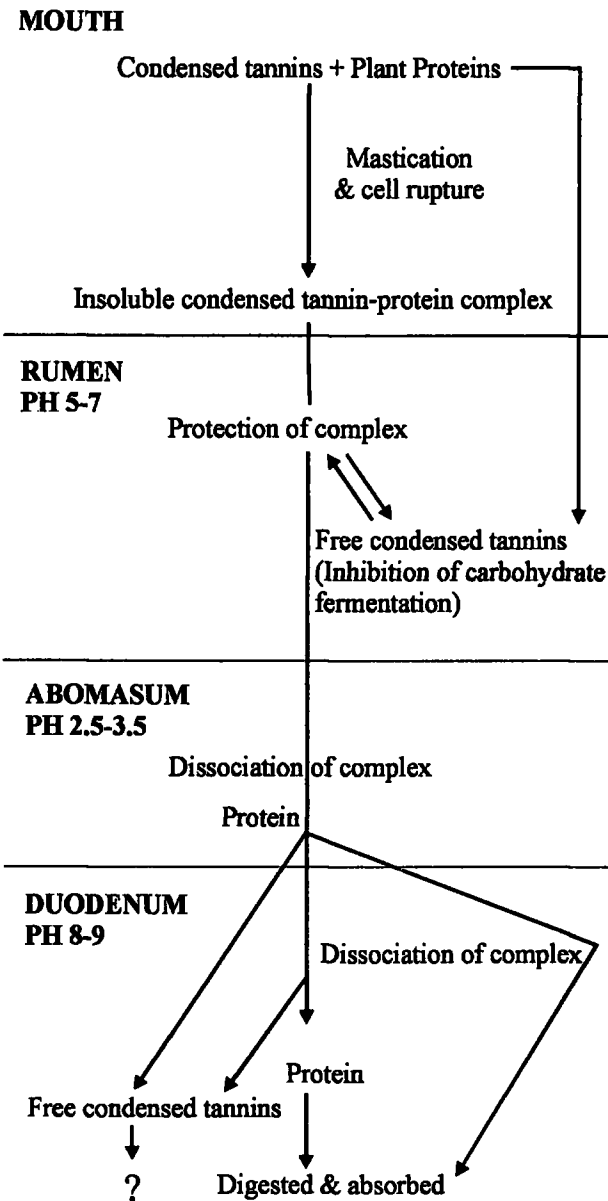


Fig 2. Condensed tannins and protein protection in the rumen (after Mangan, 1990)

secrete proline rich proteins (PRP) in saliva has been reported. (D’Mello, 1992) being absent in cattle and sheep while deer and probably goats secrete them. Thus, browsing ruminants such as deer secrete various amounts of PRP (Robbins *et al*, 1987) and are absent in the saliva of cattle and sheep (Austin *et*

al, 1989), which are predominantly grazing animals

Sheep have been shown to adapt to tannins in the diet suggesting that there are ruminant organisms that in some way detoxify tannins. Reed *et al*, (1985) showed that sheep fed tanniferous tree leaves slowly adapted to the tannin and increased their feed intake. Frutos *et al*, (2000) showed that the use of different levels of quebracho tannins as chemical additives (0,1, 5, 10, 15, or 25g with 100g of soy bean meal (SBM)) improved the digestive utilization of SBM fed to sheep. However, long term dosing of quebracho tannins did not increase the ability of the rumen microbes to degrade tannin treated SBM. In contrast, Dawsen *et al*, (1999) demonstrated the effect of feeding of quebracho tannins, a mixture of condensed tannins on dietary nutrient utilization and N retention and its effects on the gastrointestinal tract of sheep. Sheep were fed on a pelleted diet of dried grass alone or containing quebracho tannin 50 g / kg diet DM at a level sufficient to achieve an average live weight gain (AWG) of 100 g. Apparent digestibility of dry matter, N and NDF were reduced in tannin fed sheep. No evidence was obtained to suggest that rumen microorganisms can adapt to the presence of dietary tannins with prolonged feeding. Tannin fed sheep excreted more nitrogen in faeces and less in urine than the sheep fed no tannins suggesting an alternation in nitrogen metabolism with a clear indication of impaired N utilization in the gastrointestinal tract. Histological examination of samples of the GI tract after slaughtering, indicated ulceration and increased mucosal histiocytes, particularly in the ileum of most tannin fed sheep. Their observations suggested that, feeding quebracho tannin to ruminants had ruminal and post ruminal effects that, together, resulted in reduced nutrient utilization and impaired performance.

Tannins may indirectly affect rumen function by reducing rumen ammonia levels through decreased protein degradation in the rumen. Rumen ammonia levels below 80 mg N /l usually result in depressed fibre digestibility (Leng, 1990) leading to a highly significant reduction below 10 mg N/l. Thus whenever tannins are present in forages, there may be a need to supplement with non-protein nitrogen sources. Conversely, tannins in feed may have increased detrimental effects on rumen function when the basal diet is low in protein (Leng, 1990).

According to Kriaa and Thewis (1999) the addition of chestnut tannins at a lower concentration (4g/ 100 g of total nitrogen, approximately 0.4 g/kg of DM) to grass forage for growing sheep improved the nitrogen retention. The improvement of nitrogen retention is explained by the decrease in urine N,

indicating better use of absorbed N.

The addition at these low concentrations of CT to fresh grass did not alter the digestibility of its constituents or the rate of ingestion.

Molina *et al.* 1999, fed a group of lambs inoculated with an oral dose of a culture of *Eubacterium cellulosolvens*, that was able to tolerate 0.5 g/litre of purified condensed tannins (CT) from *Desmodium ovalifolium*, for three weeks containing 30% peanut skins. The control group was inoculated with auto claved bacteria. The treatment group was inoculated with the actively growing bacteria. Daily DM intake was higher ($P < 0.05$) in the control group while the digestibility of DM, CP, and NDF were similar with both treatments. However, CP intake, CP retention and CP retention/CP intake ratio were higher for animals inoculated with live bacteria than the control. To investigate carry-over effects, the animals continued to receive the high CT diet but no supplemental bacteria were given. DM and CP intake, as well as the feed: gain ratio, were similar in two groups.

Hanley *et al.* (1992) found that protein precipitation assays were useful indicators of the inhibition by tannins of protein and dry matter digestibility in deer fed on seven tannin containing forages and twigs from one browse species. Bhatta *et al.* (2000) reported the effect of feeding tamarind (*Tamarindus indica*) seed husk (TSH) as a source of tannin on dry matter intake, digestibility of nutrients and production performance of crossbred dairy cows in mid-lactation. Cows were divided into 3 groups of 3 each (group 1-compounded feed mixture only (CFM), group 2- CFM +2.5%TSH; group 3- CFM + 7.5%TSH). There were no differences between the groups with respect to DMI, milk yield, solid-not-fat and lactose. There were significant difference in live weight gain and milk protein content between group 1 and group 3. CP digestibility differed among the three groups with significant differences in faecal and urinary nitrogen excretion. It was concluded that TSH tannin at a low concentration has a beneficial effect on the performance of crossbred lactating cows.

The effects of different tannin sources on feed consumption, live weight gain and mohair production and quality of Akkaraman sheep and Angora goats were studied by Imik and Seker (1999) Eighteen month old white Karaman sheep were fed with diets containing oat hay, oak leaves or broomcorn (*Sorghum bicolor*) with 0.292, 8.02 and 3.22% tannin/DM respectively. Digestibility of organic matter in broomcorn was lower than that of oat hay and oak leaves, while CP digestibility of oat hay (67.43%) was higher than that of oak leaves

(21.75%) and broomcorn (15.71%). No difference in live weight gain or mohair production and quality was observed in groups of angora goats fed with different combinations of oat hay, oak leaves and broomcorn.

Two grazing trials conducted by Harris *et al.* (1998) with Friesian cows in mid lactation showed milk yields were higher on Birds foot trefoil (*Lotus corniculatus*)-dominant pasture (19.7 and 16.7 litres/cow/day) than on white clover-dominant (17.8 and 15.4 litres/cow/day) or perennial ryegrass-dominant (13.0 and 11.7 litres/cow/day) pastures. Increased milk production on the lotus and clover was attributed to the higher nutritive value of legume based pasture compared with rye grass, and to higher dry matter intakes. Cows grazed lotus also had improved feed conversion efficiency compared with those grazing either rye grass or clover indicated that the presence of condensed tannins in the lotus may have contributed to the improved efficiency. Milk protein concentration was consistently higher on than lotus (3.36 and 3.35%) than on the ryegrass (3.115 and 3.21%) or clover (3.30 and 3.21%) in both experiments.

Thus the forgoing evidence suggest that a little tannin (2%-4%) can protect protein of forages and allow a high efficiency of feed utilization by the animal, where as too much tannins can be highly detrimental (5%-9%) leading first to reduced intake, and at high levels (>9%) the animals may have complications even leading to deaths.

Rumen quality assessment of tannin containing forages

A study of two fodder species *Gliricidia sepium* and *Calliandra calothyrsus*, was undertaken to investigate the degradation of leaf protein (crude and soluble protein) by rumen microbes during *in vitro* fermentation and the effect of total tannin content on that degradation. Differences in DMD and protein degradation were observed between the two leaf species. *C. calothyrsus* after extraction with 70% acetone, showed greater DMD and crude protein losses of 40% and 20% respectively. The cumulative gas production of *C. calothyrsus* increased after extraction with 70% acetone. It was concluded that the differences in protein degradation characteristics were mainly due to the inhibitory effects of tannins. The apparent increased resistance of glycoproteins to degradation appeared to be relatively of minor importance to the nutritive value of the fodder (Whetton *et al.* 1997).

The quality of the same two species was assessed *in vitro* in relation to their extractable tannin contents

(Seresinhe and Iben, 2003). *In vitro* dry matter digestibility of low CT species was higher than in *C. calothyrsus*, the medium CT species.

Some detailed work of Tanner *et al.* (1995) has described the effects of proanthocyanidins (condensed tannins), purified from forage legumes, and on the degradation of leaf protein incubated with strained rumen fluid *in vitro*. The metabolism of ribulose biphosphate carboxylase was monitored by SDS-PAGE. It was shown that the presence or absence of proanthocyanidins markedly affected the metabolism of this protein.

Hee-Dong Bae *et al.* (1993) investigated four colorimetric methods, vanillin-HCl, Prussian blue, butanol-HCl, and H₂SO₄ for their suitability for measurement of condensed tannins in media used for the culture of rumen bacteria. Of the four, only the H₂SO₄ procedure was suitable for quantifying condensed tannins in both the vanillin -HCl and Prussian blue method. In contrast, the H₂SO₄ method was not subject to a loss in chromophore yield from water or to interference from medium components. The chromophore formed was stable, and the assay enabled microgram quantities of condensed tannins to be measured in microbial medium. Condensed tannins in bovine serum albumin complexes were readily quantified by the H₂SO₄ method. Thus, the potential exists for the H₂SO₄ method to quantify condensed tannins in both condensed tannin-bacterial cell and condensed tannin-bacterial enzyme complexes.

Wood *et al.* (1995) investigated the intraspecific differences in ash, crude protein contents and protein precipitation activity of extractable tannins in three *Artocarpus lakoocha* and three *Quercus semecarpifolia* (Nepalese tree fodder) harvested at bimonthly intervals (3 times). Much of the variation within species related to leaf age and position appeared to be random in nature. Protein contents generally fell and ash content rose over the period, but the trend was generally not statistically significant ($P > 0.05$). Some significant ($P < 0.05$) tree-to-tree differences were found in these components. Variability in leaf protein precipitation activity was significant ($P < 0.05$) within trees, between trees and between bimonthly samples.

IVDMD of forage legumes and tree fodders are also presented in Table 1, 2 & 7. Between CT containing legumes *A. americana* (Table 1) and *G. sepium* and *Leucaena* spp (Table 2) had high IVDMD approaching 70%. Although these legumes were also relatively high in CP%, it was not always that the higher CP% was associated with higher IVDMD. Generally, the species devoid of tannins tended to have higher IVDMD.

The effect of polyethylene glycol (PEG) addition on the digestibility of proteins.

Several methods have been adopted to overcome the nutritional problems associated with high tannin, legume grains, agricultural and industrial waste by-products (Kumar & Singh, 1984). Polyethylene glycol (PEG) incorporation in the feed is one such method, which is known to preferentially bind with condensed tannins and thus prevent the formation of potentially indigestible tannin-protein complexes (Jones 1965). Seresinhe and Iben (2003) reported that *in vitro* gas production and IVDMD increased ($P < 0.05$) with increasing levels of PEG from 0 to 10g/100g, especially for *C. calothyrsus* which had a higher CT content (5.05%) compared with *G. sepium* (0.57%). Application of PEG resulted in higher microbial masses for both species, which has also been reported by Getachew *et al.* (2001).

Palmer and Jones (2000a) used a modified two stage *in vitro* digestion method to investigate the effects of rate of PEG addition (0-1100 mg/g DM) on dry matter (IVDMD) and nitrogen digestibility (IVND) of freeze dried leaf material from actively grown shoots of *Calliandra calothyrsus* (Calliandra) and *Leucaena leucocephala* (Leucaena). For both species, IVDMD and IVND increased asymptotically with PEG rate; the IVND response being markedly greater for Calliandra. There was a three-fold greater amount of PEG bound to the residue after stage 1 digestion with Calliandra than with Leucaena (3 fold). The presence of PEG in the residue which was not reduced after stage 2 digestion, resulted in a higher residue weight and therefore an underestimated IVDMD. Quantification of PEG in the residue enabled a corrected IVDMD (CIVDMD) to be estimated. PEG did not bias estimates of nitrogen digestibility in the same way. In a second experiment, estimates of CIVDMD and IVND were made after stages 1 and 2 in the presence and absence of PEG at 160 mg/g sample. For Leucaena, there were small, but significant effects of PEG, where as with Calliandra there were large responses to PEG and to timing of addition. When measured after stage 1, IVND in the absence of PEG was not significantly different to zero (compared with 57% for Leucaena) whereas with PEG, IVND was 75% (compared with 68% for Leucaena), where as with PEG, IVND was 75% (compared with 68% for Leucaena). The low IVND for Calliandra was associated with low NH₄N levels in the rumen fluid/buffer after stage 1. They suggested that about 160 mg PEG/g sample is appropriate for most studies with tropical tanniniferous shrub legumes to estimate any

deleterious tannin effect. The use of PEG to estimate this effect on IVDMD is not valid without accounting for the PEG bound to the residue. For estimation of the adverse effect of tannins on IVND the use of PEG may be appropriate.

Palmer and Jones (2000b) further investigated the effect of sample preparation on measures of *in vitro* digestibility of *Calliandra calothyrsus* leaves. Leaves were chopped fresh (FrCh), chopped and oven dried at 65C (ChOD), chopped and freeze dried (ChFD), oven dried and ground (ODGr) or freeze dried and ground (FDGr). Samples were analysed for *in vitro* dry matter and nitrogen digestibility with or without PEG addition to bind tannins. Ground samples gave higher dry matter digestibilities (IVDMD) than chopped samples; freeze dried samples had higher IDVMD than oven dried samples, and fresh leaves were intermediate. Overall, PEG increased IVDMD from 42.1 to 45.4 ($P<0.01$) and IVND from 37.0% to 66.4%. ($P<0.01$). When corrected for PEG bound in the residue, CIVDMD increased by 8.6-15.5% depending on the pre treatment. PEG improved IVND of all samples, while the improvement expressed in percentage units was greatest with the ground samples and least with the dried chopped and fresh samples. The relationship between PEG binding and IVND for the dried samples was linear ($r^2=0.96$), indicating that the pre treatment influenced the ability of PEG to penetrate and bind to tannins. Results emphasise the importance of standardisation of sample pre treatment for PEG binding studies and again demonstrated that IVDMD studies with tanniferous forage and browse species using PEG will give unreliable results unless corrected for PEG-tannin complexes in the residue.

Jones and Palmer (2000) further investigated the IVDMD and IVND of six leguminous tropical shrubs and the grass *Panicum maximum* in the presence and absence of PEG 4000. The PEG was spiked with ^{14}C -labelled PEG 4000 so that PEG remaining in the residues after digestion could be calculated. This was subtracted from the residues to calculate corrected (CIVDMD) from IVDMD. In the absence of PEG, the ranking of the species for IVDMD was: *Gliricidia sepium*>*Panicum maximum*> *Leucaena leucocephala*> *L. trichandra*> *L. pallida*> *Calliandra calothyrsus*>*Acacia boliviana*. PEG increased IDVMD for *Acacia boliviana*, decreased it for *L. pallida* and had no effect on the other species ($P>0.05$). The CIVDMD values for the legumes were all higher with PEG by a mean of 8.6% units, though the species ranking remained the same.

IVND varied from 82% for *G. sepium* to 49% for *C. calothyrsus* in the absence of PEG. The rankings were similar to those for IVDMD. PEG increased the IVND of all species except for grass and reduced the range from 86.6% with *G. sepium* to 75% to *P. maximum*. There were no condensed tannins in *P. maximum* and so PEG had no effect on IVDMD or IVND. PEG binding of the legume species was negatively related to IVND%. Their results showed that the tannins in these shrub species can have a larger effect on IVDMD and an even larger effect on IVND, consequently the potential value of *A. boliviana* and *C. calothyrsus* for improving animal production should be questioned. The digestibility of psyllid-tolerant *L. pallida* and *L. trichandra* was also shown to be lower than that of proven high quality, but psyllid susceptible *L. Leucocephala*.

Jones *et al.* (2000) investigated the digestibilities of dry matter (IVDMD) and nitrogen (IVND) of the leaves of six leguminous shrubs in the presence and absence of polyethylene glycol 4000 (PEG). The difference due to PEG acting on tannin was more marked for IVND than for IVDMD and varied with species (Table 7).

The tannin effect correlated poorly with previously published data (Jackson *et al.* 1996) on the butanol CT levels in these samples: extractable CT ($r^2=0.0007$), protein bound ($r^2=0.485$); fibre bound ($r^2=0.566$); and total CT ($r^2=0.1473$). The bound CT was positively correlated with the PEG effect ($r^2=0.578$). *A. boliviana* and *C. calothyrsus*, behaved differently to others. Omitting these from the regressions improved the relationships with both butanol extractable CT and total Butanol CT and with vanillin CT ($r^2 0.9$).

For the new pasture species, screening by using this modified *in vitro* digestion technique would avoid problems of using a known CT standard or of isolating CT standards for each species.

The effect of sainfoin (*Onobrychis vicifolia*) on digestion of alfalfa (*Medicago sativa*) was investigated *invivo* and *invitro*. Fresh alfalfa and sainfoin were incubated in an artificial rumen (RUSITEC) in ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 (as fed). Disappearances of DM and N from sainfoin were 77% and 65% of those from alfalfa, respectively. Protease and endoglucanase activities, $\text{NH}_3\text{-N}$ and methane production decreased ($P<0.05$) as sainfoin increased. Bacterial numbers and microbial outputs were unchanged, but cells incorporated more $^{15}\text{NH}_3\text{-N}$ as sainfoin in the diet increased. Chopped leaves (100:0, 95:5 and 90:10 alfalfa :sainfoin) were incubated for 40 hours with diluted rumen fluid containing 0 or 50 mg PEG, which binds tannin. Gas and volatile fatty acid

Table 7. The effect of PEG 4000 on the *invitro* dry matter and nitrogen digestibilities (IVDMD & IVND) of six tropical forage species^a.

Species	Cultivar of CPI ^b	IVDMD -PEG	IVDMD +PEG	IVND -PEG	IVND +PEG
<i>Acacia boliviana</i>	40175	46.2	49.3	50.9	84.7
<i>Calliandra calothyrsus</i>	115690	49.3	51.5	47.7	79.8
<i>Gliricidia sepium</i>	60796	75.5	75.3	80.2	85.8
<i>Leucaena trichandra</i> (<i>diversifolia</i>)	46568	57.9	59.7	67.9	83.87
<i>Leucaena leucocephala</i>	cv. Cunningham	67.3	67.3	76.3	86.2
<i>Leucaena pallida</i>	84581	57.3	54.2	68.1	79.9

^aLSD for the species x PEG interaction : 5% 2.16; 1% 2.94%

^bCommonwealth plant interaction number

Source : Jones and Palmer (2000).

productions were similar across treatments, but including 10% sainfoin without PEG reduced ($P < 0.05$) NH_3 concentrations between 8 and 24 hours. Sainfoin tannins reduced degradation of forage protein without affecting the digestibility of the non protein fraction. Alfalfa herbage was fed alone or with early -to full- bloom sainfoin herbage (at least 10 or 20% of *ad libitum* alfalfa intake) or with sainfoin hay or pellets, to 8 Jersey steers in cross over trials conducted over 4 years. Including sainfoin in the diet reduced ($P < 0.001$) the incidence of bloat by 45-93% in 3 to 4 years, irrespective of the form in which it was supplied (McMahon *et al.*, 1999).

McSweeney *et al.* (1999) used *in vitro* techniques to evaluate the nutritive value of a selection of tanniniferous tree and shrub legumes (*Calliandra calothyrsus*, *Leucaena leucocephala*, *L. diversifolia* and *L. pallida*) compared with Lucerne (*Medicago sativa*). PEG was also added to some *in vitro* fermentations (10 mg PEG / 50 mg plant substrate) to assess the effects of tannins on digestion of dry matter (DM), neutral detergent fibre (NDF) and nitrogen (N). Total tannin content was poorly correlated with digestibility of dry matter and nitrogen. PEG addition caused significant increase in rate and extent of DM and NDF digestibility and ammonia production for all the tannin containing shrub legumes but not for Lucerne. However, dry matter loss and fermentability of these plants appeared to be poorly correlated because PEG addition resulted in an increase in volatile fatty acid production ranging from 3.7 to 20.2% compared with a greater increase in apparent DM digestibility of 9.1-30%.

It was concluded that *in vitro* evaluation of apparent DM and N digestibility of tannin containing plants provides a poor indication of true digestion (fermentability) and thus measurements of fermentation end products (ammonia and short and branched chain fatty acids) should also be

undertaken to evaluate nutritive value. Also, addition of PEG to *in vitro* fermentations can be used to determine the effects of tannins on N digestibility. Based on the *in vitro* methods of rumen fermentation used in this study, nutritive value of the tanniniferous forages ranked as follows *L. leucocephala* > *L. diversifolia* and *L. pallida* > *C. Calothyrsus*.

Getachew *et al.* (2001) investigated the effects of application of different amounts of tannin-complexing agent, PEG, MW 6000 together with different methods of application (addition of PEG as a single dose to tannin-containing browses (*Acacia albiba*, *Acacia cyanophylla* and *Calliandra calothyrsus*). The method of application of PEG did not affect the SCFA production in *A. cyanophylla* and *A. albiba* but significantly increased in *C. calothyrsus* NH_3 N concentration was significantly lower in *A. cyanophylla* and *C. calothyrsus* when PEG was applied as a split dose compared to a single dose. The split application of PEG resulted in a higher production of microbial protein and higher efficiency of microbial protein synthesis (EMPS; $\mu\text{mol purins/mmol SCFA}$) than the single application. This study demonstrated the possibility to improve the efficiency of utilization of tannin containing browses using split application of PEG which improved rumen fermentation resulting from better synchronization of energy availability and N degradability.

Use of polyethylene glycol as an anti-tannic substance in animal feeding studies

Pintus (2000) investigated the effect of polyethylene glycol (PEG), as an antitannic substance, on feeding behaviour, intake and milk production of Sarda goats at the end of lactation. Twenty goats were allowed to browse for 7 hours daily on 5 ha of shrubland. The goats were allotted to two groups of ten each: PS receiving 50 g/day of PEG 4000 MW, and PU,

receiving no PEG supplementation. The time spent on grazing was similar in the two groups. The average intake at pasture tended to be higher in PS than PU (1366 vs. 1187 g DM/head/day, NS). The percentage of the species eaten by goats was different in the two groups. PEG-supplemented goats ate more tanniniferous species like *Pistacia lentiscus* L. whereas the control goats selected more herbaceous species. Goats from PS had higher *in vivo* crude protein digestibility, compared with PU (0.53 vs. 0.40). Milk yield (755 vs. 645 ml) and milk urea content (19.76 vs 16.46 mg/100 ml) were higher in PS goats. It was concluded, when a goat diet consists mainly of species rich in tannins, PEG alleviates their negative effect on protein digestion.

Woodward *et al.* (1999), conducted an experiment to determine what proportion of the increased milk yield and milk protein percent was due to the condensed tannins (CT) in Birdsfoot trefoil (*Lotus corniculatus*), and what proportion was due to the factors typically associated with legumes (increased herbage intake and improved forage quality). Twenty Friesian cows were housed and fed twice daily on perennial rye grass or lotus for 10 days. Five cows on each diet were also drenched with 1.2 l of 50% polyethylene glycol (PEG) 3 times per day. PEG blocks the action of CT, preventing them binding to plant proteins. Milk yields (litre/cow/daily) were higher on lotus (16.5) than on lotus + PEG (13.8), ryegrass (10.29) or ryegrass+PEG (9.9) indicating that CT contributed to 42% of the increased milk yield that resulted from feeding lotus rather than ryegrass. CT had no effect on intake, since intakes of cows fed lotus (16.8) and lotus+PEG (16.7) were similar and higher than for cows fed ryegrass (14.7) or rye grass +PEG (13.7). CT, however, accounted for all of the increase in herbage conversion efficiency as indicated by the higher efficiencies (ml FCM/MJ ME) of cows fed lotus (147) compared with those fed lotus +PEG (126), ryegrass (123) or ryegrass +PEG (127). CT accounted for 57% of the increase in milk protein percent as cows fed lotus had higher protein percent (3.61) than those fed lotus + PEG (3.44), ryegrass (3.31) or ryegrass +PEG (3.30). Herbage and CT had no effect, however, on casein or whey protein concentrations. CT had no effect on milk fat or lactose concentration but cows fed lotus had lower concentrations than cows fed ryegrass ($P < 0.05$). Overall, the trial showed legumes containing CT have potential as a forage for dairy cows.

Ingestion of condensed tannins decreases feed intake in ruminants. PEG forms high affinity complexes with tannins. In two experiments carried out on Holstein heifers, quebracho (Q) from the

Aspidosperma quebracho served as a source of condensed tannins. The aim of the study were (i) to quantify the effect of Q on feed intake and eating behaviour in cattle fed complete mixed diets (CMDS). (ii) to clarify if changes induced in ingestive behaviour and feed intake by Q in cattle can be reversed by feeding PEG and (iii) to clarify if the decrease in feed intake is associated with short-term (astringency, post- negative malaise) or longer term effects. In experiment 1, 500 g/day of Q was found to be the minimal dose that decreased feed intake in heifers. A ratio of PEG:Q equal to 1:12.5 did not fully restore feed intake. In experiment 2, 4 heifers received a random sequence of 4 rations in a Latin square design with feeding cycles of ca. 7 days: CMD containing no supplements (C), or supplemented with 625g/day of Q without PEG (Q), with 625 g/day of Q and 250g/day of PEG (Q-PEG), or with 250 g/day PEG without Q(PEG). Overall, feeding Q was associated with lowered feed intake and shorter duration of eating bouts, mainly of the first eating bout, immediately after distribution of the diet. A larger proportion of the diet was consumed subsequent to 180 minutes after distribution of Q-fed heifers. Q did not affect eating rate and the water to food ratio. The effects of Q on feed intake were achieved by feeding PEG. Heifers adapted effectively to condensed tannins by increasing the number of eating bouts and the portion of diet consumed subsequent to 180 mins after distribution, so that no differences in feed intake were noted on the last day of each feeding cycle. Data were interpreted to show that (i) negative effects of Q on feed intake derive from astringency of CT and short -term post-ingestive malaise; (ii) the increasing number of eating bouts and their wider partition throughout the day were means to preserve the ruminal environment in Q-fed heifers and (iii) PEG has the potential to neutralize negative effects of condensed tannins in cattle (Landau *et al.*, 2000).

Provenza (2000) studied the effect of the amount of supplemented PEG (0, 25, 50, 75 or 100 g; molecular weight 3350) on intake by sheep of a feed (Milo-tannin mix) containing 20% quebracho tannin. There was a linear relationship between the supplemental PEG ingested and the subsequent intake of milo-tannin feed. They also determined whether sheep self regulated intake of PEG when fed a ration that contained 0. 5. 10. 15 or 20% quebracho tannin and whether they adjusted their intake of PEG when tannin was removed from the diet. There was a positive relationship between the amount of PEG ingested and intake of feed and tannin. Sheep fed high tannin diets ate more PEG than controls. Sheep fed 20% tannin diet ate the most PEG, and the

controls ate the least PEG. Tannin limited the intake of diets, but PEG attenuated the response to a great degree. Immediately after tannin was removed from the ration, sheep that formally had been fed the 20% tannin ration ate more PEG than those fed the other rations. Ten of the sheep from 20% tannin group, 1 from 15% tannin, and 2 each from the 10 and 5% groups) continued to eat PEG for 7 days after tannin was removed from their ration. When they were tested again 6 weeks after the trial and offered tannin free diets, their intake of PEG had decreased.

A grazing experiment was conducted by Min *et al.*, (2001) in New Zealand to compare the reproductive efficiency and wool growth of ewes grazing *Lotus corniculatus* (birds foot trefoil) or *Lolium perenne* (perennial ryegrass)/ *Trifolium repens* (white clover) pasture. Half the ewes grazing *Lotus corniculatus* were given twice daily oral PEG (PEG; MW 3500) supplementation to inactivate condensed tannins (CT) in lotus. A rotational grazing system with mixed ewes (53.2±3.78 kg per ewe) were used with 75 ewes per treatment. Lotus contained 18 g total CT/kg dry matter in the feed offered. There were only trace amounts of total CT in pasture. Mean ovulation rates (ORS) at cycle 3 for CT-acting and PEG sheep grazing lotus and sheep grazing pasture were 1.79, 1.58 and 1.48 with corresponding lambing percentages being 1.69, 1.39 and 1.22 respectively. Fecundity (number of corpora lutea per ewe ovulating) at cycle 3 ovulation and lambs per ewe lambing were greater for ewes grazing lotus than pasture (P<0.01), and for CT acting than PEG sheep grazing lotus (P<0.05). Increases in fecundity were due to a lower proportion of single ovulation and a greater proportion of multiple ovulations. CT increased reproductive efficiency by increasing fecundity and by reducing embryonic loss. Rumen ammonia and blood plasma urea concentrations were lower for ewes grazing lotus than pasture (P<0.01), and were generally lower for CT-acting than PEG sheep grazing lotus. The nutritional treatments had little effect on plasma ammonia concentration. Organic matter intake (OMI) (P<0.05), clean wool production (P<0.01) and staple length (P<0.01) were significantly higher for ewes grazing lotus than pasture. Live weight gain (LWG) was low, and lower for sheep grazing lotus pasture. There was no difference in OMI and LWG due to CT in lotus, but wool length and clean wool production were higher (P<0.01) for CT-acting than PEG supplemented ewes (CT not acting). It was concluded that grazing lotus during mating increased the efficiency of reproduction and clean wool production, with a component due to the action of CT.

Use of tannins for improvement of silage quality

Four hybrid forage sorghum silages with different tannin concentrations and moisture in the stem were ensiled in laboratory silos made of PVC tubes (Goncalves *et al.*, 1999). Silos were opened 1 (P2), 7 (P3), 14 (P4) and 28 (P5) and 56 (P6) days after ensiling, to evaluate fermentation patterns and other parameters. High tannin sorghums had higher dry matter contents.

Further, Borges *et al.* (1999) measured alcohol soluble carbohydrates (ASC), starch and structural carbohydrates were in above silage samples. ASC were highly consumed from P1 to P3. FDA was higher in moist stem hybrids. Lignin was higher in high tannin sorghum silage than in the low tannin ones.

Salawu *et al.* (1999) studied the effect on silage composition of ensiling perennial rye grass (PRG) with 3 commercial tannins (mimosin, myrabolone and quebracho tannins) and mobile bag disappearance of DM, N and true protein due to the addition of these tannins or a combination of tannin plus formic acid, formaldehyde alone, formic acid alone or a combination of aflatoxin. In experiment 1, PRG was from the third cut with a mean oven DM content of 200g/kg. All silage were prepared on a small laboratory scale (500g), with the additives added in 20ml aliquots / kg herbage fresh weight. Tannins were added at the rate of 5 to 50 g/kg herbage DM in experiment 1. Treatment with tannins reduced the soluble nitrogen (SN) and ammonia content of the silages. In experiment 2, the tannins also reduced degradation of silage nitrogen and true protein in the rumen. However, the tannins were not as good as formaldehyde in protecting silage proteins, both during ensiling and in the rumen. Neither were they better than formic acid in enhancing silage quality. For both the tannins and formaldehyde, formic acid addition further reduced the SN content as a result of the combined effect of rapid acidification and protein binding.

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