Muresk Institute of Agriculture

The Value of *Acacia Saligna* as a Source of Fodder for Ruminants

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Declaration

I, Delwyn Margaret Howard certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree.

I certify that, to the best of my knowledge, any help in preparing this thesis, and all sources used, have been acknowledged.

Delwyn Margaret Howard

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Dedication

To my sister … I miss you Shez (1962-1997).

Abstract

Three pen trials were conducted to evaluate the value of *A. saligna* as a source of feed for ruminants.

In Trial 1 *A. saligna* was inadequate as the sole source of nutrients for sheep. Furthermore, the level of detannification achieved in Trial 1, with the addition of PEG 4000 or PEG 6000, failed to improve the diet sufficiently. The antinutritional effects on the animals were largely attributed to the excessive biological activity of the phenolics in the *A. saligna* leaves. Feeding of these leaves, without PEG, had a definite defaunating effect on the ruminal fluid.

The ruminal ammonia levels were all well below the threshold for maximal microbial growth.

Given the results of Trial 1, the second trial was undertaken to determine if *A. saligna* was more useful as a supplement rather than a basal diet.

The benefits of including *A. saligna* as a supplement to a basal diet of lupins and wheat straw were not clear. The benefits of including a detannification agent with the *A. saligna* were also not evident. Ruminal ammonia levels were much higher than in Trial 1 and animals generally maintained weight.

Trial 2 revealed that the sheep were capable of consuming significantly more *A. saligna* than they did in Trial 1, but it was not clear whether this was due to the basal diet providing adequate nutrients or if it was due to differences in the *A. saligna* fed in the respective trials. Total phenolics, CT and PPC were considerably lower than those of Trial 1.

Trial 3 was designed to investigate the use of *A. saligna* as the basal source of nutrients, with or without a supplement of N in the form of urea or PEG.

Total phenolics, CT and PPC were lower than those of Trial 1, but higher than those of Trial 2. Animals consumed more *A. saligna* than in Trial 2 and generally maintained weight.

The results from Trial 3 suggest that *A. saligna* could be a useful feed source for ruminants. The substitution of straw with *A. saligna* indicates that its incorporation into a grazing system could significantly decrease grazing pressure on dry summer pastures.

In Trial 3 goats were not shown to have a superior ability than sheep in utilising *A. saligna* as a source of nutrients.

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Abbreviations

CP	crude protein
CT	condensed tannins
d	day
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
g	gram
h	hour
H_2SO_4	sulphuric acid
HT	hydrolysable tannins
kg	kilogram
kJ	kilojoule
L	litre
LWG	live weight gain
ME	metabolisable energy
mg	milligram
min	minimum
mL	millilitre
mm	millimetre
MP	metabolisable protein
N	nitrogen
NaCl	sodium chloride
NH_3-N	ammonia nitrogen
NH_4	ammonium

$(NH_4)_2SO_4$	di-ammonium sulphate
NPN	non-protein nitrogen
NPS	nitrogen, phosphorous, sulphur
OM	organic matter
OMD	organic matter digestibility
P	phosphorous
PEG	polyethylene glycol
PPC	protein precipitation capacity
PRP	proline rich proteins
S	sulphur
TPC	tannin-protein complexes
v	volume
VFA	volatile fatty acids
w	weight
μm	micrometer
%	percent
°C	degrees celcius

Species

Acacia saligna

Acacia brevispica

Acacia seyal

Acacia sieberiana

Andansonia digitata

Calliandra calothyrsus

Calatropis procera

Ceratonia siliqua

Desmodium ovalifolium

Flemingia macrophylla

Glyricidia sepium

Hedysarum coronarium

Lespedeza cuneata

Leucaena leucocephala cv Cunningham

Lotus pedunculatus

Lotus corniculatus

Onobrychus viciifolia

Pistacia lentiscus

Pithecellobium dulce

Quercus calliprinos

Sericea lespedeza

Sesbania sesban

Terminalia oblongata

Section 1: Introduction

As part of the battle against land degradation/desertification there is an urgent need to develop sustainable grazing systems.

Acacia saligna, a native to Western Australia has been widely acknowledged as a useful species for land conservation. It has been used extensively in the rehabilitation of rangelands, in soil and sand dune stabilisation, and reforestation in semi-arid and arid zones. More recently, there has been a focus on *A. saligna* as a potential source of fodder for ruminants, particularly in semi-arid/arid regions.

If it can be shown that *A. saligna* is not only a useful species for conservation purposes, but can also provide a valuable feed source for ruminants, then the implications concerning its incorporation into agricultural systems ought to be significant.

Traits of *A. saligna* include:

- Ease of establishment
- Ability to grow well on poor soils/adaptable to a wide range of soils
- Salt tolerant
- Frost tolerant
- Drought tolerant
- Persistent under grazing/cutting
- High production of edible biomass
- Palatable
- Relatively high protein content.

The common conclusion drawn by researchers regarding the value of *A. saligna* as a source of fodder for ruminants is that it is inadequate as the ruminant's sole source of nutrients. This is largely attributed to its condensed tannin content that has been shown to have an inverse relationship with voluntary intake, digestibility and nitrogen retention in ruminants.

With so many desirable attributes of *A. saligna* as a fodder tree, one would surely consider it a challenge to overcome its limitations in becoming a valuable source of feed.

Whilst information on the nutritive value of *A. saligna* is available, numerous factors influence its feed value, including environmental. Therefore, if *A. saligna* is to be part of a sustainable grazing system in Western Australia, there needs to be more information concerning its feed value given the conditions that prevail in that state.

In this thesis the potential feed value of *A. saligna* for ruminants is investigated. The design of trials has borne in mind the paddock situation in which animals would have free access to *A. saligna*, in contrast to the cut and carry systems where access could be restricted. In addition, improvements to the value *A. saligna* as a feed have focused on supplements to the ruminant in preference to any pretreatment of *A. saligna*, in consideration of labour requirements.

Section 2: Literature Review

2.1 Scope of the review

Condensed tannins (CT) are considered the primary antinutritional factor of *A. saligna* (Reed and Soller, 1987). Hence, although reference will be made to both hydrolysable tannins (HT) and CT, and tannins in general, this review will focus on CT and their effect on ruminant nutrition and at possible means of overcoming nutritional limitations imposed by their presence.

In preference to the citing of specific levels of CT, references will be made to the relative levels of CT due to the difficulty in comparisons imposed by factors such as different extraction methods, assays and standards used to determine tannin levels and differences in tannins between species (Makkar and Becker, 1994; Mueller-Harvey and McAllan, 1992; Deshpande et al., 1986).

Low, medium and high CT concentrations will generally refer to total CT concentrations of less than 1% DM, 2-4% DM and 7% DM or greater, respectively, using CT extracted from *Lotus pedunculatus* as the standard (T.N. Barry, personal communication, 2001).

'Bound' CT will refer to that which is bound within the plant and is insoluble, in contrast to soluble CT that is free to bind within the ruminant following mastication.

2.2 Introduction

Browse species play a major role in providing feed for ruminants in arid and semi-arid regions, particularly during the dry season when poor quality roughage and crop residues prevail (Kibon and Orskov, 1993; Ahn et al., 1989).

During dry periods forage trees remain green and maintain a relatively high crude protein (CP) content (D'Mello, 1992). Their foliage may be used as a protein and energy supplement when animals are given low quality roughage (Reed et al., 1990). However, legume trees and shrubs contain a wider range of antinutritional factors than more conventional fodder species (D'Mello, 1992). Hence, although they may contain adequate concentrations of nutrients, the presence of secondary plant compounds could present major constraints to their use (Dzowela et al., 1987).

Many browse species are associated with deleterious effects on livestock either via toxic compounds or through antinutritional factors that can reduce food intake and nutrient utilisation. The primary antinutritional agent in *Acacia* species and many other browse species appears to be the CT (D'Mello, 1992). The CT are widely distributed in the leaves of trees and shrubs, but occur in the leaves and stems of only a small number of specialised non-woody forage legume plants (Barry, 1989).

2.3 Chemistry and occurrence of tannins

2.3.1 Hydrolysable and condensed tannins

Secondary compounds in plants are compounds that are not involved in the plant's primary metabolism (Lowry et al., 1996). Although once considered not to play an indispensable role in plant life, they are now known to be essential to plant life, many of them providing a defense mechanism against bacterial, viral and fungal attack, analogous to the immune system of animals (Deshpande et al., 1986).

Phenolic compounds are the major group of secondary compounds in plants (Lowry et al., 1996). These include the polyphenols (also referred to as "flavonoids") that are found in nearly every species of higher plants (Deshpande et al., 1986). Tannins are a group of polyphenols that are commonly found in browse plants (D'Mello, 1992).

Tannins are distinguishable from other polyphenols by their ability to precipitate proteins (although they also complex with starch and cellulose) (Reed, 1995). They can only be distinguished from non-tannin phenolics by a protein precipitation method (Lowry et al., 1996).

Tannins comprise two major classes: i) HT which after hydrolysis yield carbohydrates and phenolic acids, and ii) CT which are non-hydrolysable and are resistant to hydrolysis and are oligomers of flavan-3-ols and flavan-3,4-diols (Salunkhe et al., 1990).

2.3.2 Occurrence of tannins in plants

Tannins have widespread occurrence in higher plants. They are not known to have any physiological functions (Zucker, 1983, as cited by Getachew, 1999).

Generally, leaves of browse species contain both HT and CT (Kumar and Vaithiyanathan, 1990). However, the major class in *A. saligna* leaves is CT (Reed and Soller, 1987).

The forage CT can be categorised as soluble, protein bound or fibre bound (Terrill et al., 1992a). Tannins bound to proteins or fibre in the leaves may render these indigestible, while soluble tannins can form complexes with dietary proteins following mastication (Vaithiyanathan and Kumar, 1993) as well as endogenous proteins including enzymes (Kumar and D'Mello, 1995).

The majority of the CT occurs in the vacuole of plant cells, hence their high solubility. Jackson et al. (1996) found a number of CT-containing browse species contained 70-95% of total CT in the form of soluble CT. Exceptions included *Flemingia macrophylla* where only 60% of its CT was soluble, and *Glyricidia sepium* in which almost all was bound CT.

Soluble tannins are released with cell breakdown during mastication and are then able to bind with dietary and endogenous proteins in the gut and to a lesser extent with fibre (Kumar and D'Mello, 1995; McAllister et al., 1994; Vaithiyanathan and Kumar, 1993; McLeod, 1974).

2.3.3 Factors affecting concentrations of CT in plants

Several factors affect both the levels and solubility of CT in leaves.

The amount of CT found in foliage may vary with genotype (Baldwin et al., 1987) eg, different *Lotus* species tend to have different concentrations of CT (Barry and McNabb, 1999).

Tannin levels and extractability change dramatically through the growing season (Hagerman, 1988). Baldwin et al. (1987) found that CT levels were higher in late summer than in winter in birch and maple trees.

The content of CT of tree and shrub foliage can vary due to the age of the plant and the age of the foliage. Degen et al. (1997) found that although foliage from older *A. saligna* trees had a higher total tannin content, their CT content was only half the level of CT found in the foliage from young trees. Makkar et al. (1991) found that CT increased with maturity of leaves in a number of oak species studied. However, the level of total soluble phenols was higher in younger leaves in some species, yet lower in younger leaves of others, with protein precipitation capacity (PPC) showing a similar trend to the total soluble phenols.

Low soil fertility and acidity are associated with higher CT levels in *L. pedunculatus*. The level of CT can be reduced by rectifying soil nutrient deficiencies or by increasing soil pH (Barry and Duncan, 1984; Kelman and Tanner, 1990).

The position of leaves in the canopy, browsing, ambient temperature, and sunlight can all influence CT levels in leaves (Furstenburg and van Hoven, 1994). High temperature stress has been shown to lead to greater levels of CT in the leaves of *Lotus* (Lees et al., 1994).

The solubility of CT is dependent on many factors such as seasonal changes in leaf morphology and moisture content, and chemistry of the CT such as molecular weight (Swain, 1979) as well as the method of preservation chosen eg, lyophilization, drying at ambient or ellevated temperatures, and the assays used to extract the tannins (Hagerman, 1988).

2.4 Effects of tannins on nutritive value of ruminant feeds

The ability of tannins to form strong complexes with proteins is the most important aspect of their antinutritional effects. Tannins bind with at least four groups of proteins in the ruminant: dietary proteins, salivary proteins, endogenous enzymes and gut microbes including microbial enzymes (Hagerman and Butler, 1981).

The effects of CT, such as inhibition of feed intake and digestion by ruminants are usually ascribed to their ability to bind to proteins (D'Mello, 1992). The strength of the tannin-protein complexes (TPC) depends on characteristics of both the tannin and protein (Haslam, 1989).

The HT and CT differ in their nutritional significance and toxic effects. Both precipitate protein. While CT are not readily degraded in the gut, HT undergo microbial and acid hydrolysis with the release of simpler phenolics. These are absorbed and can cause toxicity (Murdiati et al., 1992). While CT reduce forage quality, the HT cause poisoning in animals if sufficient quantities are consumed (Zhu et al., 1995).

McSweeney et al. (1988) found that although sheep were sensitive to the toxicity of HT present in the browse tree *Terminalia oblongata*, the digestion of nitrogen (N), organic matter (OM) and cell wall constituents remained unaffected. In contrast, CT have detrimental nutritional effects such as reducing feed intake, reducing feed digestibility and increasing faecal N excretion (Reed and Soller, 1987). On the other hand CT can be of benefit in the prevention of bloat (Jones et al., 1973), in the protection of feed protein against degradation in the rumen (Barry et al., 1986) and by increasing N retention (Robbins et al., 1991) under some conditions.

2.4.1 Nitrogen metabolism

Tree and shrub legume foliages are usually high in N content. However, the rumen degradability of N varies depending upon the linkages with secondary compounds such as CT (Haryanto and Djajanegara, 1993).

The CT can affect N metabolism in ruminants in both positive and negative ways. The CT may enable dietary protein to escape (undegraded) from the rumen for digestion in the lower digestive tract (Barry et al., 1986) or for excretion (as TPC) with faeces, hence reducing the protein available to the animal (Woodward and Reed, 1997). The CT may increase the recycling of N into the rumen (Barry et al., 1986), decrease the rate of fermentation and increase the efficiency of microbial protein synthesis (Makkar et al., 1995a), although McNeill et al (2000) found the presence of CT to cause no change in the efficiency of microbial synthesis.

2.4.1.1 Protein degradation

The formation of TPC has been shown to protect dietary protein from ruminal fermentation (Barry and Duncan, 1984). The formation of the TPC and N fermentation were found to be positively correlated with the presence of soluble CT rather than bound CT (Barahona et al., 1997; Rittner and Reed, 1992). Bound CT are considered unsuitable to protect dietary protein because they are not hydrolysed by acids or enzymes in the ruminant (Zelter et al., 1970, as cited by Woodward and Reed, 1997).

In ruminants consuming high quality fresh forages, a high proportion of the protein ingested may be degraded in the rumen. The portion of dietary protein that escapes to the small intestine for absorption may be inadequate to meet the total metabolisable protein needs for high levels of animal production (Douglas et al., 1995). In other words, amino acid uptake in the small intestine may be limited in temperate forages containing high levels of total and soluble N (Meissner et al., 1993).

In temperate legumes with a high digestibility and high rumen degradability of feed protein, metabolisable protein requirements of high producing animals may not be always met. In such conditions low levels of CT may be beneficial by reducing rumen degradability of feed protein (Terrill et al., 1992a). Conversely, with excess CT in the diet there can be a complete absence of soluble protein in the rumen, which reduces microbial protein synthesis and metabolisable protein supply to the animal (Jones and Mangan, 1977).

Numerous trials investigating the effects of CT in browse species on ruminant nutrition include straw in the basal diet. Compared to low CT browse, N digestibility is reduced where browse contains high levels of CT eg, *A. saligna* (Degen et al., 1995), *A. brevispica* (Woodward and Reed, 1997), *A. seyal* (Ebong, 1995) and *F. macrophylla* (Fassler and Lascano, 1995). With a diet of ad libitum *A. saligna*, TPC that are formed in the digestive tract appeared in the faeces of sheep and to a lesser extent goats, originating from soluble tannin in the feed (Degen et al., 1995).

2.4.1.2 Nitrogen absorption and amino acid supply to the small intestine

The absorption of essential amino acids from the small intestine limits productivity in ruminants fed entirely on diets of high quality fresh forages ad libitum (Barry, 1981). The CT in *Lotus* can reduce the degradation of proteins in the rumen and increase essential amino acid absorption in ruminants fed fresh forages (Barry and McNabb, 1999), provided they are not in excess.

An increase in flow of metabolisable protein or essential amino acids to the small intestine has been observed in animals grazing forages of high CT content compared to those grazing a low CT diet (Wang et al., 1994; McNabb et al., 1993; Waghorn et al., 1990; Barry and Manley, 1984; John and Lancashire, 1981). With *L. pedunculatus* the relationship between CT concentration and increased duodenal flows of metabolisable protein was linear (Barry et al., 1986). Benefits from feeding forages of high CT will be evident only where there is adequate rumen degradable N to meet microbial needs (Leng, 1992) and where the increase in bypass protein supply is not offset by a decrease in microbial protein flow to the small intestine.

Meissner et al. (1993) found that although tannin in forages significantly increased metabolisable protein passage to the small intestine, compared with forages with no tannin, the digestibility of N in the small intestine was reduced. However, Harrison et al. (1973) observed that apparent and true digestibility of protein were lower in sheep fed dried *Onobrychis viciifolia*, a high CT species, compared to a low CT diet, but urinary loss of N, due to less ammonia absorption, was reduced, such that N retention was not significantly different between the two groups.

2.4.1.3 Nitrogen retention

Phenols have a varying effect on N retention and intake depending on the basal diet, including dietary CP content (Holechek et al., 1990).

An investigation of a range of forage species (including fresh and conserved pastures, and shrubs) indicated that rumen ammonia concentrations were positively correlated with the N content of forage. Ruminal fermentation of the tannin-containing forages resulted in much lower ammonia concentrations than ruminal fermentation of forages without tannins (Meissner et al., 1993). A negative correlation between CT content and rumen ammonia concentration has been observed by others, with the inclusion of CT-containing browse species in the ruminant's diet eg, *A. saligna* (Ben Salem et al., 1997), *A. seyal* (Ebong, 1995) and *A. brevispica* (Woodward and Reed, 1997).

Reduced ruminal ammonia concentrations have been attributed to lower rumen solubility and reduced deamination of plant proteins when CT are present (McNabb et al., 1993; Terrill et al., 1992a). Barry et al. (1986) observed only a slight decline in rumen ammonia concentration in sheep consuming *L. pedunculatus* containing high levels of CT compared to *L. pedunculatus* containing lower CT concentrations. They concluded that this was due to an increase recycling of N into the rumen. However, it may have also been due to differences in bound/free CT content.

Tannins may increase the efficiency of urea recycling to the rumen (Reed, 1995). Sheep fed *L. pedunculatus* have been found to have lower plasma urea concentrations, a more rapid plasma urea turnover rate and a higher irreversible loss than sheep receiving *L. pedunculatus* with polyethylene glycol (PEG) which preferentially binds to tannins and therefore avoids the formation of TPC (Waghorn et al., 1994). A correlation was shown in sheep and goats between increasing content of CT in the diet and reduced plasma urea N, when *A. brevispica* (high CT) was fed as a supplement to a poor quality basal diet, compared to the inclusion of *Sesbania sesban* (low CT) as the supplement (Woodward and Reed, 1997).

Higher concentrations of CT were associated with increased N retention in sheep fed *L. pedunculatus* or *O. viciifolia* compared to a low CT diet (Barry et al., 1986; Harrison et al., 1973). However, high CT concentrations in a number of browse species, when fed as supplements to straw, have been associated with a reduced N retention eg, *A. saligna* (Ben Salem et al., 1997; Reed et al., 1990). *A. seyal* (Ebong, 1995), *A. brevispica* (Woodward and Reed, 1997) and *F. macrophylla* (Fassler and Lascano, 1995). These divergent results may be due to the differences in soluble N or digestibility of the basal diets, as well as actual content and composition of CT.

Increased faecal N and reduced urinary N have been shown to correspond with increasing levels of CT in the diet. For example, animals were given a basal diet of ad libitum teff straw and supplemented with either *A. brevispica* that contains a high concentration of CT, or *S. sesban* that does not. Although N retention was much lower for the diet containing *A. brevispica*, the N balance remained positive, the reduced urinary N loss compensating the higher faecal N loss (Woodward and Reed, 1997).

Goats and sheep fed air-dried *A. saligna* or *A. salicina* ad libitum were in negative N balance, attributed mainly to high urinary N excretion (Degen et al., 1997; Abou El Nasr et al., 1996). In sheep fed *A. saligna* ad libitum plus 400 g/d barley, faecal N excretion decreased by 54%, relative to sheep supplemented also with PEG, while urinary N increased. The net result was about a three-fold higher N retention in sheep given PEG (Ben Salem et al., 1999).

A. saligna was compared to *S. sesban*, as a supplement to ad libitum teff straw. There was a correlation between high CT in the diet and faecal N loss and lower urinary N loss. However, with *A. saligna* N retention was negative and animals lost weight. Conversely, even though *A. seyal* contains a similar level of soluble phenolics as *A. saligna*, it contains a much lower level of bound CT and its use in the same trial resulted in a positive N retention by sheep and an increase in their live weight. *A. seyal* may have increased the recycling of endogenous N to the rumen, as well as the microbial utilisation of endogenous N (Reed et al., 1990; Reed and Soller, 1987).

By increasing the proportion of *F. macrophylla* (high CT) in the diet of sheep, urinary N was reduced implying that rumen ammonia losses were reduced due to protection of dietary protein by CT. However, there was also an increase in faecal N that suggests that some TPC remains indigestible. Not only is the consideration of tannin level in the plant important, but also the plant's digestibility (Fassler and Lascano, 1995).

2.4.2 Effect of pH

In addition to the initial binding of tannins to protein at the time of mastication, further binding may take place in the rumen, rendering these complexes undegradable by the ruminal microbial population (Jones and Mangan, 1977). Although CT that is bound to protein is generally insoluble, in some circumstances it can be soluble (T.N. Barry, pers. comm. 2001).

The TPC are stable and bound at rumen pH 5.5-7, but they are unstable in the acid environment (pH 2-3) of the abomasum. Hence the TPC may be disrupted, allowing the protein to become available for digestion and absorption in the small intestine (Jones and Mangan, 1977; Barry and Manley, 1984). However, where a higher faecal N is associated with increased CT in the diet (Woodward and Reed, 1997; Ben Salem et al. 1999; Reed et al., 1990) it would suggest, in those instances, that little (if any) dissociation of the TPC does in fact occur.

It is presumed that if CT is released post-ruminally, it will not exert any deleterious effects subsequently, since pH conditions do not allow further reactions with dietary or endogenous proteins (D'Mello, 1992).

The results of Diaz Hernandez et al. (1997) dispute the sole importance of pH in respect of its effects on TPC formation or dissociation. Using a range of purified plant CT (including *A. saligna*) it was shown that the binding of the CT with rubisco (a major protein in forages) occurred only in the pH range 3-7. Above pH 7, almost no precipitation occurred. Once formed, the TPC was shown to be largely unaffected by a wide range of pH (3-9). However, the addition of abomasal and intestinal fluids released about half the protein in the complex. It was concluded that any dissociation of the complex post-ruminally would probably be due to the proteolytic activity in digesta, rather than change in pH per se.

2.4.3 Carbohydrate metabolism

Lascano et al. (1995), as cited by Barahona et al. (1997), showed a negative correlation between dry matter digestibility (DMD) in vitro and the concentration of soluble CT in a range of tropical legumes. A high ruminal pH and low concentrations of volatile fatty acids in the presence of CT in the diet are indicators of depressed rumen fermentation (Silanikove et al., 1996a).

Feeding of high-tannin forage to ruminants can induce a deficiency of rumen-degradable N, thus indirectly impairing the fermentation of structural carbohydrates (D'Mello, 1992). CT not bound to protein can inhibit the fermentation of structural carbohydrates in the rumen by forming indigestible complexes with cell wall carbohydrates, rendering them undegradable. It can form complexes with microbial enzymes, rendering them inactive (Gamble et al., 1996).

Using three browse species i.e. *Adansonia digitata*, *Pithecellobium dulce* and *Calotropis procera*, as supplements to a basal diet of 85% rice straw and 15% peanut cake, Fall Toure et al. (1998) noted a significant negative correlation between in vivo DMD in sheep and total tannin content of the diets. In a similar study, sheep were fed graded amounts of *A. aneura* leaves in addition to a low-quality sorghum stubble diet. The DMD of the diet was inversely related to the level of inclusion of *A. aneura* leaves and this was attributed to its CT content (Elliott and McMeniman, 1987).

Although the majority (i.e. 70-95%) of CT in a number of browse species is soluble, it is the high content of bound CT in some species eg, *A. brevispica*, *F. macrophylla* (Woodward and Reed, 1997; Jackson et al., 1996) and oak (Bederski et al., 1992), with which their low digestibility is primarily associated (see Section 2.3.3 regarding soluble, protein bound or fibre bound CT)Four tree legumes i.e. *A. saligna*, *A. seyal, A. sieberiana* and *S. sesban*, were fed as supplements to a basal diet of teff straw. The digestibility of OM and fibre fractions was lowest for sheep fed *A. saligna,* the supplement with the highest content of bound CT (Reed et al., 1990). It appears that both the bound and soluble CT of *A. saligna* are responsible for its poor utilisation by ruminants (Reed et al., 1990; Reed and Soller, 1987; Degen et al., 1995).

It was the soluble CT that was considered to be most likely responsible for the depressive effects in sheep fed high CT *L. pedunculatus* (Barry and Duncan, 1984). The CT in *L. pedunculatus* were shown to depress the rumen digestion of hemicellulose and readily fermentable carbohydrates, yet increase their post-ruminal digestion (Barry et al., 1986). This suggests that a compromise between positive and negative effects of CT might be obtained at particular CT concentrations in the diet. The CT in *O. viciifolia* did not impair rumen digestion of carbohydrates (Barry and Manley, 1984), neither did the CT in *L. corniculatus* (Waghorn et al., 1987). *O. viciifolia* and *L. corniculatus* contain lower concentrations of CT than *L. pedunculatus*, whereas high levels of soluble CT in *L. pedunculatus* could have impeded microbial rumen carbohydrate digestion (Barry and McNabb, 1999).

Barry et al. (1986) concluded that increasing the soluble CT concentration in *L. pedunculatus* had no effect on the rate at which carbohydrates were degraded in the rumen, but increased the rate at which their undegraded residues left the rumen. The latter appeared to be the principal mechanism by which rumen digestion as a proportion of total digestion was reduced at high dietary CT concentrations.

Makkar et al. (1995b) suggested that the rate of digestion was affected to a greater extent by the presence of tannins, than was the potential extent of digestion.

Jackson et al. (1996) emphasised the need to consider not only tannin levels but also digestibility of the plant. Similarly, Lowry et al. (1996) reported that it is important to distinguish between high-phenolic plants with a composition that is otherwise of high feed quality and high-phenolic plants that are also highly fibrous. For example, browse species with medium concentrations of soluble CT (eg, *Leucaena leucocephala* cv Cunningham) may have relatively high cell wall degradability and thus provide both bypass protein and energy. *F. macrophylla* has a similar level of soluble CT but a very low cell wall digestibility (Jackson et al., 1996), hence its overall nutritive value is much inferior. *A. saligna* and *A. seyal* contain similar high concentrations of soluble CT, however, *A. saligna* contains considerably more bound CT and is less digestible than *A. seyal* (Reed et al., 1990).

Feeding of three browse species to goats indicated that the DMD and OMD of the leaves were not correlated with the content of soluble CT but rather with high lignin content of the cell wall (Goromela et al., 1997). Ndlovu and Nherera (1997) studied seventeen Zimbabwean browse species and found that the presence of polyphenolics was poorly correlated with gas production whilst cell-wall constituents were negatively correlated to the rate and extent of gas production.

The synthesis of CT and lignin in plant tissues involves, to a large extent, common biochemical pathways (Swain, 1979). Consequently plants containing high levels of CT tend also to be highly lignified. Similarly, environmental stresses placed on plants that elevate CT concentrations also tend to elevate lignin content (Barry, 1989).

The poor digestibility of *A. saligna* (fresh or dried) is attributed to its content of CT, fibre bound-N and lignin (Ben Salem et al., 1997; Abou El Nasr et al., 1996). Ben Salem et al. (1997) estimates 20% of total N in *A. saligna* to be bound to fibre.

2.4.4 Palatability and voluntary feed intake

Barry and Duncan (1984) concluded that high concentrations of CT in *L. pedunculatus* depressed metabolisable energy intake of sheep due to depressions in both the voluntary intake and OMD.

Tannins may reduce intake of forage legumes by decreasing palatability or by negatively affecting digestion. Palatability has been thought to be associated with CT concentration (Jones et al., 1976) due to astringency. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. This may increase salivation and decrease palatability (Reed, 1995).

The relationship between CT content and palatability is unclear. Abou El Nasr et al. (1996) and Chriyaa et al. (1997a) suggested that the low voluntary intake of *A. saligna* is an indication of its lack of palatability. Low palatability of most varieties of sorghum is believed to be the consequence of high tannin content (Bate-Smith and Rasper, 1969). Ebong (1995), however, found no difference in the acceptability of three browses ranging from low (*S. sesban*) to high CT content (*A. seyal*). Flowers of *L. corniculatus* and *Hedysarum coronarium* which both contain high concentrations of CT were eaten readily suggesting that high concentrations of CT did not have an adverse effect on palatability (Douglas et al., 1999).

Waghorn et al. (1994) claim that decreased ruminal turnover and rate of digestion was more important than palatability in reducing intake of sheep fed purified diets of *L. pedunculatus* in comparison to sheep fed *L. pedunculatus* with PEG. They based this conclusion on the observation that sheep selected leaves over stems, although the leaves were higher in CT. Also, saliva production was not different between treatments.

The general conclusion appears to be that low dry matter digestibility (DMI) is principally associated with the inhibitory effects of the high CT on digestion (Chriyaa et al., 1997a; Odenyo et al., 1997; Degen et al., 1997; Degen et al., 1995; Reed et al., 1990) rather than palatability associated with CT.

2.4.5 Effects of condensed tannins on animal production

The CT have been reported to have both positive and negative effects on animal production.

The CT in *H. coronarium* (medium CT) were associated with a superior rate of wool growth in sheep, apparently due to increased post-ruminal supply of amino acids, compared to those grazing the same pastures but dosed daily with PEG (Terrill et al., 1992a). In contrast, using *H. coronarium* with a high concentration of CT, a negative effect of CT on nutrient supply to lambs was demonstrated by Douglas et al. (1999). The high CT content in *L. pedunculatus* was shown to have a negative influence on wool growth and live weight gain, with both parameters being superior in sheep dosed daily with PEG (Barry, 1985).

The live weight gain and wool growth by sheep fed *A. aneura* were improved significantly when supplemented with PEG, thus implicating CT as the primary inhibitory factor on animal performance (Pritchard et al., 1992; Pritchard et al., 1988).

The CT in *L. corniculatus* did not affect wool growth, live weight gain or carcass characteristics (Douglas et al., 1999), contrary to other reports with *L. corniculatus* (Waghorn and Shelton, 1997; Douglas et al., 1995), Contrasting outcomes are likely to be due to differences in CT content as indicated by Douglas et al. (1999).

Low growth rates (or loss of body weight) and low intakes have been observed in animals eating leaves of *A. saligna* (fresh or dried), either as a sole diet or as an ad libitum supplement to straw. The negative effects were due to a combination of reduced intake and low digestibility of nutrients, attributed mainly to the high CT content (Chriyaa et al., 1997b; Degen et al., 1997; Abou El Nasr et al., 1996; Degen et al., 1995). Similar effects were observed in sheep and goats fed dried *A. salicini*, another shrub with a high CT content (Degen et al., 1997).

Foliage from *A. saligna* was dried and 330 g/d offered (not all was eaten) with ad libitum teff straw to yearling intact male sheep for 91 days (Reed et al., 1990). The sheep lost weight throughout the trial even though their N intake was greater and their total intake similar to sheep whose live weight increased when fed teff straw plus 1% urea. In contrast, no significant difference in weight gain was evident between treatments when sheep were given *A. seyal*, compared with those supplemented with noug cake (an oilseed mal from the niger plant, *Guizotia abyssinica*) or urea. The different responses to *A. seyal* and *A. saligna* were largely attributed to the differences in phenolic content of the species. Both had similar high levels of soluble phenolics but *A. seyal* had a much lower level of bound CT. Hence *A. seyal* is likely to be digested to a greater extent than *A. saligna*, with a greater extent of N recycling to the rumen, as was indicated by the higher excretion of microbial and endogenous N in the faeces of sheep that consumed *A. seyal.*

Improved production may be expected when feeds containing medium to high levels of CT are combined with readily degradable, high protein feeds. Readily degradable feeds can result in ammonia being released in the rumen at a rate that exceeds the capability of the microbes to utilise it in the synthesis of microbial protein. By including in the diet, a high-tannin feed of lower protein content, the protein solubility of the total diet may be reduced, increasing the availability of bypass protein and possible production (Nsahlai et al., 1999), assuming the TPC dissociates post-ruminally.

2.4.5.1 Bloat

Bloat is caused by a very high solubility of forage proteins leading to the formation of a stable foam in which fermentation gases are entrapped (Mangan, 1988)]. CT-containing legumes are known to eliminate bloat because of their protein-precipitating properties (Jones et al., 1973).

Bloat occurs readily when ruminants graze low-tannin pasture legumes such as alfalfa and clover, but plants such as *O. viciifolia* and *Lotus* are considered safe in this respect due to their CT content (Jones and Mangan, 1977; Mangan, 1988).

A minimum plant CT concentration (about 0.5% of DM, according to Li et al. (1996)) is required to render the plant 'bloat-safe'. Most common legumes and grasses used in temperate agriculture contain only trace amounts of CT, well below the minimum required (Barry and McNabb, 1999).

2.4.5.2 Protozoa

Ben Salem et al. (1997) found that the supplementation of a lucerne hay based diet with graded amounts of *A. saligna* caused a linear decrease in the concentration of protozoa in rumen fluid. Odenyo et al. (1997) tested 5 browse species including *A. saligna* as a supplement to maize stover. The inclusion of *A. saligna* decreased protozoal numbers but had no effect on fibre degradation. It was not clear whether this effect was due to direct toxicity on protozoa or insufficient nutrients, perhaps resulting from tannin complexes or reduced DM digestibility.

2.5 Proline-rich proteins - a defense mechanisms against dietary tannins

During mastication, CT bind with proline-rich proteins (PRP) in saliva, in preference to other proteins, including dietary proteins (Hagerman and Butler, 1981). The tannin-PRP complex is claimed to resist endogenous or microbial enzyme attack in the digestive tract (Robbins et al., 1987). The indigestible tannin-PRP complex passes through the digestive tract and is excreted in the faeces (Austin et al., 1989). The PRP were found to contain very low levels of essential amino acids (Mole et al., 1990) hence the advantage of their excretion rather than excretion of other TPC (Robbins et al., 1991).

Saliva from mule deer, a browser, was found to be more proline rich than the saliva of sheep, and had a greater tannin-binding capacity (Robbins et al., 1987). This suggests that the production of PRP is an adaptation to provide a means of defense for deer against CT. Domesticated sheep and cattle do not appear to produce CT-binding proteins in their saliva. However, it cannot be concluded that they are unable to produce these proteins as perhaps a longer period of, or increased exposure to tannin is necessary to induce their production (Austin et al., 1989).

In some cases the PRP can be highly specific and only bind the type of CT that is present in the normal diet while not binding other types of CT (Hagerman and Robbins, 1993).

Robbins et al. (1991) found that digestibility of plant fibre by sheep was reduced significantly when quebracho tannin (a complex mixture of CT and non-tannin phenolics) was added to their basal diet. The sheep did not produce tannin-binding salivary protein, in contrast to mule deer that did produce tannin-binding salivary protein, thus reducing faecal-N losses per unit of ingested tannin.

Goats are known for their selection of browse species in preference to pasture species (Kababia et al., 1992). CT are widely distributed in the leaves of trees and shrubs, but only occur in the leaves and stems of a small number of herbaceous legume plants (Barry, 1989). One might therefore expect that goats would have a higher tolerance to CT than species such as sheep that prefer to graze pasture. However, goats do not appear to produce PRP in saliva as a defense against tannins. Instead they appear to detoxify tannins or their degraded products (Distel and Provenza, 1991) with active tannase in the rumen (Begovic, 1978, as cited by Kumar and D'Mello, 1995).

2.6 Manipulation of condensed tannin concentration to improve the efficiency of rumen digestion

Barry (1989) emphasised the need to define the concentration of forage CT that will improve the efficiency of N utilisation without depressing rumen fibre digestion and feed intake. The optimum concentration of CT for the digestion of fresh legume diets by ruminants is likely to be highly dependent upon the molecular weight and reactivity of the CT present in the plant (Barry and Manley, 1984), as well as the protein and energy composition of the forage.

By varying the amount of PEG fed to sheep on a diet of *L. pedunculatus*, Barry et al. (1986) observed that an increasing CT concentration caused linear increases in postruminal plant protein digestion but caused linear reductions in the apparent digestibility of plant DM. They subsequently defined a range in which the level of CT represented a nutritionally beneficial balance between improving the efficiency of N digestion and depressing ruminal carbohydrate digestion.

Low levels of CT (1-2% of DM) in temperate legumes of relatively high digestibility (eg, *Lotus*) may be nutritionally beneficial where protein requirements of the animal are not met with basal forage diets (Terrill et al., 1992a). Miller and Ehlke (1994) demonstrated that CT in *L. corniculatus* may reduce ruminal protein degradation with little or no corresponding reduction in DM digestibility. Low concentrations of CT in *L. corniculatus* have been shown to increase the quantities of essential amino acids absorbed from the small intestine of sheep without affecting voluntary intake (Waghorn et al., 1987).

Feeding high tannin browse species *A. digitata* or *P. dulce* as supplements to a basal diet of 85% rice straw and 15% peanut cake, Fall Toure et al. (1998) determined the optimum level of browse inclusion for each species for maximum DM digestibility i.e. 15-30% for *A. digitata* and 50% for *P. dulce*, corresponding to DM digestibility of 47.1% and 51.3%, respectively.

In sheep fed 700 g DM/d lucerne hay Ben Salem et al. (1997) noted that the inclusion of 75 or 150 g DM of *A. saligna* had no effect on nutrient digestibilties, whereas with further levels (>150 g/d) nutrient digestibilities were substantially reduced. Although perhaps feasible in a cut and carry system, controlling the proportion of browse in the diet in a paddock situation would undoubtedly prove difficult.

Using CT extracted from *L. pedunculatus* as the standard, Jackson et al. (1996) suggested that browse containing total CT levels of 5% of DM could be used as browse or fed in medium quantities of the diet in supplementary cut and carry systems. If CT levels are in excess of this level then they should perhaps be fed as supplements at low proportions to dilute the CT concentrations.

Most of the CT in *G. sepium* is bound within its leaves. It has been shown to be a useful supplement (up to 30% daily intake) for increasing animal production where low protein feeds are fed in the tropics (Preston and Leng, 1987).

Perhaps it is reasonable to determine 'beneficial' levels of CT in each given set of circumstances but because so many variables are involved, even within the one plant species, claiming beneficial levels beyond specific feeding applications may well be inappropriate. One might simply concede that too much CT is harmful, that too little is of no benefit and that somewhere 'in between' is useful. The most appropriate generalisation regarding CT and ruminant nutrition might be that a balance is necessary between the positive effects of CT in improving the efficiency of N digestion and suppressing bloat (see Section 2.4.5.1) and their negative effects in depressing N balance and rumen fermentation of structural carbohydrates (Barry et al., 1986).

2.7 Detannification

A number of methods of detannification has been investigated. Although a degree of success has been achieved, widespread application has often been hindered by factors such as practical limitations or economic viability (Miller et al., 1997).

2.7.1 Polyethylene glycol

Tannins bind to PEG in preference to protein (Jones and Mangan, 1977). PEG, particularly that of molecular weight 4000 (PEG 4000) has been widely used in the studies of tannin-rich forages eg, Ben Salem et al. (1999); Degen et al. (1998); Barahona et al. (1997); Miller et al. (1997); Silanikove et al., (1997); Pritchard et al., (1992).

Using the in vitro gas production technique, inclusion of PEG in samples with tannin, increased gas production, indicating a binding of tannins by the PEG. At near neutral pH values, in vitro, PEG 6000 was found to have a superior capacity to bind with tannins than PEG of various other molecular weights, including 4000 (Makkar et al., 1995a).

PEG has been applied by spraying onto tannin-rich browse, infusion into the rumen and by oral drenches (Ben Salem et al., 1999; Pritchard et al., 1992; Terrill et al., 1992a).

Degen et al. (1998) observed an increase in DMI and live weight gain in lambs consuming a diet of *A. saligna* and supplemented with PEG. Ben Salem et al. (1999) showed that spraying of *A. saligna* leaves with PEG improved the digestibilities of CP and fibre and increased N retention and live weight gain of sheep, although it did not increase DMI.

Positive responses to supplementation with PEG including increased DMI, digestibility, wool growth and live weight gain (or reduced live weight loss) have been shown in numerous studies involving tannin-rich species such as *A. saligna* (Degen et al., 1998), *F. macrophylla* (Barahona et al., 1997), *Quercus calliprinos* (Silanikove et al., 1996a), *Pistacia lentiscus*, *Ceratonia siliqua* (Silanikove et al., 1994), *A. aneura* (Pritchard et al., 1988), and *L. pedunculatus* (Barry and Duncan, 1984). The use of PEG has been associated with increased concentrations of ruminal ammonia and volatile fatty acids (Silanikove et al., 1996a), when included in a tannin-rich diet.

F. macrophylla and *Desmodium ovalifolium* had similar, high concentrations of soluble CT. PEG, sprayed onto both species after having been chopped and sun-dried, reduced concentrations of soluble CT in the leaves and stems. Increases in total forage intake in response to PEG were accompanied by increases in fibre digestibility (Barahona et al., 1997).

Silanikove et al. (1997) recommended that supplementing tannin-rich leaves with high protein supplements should be done in combination with PEG, otherwise a considerable portion of the protein supplement will be wasted due to interaction with tannins. Although PEG is undoubtedly a useful tool for studying the effects of CT under trial conditions, economic factors may well inhibit its commercial application, in which case alternative, less costly compounds should be investigated.

2.7.2 Pretreatment with chemicals

Significant reductions in tannin levels have been achieved with treatments involving moist, alkaline conditions. This has been shown with high-tannin sorghum grain (Price et al., 1979; Waichungo and Holt, 1995) and oak leaves (Makkar and Singh, 1992).

Wah et al. (1977) induced a 100% reduction in assayable tannin in salseed meal after treatment with moisture and 4% CaO. Similarly Price et al. (1979) reduced the amount of tannin in ground high-tannin sorghum grain by 99% by treating with moisture and 5% CaO. The reduction was almost instantaneous. CaO can be fed to animals and is therefore worthy of investigation for potentially increasing the use of high tannin browse species. (Kahn)Significant reductions of tannins in salseed meal were also achieved by treating with urea (3, 4 and 5% urea w/v) and storing in moist conditions in sealed polythene bags for 0-4 weeks (Bhakt et al., 1993). Treatment of high moisture sorghum with urea increases grain pH due to hydrolysis of urea to ammonia and has been shown to be effective in preservation of stored grain. Russell and Lolley (1989) showed that under conditions where urea is an effective preservative of high moisture sorghum, CT is deactivated completely.

Temperature and moisture were found to have an important role in the inactivation of tannins where oak leaves were stored. After five days of storage in the presence of 4% urea and high moisture there was a substantial decrease in the levels of total tannins, CT and protein PPC. Beyond five days of storage DM digestibility declined, possibly due to higher lignification and other changes in cell-wall constituents (Van Soest, 1982, as cited by Makkar and Singh, 1993). Longer periods of storage, although further reducing tannin content could therefore offset the advantage of detannification (Makkar and Singh, 1993).

Ferric chloride (FeCl) reacts with phenolic compounds in alkali to form complexes (Wesp and Brode, 1934, as cited by Hagerman and Butler, 1989). By storing sal-seed meal with 1% FeCl, tannin levels can be significantly reduced. Significant reductions of tannins in salseed meal can also be achieved by treating with ferrous sulphate ($FeSO_4$) (Bhakt et al., 1993). Tannin levels in oak leaves were reduced by 85% by treating with $FeSO_4$ (Makkar and Singh, 1992).

Makkar and Singh (1992) reduced tannin levels in oak leaves by up to 70% by treating with various organic solvents. By treating with the oxidising agents $KMnO_4$ and $K_2Cr_2O_7$, tannin levels were reduced by approximately 95%.

Although various chemical treatments have been shown to be effective in reducing tannin in feeds, cost and labour requirements make their use impractical or uneconomic.

2.7.3 Drying

Ben Salem et al. (1997), as cited by Ben Salem et al. (1999) found that when fed with a good quality roughage such as lucerne hay, field drying of *A. saligna*, compared with fresh foliage, increased the DMI of *A. saligna* by sheep. However, it did not significantly affect digestibility or ruminal fermentation. In another study, Ben Salem et al. (1999) found that although drying of *A. saligna* foliage slightly reduced its CT and increased the DM and OM digestibility, its influence on DMI (as a supplement to 400 g/d/h barley) in growing sheep was negligible. The live weight gain was not significantly different ($P > 0.05$) between those fed fresh or dried *A. saligna* foliage.

Terrill et al. (1989) observed that field-drying of high-tannin *Lespedeza cuneata* decreased its assayable tannin concentration resulting in improved intake and increased N and fibre digestibility. Low-tannin *L. cuneata* did not show similar effects.

Oven-drying at 60°C markedly reduced the tannin content of *Calliandra* and *Gliricidia* and when fed as supplements to straw, they significantly increased the voluntary consumption of straw, increased DM digestibility, decreased faecal N excretion and increased N balance (Ahn et al., 1997).

Oven-drying at 50°C caused a reduction in tannin of *A. aneura*, *A. angustissima*, *A. chinensis* and *Calliandra calothyrsus* (high CT) thus improving N utilisation in the rumen (Ahn et al., 1989). However, Palmer and Schlink (1992) reported that fresh *C. calothyrus* was more rapidly digested than wilted, dried or freeze-dried material and recommended the use of fresh forages for their maximum utilisation.

Wilting or drying of oak leaves had no effect on removal/inactivation of tannins. Drying conditions also had no effect on the contents of fibre, fibre-linked tannins, cellulose and lignin (Makkar and Singh, 1991). Robbins et al. (1987) found that a substantial fraction of phenolic/tannin compound remained soluble after drying, and relative differences in protein digestion among forages were not changed greatly by drying.

Conflicting reports on drying and its inactivation of tannin may be due to differences in initial moisture levels in the leaves and the different chemical nature of tannins (Makkar and Singh, 1991). Similarly, moisture plays an important role in the damaging effect of heating to forage constituents (Van Soest, 1965, as cited by Makkar and Singh, 1991).

2.7.4 White rot fungi and bacteria

White rot fungi are well documented in their ability to degrade lignins (Kerem et al., 1992; Rolz et al., 1986). Lignins are a group of polyphenols related to tannins (Swain, 1979) hence the investigations of using white rot fungi to degrade CT.

The fungus *Sporotrichum pulverulentum* effectively degrades CT in *Quercus. incana* leaves but not without a substantial removal of rumen digestible carbohydrates (Makkar et al., 1994). *Ceriporiopsis subvermispora* and *Cyathus stercoreus* have been shown to preferentially degrade the lignin component of plant cell walls thereby improving the digestibility of the residual plant fibre (Akin et al., 1995). Using leaves of *S. lespedeza* Gamble et al. (1996) were able to show that both of these fungi are also capable of degrading CT.

Research on white rot fungi has been based on growing the fungi on sterilised plant material. Until it can be cultured successfully on living plants it has limited practical application, other than perhaps treating of harvested leaves.

Studies have identified the ability of some rumen microbes to degrade HT (Nelson et al., 1995; Murdiati et al., 1992; Osawa and Mitsuoka, 1990). Skene and Brooker (1995) isolated a *Selenomonas* species which is able to degrade CT. Others, although not active in the degradation of CT, are able to tolerate high concentrations of CT eg, *Streptococcus caprinus*, found in feral goats consuming *A. aneura* (Brooker et al., 1994). Rumen fluid, containing *S. caprinus*, was transferred from feral goats to sheep. It was apparent that this improved the utilisation of *A. aneura* leaves by the sheep (Miller et al., 1995). However, inoculation of sheep by pure cultures of *S. caprinus*, grown in vitro, was ineffective in improving their digestion of *A. aneura* (Miller et al., 1996).

The manipulation of rumen microbial populations has the potential to improve the utilisation of high tannin feeds but more research in this area is needed.

2.8 Nutrient supplements

The use of various nutrient supplements have been shown to be useful in counteracting the antinutritional effects of CT when present in stock feeds.

2.8.1 Nitrogen

Adequate ammonia levels in the rumen are essential for efficient microbial activity (Leng, 1992). If ammonia levels decline below the threshold of 50 mg/L, microbial growth and thus rumen fermentation will be compromised (Satter and Slyter, 1974). Under these conditions supplementation with NPN such as urea, or other sources of rumen degradable protein is likely to be advantageous (Leng, 1992).

If a significant amount of dietary protein becomes complexed with CT, rumen ammonia levels may decline below 50 mg/L, therefore reducing microbial activity and consequently microbial protein synthesis. If there is surplus CT the protein supplement may still not overcome the problem as the surplus CT might complex with the supplemental protein.

The protein-binding capacity of tannin-containing leaves may considerably exceed the protein content in the leaves. Thus, tannins ingested with the browse may also affect the protein utilisation of supplementary dietary protein (Silanikove et al., 1997), (hence the advantage of a supplement NPN with which CT do not bind). To reduce the likelihood of an N deficiency when feeding high tannin feeds Barry and McNabb (1999) recommended combining the CT-containing forage with feed that has low levels of CT but a higher protein content.

Both urea and cottonseed meal supplements have been shown to significantly increase the intake and live weight gain of sheep consuming an *A. aneura* diet supplemented with molasses and phosphorus (McMeniman et al., 1981; McMeniman, 1976). However, this response was not necessarily through any effect on CT.

Goodchild and McMeniman (1994) fed graded amounts of *A. aneura* as a supplement with sorghum stover, with or without urea. The differences in intake between sheep supplemented with urea and those not supplemented, decreased as the browse level increased. McMeniman et al. (1981) emphasised the need to clarify when a non-protein N (NPN) supplement is beneficial to sheep consuming an *A. aneura* diet. For example, the digestibility of the diet should be considered as the more indigestible the diet, the less the intake response after elevation of ruminal ammonia concentration (Hunter and Siebert, 1985).

2.8.2 Energy

High tannin feeds can limit protein availability and therefore animal production by forming TPC. If N availability is improved with supplements such as PEG or urea, animal productivity may then be limited by the energy content of the high tannin feeds (Pritchard et al., 1992).

Fassler and Lascano (1995) recommended combining tannin-free, highly digestible legumes with tannin-containing legumes. Such a combination could yield benefits due to protein protection from ruminal degradation, while also providing adequate energy.

Craig et al. (1991) claimed that a number of *Acacia* species would be inadequate as sole diets for ruminants because they would provide insufficient energy for sheep, due to their low digestibility that was primarily associated with high lignin content. Abou El Nasr et al. (1996) recommended *A. saligna* be fed with an energy supplement such as barley grain. Dumancic and Le Houerou (1980) found that sheep could at least maintain body mass grazing on rangeland for 79 days from the end of summer, with access to an *A. saligna* plantation and supplemented with barley grain.

It is necessary to understand which nutrients are limiting when feeding high tannin feeds in order to determine appropriate supplements. In some instances it may be appropriate to include a supplementary source of dietary N or energy or both. In addition, there needs to be an understanding concerning the benefits of increasing nutrient supply directly and achieving this through effects on CT.

2.8.3 Minerals

Several trials have reported an increase in animal production when sheep given a basal diet of tannin-rich browse were supplemented with minerals eg, Gartner and Niven (1978); Entwistle and Baird (1976); McMeniman et al. (1981). These indicate that the content of some minerals in the browse may be either insufficient or that their availability is inhibited.

Phosphorous is considered to be the mineral most deficient in browse fodder (Dann and Low, 1988). In the pen trial of McMeniman (1976) DMI by sheep fed *A. aneura* was increased when supplemented with phosphorous, although they still lost weight, indicating inadequate nutrient supply. The DMI response was further enhanced with a molasses (which is high in energy and sulfur) supplement and sheep gained weight.

Sulfur is a component in the essential amino acids methionine and cysteine/cystine. Sulfur is also essential for the synthesis of microbial protein. Its utilisation is interrelated with the utilisation of copper and molybdenum (Haryanto and Djajanegara, 1993). Most sulfur in plants is present in protein as sulfur-containing amino acids. Therefore, when plants contain high levels of CT, both amino acids and sulfur availability may become restricted for metabolisable protein synthesis (Gartner and Hurwood, 1976).

Entwistle and Baird (1976) observed a significant increase in DMI by sheep consuming *A. aneura* plus phosphorous, when supplemented with molasses. However, because the greatest response was with the first 50 g of molasses they suggested the sulfur component in molasses to be largely responsible, as the energy component at such a level would likely to have been insignificant.

Gartner and Niven (1978) noted a response in sheep fed *A. aneura*, when supplemented with various sulfur supplements. However, although each supplement provided similar amounts of sulfur, the response was greatest with the 50 g DM molasses supplement. This implied that an element other than sulfur contained in the molasses induced the additional response, possibly cobalt as *A. aneura* is marginal in this element. Results of McMeniman et al. (1981) supported those of Gartner and Niven (1978). However, their results indicated that the additional response to molasses was not due to cobalt although they admit that the trial may have been conducted over too short a period to induce a deficiency.

DMI, live weight gain and wool growth improved when sheep given *A. aneura* were supplemented with PEG. These measures were further increased when nitrogen, phosphorous and sulfur (NPS) were included as a supplement, in conjunction with the PEG. NPS alone did not significantly alter DMI but reduced live weight loss (Pritchard et al., 1992).

Calcium and sodium have been implicated in the improved productivity of sheep consuming *A. aneura*, when supplemented with calcium sulphate or sodium sulphate (Hoey et al., 1976; Gartner and Niven, 1978).

2.9 Conclusion

There is a lack of uniformity in the way in which information concerning tannins is presented in the literature. Often there is a reference to tannins in general, without distinguishing between CT and HT. Furthermore, where reference is made to CT, there is often no differentiation between bound or soluble CT, and no mention of its PPC. Greater detail would enable more useful interpretation of the information in the literature.

If plant species containing CT are to be utilised successfully as sources of feed for ruminants then a greater understanding of the factors that influence CT content and activity within a plant species is necessary. This, together with knowledge of the interaction between CT levels and nutrients in animal feeds, would assist in the development of management strategies.

The following chapters describe three pen trials that were conducted in order to gain some understanding of the value of *A. saligna* as a source of fodder for ruminants. It is hoped that they will provide a basis for further research of this particular species.

Section 3: Trial 1

3.1 Introduction

A. saligna, a native to Western Australia, has been widely acknowledged as a useful species for land conservation. More recently, there has been a focus on *A. saligna* as a potential source of fodder for ruminants. A common conclusion of researchers is that the presence of CT is the primary factor inhibiting its value as a feed source. Its poor digestibility is attributed mostly to CT, but also to fibre bound-N and lignin.

Most research of *A. saligna* tends to involve plant material grown in arid/semi-arid regions. However, it is also known to grow prolifically in areas of higher rainfall eg, south west Western Australia where annual rainfall can exceed 1000 mm and in climates ranging from cool to tropical.

If the limitations to *A. saligna* being a worthwhile source of fodder for ruminants could be overcome then it could serve a dual role of conservation and animal feed. If so, the implications concerning its incorporation into agricultural systems ought to be significant.

The aims of Trial 1 were:

- To evaluate the value of *A. saligna* as a sole source of nutrients for sheep.

  In many parts of rural Australia, particularly in autumn, supplementary feeding of stock is necessary to address the feed deficit in the paddock. If incorporated into the farming system, it may be that the foliage from the *A. saligna* provides almost the entire feed for the grazing ruminant, with available pasture being negligible. In such a scenario, it would be reassuring to know that the *A. saligna* was providing adequate nutrients to the animals.

- To evaluate the effect that partial detannification of *A. saligna* might have on its value as a source of nutrients for sheep.

It is evident from a number of studies that where a high tannin feed is the main source of fodder, detannification is beneficial in terms of nutrient availability to the ruminant.

- To compare the effects of using either PEG 4000 or PEG 6000 as a detannification agent in vivo.

  PEG 4000 appears to be the major detannification agent used in trials involving high tannin feed despite the fact that PEG 6000 has been shown to be more effective, in vitro. For this reason it was of interest to compare the two, in vivo.

3.2 Materials and methods

The feeding trial was conducted during April-June 1999.

3.2.1 Housing and care of animals

Approval was received from Curtin University of Technology Animal Experimentation Ethics Committee prior to the commencement of each trial.

Each feeding trial involved six mature Merino wether sheep. All animals were fitted with a permanent rumen cannula.

Each animal was housed in a metabolism cage that facilitated the separation of faeces and urine. Prior to the commencement of each feeding trial the animals were treated for internal parasites.

Rumen cannulae were inspected daily. The condition of the animals was monitored regularly.

3.2.2 Experimental design

The experiment was based on a Latin square design. Animals were randomly allocated to one of three dietary treatments. Because each trial consisted of 3 dietary treatments, each trial was comprised of 3 sets of sampling periods.

Each of the three experimental periods was of 21 d duration, made up of 13d for diet adaptation followed by 1 d of sampling of ruminal fluid and 7 d of recording of feed intake and faecal and urinary output.

3.2.3 Harvesting of *A. saligna*

Once a week, *A. saligna* was lopped from a three year old plantation (grown directly from seed, sown in 1996). Only foliage less than 12 months old was used. The plantation was located on a commercial farm at Gidgegannup, approximately 50 km north east of Perth, Western Australia. The climate of the area is described as Mediterranean with an average annual rainfall of 923 mm (P. Hanson, Agriculture Western Australia, personal communication 2001). The soil in which the *A. saligna* was growing may be described as sandy gravel (Moore 2001).

After harvest, material was stored at -18°C pending feeding.

During the adaptation period branches of *A. saligna* were removed from the freezer room, tied together and hung up for its provision to the animals. However, throughout the 7 d sampling period each morning's feed consisted of leaves only. On d 1 of each sampling period, a random sample of approximately 1 kg (fresh weight) of leaves was collected and stored at -18°C, pending chemical analysis.

3.2.4 Treatments

Three treatments were used:

1. Control: ad libitum access to *A. saligna* (basal diet);
2. PEG 4000: basal diet + 25 g/d PEG 4000;
3. PEG 6000: basal diet + 25 g/d PEG 6000.

Where PEG was used it was dissolved in water (1:1 w/v) and administered as an oral dose immediately prior to feeding.

The content and biological activity of CT within the *A. saligna* was unknown prior to the trial (as the appropriate methodology had not yet been developed). Therefore, it was not possible to determine the extent of detannification that might occur with any particular level of PEG administered. Therefore, the dose rate was based on rates used by Silanikove et al. (1994) with the expectation that partial detannification would occur and some benefits of its use would become apparent.

Ideally the content and biological activity of the CT within the *A. saligna* together with the live weights of the animals should have been measured to determine the appropriate dose rate.

3.2.5 Experimental data

3.2.5.1 Feed and water intake

Animals had free access to fresh water which was recorded daily during the sampling period.

During the sampling period, animals were fed every morning, following the collection of faeces and urine. Feed intake was determined by subtracting the DM weight of daily feed refusal from the amount of feed offered (DM weight). All refusals of *A. saligna* from each animal was collected daily and stored at -18°C pending oven drying at 55°C for determination of DM of individual samples and therefore DMI of individual animals.

3.2.5.2 Faecal and urine sample collection

The weight of faeces excreted from each animal was measured, after which a 10% aliquot of each animal's daily faecal output was stored at -18°C pending oven drying at 55°C. After drying, samples were then pooled for each animal and ground through a 1 mm screen and stored for later analysis (OM and N).

Urine from each animal was collected into 10 mL (37%) HCl. The amount voided was recorded and a 10% aliquot of each animal's daily urine output was collected and stored at 4°C. At the end of each sampling period urine samples were pooled for each animal and stored at 4°C for N analysis.

3.2.5.3 Ruminal fluid sampling

Samples of ruminal content were obtained via the rumen cannula, just prior to feeding and thereafter, at approximately 3 h intervals for 24 h using a sampling suction probe. Fluid was strained through a 100 μm sieve and then pH was measured. The pH meter was calibrated prior to each analysis using buffer solutions of pH 7 and 4.

Sixteen mL of strained ruminal fluid were placed in specimen bottles containing 0.2 mL of 18M H_2SO_4. The samples were then stored at -18°C pending chemical analyses.

Four mL of strained ruminal fluid were added to specimen containers containing 16 mL formal saline solution (0.9% NaCl, 4% formaldehyde). These were further filtered to remove feed matter, through 2 layers of stocking material, and stored at room temperature for counting of protozoa.

3.2.6 Analytical procedures

3.2.6.1 Dry matter content

Samples of *A. saligna* foliage were dried in a forced-air oven at 35°C until constant weight to determine DM contents. Because the analyses of the samples included determination of tannins, the samples were dried at 35°C to minimise the loss of tannins through excessive heat (H. Makkar, personal communication 1999). The samples were then ground through a 1 mm screen, a 200 g subsample from each was taken and combined into a single sample and stored at room temperature pending chemical analyses.

Feed refusals and faeces were dried in a forced-air oven at 55°C until constant weight was reached and DM determined. Where applicable, the weight of PEG was subtracted from the faecal weight in determining DMD. All determinations were carried out in duplicate.

3.2.6.2 Ash and organic matter

Duplicate samples of approximately 5 g (known weight) of dried material (combined samples of feed and faeces, respectively) were ignited in a muffle furnace at 550°C for 6 h. Following removal from the furnace the residue was placed in a desiccator, cooled and then weighed. The ash content was expressed as a percentage of the original sample DM weight and the organic content (OM) calculated.

3.2.6.3 Crude protein

Crude protein was determined for duplicate samples of known weight (approximately 1 g) of combined samples of feed and faeces, respectively. Total N was determined using the Kjeldahl oxidation procedure (Mossberg, 1979).

The N content was multiplied by 6.25 to give the CP content, expressed as a percentage of the DM.

3.2.6.4 Ammonia concentrations

Ruminal fluid was centrifuged (3000 g for 10 min) and analysed for ammonia concentrations. The procedure used an automated segmented flow technicon instrument and was based on the modified Berthelot reaction (Searle, 1984).

After every 22 samples, 2 samples were repeated and 2 controls were read with the standard curve determined after every 25 readings. The standard curve was developed from standards of 0.00, 0.25, 0.5, 1.25, 2.5, 5, 10 mg/L NH_4, using the stock standard solution of $(NH_4)_2SO_4$ (500 mg/L NH_4). Most samples were diluted with deionised water (1:10 or 1:100) to enable their reading within the standard curve.

3.2.6.5 Protozoa

Using a 1 mL pipette, ruminal fluid was placed on the counting chamber. Protozoa were counted using an objective lens with a 40 X magnification. Two fields were counted. Using the chamber specifications (volume = 1000 mm^3, depth = 0.2 mm, area = 16 mm^2), calculations were made as follows:

No. of protozoa per mL ruminal fluid = average count / v x d x a.

3.2.6.6 Tannins

Total extractable phenolics, CT and PPC were analysed according to the methods described in (FAO/IAEA, 2000).

3.2.7 Statistical analysis

An analysis of variance was carried out using the Genstat statistical package. Where there was censored data i.e. < x (eg, ruminal ammonia < 2.75 mg/L), that data has been assumed to = x (eg, ruminal fluid = 2.75 mg/L). The analysis of variance included calculated values for missing data as well as the raw data values for attributes. The standard deviations, however, were calculated using raw data only.

3.3 Results

The nutrient analysis of *A. saligna* foliage is shown in Table 3.1. The value for metabolisable energy has been obtained from a source of *A. saligna* other than in the present trial, therefore may not represent the true value of this trial.

Table 3.1 Composition of *A. saligna* foliage

DM (g/kg As fed)	350
OM (g/kg DM)	927
ME (kJ/kg DM)	5.1[1]
CP (g/kg DM)	114
Total extractable phenolics[2] (g/kg DM)	94.5
Condensed tannins[3] (g/kg DM)	26.9
PPC[4] (%DM)	0.044

[1] Degen et al. (1997) *

[2] as tannic acid equivalent

[3] as leucocyanidin equivalent

[4] as tannic acid equivalent

The DMI of *A. saligna* (Table 3.2) was greater ($P < 0.05$) in sheep supplemented with either PEG 4000 or PEG 6000 compared to the control. However, the difference in intake was not significant ($P > 0.05$) between those supplemented with either PEG 4000 or 6000.

Table 3.2 Intake and digestibility of *A. saligna* offered to sheep with or without a supplement of PEG 4000 or PEG 6000

	Treatment			
	Control	PEG 4000	PEG 6000	Significance
DMI (g/d)	187[a]	499[b]	463[b]	*
	(57)	(101)	(88)	
DMD (%)	31.3[a]	36.8[b]	37.8[b]	**
	(7.9)	(9.1)	(7.7)	
OMD (%)	30.4[a]	32.1[b]	33.0[c]	**
	(7.8)	(9.1)	(8.3)	

*P < 0.05; **P < 0.01.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

The DMD of *A. saligna* foliage was not different ($P > 0.05$) between animals supplemented with PEG 4000 or PEG 6000 but both of these groups were greater ($P < 0.01$) than the control group.

The OMD was significantly different ($P < 0.001$) between all treatments with PEG 6000 being the greatest, followed by PEG 4000 and the control group.

Animals were not weighed throughout the trial. However, a loss in body condition was obvious, in particular in the control group. One animal had to be withdrawn from the control treatment due to inappetance.

There was no significant difference ($P > 0.05$) between the treatment groups in terms of their water intake, being 0.68, 0.80 and 0.83 L/d for the control, PEG 4000 and PEG 6000 groups, respectively.

Intake of N (Table 3.3) was greater ($P < 0.05$) in sheep supplemented with either PEG 4000 or PEG 6000 than the control. The difference in N intake was not significant ($P > 0.05$) between those supplemented with either PEG 4000 or PEG 6000.

Table 3.3 Nitrogen intake and balance in sheep offered *A. saligna* with or without a supplement of PEG 4000 or PEG 6000

	Treatment			
N (g/d)	Control	PEG 4000	PEG 6000	Significance
N intake	3.4[a]	9.1[b]	8.4[b]	*
	(1.03)	(1.83)	(1.61)	
Faecal N	4.8	7.1	6.3	NS
	(0.86)	(2.63)	(1.76)	
Urine N	3.1	2.5	2.4	NS
	(0.41)	(0.57)	(0.61)	
N balance	-4.5[a]	-0.5[b]	-0.3[b]	**
	(0.52)	(1.00)	(0.87)	

*P < 0.05; **P < 0.01; NS, not significant.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

There were no significant differences ($P > 0.05$) in either the faecal or urinary N output between any of the treatment groups. High standard deviations eg, faecal N, PEG 4000 group, suggests possible significant differences between animals within the treatment.

All treatment groups were in negative N balance. N balance was not significantly different between animals supplemented with PEG 4000 or PEG 6000 but both of these groups were different ($P < 0.01$) to the control group.

Neither the average nor maximum pH of ruminal fluid of the control group was different ($P > 0.05$) to those supplemented with PEG (Table 3.4). However, the minimum pH for the control group was significantly higher ($P < 0.05$) than for either of the PEG treatments. The pH measurements for the PEG groups were the same ($P > 0.05$).

Table 3.4 Ammonia levels and pH of ruminal fluid in sheep offered *A. saligna* with or without a supplement of PEG 4000 or PEG 6000

	Treatment			
	Control	PEG 4000	PEG 6000	Significance
NH_3-N ave.	3.52^a	10.23^b	9.27^b	*
(mg/L)	(0.50)	(4.58)	(3.82)	
NH_3-N min.	2.79	6.5	8.46	NS
(mg/L)	(0.10)	(2.81)	(5.26)	
NH_3-N max.	5^a	13.5^b	14^b	*
(mg/L)	(0.89)	(6.28)	(7.07)	
pH ave.	7.6	7.0	7.0	NS
	(0.4)	(0.3)	(0.4)	
pH min.	7.4^a	6.6^b	6.6^b	*
	(0.5)	(0.4)	(0.4)	
pH max.	7.8	7.4	7.5	NS
	(0.3)	(0.2)	(0.3)	

*$P < 0.05$; NS, not significant.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

The lowest ruminal ammonia levels (average, minimum and maximum) were observed for the control group. The minimum ammonia levels were not significantly different ($P > 0.05$) between any of the treatments. However, both the average and the maximum ammonia levels were lower ($P < 0.05$) in the control group compared with those in either of the PEG treatment groups in which these parameters were similar ($P > 0.05$).

Protozoa were present in abundance in ruminal fluid only until the animals had undergone the control treatment, after which there was virtually no protozoa at all present (Table 3.5).

Table 3.5 The number of protozoa in ruminal fluid (per mL) and its relationship with the order of treatment

		Trial Period		
	S = sheep	1	2	3
Animals	S1 & S2	PEG 6000	PEG 4000	Control
Protozoa (x 10^5)		> 0.6	> 0.6	0
Animals	S3 & S4	PEG 4000	Control	PEG 6000
Protozoa (x 10^5)		> 0.6	0	0
Animals	S5 & S6	Control	PEG 6000	PEG 4000
Protozoa(x 10^5)		0	0	0

3.4 Discussion

3.4.1 Composition of foliage

The DM of *A. saligna* foliage recorded in this trial (350 g/kg) is lower than those recorded by Abou El Nasr et al. (1996) and Ben Salem et al. (1999) (435 g/kg and 392 g/kg, respectively). The OM recorded in the present trial (927 g/kg) is similar to the higher value in the range of values (776 - 928 g/kg) reported in other trials (Degen et al., 1995; Ben Salem et al., 1997; Degen et al., 1997; Ben Salem et al., 1999), the lower figure indicating the OM of foliage from *A. saligna* trees that were less than 12 months old, the higher figure from mature trees.

The CP reported in this trial (114 g/kg) is within the range of CP reported elsewhere for *A. saligna* foliage i.e. 105 g/kg - 132 g/kg (Ben Salem et al., 1999; Ben Salem et al., 1997; Chriyaa et al., 1997a; Degen et al., 1997; Abou El Nasr et al., 1996; Degen et al., 1995).

There are very few reports of total phenolics and CT for *A. saligna* foliage in the literature. Of these there is a lack of uniformity in standards used, therefore hindering comparisons. Degen et al. (1997) and Degen et al. (1995), however, report total phenolics and CT as tannic acid and leucocyanidin equivalent, respectively, as used in the present trial. Their total phenolics ranged from 103 g/kg (young trees) to 150 g/kg (mature trees), with CT ranging from 83 g/kg (mature trees) to 156 g/kg (young trees). Total phenolics (94.5 g/kg) and CT (26.9 g/kg) in the present trial were both lower than these values. Although the CT in the present trial is indicated to be considerably lower than in these other trials, such comparisons may not be truly indicative of differences as many factors can influence the values such as extraction methods, assays and standards used (see Section 2.1).

The determination of PPC of the phenolics was useful for the purpose of comparisons between trials within this thesis. It is not only the content of phenolics/CT that is significant but also the biological activity. A lack of PPC determination by others, however, prevents comparisons with other authors' data.

3.4.2 Feed intake, digestibility and palatability

The DMI of *A. saligna* by the sheep that were not supplemented with PEG were lower than those reported by Abou El Nasr et al. (1996). Where fresh *A. saligna* was the sole feed for rams, the DMI of *A. saligna* exceeded 800 g/d. Their higher DMI corresponded to a higher DMD of 54.2% compared to 31.3% in the current trial. Neither CT concentration nor its activity is reported for the former trial but such factors are expected to largely explain the differences in DMI between that trial and the present one.

In trials of Degen et al. (1995) and Degen et al. (1997) the DMI of air-dried foliage from mature *A. saligna* trees was approximately 200-250 g/d. Both the DMD and OMD in these trials were 31-35%. These figures are comparable to those in the current trial but the experimental animals in the current trial were likely to be significantly heavier than the animals used by Degen. However, in Degen et al. (1997), where foliage was harvested from young trees (8 months old) DMI was less than 150 g/d, despite both DMD (38.3%) and OMD (39.8%) being higher than those harvested from mature trees (32.3% and 33.8% for DMD and OMD, respectively). This was attributed mainly to the much higher CT content of the foliage from the younger trees compared to those obtained from the mature trees, the age of the tree being just one of many factors which may affect its CT content.

The foliage used in the current trial consisted of foliage less than 12 months of age, from 3 year old trees. The foliage from 'mature' trees in the trial of Degen et al. (1997) were also obtained from 3 year old trees that had been cut in each of the previous 3 years.

Low growth rates (or loss of body weight), together with low intakes, were observed in animals eating leaves of *A. saligna* (fresh or dried) as a sole diet (Degen et al., 1997; Abou El Nasr et al., 1996; Degen et al., 1995). Clearly, none of the sheep could be maintained by a diet of *A. saligna* only, in the present trial.

The DMI of *A. saligna* was significantly improved, as were both the DMD and OMD, where either PEG 4000 or 6000 was administered. PEG 6000 increased OMD to a greater extent than did PEG 4000. Positive responses to PEG including DMI, digestibility, wool growth and liveweight gains (or reduced liveweight loss) have been evident in numerous studies involving tannin-rich species such as *A. saligna* (Degen et al., 1998), *F. macrophylla* (Barahona et al., 1997), *Q. calliprinos* (Silanikove et al., 1996a), *P. lentiscus*, *C. siliqua* (Silanikove et al., 1994), *A. aneura* (Pritchard et al., 1988) and *L. pedunculatus* (Barry and Duncan, 1984).

Despite PEG alleviating to some extent the inhibiting effects of CT on the utilisation of *A. saligna* by the sheep, it was evident that this was not sufficient to render the diet adequate for maintenance (as evidenced by visual weight loss in all animals). Jackson et al. (1996) and Fassler and Lascano (1995) emphasised the need to consider not only the tannin levels but also the digestibility of the plants.

Besides negatively affecting digestion, tannins may reduce intake of forage legumes by decreasing palatability. It has been suggested that astringency may increase salivation and decrease palatability. Astringency is the sensation caused by formation of complexes between tannins and salivary glycoproteins (Reed, 1995).

Abou El Nasr et al. (1996) and Chriyaa et al. (1997a) suggested that a lack of palatability may have contributed to the low DMI of *A. saligna* observed. However, in the present trial palatability did not appear to be a problem as all animals readily accepted the *A. saligna* from the start of the initial adaptation period. The low DMI may have been principally associated with the inhibitory effects of the high CT on digestion (Chriyaa et al., 1997a; Degen et al., 1997; Reed et al., 1990), with palatability having a minor influence on DMI.

High palatability of *A. saligna* is apparent in Plate 3.1.

Plate 3.1 Yearling goats enjoying 10 month old *A. saligna*, Gidgegannup, Western Australia

3.4.3 Body condition and N balance

The N intake of sheep dosed with PEG 4000 or PEG 6000 was similar and at least twice the N intake of the control.

N excretion in faeces and urine were similar in sheep dosed with either PEG 4000 or 6000. As a proportion of N intake, faecal N in both PEG groups was approximately 45% less than the control group where faecal N exceeded N intake. Similarly, Ben Salem et al. (1999) noted that faecal N from sheep fed PEG treated *A. saligna* (plus 400 g/d of barley) was 56% lower than where the acacia was not treated with PEG. However, in contrast to their trial in which the treating of *A. saligna* with PEG increased urinary N in sheep, in the present trial there was a significant reduction in the urinary N for both groups administered with PEG.

The very high faecal N in the control indicates very strong CT activity resulting in dietary N being excreted in the faeces as tannin-protein complexes. The fact that faecal N exceeded N intake suggests that the CT not only have the capacity to bind all cellular proteins, but the excess has the additional effects of binding with endogenous proteins such as enzymes, as well as with gut microbial protein. Although not as high as the control, faecal N was also high for the PEG groups. This suggests that a higher rate of PEG might have had further benefits.

Reduced urinary N is a necessary consequence of decreased N absorption caused by high CT contents of feed, as evident in work such as that of Harrison et al. (1973), Fassler and Lascano (1995), Reed and Soller (1987) and Woodward and Reed (1997). The reduced urinary N is consistent with a reduction in ruminal ammonia losses, due to protein protection by CT (Fassler and Lascano, 1995). In some cases this effect is sufficient to maintain an adequate N balance (Woodward and Reed, 1997) while at other times it is not (Reed and Soller, 1987; Reed et al., 1990). In the present trial there were no significant differences in urinary N output, although the control group had the highest level of urinary N excretion.

All groups were in negative N balance, in particular the control group. Weight loss in the control animals could be expected to be considerably greater than those in the PEG groups, as was visually evident.

Sheep fed solely on air-dried *A. saligna* or *A. salicina* ad libitum were in negative N balance, attributed mainly to high urinary N which in turn was attributed possibly to an imbalance of high N relative to a low energy in the rumen (Degen et al., 1997).

The high urinary N in the controls in Trial 1 is the result of tissue breakdown under conditions where there is no net dietary N being absorbed. PEG supplements released sufficient dietary N for digestion and absorption to balance that being lost by normal catabolism, thus sheep were generally in N equilibrium.

As in the present trial, high CT concentrations in a number of browse species (when fed as supplements to straw) have been associated with a reduced N retention eg, *A. saligna* (Ben Salem et al., 1997; Reed et al., 1990), *A. seyal* (Ebong, 1995), *A. brevispica* (Woodward and Reed, 1997) and *F. macrophylla* (Fassler and Lascano, 1995). The reduced N retention might be due to the lack of soluble N or low digestibility in the basal diets. The addition of PEG in the present trial increased the supply of ruminal N concentration as well as increasing DM digestibility, hence the improved (although still inadequate) N balance.

It would appear in this trial that the principle effect of CT on protein metabolism was to enable protein to escape digestion while bound to tannin-protein complexes, passing through as faecal N (Woodward and Reed, 1997). This was also evident in the work of Degen et al. (1995) in which ad libitum *A. saligna* was fed to sheep and goats. In the control animals, the CT may have also bound with endogenous proteins resulting in faecal N exceeding N intake.

Tannins bind to polyethylene glycol (PEG) in preference to protein (Jones and Mangan, 1977). The addition of PEG to the diet in this trial improved the N retention (although there were no differences between PEG 4000 and PEG 6000). PEG has been shown to have positive effects on digestible N and N retention in other trials involving high tannin feeds (Ben Salem et al., 1999; Barry and Duncan, 1984).

3.4.4 Ruminal ammonia concentration and pH

Ruminal fluid was stored at -18°C. An electrical failure resulted in samples thawing and warming up. The duration of the failure and the extent of the warming were not known thus the possible impact on ammonia results could not be evaluated. It is expected that the relativity in the results would remain, however, absolute values may have been altered and may account for the low levels recorded.

Although PEG increased average ruminal ammonia levels, in all treatment groups, ammonia levels were well below the threshold (50 mg/L) for maximal microbial growth (Satter and Slyter, 1974). Average ammonia levels were less than 11 mg/L. Such extremely low levels would have a profound effect on microbial activity, with serious repercussions for DMI and rumen functions.

The high PPC could have rendered the CP virtually completely unavailable, both ruminally and post ruminally (as indicated by high faecal N), with N recycling being negligible. This, together with the very low DMD and OMD indicates that the diets were all considerably below maintenance, despite the inclusion of PEG.

Meissner et al. (1993) found that ruminal fermentation of tannin-containing forages resulted in much lower ammonia concentrations than ruminal fermentation of forages without tannins. Studies with a number of high tannin browse species have supported this observation eg, *A. saligna* (as a supplement to lucerne hay) (Ben Salem et al., 1997), *A. seyal* (Ebong, 1995) and *A. brevispica* (Woodward and Reed, 1997).

Reduced ruminal ammonia concentrations in response to tannin consumption have been attributed to lower solubility and reduced deamination of plant proteins when CT are present (McNabb et al., 1993; Terrill et al., 1992a). One would therefore expect that the binding of CT by the addition of PEG would elevate the ruminal ammonia concentrations as demonstrated in the present trial and the trial undertaken by Silanikove et al. (1996a). Despite the improvement in ammonia concentration with PEG, it was still extremely low. It is possible that a higher dose of PEG would have improved ruminal ammonia concentrations further, to a level that is not limiting DMI and rumen functions. Alternatively, supplementation of the *A. saligna* diet with a soluble source of N (eg, urea or lupins) would likely be advantageous.

The only effect that PEG had on ruminal pH was lowering the minimum pH. The decrease in pH with the addition of PEG to the diet may reflect higher production of VFA due to improved rumen fermentation (Woodward and Reed, 1997), the activity of CT imposing an indirect rather than a direct influence on rumen pH through a depression in rumen fermentation.

3.4.5 Protozoa

Ben Salem et al. (1997) supplemented a lucerne hay based diet with graded amounts of *A. saligna*, noting a linear relationship between the inclusion of *A. saligna* and protozoa numbers in ruminal fluid. Odenyo et al. (1997) included *A. saligna* as a supplement to maize stover and also observed an associated decrease in protozoa numbers. However, defaunation did not occur in either instance.

Odenyo et al. (1997) suggested that a decrease in protozoa numbers could be due to direct toxicity on protozoa or insufficient nutrients, perhaps resulting from tannin complexes or reduced DM digestibility.

In the present trial, the marked effect that the control diet had on protozoa numbers, in the absence of PEG, strongly indicates that it was due primarily to the high PPC.

3.5 Implications

The results of this trial indicate that *A. saligna*, harvested from a 3 year old plantation, could not be used as a sole diet to maintain the weight of sheep.

The inclusion of either PEG 4000 or PEG 6000 in the diet improved the utilisation of *A. saligna*. However, the animals remained in negative N balance and the diets were still considered submaintenance. Perhaps a greater dose of PEG might have resulted in further improvements in nutrient digestion and utilisation.

The level of phenolics (CT, in particular) in the present trial was less than values reported in the literature. However, the results of this trial suggest that their biological activity was excessive, such that the benefit of PEG was limited. This reinforces the need to not only consider the level of phenolics present, but also their biological activity, such as the PPC.

The benefits induced by PEG 4000 were generally not different to those resulting from the use of PEG 6000.

The order of treatment had an obvious effect on the number of protozoa in ruminal fluid. This fact alone, highlights the weakness of the experimental design, in that animals remained in designated pairs throughout the entire trial.

The ruminal ammonia levels were all well below the threshold for maximal microbial growth. One purpose of Trial 2 was therefore to investigate the use of *A. saligna* as a supplement to a basal diet that provided a source of soluble N (i.e. lupins). Trial 2 is described and discussed in Section 4.

Section 4: Trial 2

4.1 Introduction

Although PEG supplementation in Trial 1 did result in a doubling of intake and permit N balance, it still did not provide a maintenance ration and thus it is assumed that the nutritive content/value of *A. saligna*, even after overcoming (perhaps only partially) the effects of CT, is not adequate to support animal maintenance. Given these results, the second trial was undertaken to determine if *A. saligna* was more useful as a supplement rather than a basal diet, assuming the high CT content of *A. saligna* would be of benefit when fed with a source of soluble N

A sub-maintenance diet of lupins (*Lupinus angustifolius*) and wheat straw was chosen on the basis that this would closely resemble what stock would be fed during the traditional summer/autumn feed deficit that occurs in Western Australia. It is during this season that *A. saligna* grows best, thus providing a source of green feed to grazing animals.

The aims of Trial 2 were:

- To assess the value of *A. saligna* as a supplement to a basal diet of lupins and wheat straw.

  Given the high content and biological activity of CT in the *A. saligna* used in Trial 1, it was assumed that, in addition to binding with the protein from the *A. saligna*, excess CT would bind with protein from the lupins. Increasing the bypass protein component of lupins (by TPC formation) should improve their efficiency of use resulting in improvements in production i.e. wool growth and live weight gain.

- To determine whether or not the addition of PEG would further improve the expected benefits of the provision of *A. saligna* to a basal diet of lupins and straw.

If excess CT is present in the *A. saligna*, then its binding with lupins could result in an inadequate supply of soluble N. By binding some of the CT with PEG, such a risk is reduced. It is possible that additional soluble protein would be provided by the *A. saligna* and this, together with a potential bypass protein component, could improve production.

4.2 Materials and methods

The feeding trial was conducted during October-December 1999.

Details concerning materials and methods are described in Section 3.2. Changes to the materials and methods, which relate to Trial 2 included:

- Water intake was not measured.
- Animals were weighed at the start and end of each trial period.
- The duration of each of the three trial periods was 28 d to enable measurement of wool growth. Feed intake, faeces and urine output were measured in week 3 and ruminal parameters were measured during week 4.

4.2.1 Wool samples

To enable the measurement of wool growth dye-bands were applied on the midside of each sheep at the start and end of each treatment period (28 d). Three weeks after the conclusion of the trial the wool was removed at skin level with Oster clippers (Groom Master Model 15-75) with wool growth, during each of the three trial periods, evident from the appearance of the 4 dye-bands.

Fibre diameter was measured using a Syrolan laser scan and linear wool growth was measured using an Agritest length and strength tester.

The dye-band solution was made by adding 0.2 mL hydrogen peroxide and 0.2 g N-1-Naphthylethylenediamine dihydrochloride to 10 mL deionised water.

4.2.2 Treatments

The basal ration was calculated to meet metabolisable energy demands for maintenance for a 64 kg wether (AFRC 1992). A ration comprising of 500 g (air dried) lupins (96% DM, 32% CP) and 300 g wheat straw (95% DM, 2% CP) provided adequate metabolisable energy but a shortfall in metabolisable protein (Table 4.1).

Table 4.1 Formulation for basal diet for 64 kg wether sheep

	ME (MJ/d)	MP (g/d)
Demand	7.7	70
Supply	8.9	55
Balance	1.2	-15

The three dietary treatments were:

1. Control: 500 g/d lupins plus 300 g/d wheat straw (basal diet);
2. Basal diet plus ad libitum access to *A. saligna*;
3. Basal diet + ad lib access to *A. saligna* + 25 g/d PEG 6000 (note: PEG 6000 was used because there was an excess available from Trial 1).

See Section 3.2.4 regarding administration and dose rate of PEG.

4.3 Results

The nutritive value of the 3 feeds is shown in Table 4.2. The values for metabolisable energy have been obtained from feed sources other than in the present trial, therefore may not represent the true values of the feeds used in this trial.

Table 4.2 Nutritive value of feeds used in the 3 dietary treatments

	Lupins	Wheat Straw	*A. saligna*
DM (g/kg As fed)	960	950	340
OM (g/kg DM)	972	959	919
ME (kJ/kg DM)	14.2[1]	6.1[1]	5.1[2]
CP (g/kg DM)	321	20	144
Total extractable phenolics[3] (g/kg DM)			50.4
Condensed tannins[4] (g/kg DM)			13.5
PPC[3] (%DM)			0.014

[1] AFRC (1992)
[2] Degen et al. (1997)
[3] as tannic acid equivalent
[4] as leucocyanidin equivalent

The provision of *A. saligna* resulted in a reduced ($P < 0.05$) intake of straw compared to the control group, the preference for *A. saligna* over straw being obvious at feeding time (Table 4.3). The PEG supplement did not affect straw intake or the consumption of *A. saligna*.

Table 4.3 Intake and digestibility, by sheep, of lupins and straw, with or without ad libitum *A. saligna*, with or without a supplement of PEG 6000

	Treatment			
	Control	+ *A. saligna*	+ PEG 6000	Significance
Ave. live weight (kg)	63.0 (10.4)	64.9 (9.0)	62.8 (9.7)	NS
DMI (g/d)				
Lupins	480	480	480	
Straw	271[a] (25)	162[b] (62)	161[b] (74)	*
A. saligna		994 (178)	999 (112)	NS
Total (g/d)	751[a] (83)	1636[b] (231)	1640[b] (165)	***
DMD total feed (%)	76.1[a] (4.0)	53.2[b] (3.2)	54.7[b] (3.8)	***
OMD (%)	77.9[a] (3.8)	54.2[b] (3.2)	55.8[b] (3.8)	***

*P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.
Values within rows with different superscripts are significantly different.
Values within brackets are standard deviations.

Both the DMD and OMD were significantly higher ($P < 0.001$) in the control group than in the groups receiving *A. saligna*. The DMD and OMD of either group receiving *A. saligna* were similar ($P > 0.05$). Both DMD and OMD were >76% for the control group but only 53-56% for the other two groups.

Based on a lupin digestibility of 90% (Petterson and Mackintosh, 1994) and digestibility of wheat straw of 41% (NRC 1984), the DMD of the *A. saligna* component of the diets was approximately 39%.

N intake was significantly lower ($P < 0.001$) in the control group compared to the other two groups (Table 4.4). The addition of *A. saligna* to the diet significantly increased ($P < 0.001$) faecal N, the inclusion of PEG having no influence on this parameter. Urinary N was highest in the group supplemented with PEG, although this was only significantly different ($P < 0.05$) to the other group with access to *A. saligna*.

The N balance was positive in all groups and did not differ between treatments.

Table 4.4 Nitrogen intake and balance in sheep fed lupins and straw, with or without ad libitum *A. saligna*, with or without a supplement of PEG 6000

	Treatment			
N (g/d)	Control	+ *A. saligna*	+ PEG 6000	Significance
N intake	26.2^{a}	48.3^{b}	48.4^{b}	***
	(3.15)	(6.08)	(4.57)	
Faecal N	4.2^{a}	29.0^{b}	24.8^{b}	***
	(0.98)	(4.55)	(2.03)	
Urine N	15.92^{ab}	14.0^{a}	17.2^{b}	*
	(2.53)	(2.47)	(4.02)	
N balance	6.1	5.3	6.4	NS
	(1.92)	(3.20)	(3.61)	

*P < 0.05; ***P < 0.001; NS, not significant.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

The dietary treatments had no effect on either average ammonia concentrations or pH of ruminal fluid (Table 4.5). No defaunating activity was evident from any of the dietary treatments as protozoa were present in all samples regardless of treatment.

Table 4.5 Ammonia levels and pH of rumen fluid from sheep fed lupins and straw, with or without ad libitum *A. saligna*, with or without a supplement of PEG 6000

	Treatment			
	Control	+ *A. saligna*	+ PEG 6000	Significance
NH_3-N ave. (mg/L)	240 (65)	172 (73)	189 (26)	NS
NH_3-N range[1] (mg/L)	212-288	136-229	167-209	
pH ave.	6.7 (0.0)	6.7 (0.1)	6.7 (0.2)	NS
pH range[1]	6.6-6.8	6.5-6.9	6.5-6.9	

NS, not significant.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

[1] The diurnal variation over a 24 h period.

Wool parameters did not vary between treatments (Table 4.6). The possibility that 28 d was not a sufficient time period to avoid lag effects on wool growth may have obscured the results. Effects on LWG (data not shown) were obscured by the effects of gutfill and the short time frame of the trial.

Table 4.6 Production responses by sheep fed lupins and straw, with or without ad libitum *A. saligna*, with or without a supplement of PEG 6000

	Treatment			
	Control	+ *A. saligna*	+ PEG 6000	Significance
Wool growth (μm/d)	304	277	278	NS
	(63)	(122)	(77)	
Fibre diameter (μm)	22.37	22.35	22.35	NS
	(1.33)	(1.75)	(1.11)	

***P < 0.001; NS, not significant.

Values within rows with different superscripts are significantly different.

Values within brackets are standard deviations.

4.4 Discussion

4.4.1 Composition of foliage

The DM of *A. saligna* foliage recorded in this trial (340 g/kg) is lower than those recorded by Abou El Nasr et al. (1996) and Ben Salem et al. (1999) (435 g/kg and 392 g/kg, respectively), but similar to that of Trial 1. The OM recorded in the present trial (919 g/kg) is similar to that of Trial 1 as well as the higher value in the range of values (776 - 928 g/kg) reported in other trials (Degen et al., 1995; Ben Salem et al., 1997; Degen et al., 1997; Ben Salem et al., 1999).

The CP reported in this trial (144 g/kg) exceeds the range of CP reported elsewhere for *A. saligna* foliage i.e. 105 g/kg - 132 g/kg (Ben Salem et al., 1999; Ben Salem et al., 1997; Chriyaa et al., 1997a; Degen et al., 1997; Abou El Nasr et al., 1996; Degen et al., 1995) as well as Trial 1 (114 g/kg).

Total phenolics and CT (50.4 g/kg and 13.5 g/kg, respectively) in *A. saligna* foliage used in the present trial are considerably less than the values reported by Degen et al. (1997) and Degen et al. (1995) (103-150 g/kg total phenolics and 83-156 g/kg CT). They are also considerably less than those reported in Trial 1 (94.5 g/kg and 26.9 g/kg, respectively). This reinforces the belief that many factors can influence the values of these parameters within a species (see Section 2.1).

4.4.2 Intake and digestibility

None of the animals consumed all the straw that was on offer. With access to *A. saligna* the intake of straw was greatly reduced. The consumption of *A. saligna* was > 990 g/d, regardless of PEG administration. In contrast, DMI of *A. saligna* in Trial 1 was < 200 g/d without PEG and < 500 g/d with PEG. Several factors could have contributed to such a difference, however, the most significant difference is probably the PPC which is 4 times greater in Trial 1 than in Trial 2.

In a trial in which *A. saligna* was offered as a supplement to lucerne hay, the intake of *A. saligna* was comparable to the current trial i.e. 600 g/d (Ben Salem et al., 1997). However, in other trials where *A. saligna* served as a supplement to a diet of straw, the intake of *A. saligna* by adult sheep was considerably lower eg, 170 g/d (Reed et al., 1990) and < 200 g/d (Chriyaa et al., 1995). The differences could be attributed to differences in body weight in respective trials, as well as differences in the rumen degradable N in the basal diets, CT levels and PPC of the *A. saligna*.

In contrast to the suggestions of Abou El Nasr et al. (1996) and Chriyaa et al. (1997a), the high intakes of *A. saligna* in the present trial indicate that the *A. saligna* was highly palatable.

The digestibility of the diet (both DM and OM) was greatly decreased with the inclusion of *A. saligna*. This was also evident where graded amounts of *A. saligna* were included in a diet of good quality lucerne (Ben Salem et al., 1997). The DMD of the *A. saligna* component was approximately 39% which is similar to that reported by Degen et al. (1997) for *A. saligna* foliage from young trees, and not greatly higher than in Trial 1.

PEG had no benefit to either DM or OM digestibility in contrast to the benefits gained in Trial 1 and in the trial of Silanikove et al. (1997). In the trial of Silanikove et al. (1997), improvements in digestibility were observed with the addition of PEG to a diet of high tannin oak leaves with or without either a cereal or soya concentrate. This suggested that the tannin in the oak leaves had the capacity to precipitate more protein than was contained within the oak leaves. (This explanation could also apply to Trial 1 in which the CP and PPC of the leaves were lower and higher, respectively, than the leaves in Trial 2.) Consequently the protein utilization of the supplementary food could also be impeded.

Given the markedly lower levels of total phenolics, CT and PPC in the diet of Trial 2 the PEG is unlikely to have had a significant impact on digestibility as it did in Trial 1.

4.4.3 Nitrogen balance

The differences in N intake reflect the intake of freely available *A. saligna* in addition to the basal diet of lupins and straw. However, while N intake was significantly increased with *A. saligna*, faecal N also increased to the extent that the retention of the additional N was negligible.

Furthermore, where *A. saligna* was included, urinary N was substantially lower but only in the absence of PEG. A decrease in urine N is probably a function of lower NH_3, which in turn is likely to have been in response to higher levels of CT in the diet associated with the *A. saligna*. Such a relationship has been observed elsewhere involving high-tannin species eg, *F. macrophylla* (Fassler and Lascano, 1995), *O. viciifolia* (Harrison et al., 1973), *A. brevispica* (Woodward and Reed, 1997), *A. saligna* and *A seyal* (Reed and Soller, 1987; Reed et al., 1990).

The addition of PEG in the current trial tended to reduce faecal N while increasing urinary N, as it did in the trial of Ben Salem et al. (1999). This supports the evidence of the correlation of CT with faecal N and urinary N as the PEG is expected to have bound some of the CT in the *A. saligna*.

A reduced urinary N is often a mechanism by which animals compensate the higher faecal N with increasing CT level in the diet. In this trial the compensation was adequate, such that the N balance was the same for all treatment groups. All were in positive N balance, in contrast to the animals in Trial 1 that were all in negative N balance.

Of the diet provided in this trial, the *A. saligna* component alone comprised a higher CP and a much lower PPC than in Trial 1. Even without consideration of the basal diet of lupins, this alone could account for the positive N balance of all animals in Trial 2, compared to the negative N balance in Trial 1. The lower CP in the diet and higher PPC in Trial 1 could have rendered the CP virtually completely unavailable.

4.4.4 Ammonia concentration, pH and protozoa in ruminal fluid

Ruminal fluid was stored at -18°C. An electrical failure resulted in samples thawing and warming up. The duration of the failure and the extent of the warming were not known thus the possible impact on ammonia results could not be evaluated. It is expected that the relativity in the results would remain, however, absolute values may have been altered.

All measurements of ruminal ammonia exceeded the threshold (50 mg/L) for optimum microbial growth (Satter and Slyter, 1974).

Although the addition of *A. saligna* increased the N intake, it tended to decrease ruminal ammonia concentrations, indicating a decrease in rumen degradable protein. This effect could be due to both the low digestibility of *A. saligna* and the binding of protein by CT.

Dietary treatments had no effect on either pH or average ammonia concentrations of ruminal fluid. Ruminal ammonia concentrations were adequate with the basal diet alone, therefore a response to *A. saligna* supplementation, in terms of increased ruminal ammonia, would be unlikely.

In Trial 1 N recycling could have been negligible due to low CP and high PPC, hence the lower ruminal ammonia levels compared to Trial 2.

In contrast to Trial 1, no defaunation in the present trial was evident, suggesting that the content and biological activity of CT in the diets of Trial 1 were much greater that in the present trial. The PPC in the present trial supports this assumption.

4.4.5 Live weight and wool growth

The absorption of essential amino acids from the small intestine has been identified as limiting productivity in ruminants fed entirely on diets of high quality fresh forages ad libitum (Barry, 1981). Condensed tannins are able to reduce the degradation of proteins in the rumen and increase essential amino acid absorption in ruminants fed fresh forages (Barry and McNabb, 1999).

The provision of *A.saligna* had no impact on ruminal ammonia concentrations or the N balance of each group. This would indicate that the apparent weight loss by the control group was due more to the effect of gutfill when access to *A. saligna* was allowed and conversely when it was not, rather than an improved availability of nutrients when *A. saligna* was provided.

The weight loss in Trial 1 was visually evident. The *A. saligna* in Trial 1 was clearly an inferior source of feed compared to the *A. saligna* in Trial 2. This fact alone would account for the better responses in Trial 2, without consideration of the lupin component as well.

There are contrasting reports concerning the effect of CT on wool growth from a negative (Barry, 1985) or neutral influence (Douglas et al., 1999) to a positive response (Douglas et al., 1995; Waghorn and Shelton, 1997).

In contrast to the observations of Wang et al. (1996), the lack of effect of *A. saligna* on wool parameters could suggest that essential amino acid supply was limiting body growth but not wool growth. The lack of response in wool growth to the addition of *A. saligna* could reflect a lack of increase in post ruminal supply of S-containing amino acids and/or an imbalance of protein and energy post-ruminally. A response to bypass protein (eg, as with TPC) depends on the post-ruminal energy status. In addition, the PPC of the phenolics in this trial may have been insufficient to result in the formation of (dietary) bypass protein. The lack of effect on wool growth corresponded to a lack of change in N retention.

4.5 Implications

In Trial 2 there was no benefit of including *A. saligna* as a supplement to a basal diet of lupins and wheat straw. Any advantage might have been more evident had the basal diet not supplied adequate metabolisable energy and had the trial been conducted over a greater period of time. The effect of gutfill also obscured the effects of providing *A. saligna*. To evaluate "true" effects on live weight, an experimental design other than a latin square would be required.

In the present trial, where *A. saligna* was fed in combination with lupins and straw, there was no benefit in including a detannification agent. In effect, the lupins may have acted similarly to PEG by inhibiting free tannins and increasing N availability for the animals both directly and indirectly. Given the considerably lower levels of total phenolics, CT and PPC, compared to reports in the literature and those of Trial 1, one might have cause to wonder if the *A. saligna* used in this trial could be regarded a 'high tannin' feed.

Trial 2 did reveal that the sheep were capable of consuming significantly more *A. saligna* than they did in Trial 1 but it was not clear whether this was due to the basal diet providing adequate nutrients or if it was due to differences in the *A. saligna* fed in the respective trials - probably both. Certainly there were significant differences in the total phenolics, CT and PPC of the *A. saligna* foliage used in the respective trials, although DMD was not greatly different between the two sources.

A more informative trial would have included urea as the source of soluble N (assuming soluble N is the major nutrient limiting microbial growth and activity) rather than lupins, with *A. saligna* as the basal diet. In that instance, the N component could have been evaluated without the effects of additional energy (as with lupins). In addition, the effects of gutfill would have been eliminated.

Given the shortfalls of Trial 2, Trial 3 was designed to investigate the use of *A. saligna* as the basal source of nutrients, with or without a supplement of N in the form of urea or higher rates of PEG.

Section 5: Trial 3

5.1 Introduction

It was shown in Trial 1 that *A. saligna* was inadequate as the sole source of nutrients for sheep. It was also shown that the level of detannification achieved in Trial 1, with the addition of PEG 4000 or PEG 6000, failed to improve the diet sufficiently.

Given the results of Trial 1, the second trial was undertaken to determine if *A. saligna* was more useful as a supplement rather than a basal diet. The benefits of including *A. saligna* as a supplement to a basal diet of lupins and wheat straw were not clear. The benefits of including a detannification agent with the *A. saligna* were not evident.

Trial 2 revealed that the sheep were capable of consuming significantly more *A. saligna* than they did in Trial 1, but it was not clear whether this was due to the basal diet providing adequate nutrients or if it was due to differences in the *A. saligna* fed in the respective trials.

Trial 3 was designed to investigate the use of *A. saligna* as the basal source of nutrients, with or without a supplement of N, in the form of urea or PEG. Urea, rather than lupins was used as the N supplement to avoid the influence that additional metabolisable energy (and other components of lupins) may have on results. Providing a basal diet of *A. saligna* would also potentially eliminate the effects that gutfill may have had in Trial 2.

Wheat straw was included in the basal diet to reflect a paddock situation in which plantations of *A. saligna* are interspersed with dry pasture during the summer/autumn period of Western Australia.

Goats were also included in this trial for the purpose of comparing their responses with those of sheep. Some researchers (eg, Silanikove et al., 1997; Degen et al., 1995) have indicated that goats are better able than sheep to tolerate high tannin feeds.

5.2 Materials and methods

The general materials and methods are presented in Section 3.2. Further information, regarding the time frame of the trial and wool measurements, is described in Sections 4.2 and 4.2.1. The feeding trial was conducted during April-June 2000.

5.2.1 Plant material

An alternative source of *A. saligna*, to that used in the previous trials, was sought due to the original source being required for 'on farm' utilization. The *A. saligna* for Trial 3 was sourced from Bakers Hill, approximately 80 km north east of Perth, Western Australia. The climate of the area is described as Mediterranean with an average annual rainfall of 622 (P. Hanson, Agriculture Western Australia, personal communication 2001). The soil in which the *A. saligna* was growing may be described as sandy gravel (Moore, 2001).

Branches were cut from mature trees (5-6 year old, see Plate 5.1) and then fed through a mechanical leaf stripper (McMeniman, 1975). The *A. saligna* offered to the sheep and goats consisted of leaves (mostly whole) and small twigs. Material was harvested an average of 5 times each week. After harvesting, material was stored at -18°C pending feeding.

Plate 5.1 Mature *A. saligna*, Bakers Hill, Western Australia

On day 1 of each sampling period, approximately 1 kg (fresh weight) of leaves was randomly collected from each 20 kg batch for chemical analyses.

5.2.2 Diets and experimental animals

In addition to the six merino wethers used in the previous trials, six mature boer cross wether goats, fitted with permanent rumen cannulae, were included in this feeding trial.

The three dietary treatments were:

1. Control: ad libitum *A. saligna* + 400 g/d wheat straw (95% DM) (basal diet);
2. Basal diet + plus 50 g/d PEG 4000;
3. Basal diet plus 1% (on a DM basis) urea sprayed onto the straw and *A. saligna* 30 min prior to feeding.

The quantity of *A. saligna* to be treated with urea was weighed and then spread out on a clean tarpaulin on the floor. The urea was dissolved in water (1:20 w/v, respectively) and then sprayed over the *A. saligna* which was turned several times during the spraying to encourage even coverage. The straw was similarly treated where applicable.

See Section 3.2.4 regarding administration of PEG.

There was no prior knowledge of the content or biological activity of the CT in the *A. saligna*, therefore, the dose rate was based on the results of the previous trials. Because Trial 2 demonstrated that sheep were capable of consuming almost double that consumed in Trial 1 (where animals were dosed with PEG), the dose rate of PEG in the third trial was double the dose rate in the previous trials i.e. to 50 g/head/d.

5.3 Results

The nutritive value of the 2 feeds is shown in Table 5.1. The values for metabolisable energy have been obtained from feed sources other than in the present trial, therefore may not represent the true values of this trial.

Table 5.1 Nutritive value of the basal diet

	Wheat Straw	*A. saligna*
DM (g/kg As fed)	920	350
OM (g/kg DM)	975	943
ME (kJ/kg DM)	6.1[1]	5.1[2]
CP (g/kg DM)	26	138
Total extractable phenolics[3] (g/kg DM)		73.8
Condensed tannins[4] (g/kg DM)		24.6
PPC[3] (% DM)		0.022

[1] AFRC (1992)

[2] Degen et al. (1997)

[3] as tannic acid equivalent

[4] as leucocyanidin equivalent

Sheep supplemented with PEG consumed more *A. saligna* than either the control group or those supplemented with urea ($P < 0.05$, Table 5.2). This was reflected in the differences in the total DMI ($P < 0.05$). All sheep readily consumed the *A. saligna* in preference to straw. The consumption of straw did not differ ($P > 0.05$) amongst treatment groups. The animals consumed < 25% of the straw offered, the addition of urea failing to increase the intake of straw.

Both the DMD and OMD were higher ($P < 0.05$) where PEG was included in the diet compared to the other two treatments ($P > 0.05$).

In goats there was no significant difference ($P > 0.05$) between any of the treatment groups in the parameters of DMI, DMD or OMD.

There was no significant difference ($P > 0.05$) in DMI, DMD or OMD between sheep and goats in corresponding treatment groups.

The sheep supplemented with urea had a higher N intake than the other two groups ($P < 0.001$, Table 5.3). Faecal N from both the control and the urea treatment groups was greater ($P < 0.001$) than for the PEG group. The urinary N was significantly different ($P < 0.01$) between all groups, the greatest being for the PEG group, followed by the urea and then the control group. Both faecal and urinary N displayed the same trends for goats as they did for sheep.

All sheep were in positive N balance, the greatest being for the PEG group, which was significantly higher ($P < 0.05$) only than the control sheep.

In goats the N balance was significantly higher ($P < 0.01$) for both the PEG and urea groups compared to the control group. All groups were in positive N balance.

There was no significant difference ($P > 0.05$) in the N balance ($g/kg^{0.75}$) in sheep and goats in corresponding treatment groups.

Table 5.2 Intake and digestibility of *A. saligna* and straw offered to sheep and goats, with or without a supplement of PEG 4000 or 1% urea

	Sheep				Goats				Between species
	Control	+PEG	+ urea	Significance	Control	+PEG	+ urea	Significance	LSD
Average LW (kg)	66.6	66.4	67.5	NS	47.5	47.8	47.5	NS	
	(10.7)	(9.9)	(10.6)		(3.6)	(3.3)	(4.6)		
DMI (g/d)									
A. saligna	1287[a]	1389[b]	1295[a]	*	1091	1173	1134	NS	
	(200)	(126)	(238)		(119)	(100)	(255)		
($g/kg^{0.75}/d$)	55	60	55	NS	60	64	63	NS	10.67
	(8.6)	(5.4)	(10.1)		(6.6)	(5.5)	(14.1)		
(% $kg^{0.75}$)	5.5	6.0	5.5		6.0	6.4	6.3		
Straw	75	72	82	NS	34	33	38	NS	
	(44)	(28)	(52)		(7)	(18)	(25)		
($g/kg^{0.75}/d$)	3.2	3.1	3.5	NS	1.9	1.8	2.1	NS	1.65
	(0.4)	(1.0)	(1.4)		(0.4)	(1.0)	(1.4)		
Total	1362[a]	1461[b]	1377[a]	*	1125	1206	1172	NS	
	(175)	(107)	(205)		(116)	(100)	(261)		
($g/kg^{0.75}/d$)	58.4	62.8	58.4		62.2	66.4	64.8		10.23
	(7.5)	(4.6)	(8.7)		(6.4)	(5.5)	(14.4)		
DMD (%)	48.2[a]	55.2[b]	49.0[a]	*	51.2	54.2	52.2	NS	4.60
	(2.6)	(4.7)	(2.8)		(3.3)	(4.9)	(4.8)		
OMD (%)	49.7[a]	56.6[b]	50.9[a]	*	53.1	56.0	54.1	NS	4.42
	(2.4)	(4.6)	(2.5)		(3.3)	(5.0)	(4.6)		

*P < 0.05; NS, not significant.
Values within rows with different superscripts are significantly different.
Values within brackets indicate standard deviations.

Table 5.3 Nitrogen intake and balance in sheep and goats fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

	Sheep				Goats				Between species
N (g/d)	Control	+PEG	+ urea	Significance	Control	+PEG	+ urea	Significance	LSD
N intake	28.7^{a}	31.0^{a}	35.3^{b}	***	24.2^{a}	26.1^{ab}	30.6^{b}	***	
	(4.31)	(2.68)	(6.05)		(2.62)	(2.18)	(6.88)		
(mg/kg$^{0.75}$/d)	1232^{a}	1333^{a}	1498^{b}	***	1340^{a}	1434^{ab}	1692^{a}	***	
	(185)	(115)	(257)		(145)	(120)	(380)		
Faecal N	21.2^{a}	13.4^{b}	22.2^{a}	***	16.4^{a}	10.2^{b}	16.6^{a}	***	
	(2.91)	(2.56)	(3.65)		(2.41)	(1.98)	(4.05)		
(mg/kg$^{0.75}$/d)	911^{a}	575^{b}	943^{a}	***	909^{a}	562^{b}	917^{a}	***	
	(125)	(110)	(155)		(133)	(109)	(224)		
Urine N	6.2^{a}	12.2^{c}	8.6^{b}	***	5.8^{a}	9.8^{c}	7.7^{b}	**	
	(1.49)	(2.49)	(3.53)		(1.18)	(1.62)	(2.44)		
(mg/kg$^{0.75}$/d)	268^{a}	523^{b}	366^{c}	**	318^{a}	538^{b}	426^{c}	**	
	(85)	(105)	(86)		(65)	(89)	(135)		
N balance	1.2^{a}	5.5^{b}	4.4^{ab}	**	2.0^{a}	6.1^{b}	6.3^{b}	**	
	(1.98)	(2.44)	(2.03)		(1.45)	(2.15)	(3.02)		
mg/kg$^{0.75}$/d	53^{a}	235^{b}	189^{b}	***	113^{a}	335^{b}	349^{b}	***	127.7
	(85)	(105)	(86)		(80)	(118)	(167)		

**P < 0.01; ***P < 0.001.
Values within rows with different superscripts are significantly different.
Values within brackets indicate standard deviations.

Table 5.4 Ammonia concentrations and pH of ruminal fluid from sheep and goats fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

	Sheep				Goats				Between species
	Control	+PEG	+ urea	Significance	Control	+PEG	+ urea	Significance	LSD
NH_3-N ave. (mg/L)	15[a]	48[b]	47[b]	**	21[a]	66[b]	51[b]	**	18.84
	(9)	(24)	(21)		(12)	(27)	(26)		
NH_3-N range[1] (mg/L)	9-21	24-97	27-72		15-28	32-127	23-102		
pH ave.	6.9	6.8	6.8	NS	7.0[a]	6.7[b]	6.8[b]	**	0.17
	(0.2)	(0.1)	(0.1)		(0.2)	(0.1)	(0.1)		
pH range[1]	6.7-7.1	6.6-7.0	6.7-7.0		6.8-7.2	6.5-6.9	6.6-7.0		

**P < 0.01; NS, not significant
Values within rows with different superscripts are significantly different.
Values within brackets indicate standard deviations.
[1] The diurnal variation over a 24 h period.

Figure 5.1 Ruminal ammonia of sheep fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

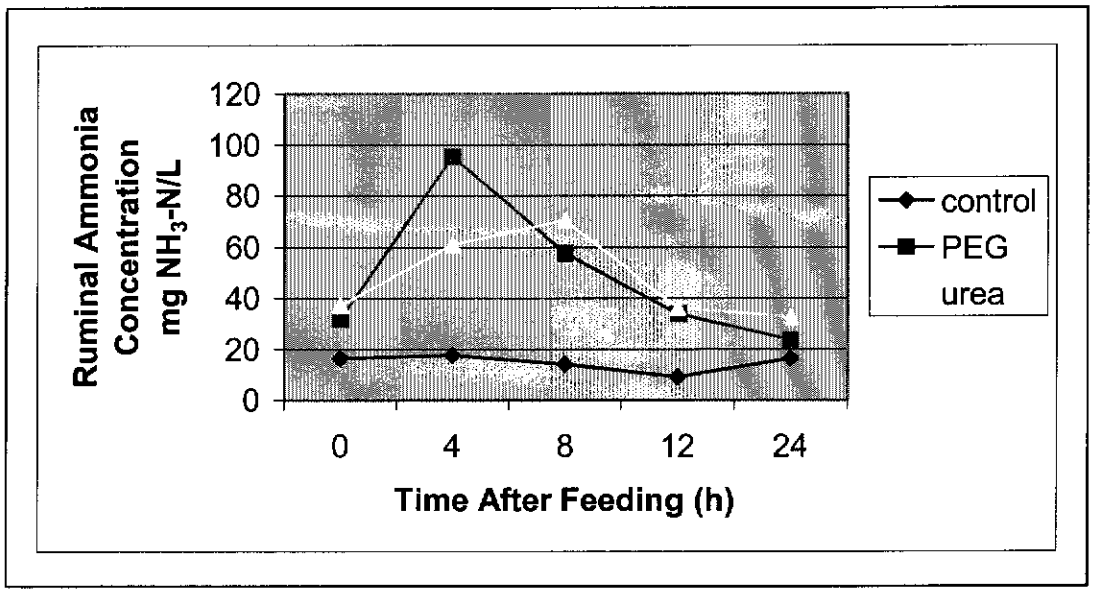
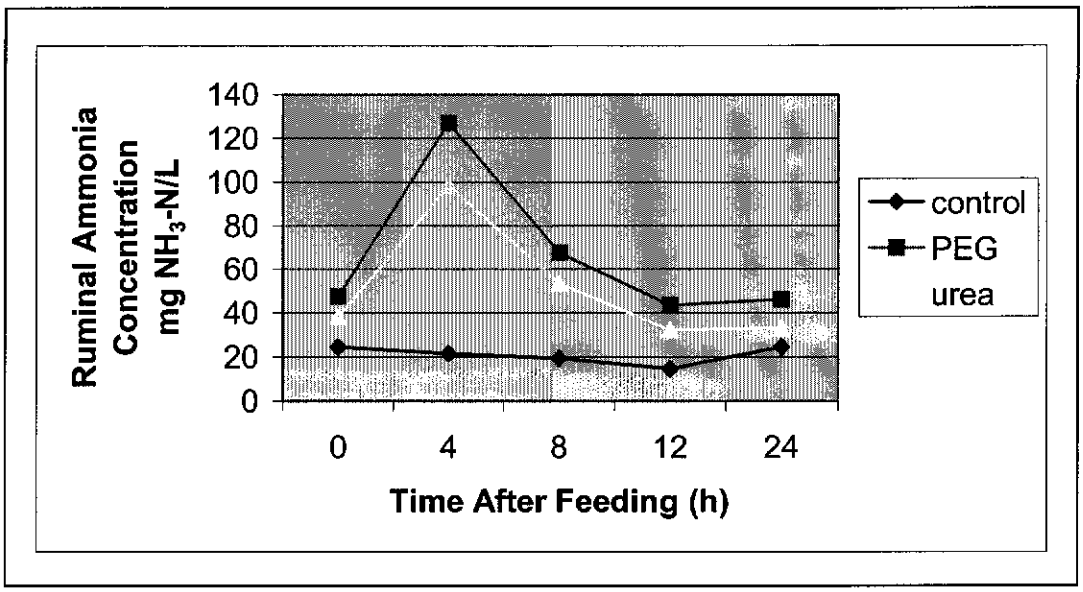


Figure 5.2 Ruminal ammonia of goats fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea



In sheep, the average ammonia concentration of ruminal fluid was lowest in the control group ($P < 0.01$), as it was for the goats (Table 5.4, Figures 5.1 and 5.2). There was no difference in the average ammonia concentration of ruminal fluid between the PEG and urea groups for either sheep or goats. There was no significant difference ($P > 0.05$) in the average pH of ruminal fluid from sheep in any of the treatment groups. However, in goats the average pH was higher ($P < 0.01$) for the control (goats) than the other two groups.

There was no significant ($P > 0.05$) difference in the average ammonia concentration or pH of ruminal fluid from sheep and goats in corresponding treatment groups.

No defaunating activity was evident from any of the dietary treatments as protozoa were present in all samples regardless of treatment.

There was no significant difference ($P > 0.05$) in the linear wool growth of any group of sheep. However, the average fibre diameter of wool from sheep supplemented with PEG was greater than either the control or the urea group ($P < 0.01$, Table 5.5).

Table 5.5 Wool growth and fibre diameter in sheep fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

	Treatment			
	Control	+PEG	+ urea	Significance
Wool growth (μm/d)	292	304	310	NS
	(42)	(37)	(43)	
Fibre diameter (μm)	20.65[a]	21.55[b]	20.73[a]	**
	(1.11)	(1.10)	(1.21)	

**P < 0.01; NS, not significant
Values within rows with different superscripts are significantly different.
Values within brackets indicate standard deviations.

There was no significant difference ($P > 0.05$) in the live weight changes of any group of sheep, as was the case with the goats. There was no significant difference ($P > 0.05$) in the live weight changes of sheep and goats from corresponding treatment groups. Overall, animals maintained live weight.

5.4 Discussion

5.4.1 Composition of foliage

The DM of *A. saligna* foliage recorded in this trial (350 g/kg) is lower than those recorded by Abou El Nasr et al. (1996) and Ben Salem et al. (1999) (435 g/kg and 392 g/kg, respectively). The OM recorded in the present trial (943 g/kg) is similar to the higher value in the range of values (776 - 928 g/kg) reported in other trials (Degen et al., 1995; Ben Salem et al., 1997; Degen et al., 1997; Ben Salem et al., 1999). The DM and OM of the *A. saligna* used in the present trial are similar to those reported in Trials 1 and 2 i.e. DM of 350 g/kg and 340 g/kg, respectively and OM of 927 g/kg and 919 g/kg, respectively. The difference between the three trials in terms of DMI and animal responses cannot be attributed to differences in DM or OM of the *A. saligna*.

Although similar to the CP of *A. saligna* in Trial 2, the CP reported in this trial (138 g/kg) exceeds the range of CP reported elsewhere for *A. saligna* foliage i.e. 105 g/kg - 132 g/kg (Ben Salem et al., 1999; Ben Salem et al., 1997; Chriyaa et al., 1997a; Degen et al., 1997; Abou El Nasr et al., 1996; Degen et al., 1995), including Trial 1 i.e. 114 g/kg. A higher N content could contribute to better animal performance.

Total phenolics and CT (73.8 g/kg and 24.6 g/kg, respectively) in *A. saligna* foliage used in the present trial are less than the values reported by Degen et al. (1997) and Degen et al. (1995) (103-150 g/kg total phenolics and 83-156 g/kg CT). They are also less than those reported in Trial 1 i.e. 94.5 g/kg and 26.9 g/kg. However, they exceed those of Trial 2 i.e. 50.4 g/kg and 13.5 g/kg. Although the PPC in the present trial is double that of Trial 2, it is half that of Trial 1. It is not the mere presence of phenolics and CT in *A. saligna* that would influence its utilisation but more their biological activity, therefore it is likely that differences in the PPC had a significant impact on the results of each Trial. Based on this parameter alone, one might expect that utilisation of *A. saligna* would be much superior in Trials 2 and 3, compared to Trial 1, as was the case.

5.4.2 Intake and digestibility

Degen et al. (1995) reported that DMI of *A. saligna*, on a metabolic body mass basis, when fed as a sole diet, was greater in goats than in sheep i.e. 1.05% and 0.80%, respectively. In the control treatment of the current trial, DMI of *A. saligna* by goats and sheep was 6.0% and 5.5%, respectively. Goats have been noted elsewhere for their higher intake of tannin-rich carob leaves compared to that of sheep (Silanikove et al., 1996a; Silanikove et al., 1994).

In Trials 1 and 3 there was a positive correlation between DMD and DMI. The intake in the present trial exceeded considerably, those reported by Degen et al. (1995) and Degen et al. (1997), as well as Trial 1. In Degen et al. (1995), leaves from mature *A. saligna* trees were compared to those from young trees, in terms of their utilisation by goats and sheep. Despite the leaves from young trees having a higher CP, higher apparent DMD and lower total phenolics, the intake by both goats and sheep was much lower than their intake of leaves from mature trees. Although the PPC was not indicated, the level of CT in the leaves from young trees was considerably higher than in leaves from the mature trees.

The DMD was greater in the present trial than in Degen's (approximately 48% compared to 31-40%, respectively), as was CP (13.8% compared to 11.1-13.2%), and total phenolics and CT were both lower. All these factors, plus the probability that PPC was lower in the present trial, would have contributed to the higher intake of *A. saligna*.

Total phenolics and CT of *A. saligna* did not greatly differ between Trials 1 and 3, however, the PPC was significantly lower in Trial 3. Although DMD and CP were greater in Trial 3, probably the main factor contributing to the higher intake in Trial 3 compared to Trial 1 was the significant difference in the PPC.

PEG improved the intake of *A. saligna* by both sheep and goats, although this was significant ($P < 0.05$) only with sheep. This corresponded to an increase in DMD and OMD, again significant only with sheep ($P < 0.05$). These results conflict with Silanikove et al. (1996b) who claimed that, when fed a high-tannin diet, goats responded better than sheep to PEG supplementation and that the amount of PEG required to elicit the maximum response in intake was lower for goats than for sheep. It is not clear why in Trial 3 the sheep responded to PEG while the goats did not. Supplementation with urea did not affect either sheep or goats in terms of *A. saligna* intake or digestibility, in the current trial. Higher rates of urea may have further improved the digestibility, hence the intake.

5.4.3 Nitrogen intake and balance

The difference in N intake by the sheep was a reflection of the additional N from the urea, not because of differences in intake. The lower faecal N with the addition of PEG, in both sheep and goats, corresponded to the higher DMD, but could also be due to lower TPC. The lower urinary N (and higher faecal N) of the control group compared to the PEG group supports the concept that urinary N is reduced to compensate increased faecal N in the presence of CT (Harrison et al., 1973; Fassler and Lascano, 1995). Faecal N from the animals supplemented with urea was similar to the control animals, yet urinary N was higher due to the greater supply of soluble N in the rumen.

In contrast to Trial 1, all animals in Trial 3 were in positive N balance, largely a reflection of the higher DMD and CP and lower PPC of the *A. saligna* in Trial 3.

5.4.4 Ruminal ammonia concentration and pH

Ruminal fluid was stored at -18°C. An electrical failure resulted in samples thawing and warming up. The duration of the failure and the extent of the warming were not known thus the possible impact on ammonia results could not be evaluated. It is expected that the relativity in the results would remain, however, absolute values may have been altered and may account for the low levels recorded.

For both sheep and goats, ammonia concentrations of ruminal fluid were significantly improved with the use of either urea or PEG indicating an improved availability of rumen degradable N. In these groups the maximum ammonia concentrations exceeded 50 mg/L, considered the minimum required to maximise microbial growth (Satter and Slyter, 1974). However, this threshold was exceeded only for a period of 8-11 h. Of those measured, ruminal ammonia levels were generally highest at 4 h post feeding. None of the measurements of ammonia for the control group approached 50 mg/L. In general, ammonia levels were higher in goats than in sheep, but this difference was not significant.

In spite of the low ruminal ammonia concentration, DMI was high in all groups, as was DMD and OMD compared to other studies reported in the literature.

The ruminal ammonia levels in Trial 3 were considerably higher than in Trial 1. A lower CP and higher PPC in Trial 1 could have rendered the CP virtually completely unavailable, both ruminally and post ruminally, with N recycling being negligible, hence the lower ruminal ammonia levels in Trial 1.

Lower pH (as in Trials 2 and 3, compared to Trial 1) indicates higher production of VFA due to improved rumen fermentation (Woodward and Reed, 1997). This supports the results of the other parameters measured which also indicate superiority in the feed value of the diets in Trials 2 and 3 compared to Trial 1.

5.5.5 Wool growth

Fibre diameter increased with PEG in sheep, indicating an increase in the postruminal supply of nutrients. This could be due to increased microbial protein synthesis as a result of improved substrate availability, due to increased N or energy.

Wool growth usually responds to an increased availability/absorption of protein/amino acids (especially S containing AA) and the responses to PEG indicate that there was a significant increase in AA absorbed, not seen when only urea was provided. Raising rumen ammonia concentrations was not sufficient to improve microbial activity. The difference between the PEG and urea treatments was that for PEG, plant proteins (including S) was available for ruminal digestion and microbial synthesis, while urea supplements only provided ammonia.

Responses in wool growth may have become more evident had it not been for the limitations of the experimental design and the short time frame.

5.6 Implications

The results from this trial suggest that *A. saligna* could be a useful feed source for ruminants. The substitution of straw with *A. saligna* indicates that its incorporation into a grazing system could significantly decrease grazing pressure on dry summer pastures.

The results from this trial do not indicate that goats have a superior ability than sheep in utilizing *A. saligna* as a source of nutrient.

The labelling of *A. saligna* as a 'high tannin' feed needs to be reconsidered as the results of this trial suggest that in some instances such categorising may be inappropriate.

Section 6: Conclusion

In Trial 1 *A. saligna* was inadequate as the sole source of nutrients for sheep. Furthermore, the level of detannification achieved in Trial 1, with the addition of PEG 4000 or PEG 6000, failed to improve the diet sufficiently to maintain the body weights of the sheep. A greater dose of PEG might have resulted in further improvements in nutrient digestion and utilisation. The antinutritional effects on the animals were largely attributed to the excessive biological activity of the phenolics in the *A. saligna* leaves. Feeding of these leaves, without PEG, had a definite defaunating effect on the ruminal fluid and the ruminal ammonia levels were all well below the threshold for maximal microbial growth.

The level of phenolics (CT, in particular) in Trial 1 was less than values reported in the literature. However, the results of this trial suggest that their biological activity was excessive, such that the benefit of PEG was limited. This reinforces the need to not only consider the level of phenolics present, but also their biological activity, such as the PPC.

Trial 2 was undertaken to determine if *A. saligna* was more useful as a supplement rather than a basal diet.

Trial 2 revealed that the sheep were capable of consuming significantly more *A. saligna* than they did in Trial 1, but it was not clear whether this was due to the basal diet providing adequate nutrients or if it was due to differences in the *A. saligna* fed in the respective trials. Total phenolics, CT and PPC were considerably lower than those of Trial 1. Ruminal ammonia levels were much higher than in Trial 1 and animals generally maintained weight.

There was no benefit of including *A. saligna* as a supplement to a basal diet of lupins and wheat straw. Any advantage might have been more evident had the basal diet not supplied adequate metabolisable energy and had the trial been conducted over a greater period of time. The effect of gutfill also obscured the effects, in terms of animal production, of providing *A. saligna*.

In Trial 2, where *A. saligna* was fed in combination with lupins and straw, there was no benefit in including a detannification agent. Given the considerably lower levels of total phenolics, CT and PPC, compared to reports in the literature and those of Trial 1, one might have cause to wonder if the *A. saligna* used in this trial could be regarded a high tannin feed.

Trial 3 was designed to investigate the use of *A. saligna* as the basal source of nutrients, with or without a supplement of N in the form of urea or PEG.

Total phenolics, CT and PPC were lower than those of Trial 1, but higher than those of Trial 2. Animals consumed more *A. saligna* than in Trial 2 and generally maintained weight. The difference in PPC is probably the key factor influencing the results of Trials 1 and 3. The lupin protein provided in Trial 2 over-rode any adverse effects of the tannins irrespective of concentration or PPC.

The results from Trial 3 suggest that *A. saligna* could be a useful feed source for ruminants. The substitution of straw with *A. saligna* indicates that its incorporation into a grazing system could significantly decrease grazing pressure on dry summer pastures.

The results from Trial 3 do not indicate that goats have a superior ability than sheep in utilizing *A. saligna* as a source of nutrient.

The difficulty in explaining the results from the 3 trials is compounded by the differences in the harvested *A. saligna* in terms of the age of the regrowth and the maturity of the trees. The *A. saligna* was sourced from separate locations at different time periods hence the potential influence of varying environmental conditions on the quality of the harvested material as a feed source. There is also the possibility of genetic variability between the sources of *A. saligna*.

Inadequacies of some of the experimental designs used (see Sections 3.5 and 4.5), together with potential differences in the *A. saligna* causes difficulty in comparing results across experiments. In addition, the use of frozen material may have resulted in variations that may not have occurred, had fresh material been used.

6.1 Future Research

There is a need to investigate the circumstances that predispose *A. saligna* to becoming a high-tannin feed. Results suggest that *A. saligna* should not be categorically considered a high-tannin feed, that in some situations, *A. saligna* may not be a high-tannin feed. Such qualification is dependent on various factors.

It would be useful to ascertain the effect(s) of cutting, transport, storage at -18°C and thawing on the concentrations, activities (PPC) and forms (free/bound) of tannins in high tannin plants to be able to relate to the nutritive value of fresh forage.

The monitoring of *A. saligna* at a particular site over a period of time eg, 1 year, could provide insight into how and why tannin, together with other nutritional aspects, changes seasonally. Ultimately there is a need to establish if the desirable *A. saligna* is somehow genetically or phenotypically different.

In situations in which tannin is not a significant impediment to the utilisation of *A. saligna*, investigation may be more appropriately directed at other causes of its low digestibility such as lignin and fibre.

It would be useful to determine the circumstances in which the supplementation with N and/or energy to a predominantly *A. saligna* diet, would be of benefit. Similarly, when would access to *A. saligna* be of benefit as a supplementary feed?

To assist researchers it would be extremely useful if analyses for phenolics were standardised. This would reduce the inefficiencies in comparisons of data from different sources where different methods of sample preparation, analyses and standards have been employed.

The standardisation of analyses should not only consider the level of phenolics present, but also their biological activity, such as the PPC.

Section 7: References

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