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The value of *Acacia saligna* as a source of fodder for ruminants

A report for the Rural Industries Research and Development Corporation

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Foreword

A. saligna (also knows as the Golden Wreath Wattle or Orange Wattle) is an extremely rugged tree which has proved to be widely adaptable to barren slopes, derelict land and arid conditions. This tree is of particular interest as a feed source for ruminants as it is drought tolerant and grows rapidly, and in the arid regions of Israel it is grown using only run-off water.

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 (N) retention in ruminants. However, 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.

This publication examines three pen trials, together with a laboratory-based trial in order to evaluate the value of *A. saligna* as a source of feed for ruminants.

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Executive Summary

Three pen trials, together with a laboratory-based trial, 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 were.

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 were, 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.

Trial 4 was designed to investigate the changes in the nutritive value of *A. saligna* over time. Mature trees (5-6 years old) were lopped in either Autumn (March), Winter (June) or Spring (September) and the newly (re)grown phyllodes were harvested at approximately monthly intervals after the initial loppings, giving samples of regrowth of varying ages. The phyllodes were analysed for their dry matter, ash and crude protein contents, organic matter digestibility (based on the gas fermentation technique), total extractable phenolics and condensed tannin levels, and the protein precipitating capacity of these tannins. Nutritive value generally declined as the plant matured, however, it was not clear what factors affected the organic matter digestibility of the *A. saligna*. Further research is required to fully understand the factors affecting its nutritive value. Examining the interactive effects of soil fertility, added nutrients, water (rainfall), season and age of regrowth would be beneficial, as all of these factors affect the nutritive value of *A. saligna*.

1. Introduction

Also known as the Golden Wreath Wattle or Orange Wattle, *Acacia saligna* is a native of Western Australia (Simmons, 1988). It has been introduced into other regions of Australia, and also into many other countries (Gutteridge, 1994), including Uruguay, Mexico, Israel, Iran, Iraq, Jordan, Syria, Greece, Cyprus and North African countries (NAS, 1980).

A. saligna is a dense bushy shrub (Gutteridge, 1994), or a small tree that grows 2-8 m tall (Simmons, 1988). It has long straggling branches (Gutteridge, 1994) that are grey to reddish brown in colour. The foliage varies in colour, either green or blue-green (Simmons, 1988), with long phyllodes (up to 20 cm) (Gutteridge, 1994), that are either curved or straight (Simmons, 1988). During spring, it is usually characterised by drooping branches that contain an abundant number of yellow flowers (NAS, 1980).

Although *A. saligna* thrives on a wide range of soil types, it is outstanding on sandy coastal plains and sand dunes, making it a plant extensively used in sand dune stabilisation (Gutteridge, 1994) and mining site rehabilitation (Simmons, 1988). It grows well in areas with annual rainfalls as low as 250 mm to as high as 1200 mm, but prefers a range of 350-600 mm. It is moderately tolerant of soil salinity (Gutteridge, 1994), as well as being tolerant of salt-laden wind (Simmons, 1988). *A saligna* grows best where the winter and summer temperature means are between 13° and 30^oC, respectively. It grows at altitudes ranging from near sea level to 300 m, although there are isolated occurrences at higher elevations (NAS, 1980).

A. saligna is an extremely rugged tree, and in Australia and North Africa it has proved to be widely adaptable to barren slopes, derelict land and arid conditions. This tree is of particular interest as a feed source for ruminants as it is drought tolerant (Nativ *et al*., 1999) and grows rapidly (NAS, 1980), and in the arid regions of Israel it is grown using only run-off water (Degen *et al*., 1995). The factors that seem to play a role in this drought adaptability are the osmotic adjustment and efficient regulation of stomatal conductance according to soil water availability and climatic conditions. *A. saligna* trees that are well established have exceptional regrowth, allowing the plant to be completely grazed without harm (NAS, 1980).

Limited research has been carried out on the nutritive value of *A. saligna*, although considerable research has been done on other species of the *Acacia* genus. *A. aneura* (mulga) is probably the most important fodder tree in Australia , partly because it is so widespread (Cremer 1990) throughout the inland areas of all mainland states. In times of drought the mulga may be the only source of nutrients, although many trials have indicated that the level of available nutrients is generally not sufficient for maintenance, growth or production (Gartner and Hurwood, 1976; Entwistle and Baird, 1976; McMeniman, 1976; McMeniman *et al*., 1981; McMeniman *et al*., 1986).

Results from a study by Abdulrazak *et al*. (2000) on *Acacia* species showed that the crude protein of acacia foliage is high enough to use as a supplement to low quality diets, and that the species are rich in most minerals. However, *Acacia* species have the disadvantage of containing phenolic compounds, which include tannins. These compounds have a negative effect on the nutritional value of the browse species, and also affect intake and digestibility (Abdulrazak et al., 2000). In addition *Acacia* species may contain high levels of proanthocyanidins and low levels of available protein (Reed, 1995).

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 (N) retention in ruminants. However, 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.

2. Literature Review

Condensed tannins (CT) are considered the primary antinutritional factor of *A. saligna* (Reed & 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.

2.1   Browse species

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 feed 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).

2.2   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 defence 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 "flavenoids") 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   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. 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). Because soluble CT have the ability to depress protein and fibre digestibility, they potentially affect animal production (Norton, 2000).

2.4   Factors affecting concentrations of condensed tannins 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). Tannin levels and extractability change dramatically through the growing season (Hagerman, 1988). 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.

Soil fertility and acidity also affect CT levels, with low pH associated with higher CT levels. 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, lyophilisation, drying at ambient or elevated temperatures, and the assays used to extract the tannins (Hagerman, 1988).

2.5   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, but both precipitate proteins. 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 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.

2.5.1 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).

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).

2.5.1.1 Protein degradation

The formation of TPC (based on soluble CT) has been shown to protect dietary protein from ruminal fermentation (Barry and Duncan, 1984). Bound CT are considered unsuitable to protect dietary protein because they are not hydrolysed by acids or enzymes (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 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) and *A. brevispica* (Woodward and Reed, 1997).

2.5.1.2 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). CT can reduce the degradation of proteins in the rumen and increase essential amino acid absorption in ruminants fed these types of diets (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). 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.

2.5.1.3 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 associated with the N content of forage in an exponential manner. Ruminal fermentation of the tannin-containing forages resulted in much lower ammonia concentrations than ruminal fermentation of forages without tannins (Meissner *et al*., 1993).

Tannins may increase the efficiency of urea recycling to the rumen (Reed, 1995). 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).

Whilst higher concentrations of CT have been associated with increased N retention in sheep fed a high CT diet (Barry *et al*., 1986; Harrison *et al*., 1973), high CT concentrations in a number of browse species, when fed as supplements to straw, have been associated with reduced N retention eg, *A. saligna* (Ben Salem *et al*., 1997; Reed *et al*., 1990), *A. seyal* (Ebong, 1995) and *A. brevispica* (Woodward and Reed, 1997). 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 (Woodward and Reed, 1997). *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).

2.5.2 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).

2.5.3 Carbohydrate metabolism

The extent of carbohydrate digestion is dependent on the characteristics of the feeds, including both the inherent degradability and rate of fermentation, and the rate of feed intake and passage (Pitt *et al.*, 1999). If protein resists degradation, or diets are deficient in protein, microbial growth in the rumen is sub-optimal, this in turn leads to retarded carbohydrate breakdown (McDonald *et al.*, 1995)

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).

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, with which their low digestibility is primarily associated. 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). Makkar *et al.* (1995) suggested that the rate of digestion is 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. 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).

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.

2.5.4 Palatability and voluntary feed intake

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 (Kumar and Singh, 1984). Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins (Butter *et al.*, 1999). 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.. Ebong (1995), however, found no difference in the acceptability of three browses ranging from low (*S. sesban*) to high CT content (*A. seyal*).

Waghorn *et al.* (1994) claim that decreased ruminal turnover and rate of digestion was more important than palatability in reducing intake of sheep fed diets containing high levels of CT.

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

2.5.5 Effects of condensed tannins on animal production

CT have been reported to have both positive and negative effects on animal production. The live weight gain and wool growth by sheep fed *A. aneura* were improved significantly when supplemented with polyethylene glycol (PEG, a detannification agent) thus implicating CT as the primary inhibitory factor on animal performance (Pritchard *et al.*, 1992; Pritchard *et al.*, 1988).

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).

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.

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). 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).

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 five 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.

2.6 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.

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 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 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). 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).

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 (Degen et al., 1998; Barahona et al., 1997; Silanikove et al., 1996a;Silanikove et al., 1994; Pritchard et al., 1988, 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.

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 Pre-treatment with chemicals

Significant reductions in tannin levels have been achieved with treatments involving moist, alkaline conditions (Price et al., 1979; Waichungo and Holt, 1995; Makkar and Singh, 1992). Significant reductions of tannins in salseed meal have been 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).

Temperature and moisture were found to have an important role in the inactivation of tannins where oak leaves were stored (Van Soest, 1982, as cited by 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). Tannin levels in oak leaves have been 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

It has been 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 (Ben Salem *et al*., 1999). 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 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, 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

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.

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.

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.

If N availability is improved with supplements such as PEG or urea, energy may become limiting for productivity (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 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.

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 (P) 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 P, 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 [S]) supplement and sheep gained weight. S is a component in the essential amino acids methionine and cysteine/cystine. S 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 S in plants is present in protein as S-containing amino acids. Therefore, when plants contain high levels of CT, both amino acids and S 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 P, when supplemented with molasses. However, because the greatest response was with the first 50 g of molasses they suggested the S 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 S supplements. However, although each supplement provided similar amounts of S, the response was greatest with the 50 g DM molasses supplement. This implied that an element other than S contained in the molasses induced the additional response, possibly cobalt as *A. aneura* is marginal in this element.

DMI, live weight gain and wool growth improved when sheep given *A. aneura* were supplemented with PEG. These measures were further increased when N, P and S (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).

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 describes three pen trials and a laboratory based trial 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.

3.   Trial 1

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, southwest 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.

3.1   Objectives

The aims of Trial 1 were:

1.   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.

2.   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.

3.   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. 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.

The experiment was based on a Latin square design. Animals were randomly allocated to one of three dietary treatments. Because each trial consisted of three dietary treatments, each trial was comprised of three sampling periods. Each of the three experimental periods was of 21 d duration, made up of 13 d 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.

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

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. The soil in which the *A. saligna* was growing was sandy gravel.

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 day 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.

Three treatments were used:

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

Because the content and biological activity of CT within the *A. saligna* was unknown prior to the trial, 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 at least 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 weights of the experimental animals, should have been measured to determine the appropriate dose rate.

Animals had free access to fresh water and intake 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).

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.

Samples of ruminal content were obtained via the rumen cannula, just prior to feeding and thereafter, at approximately 3 h intervals for 24 h. The fluid was strained through a 100 μm sieve and then pH was measured. Sixteen mL of strained ruminal fluid were placed in specimen bottles containing 0.2 mL of 18$\underline{M}$ 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 two layers of stocking material, and stored at room temperature for subsequent counting of protozoa.

Samples of *A. saligna* foliage were dried to constant weight in a forced-air oven at 35°C 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, pers comm., 1999). The samples were then ground through a 1 mm screen 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.

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. The ash content was expressed as a percentage of the original sample DM weight and the OM content calculated. The crude protein content of samples was determined using the Kjeldahl oxidation procedure (Mossberg, 1979).

Ruminal fluid was centrifuged (3000 g for 10 min) and analysed for ammonia concentrations using the modified Berthelot reaction (Searle, 1984).

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

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, and therefore may not represent the true value of the material used in this trial.

Table 3.1 Composition of *A. saligna* foliage

DM (%)	35
OM (% DM)	92.7
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.

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 inappetence.

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.

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

	Treatment			Significance
	Control	PEG 4000	PEG 6000	
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.

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. There were no significant differences (P > 0.05) in either the faecal or urinary N output between any of the treatment groups. 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).

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).

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

N (g/d)	Treatment			Significance
	Control	PEG 4000	PEG 6000	
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.

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			Significance
	Control	PEG 4000	PEG 6000	
Average NH_3-N	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)	
Average pH	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.

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 (x 10^5/mL) and its relationship with the order of treatment.

	S = sheep	Trial period		
		1	2	3
Treatment	S1 & S2	PEG 6000	PEG 4000	Control
Protozoa (x 10^5)		> 0.6	>0.6	0
Treatment	S3 & S4	PEG 4000	Control	PEG 6000
Protozoa (x 10^5)		> 0.6	0	0
Treatment	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 leucocyandin 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.

Additional information such as PPC of the phenolics would have been useful for the purpose of comparisons between trials within this report. It is not only the content of phenolics/CT that is significant but also the biological activity. However, had the PPC been determined in this trial, a lack of PPC determination by others would have prevented 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 *et al*. 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, harvested from three-year-old trees. The foliage from 'mature' trees in the trial of Degen *et al*. (1997) were also obtained from three year old trees that had been cut in each of the previous three years.

Low growth rates (or loss of body weight), together with low intakes, has been previously 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 live weight gains (or reduced live weight loss) have been evident in numerous studies involving tannin-rich species (Degen *et al*., 1998; Barahona *et al*., 1997; Silanikove *et al*., 1996; Silanikove *et al*., 1994; Pritchard *et al*., 1988; 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.

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 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) and *A. brevispica* (Woodward and Reed, 1997). 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 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 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 three-year-old plantation, could not be used as a sole diet to maintain the live 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 benefits induced by PEG 4000 were generally not different to those resulting from the use of PEG 6000

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 order of treatment had an obvious effect on the number of protozoa in ruminal fluid.

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.

4. Trial 2

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 to have any real impact on animal performance. 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.

4.1 Objectives

The aims of Trial 2 were:

1) 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.

2) 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 the general materials and methods are described in Section 3.2, although some changes were made (as follows). 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 (0.2 mL hydrogen peroxide and 0.2 g N-1-Naphthylethylenediamine dihydrochloride per 10 mL deionised water) 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 wool growth, during each of the three trial periods, evident from the appearance of the four dye-bands. Fibre diameter was measured using a Syrolan laser scan and linear wool growth was measured using an Agritest length and strength tester.

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

	Metabolisable energy (kJ/d)	Metabolisable protein (g/d)
Demand	7.7	70
Supply	8.9	55
Balance	+ 1.2	-15

The three dietary treatments were:

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

Administration and dose rate of PEG was the same as used in Trial 1.

4.3   Results

The nutritive value of the three 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.

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 4.2 Nutritive value of feeds used in the 3 dietary treatments

	Lupins	Wheat straw	*A. saligna*
DM (%)	96	95	34
OM (% DM)	97.2	95.9	91.9
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 (1993); [2] Degen *et al*. (1997); [3] as tannic acid equivalent; [4] as leucocyanidin equivalent.

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			Significance
	Control	+ *A. saligna*	+ PEG 6000	
Average 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	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 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, with 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 N intake and balance in sheep fed lupins and straw, with or without ad libitum *A. saligna*, with or without a supplement of PEG 6000

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

*$P < 0.05$; ***$P < 0.001$; 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			Significance
	Control	+ *A. saligna*	+ PEG 6000	
Average NH$_3$-N	240	172	189	NS
(mg/L)	(65)	(73)	(26)	
NH$_3$-N range[1]	212-288	136-229	167-209	
(mg/L)				
Average pH	6.7	6.7	6.7	NS
	(0.0)	(0.1)	(0.2)	
pH range[1]	6.6-6.8	6.5-6.9	6.5-6.9	

*$P < 0.05$; 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 effects of gutfill and the short time frame of the trial obscured effects on live weight gain (data not shown).

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			Significance
	Control	+ *A. saligna*	+ PEG 6000	
Wool growth	304	277	278	NS
(μm/d)	(63)	(122)	(77)	
Fibre diameter	22.37	22.35	22.35	NS
(μm)	(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 that reported for 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 that many factors can influence the values of these parameters within a species.

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 four 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 *A. saligna* is 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 utilisation of the supplementary feed 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 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 ammonia, 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 (Fassler and Lascano, 1995: Harrison *et al.*, 1973; Woodward and Reed, 1997; 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 greater N balance in Trial 2 compared to 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

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 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 than 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). CT 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). 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. This is in contrast to the observations of Wang *et al*. (1996) that CT was of benefit to wool growth but not live weight gain. 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, 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.

5. Trial 3

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.

5.1   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.

5.1.1 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' utilisation. 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 mm. The soil in which the *A. saligna* was growing was sandy gravel.

Branches were cut from mature trees (5-6 year old) 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 five times each week. After harvesting, material was stored at $-18^{o}C$ pending feeding.
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.

5.1.2 Diets and experimental animals

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

The three dietary treatments were:

♦ Control: ad libitum *A. saligna* + 400 g/d wheat straw (95% DM) (basal diet);
♦ Basal diet + plus 50 g/d PEG 4000;
♦ 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.

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. The PEG was administered in the same manner as used in Trial 1.

5.2 Results

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

Sheep supplemented with PEG consumed more *A. saligna* than either the control group or those supplemented with urea ($P < 0.05$, Table 5.2a, b). 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 ($P > 0.05$).

Table 5.1 Nutritive value of the basal diet

	Wheat straw	*A. saligna*
DM (%)	92	35
OM (% DM)	97.5	94.3
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 (1993); [2] Degen *et al.* (1997); [3] as tannic acid equivalent; [4] as leucocyanidin equivalent

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 5.3a, b). Faecal N from both the control and the urea treatment groups was greater (P < 0.001) than for the PEG group. 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 5.2a 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 | | | | Between |
	Control	+ PEG	+ urea	Significance	species LSD
Average live	66.6	66.4	67.5	NS	
weight (kg)	(10.7)	(9.9)	(10.6)		
DMI (g/d)					
- *A. saligna*	1287[a]	1389[b]	1295[a]	*	
	(200)	(126)	(238)		
- ($g/kg^{0.75}$/d)	55	60	55	NS	10.67
	(8.6)	(5.4)	(10.1)		
- (% $kg^{0.75}$)	5.5	6.0	5.5		
- Straw	75	72	82	NS	
	(44)	(28)	(52)		
- ($g/kg^{0.75}$/d)	3.2	3.1	3.5	NS	1.65
	(0.4)	(1.0)	(1.4)		
- Total	1362[a]	1461[b]	1377[a]	*	
	(175)	(107)	(205)		
- ($g/kg^{0.75}$/d)	58.4	62.8	58.4		10.23
	(7.5)	(4.6)	(8.7)		
DMD (%)	48.2[a]	55.2[b]	49.0[a]	*	4.60
	(2.6)	(4.7)	(2.8)		
OMD (%)	49.7[a]	56.6[b]	50.9[a]	*	4.42
	(2.4)	(4.6)	(2.5)		

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

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

	Goats				Between species LSD
	Control	+ PEG	+ urea	Significance	
Average live weight (kg)	47.5	47.8	47.5	NS	
	(3.6)	(3.3)	(4.6)		
DMI (g/d)					
- *A. saligna*	1091	1173	1134	NS	
	(119)	(100)	(255)		
- (g/kg$^{0.75}$/d)	60	64	63	NS	10.67
	(6.6)	(5.5)	(14.1)		
- (% kg$^{0.75}$)	6.0	6.4	6.3		
- Straw	34	33	38	NS	
	(7)	(18)	(25)		
- (g/kg$^{0.75}$/d)	1.9	1.8	2.1	NS	1.65
	(0.4)	(1.0)	(1.4)		
- Total	1125	1206	1172	NS	
	(116)	(100)	(261)		
- (g/kg$^{0.75}$/d)	62.2	66.4	64.8		10.23
	(6.4)	(5.5)	(14.4)		
DMD (%)	51.2	54.2	52.2	NS	4.60
	(3.3)	(4.9)	(4.8)		
OMD (%)	53.1	56.0	54.1	NS	4.42
	(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 5.3a Nitrogen intake and balance in sheep and goats fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

N (g/d)	Sheep				Between species LSD
	Control	+ PEG	+ urea	Significance	
N intake	28.7[a]	31.0[a]	35.3[b]	***	
	(4.31)	(2.68)	(6.05)		
(mg/kg$^{0.75}$/d)	1232[a]	1333[a]	1498[b]	***	
	(185)	(115)	(257)		
Faecal N	21.2[a]	13.4[b]	22.2[a]	***	
	(2.91)	(2.56)	(3.65)		
(mg/kg$^{0.75}$/d)	911[a]	575[b]	943[a]	***	
	(125)	(110)	(155)		
Urine N	6.2[a]	12.2[c]	8.6[b]	***	
	91.49)	(2.49)	(3.53)		
(mg/kg$^{0.75}$/d)	268[a]	523[b]	366[c]	**	
	(85)	(105)	(86)		
N balance	1.2[a]	5.5[b]	4.4[ab]	**	
	(1.98)	(2.44)	(2.03)		
(mg/kg$^{0.75}$/d)	53[a]	235[b]	189[b]	***	127.7
	(85)	(105)	(86)		

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

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

N (g/d)	Goats				Between
	Control	+ PEG	+ urea	Significance	species LSD
N intake	24.2[a]	26.1[ab]	30.6[b]	***	
	(2.62)	(2.18)	(6.88)		
(mg/kg$^{0.75}$/d)	1340[a]	1434[ab]	1692[a]	***	
	(145)	(120)	(380)		
Faecal N	16.4[a]	10.2[b]	16.6[a]	***	
	(2.41)	(1.98)	(4.05)		
(mg/kg$^{0.75}$/d)	909[a]	562[b]	917[a]	***	
	(133)	(109)	(224)		
Urine N	5.8[a]	9.8[c]	7.7[b]	**	
	(1.18)	(1.62)	(2.44)		
(mg/kg$^{0.75}$/d)	318[a]	538[b]	426[c]	**	
	(65)	(89)	(135)		
N balance	2.0[a]	6.1[b]	6.3[b]	**	
	(1.45)	(2.15)	(3.02)		
(mg/kg$^{0.75}$/d)	113[a]	335[b]	349[b]	***	127.7
	(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 5.4a 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				Between
	Control	+ PEG	+ urea	Significance	species LSD
Mean NH_3-N	15[a]	48[b]	47[b]	**	18.84
(mg/L)	(9)	(24)	(21)		
NH_3-N range[1]	9-21	24-97	27-72		
(mg/L)					
Mean pH	6.9	6.8	6.8	NS	0.17
	(0.2)	(0.1)	(0.1)		
pH range[1]	6.7-7.1	6.6-7.0	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.

Table 5.4b 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

N (g/d)	Goats				Between
	Control	+ PEG	+ urea	Significance	species LSD
Mean NH_3-N	21[a]	66[b]	51[b]	**	18.84
(mg/L)	(12)	(27)	(26)		
NH_3-N range[1]	15-28	32-127	23-102		
(mg/L)					
Mean pH	7.0[a]	6.7[b]	6.8[b]	**	0.17
	(0.2)	(0.1)	(0.1)		
pH range[1]	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
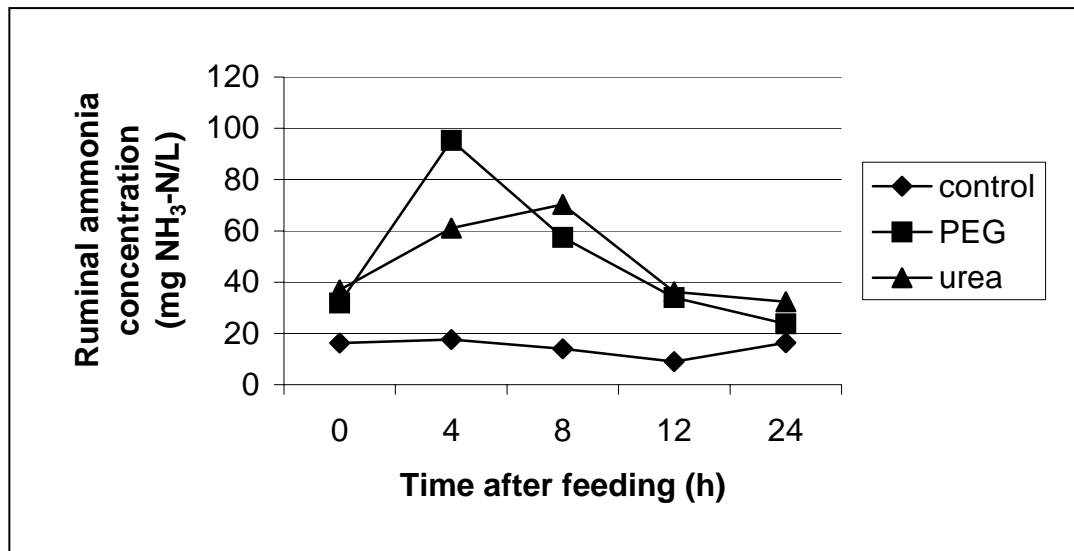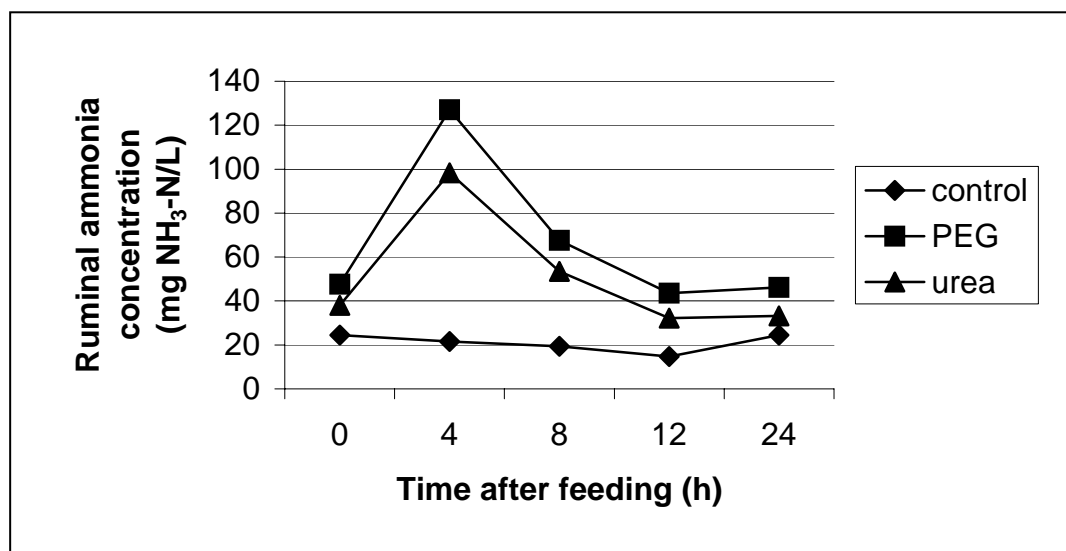

Figure 5.2 Ruminal ammonia of goats fed *A. saligna* and straw with or without a supplement of PEG 4000 or 1% urea

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 5.4a and b, Figures 5.1 and 5.2). There was no difference in the average ruminal ammonia concentration 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			Significance
	Control	+ PEG	+ urea	
Wool growth (μm/d)	292 (42)	304 (37)	310 (43)	NS
Fibre diameter (μm)	20.65[a] (1.11)	21.55[b] (1.10)	20.73[a] (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.

5.3   Discussion

5.3.1 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.

5.3.2 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).

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.

5.3.3 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.

5.3.4 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.

5.3.5 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 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.

5.4   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 utilising *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.

5.5   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 were. 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 were, but higher than those of Trial 2. Animals consumed more *A. saligna* than in Trial 2 and generally maintained weight. The difference in astringency 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 astringency.

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 utilising *A. saligna* as a source of nutrient.

The difficulty in explaining the results from the three 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*.

The labelling of *A. saligna* as a high tannin feed needs to be reconsidered as the results of Trial 3 suggest that in some instances such categorising may be inappropriate.

6. Trial 4

The results from the Trials 1, 2 and 3 indicate that animal production responses to *A. saligna* are variable, at times it is far from acceptable as a feed source, at other times it can meet the maintenance (feed) requirements of both sheep and goats. From studies on both mature phyllodes and young phyllodes, it is suggested that there is a decrease in the nutritive value of *A. saligna* with maturity (Degen *et al.*, 1997). It may well be that variation in the maturity of the *A. saligna* used in the three feeding trials was responsible for the differences in results achieved. A fourth trial was undertaken to determine how age (of regrowth) and season of growth affects the nutritive value of *A. saligna*.

Due to digestibility trials being expensive and labour intensive, attention is being focussed towards *in vitro* methods to assess the impact of polyphenolics on the nutritive value and subsequent digestibility of fodder and browse plants. These methods involve the use of microorganisms or enzymes, which create a situation similar to the digestive tract of a ruminant (Close and Menke, 1986). The gas fermentation technique is one such method. This technique is based on the method by Menke *et al.* (1979) and involves the measurement of gas production (carbon dioxide and methane), produced from the incubation of a feedstuff with rumen liquor. The principle involves incubating the feedstuff with rumen liquor for 24 h and determining describe gas production (CO_2 and CH_4) (Menke and Steingass 1988). It can be used for estimation of digestibility and/or organic matter and metabolisable energy content of straight and compound feedstuffs" (as reviewed by Close and Menke, 1986). Khazaal *et al.* (1994) used this technique to assess Mediterranean browse species, and concluded that it has good potential to use for assessment of phenolic related anti-nutritive effects in feeds. Nherera *et al.* (1999) used this technique to estimate in vivo microbial N supply of tree fodder legume-supplemented diets when fed to goats.

Rather than digestibility in the whole digestive tract, the *in vitro* gas production method is related solely to fermentation in the rumen. If digestibility of the whole tract was considered, it would include enzymatic digestion, absorption and hindgut fermentation (Williams, 2000).

Menke *et al.* (1979) found a high correlation between gas production *in vitro* and digestibility (and metabolisability) *in vivo*. It is believed that it is because there is no filtration stage in this technique, ensuring that digested and undigested material is not separated. Separation may not give the full information on digestibility. This is partly due to some indigestible or less digestible material passing through the filter, and also due to some digestible material not being extracted from the indigestible fraction on the filter.

6.1 Materials and methods

Four mature Merino wether sheep fitted with a permanent rumen cannula were used to supply the rumen fluid needed for the gas fermentation technique. Each animal was housed in a metabolism cage to facilitate easy collection of the rumen fluid. The cages were placed in a well-ventilated animal house, which was cleaned daily. Animals were fed a daily ration consisting of 600 g steam cut wheaten chaff (91.3% DM, 8.6% CP), 150 g lucerne chaff (88% DM, 16.8% CP) and 250 g lupins (91% DM, 35.9% CP). The animals were fed this diet for 18 days (adaptation period) prior to the commencement of collection of rumen fluid. Ad libitum access to clean, fresh water was provided daily.

The *A. saligna* for Trial 4 was sourced from the same location as the *A. saligna* for Trial 3 (Bakers Hill, approximately 80 km north east of Perth, Western Australia). Nine rows of mature trees (5-6 years old) were used in the regrowth study, with the rows of trees randomly allocated to three treatment groups, viz Autumn (March) cut, Winter (June) cut and Spring (September) cut. The trees were lopped according to the treatment group (the trees selected for the Autumn cut were lopped in March and those in the Winter treatment were lopped in June) and samples of the newly (re)grown phyllodes were harvested at approximately monthly intervals (actual period recorded) after the initial

lopping. Phyllodes from three trees were harvested for each sampling period, and each tree was only sampled once, resulting in samples of phyllodes of varying ages. The harvested phyllodes were stored at -18°C pending analysis.

6.1.1 Dry matter content

Duplicate samples of *A. saligna* phyllodes were dried in a fan forced oven (Contherm Digital Series 5) at 90°C until constant weight to determine average DM content. The dried samples were stored in sealed containers for subsequent determination of crude protein and ash contents.

Identical samples of the phyllodes were also freeze dried (Cryodos 50 Hz Freeze Drier Unit) for subsequent determination of *in vitro* organic matter digestibility (using the gas fermentation technique), total phenolics content, percentage condensed tannins and protein precipitable phenolics as a percentage of total phenolics. The freeze-dried samples were stored frozen and thawed in a desiccator prior to any analyses.

The total phenolics content, percentage condensed tannins and protein precipitable phenolics as a percentage of total phenolics were determined by the Chemistry Centre of Western Australia according to the methods outlined in the manual by FAO and IAEA on the *Quantification of Tannins in Tree Foliage* (FAO/IAEA, 2000).

6.1.2 Ash and organic matter contents

The oven dried material was ground using a 1 mm sieve and approximately 6 g (known weight) of the material was ignited in a muffle furnace at 550°C for 6 h. Following removal from the furnace, the residue was cooled in a desiccator and then weighed. The ash content was expressed as a percentage of the original sample DM. Organic matter was calculated as the difference in weight between the original sample and the ashed material. All determinations were carried out in duplicate.

6.1.3 Crude protein content

Crude protein was determined using the oven dried and ground (1 mm sieve) samples. Total N was determined on duplicate samples using the Kjeldahl oxidation procedure (Mossberg, 1979).

6.1.4 In vitro organic matter digestibility

In vitro organic matter digestibility was determined using the gas fermentation technique (tannin bioassay) described by Menke *et al*. (1979) and FAO/IAEA (2000).

6.1.4.1 Sample preparation

The freeze-dried phyllodes were ground using a 0.1 mm sieve.

6.1.4.2 Reagents

Bicarbonate buffer solution: 35 g sodium bicarbonate ($NaHCO_3$) and 4 g ammonium carbonate (NH_4HCO_3) was dissolved in approximately 500 mL distilled water and then made up to 1 L volume with distilled water.

Micromineral solution: 13.2 g calcium chloride ($CaCl_2\ 2H_2O$), 10 g manganese chloride ($MnCl_2.4H_2O$), 1 g cobalt chloride ($CoCl_2 \cdot 6H_2O$), and 8 g ferric chloride ($FeCl_3\ 6H_2O$) was dissolved in approximately 50 mL distilled water and then made up to 100 mL volume with distilled water.

Macromineral solution: 5.7 g disodium hydrogen phosphate (Na_2HPO_4), 6.2 g potassium dihydrogen phosphate (KH_2PO_4) and 0.6 g magnesium phosphate ($MgSO_4$ 7 H_2O) was dissolved in approximately 500 mL distilled water and then made up to 1 L volume with distilled water.

Resazurine solution: 0.1 g resazurine was dissolved in 100 mL distilled water.

Reducing solution: 996 mg sodium sulphide ($Na_2S.9H_2O$) was dissolved in 94 mL distilled water and then 6 mL of 1 N sodium hydroxide solution (4 g sodium hydroxide dissolved in 100 mL distilled water) was added.

6.1.4.3 Preparation of syringes

The phyllode samples were incubated in triplicate on two different days (with different batches of rumen fluid), yielding six parallel measurements.

Approximately 200 mg (known weight) of sample was weighed into a tared weigh boat was then transferred into a 100 mL calibrated glass syringe. The syringes had the following dimensions: 6 mm external diameter; approximately 200 mm in length; with a calibrated volume of 100 mL. A silicone tube (50 mm in length and 5 mm in internal diameter) and clip (to seal the tubing) was put on the capillar mouthpiece.

The syringes were prepared the day before the incubation and kept in the oven at $39^{o}C$.

6.1.4.4 Preparation of rumen fluid buffer solution

Rumen fluid samples were collected from the four donor animals prior to feeding. The samples were collected using a nylon stocking covered sampling probe attached to a 50 mL syringe. The strained fluid was quickly transferred into a warm thermos flask ($39°C$) filled with CO_2. The thermos was closed prior to transporting back to the laboratory. Sampling and transfer back to the laboratory was done as quickly as possible to minimise cooling of the rumen fluid (which would result in death of some of the rumen microbes).

Prior to the collection of the rumen fluid the following solutions were mixed in a 3 L wolffe flask in the following order: 210 mL bicarbonate buffer solution; 105 mL macromineral solution; 0.05 mL micromineral solution; 0.5 mL resazurine ; and 325 mL distilled water (these volumes were sufficient for 20 syringes [40 mL/syringe) plus 10% extra). The wolffe flask was kept in the water bath at $39^{o}C$, and the contents were flushed continuously with CO_2 and kept stirred using a magnetic stirrer. After about 5 min, 20 mL of reducing solution was added.

Once the mixture has been reduced (blue colour of dye changed to pink and then to colourless), 220 mL of the strained rumen fluid was added. This mixture was kept stirred and flushing with CO_2 for another 10 min.

40 mL of the rumen fluid medium-mixture was transferred into each warmed syringe using an automatic dispenser. Any gas bubbles in the syringe were removed, the plastic clip on the silicon tube closed, the position of the piston recorded, and the syringe suspended in the water bath. All of the syringes were gently shaken every hour for the first 4 h and then every 2 h. After 12 h the syringes were not shaken. The gas volume was recorded after 2, 4, 6, 8, 10, 12 and 24 h.

Differences in the composition and activity of the rumen fluid were controlled by two parallel measurements: (1) incubation of rumen fluid and medium mixture without substrate (Blank test Gb_0); and (2) incubation of a standard hay meal (200 mg DM; Hohenheim hay standard) which should give a mean gas production of 44.16 mL within 24 h (Gb_H). From these measurements it was possible to correct for each series of determinations using the correction factor $44.16/(Gb_H – Gb_O)$.

6.1.4.5 Calculations

Gas production
The mean gas production of blank tests (Gb_O) was subtracted from the gas production of samples and standards measured with the same batch of ruminal fluid. This net gas production had to be corrected for differences in sample weight (W. mg DM) when different from 200 mg dry matter.

Gas production (Gb) is defined as the total increase in volume ($V_{24} - V_0$) minus the blank (Gb_o), multiplied by the sample weight correction factor (200/W) and by the mean standard correction factor (F_H)

$$Gb \text{ (mL/200 mg DM, 24 h)} = [(V_{24} - V_o - Gb_o) * 200 * F_H]/W$$

where :
V_o = position of the piston at the beginning of incubation
V_{24} = position of the piston after 24 h of incubation
Gb_0 = mean gas production after 24 h incubation of rumen fluid and medium mixture without substrate
F_H = 44.16 / ($Gb_H - Gb_o$); roughage correction factor
W = weight of the test sample in mg DM.

Organic matter digestibility
The digestibility of organic matter (dO, %) was calculated from the gas production (Gb) and the content of crude protein (XP, g/kg DM) and crude ash (XA, g/kg DM):

$$dO = 14.88 + 0.889 \, Gb + 0.045 \, XP + 0.065 \, XA$$

6.2   Results

The nutritive value of *A. saligna* following cutting in either autumn, winter or spring are shown in Tables 6.1, 6.2 and 6.3, respectively. Unfortunately there is limited data available for the winter and spring cuts (as cattle broke through the fence surrounding the plot, consuming the plant material), but generally as age of the regrowth increased, the levels of CP (see Figure 6.1) and total extractable phenolics decreased whilst the ash content increased. The effect of age of regrowth (maturity) on OMD is less clear, with considerable variations occurring, as shown in Figure 6.2.

Table 6.1: The nutritive value of *A. saligna* over a period of 309 d following lopping in autumn

Age of regrowth (d)	DM (%)	Ash (%)	CP (%)	OMD (%)	TEP[*][1] (g/kg DM)	CT[2] (g/kg DM)	PPC[1] (% DM)
21	-	4.7	22.1	32.2	-	-	-
60	34.1	4.3	17.5	30.2	164.0	16.1	0.041
91	31.9	6.8	17.9	33.5	112.0	9.9	0.021
120	25.5	7.4	18.8	24.6	88.5	7.7	0.011
148	32.6	8.8	19.0	31.6	98.4	11.0	0.029
185	34.4	9.0	19.2	36.5	99.1	13.6	0.017
212	-	8.2	16.2	25.3	75.0	9.2	0.008
246	31.4	8.4	17.5	24.2	69.0	8.3	0.008
277	33.7	8.4	15.1	19.6	114	13.3	0.034
309	41.5	9.0	13.1	28.9	104	12.8	0.024

* Total extractable phenolics, [1] a tannic acid equivalent, [2] as leucocyanidin equivalent

Table 6.2: The nutritive value of *A. saligna* over a period of 218 d following lopping in winter.

Age of regrowth (d)	DM (%)	Ash (%)	CP (%)	OMD (%)	TEP[*1] (g/kg DM)	CT[2] (g/kg DM)	PPC[1] (% DM)
93	-	5.6	-	-	-	-	-
121	24.5	6.6	20.8	32.0	129.0	11.3	0.027
155	22.2	7.3	19.8	35.0	62.5	7.2	0.006
186	38.1	7.4	16.7	39.4	75.2	6.8	0.016
218	34.7	9.2	14.9	33.1	115.0	13.0	0.04

* Total extractable phenolics, [1] a tannic acid equivalent, [2] as leucocyanidin equivalent

**Table 6.3:** The nutritive value of *A. saligna* over a period of 125 d following lopping in spring.

Age of regrowth (d)	DM (%)	Ash (%)	CP (%)	OMD (%)	TEP[*1] (g/kg DM)	CT[2] (g/kg DM)	PPC[1] (% DM)
29	-	5.8	22.8	37.1	182.0	15.9	0.063
62	27.2	6.5	24.7	37.8	144.0	18.9	0.038
93	28.8	7.7	20.2	40.5	93.5	10.8	0.016
125	33.5	7.8	14.2	39.5	105.0	12.8	0.025

* Total extractable phenolics, [1] a tannic acid equivalent, [2] as leucocyanidin equivalent

Figure 6.1: The change in crude protein levels of *A. saligna* regrowth following lopping in either autumn, winter or spring.
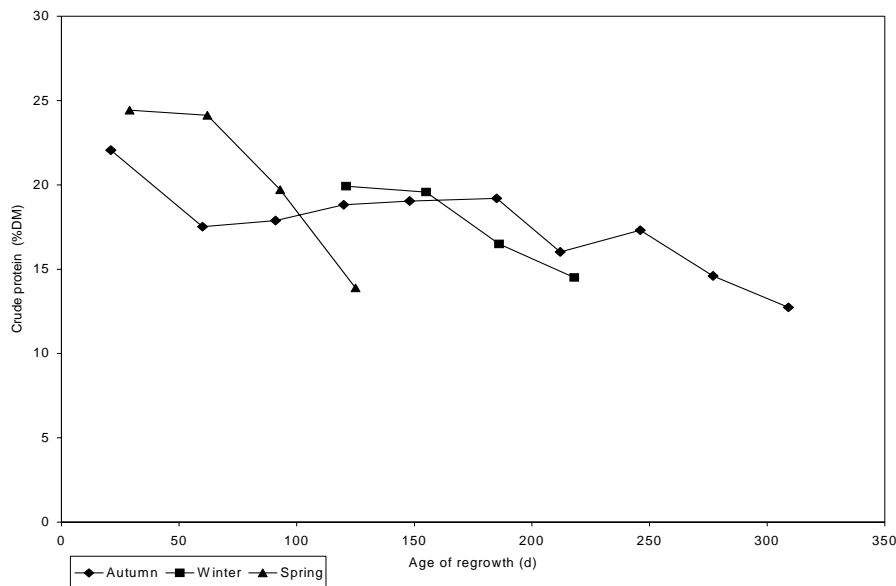
Regardless of when the *A. saligna* was originally lopped, the CP content of the regrowth declined over time.

Figure 6.2: The change in OMD of *A. saligna* regrowth following lopping in either autumn, winter or spring.
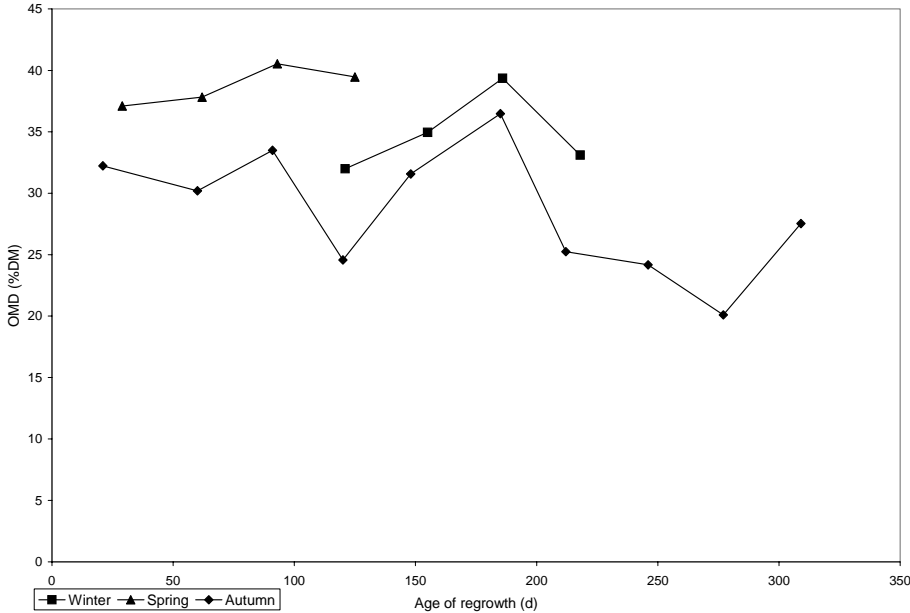
Unfortunately it was not possible to directly compare the nutritive value of the regrowth at the same age across the three seasons. The relationship between PPC and total extractable phenolics is shown in Figure 6.3. It is described by a positive linear regression (r^2=0.85, $y = 5.750 + 201.085x$).

Figure 6.3 Relationship between PPC and total extractable phenolics of *A. saligna*
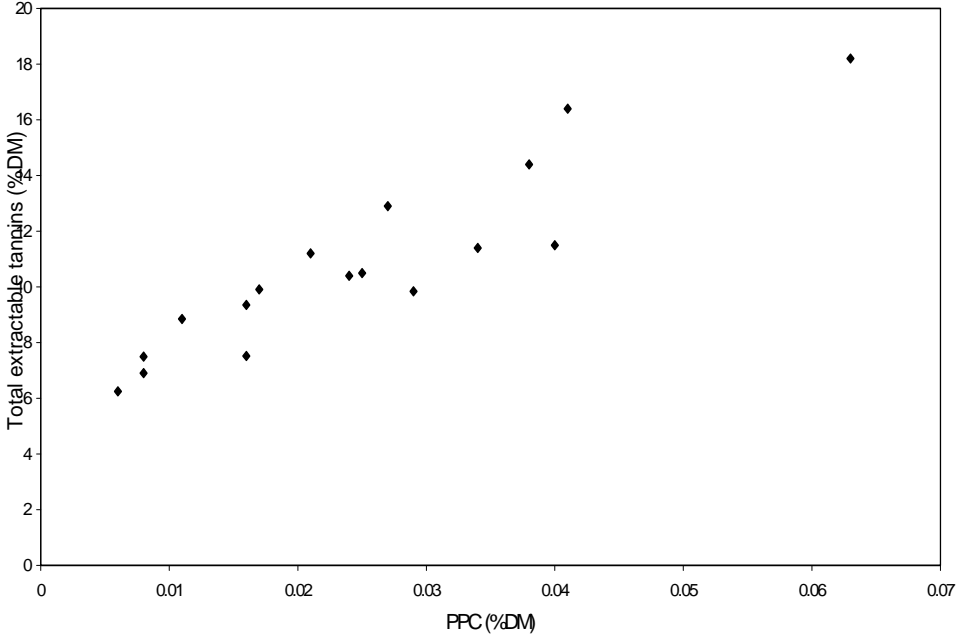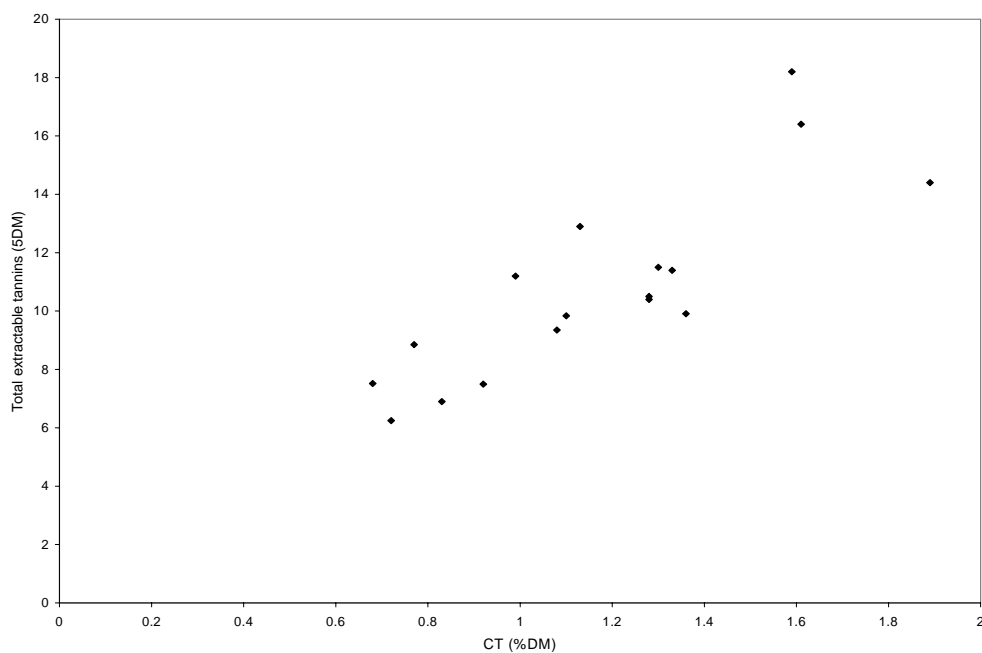
Figure 6.4 shows the relationship between CT and total extractable phenolics. There tends to be a positive linear relationship between the two measures ($r^2 = 0.70$, $y = 1.304 + 8.099x$).

Figure 6.4 Relationship between CT and total extractable phenolics of *A. saligna*

In Figure 6.5, the relationship between PPC and OMD is shown. There was no apparent relationship between the two parameters ($r^2 = 0.03$).

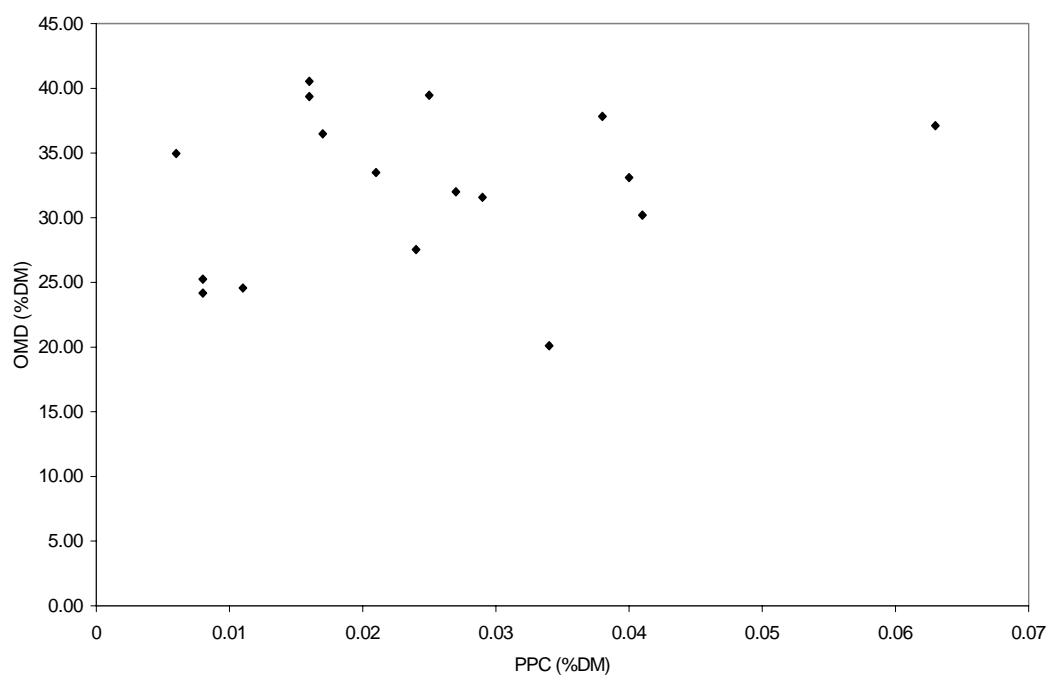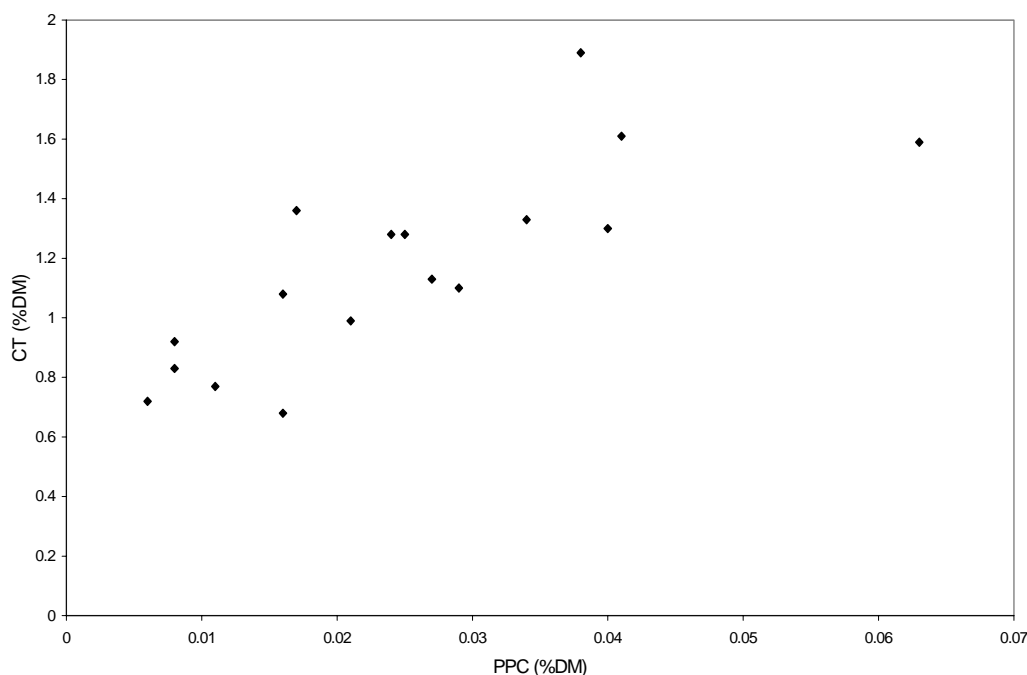**Figure 6.5** Relationship between PPC and OMD of *A. saligna*

Figure 6.6 shows the relationship between PPC and CT. There tends to be a positive linear relationship between the two parameters ($r^2 = 0.63$, $y = 0.722 + 17.88x$).

Figure 6.6 Relationship between PPC and CT of *A. saligna*

6.3   Discussion

The chemical composition of *A. saligna* is a major factor limiting its use as a supplementary feed for ruminants (Degen *et al*. 1997). In the current study large differences occur in its nutritive value as a result of changes in maturity. The effects of the season in which it is lopped is less clear, due to the limited number of regrowth results available from the winter and spring cuts.

6.3.1 Crude protein

The CP content of *A. saligna* ranged from 13.05 – 24.71%. This is generally higher than the rage of CP reported elsewhere for *A. saligna* foliage i.e 10.5 – 13.2% (Ben Salem *et al*., 1999; Ben Salem *et al*.,1997; Degen *et al*., 1997, Abou El Nasr *et al*., 1996; Degen *et al*., 1995).

The CP content of *A. saligna* phyllodes decreased with maturity (see Figure 6.1), which is consistent with previous studies on *A. saligna* (Degen *et al*., 1997), and also various oak species (Makkar and Singh 1991). In a study conducted on birch (*Betula pendula* Roth.) twigs by Thomas *et al*. (1985), it was found that CP levels increased before declining and then levelling out. In the current study, CP levels of the autumn cut regrowth decreased at a constant rate, whereas in the spring cut regrowth, the CP dropped off very rapidly after 50 d. Although the CP levels decreased over time with the winter cut regrowth, it did not decline as rapidly as the spring cut regrowth.

The season in which *A. saligna* is lopped appears to have an effect on the CP content of the regrowth. As shown in Figure 6.1, the CP content of early (young) regrowth from a spring cut was higher than that from an autumn cut, however, it could not be determined whether this difference was consistently maintained due to the limited data available for the spring cut regrowth. The season in which the *A. saligna* was lopped appears to confound the results on the effects of maturity on CP levels.

Season may also impact on the CP content of *A. saligna* in other ways. As average rainfall decreases so too does the CP content of *A. saligna* (Degen *et al*., 1995). In the current study the CP content of *A. saligna* was always greater than 13% whereas Degen *et al*. (1995) report a CP content of 12.5% for *A. saligna*. The trial conducted by Degen *et al*. (1995) was in Israel, with a mean yearly rainfall of 117 mm. The *A. saligna* used in present study was sourced from an area where the average yearly rainfall is 622 mm.

Degen *et al*. (1997) suggest that the age of the (original) plant is a further variable that may also confound the effect of age of regrowth on CP levels. They compared CP levels of *A. saligna* phyllodes from mature trees and young trees. The mature trees were ones that had been planted for three years and lopped every year, and the younger trees were ones that were planted and lopped after 6 months. Phyllodes were harvested in spring. The mature trees had a CP level that was 16% lower than the young trees.

6.3.2 Organic matter digestibility

OMD of *A. saligna* ranged from 19.62 - 40.54%. This is generally lower than the range of 30.4 - 54.2% reported for *A. saligna* used for the feeding trials. OMD in the current study was determined *in vitro* whilst for the feeding trial, OMD was determined *in vivo*.

The effect of age on the OMD of *A. saligna* is unclear, with there being no consistent trend in OMD over time (see Figure 6.2). Season, however, does appear to have an effect, with the OMD of the autumn cut regrowth being consistently lower than that of both winter and spring cut regrowth. Palo *et al*. (1985) also found, in a study on birch (*B. pendula* Roth.), OMD was affected by seasonal variation, and CP and OMD reached their maximum at the same stage, before declining accordingly. The relationship between CP and OMD reported by Palo *et al*. (1985) was not evident in the current study.

6.3.3 Ash

The (overall) average ash content of *A. saligna* was 7.7%, which is similar to the value of 7.2% reported by Degen *et al.* (1995). As the age of regrowth of *A. saligna* increased so too did the ash content (see Tables 6.1, 6.2 and 6.3). This is in contrast, however, to the findings of Degen *et al.* (1997), where the ash content of young phyllodes was twice as high as those of mature phyllodes.

6.3.4 Total extractable phenolics

In the initial stages of regrowth the levels of total extractable phenolics in *A. saligna* declined, however, with further increases in maturity the levels increased (see Tables 6.1, 6.2 and 6.3). Degen *et al*. (1997) also found that levels were lower in young phyllodes (10.3%) compared to mature phyllodes (15.0%). In general, the range in the levels of total extractable phenolics across all ages (6.25% - 18.20%) in the current study was greater than that reported by Degen *et al*. (1997).

6.3.5 Condensed tannins

CT levels ranged from 0.58 to 1.89%. These levels are substantially less than the levels of 12.52% and 8.3% reported by Degen *et al*. (2000) and Degen *et al*. (1995), respectively, but similar to values of 1.35-2.46% for the material used in the feeding trials (see Tables 3.1, 4.2 and 5.1). Plant genotype and the level of soil fertility affect the concentration of CT in plants (Lowther et al. 1987). The studies by Degen *et al*. (2000; 1995) were carried out in Israel, and thus it is possible that the differences in CT levels were due to variations in the plant genotype and soil fertility. Variations in the assays used to extract tannins also affects CT concentrations (Hagerman 1988), and may have contributed the variations in reported CT concentrations.

There was no clear relationship between maturity and CT levels of *A. saligna*. This is not in agreement with Degen *et al*. (1997), who found that CT levels were higher in young phyllodes (12.2% DM) than mature phyllodes (0.88% DM). In contrast, a study by Makkar and Singh (1991) on various oak species, showed that CT levels were higher in mature leaves (1-year-old leaves) compared to young leaves (approximately 15 d old).

As the level of total extractable phenolics in *A. saligna* increased, so too did the level of CT (see Figure 6.4; $r^2 = 0.70$). This is in contrast to Degen *et al*. (1997) who found that the CT level in foliage from young trees was twice the level of CT found in older trees, even though the older trees had a higher total tannin content.

6.3.6 Protein precipitating capacity

PPC levels of *A. saligna* ranged from 0.006 – 0.063. This is similar to the range of 0.014 – 0.044 reported previously for the *A. saligna* used in the feeding trials (see Tables 3.1, 4.2 and 5.1). As was the case with total extractable phenolics and CT, there was no clear relationship between PPC and age of *A. saligna*. In contrast, Makkar *et al*. (1988) found that as maturity of oak (*Quercus incanca*) increased PPC decreased.

There was a positive linear relationship between PPC and total extractable phenolics ($r^2 = 0.85$) but the relationship between PPC and CT ($r^2 = 0.63$) was less clear. Similarly, Makkar *et al*. (1988) found a positive relationship between PPC and total polyphenols ($r^2 = 0.973$), and only a poor relationship between PPC and CT ($r^2 = -0.798$).

Neither PPC, total extractable phenolics nor CT had any effect on the OMD of *A. saligna*. Goromela *et al* (1997) also found that OMD and CT were not correlated.

6.4   Conclusion

The nutritive value of *A. saligna* was affected by many factors, including the season in which it was pruned/lopped and the age of the subsequent regrowth. Generally as the plant matured, its nutritive value decreased, however, the season in which the *A. saligna* was lopped appears to have confounded this effect. Regrowth from spring lopped *A. saligna* generally had a higher CP and OMD than regrowth from autumn or winter lopped trees, although this difference became much less pronounced as the plant matured.

OMD was not correlated with either CP, total extractable phenolics, CT or PPC levels in *A. saligna*. As total extractable phenolics increased, so too did CT and PPC levels, but the impact of this on the nutritive value of *A. saligna* was not clear.

6.5   Limitations

During the sampling procedure, all samples were taken on the same date, which meant that the age of regrowth varied depending on when the trees were lopped. This meant that there was not comparative data for all ages of regrowth across the seasons.

The small number of samples for the winter and spring cuts limited a full examination of the effect of age of regrowth and season of cutting on the nutritive value of *A. saligna*. Ideally a time trace study providing data for at least twelve months regrowth for each season of lopping is needed to fully examine the relationships. Extending the study to lopping every month of the year and sampling of subsequent regrowth would provide the most detailed examination.

7. Future Research Needs

From the research reported, it has revealed that more research is required on *A. saligna* to fully understand the factors affecting its nutritive value. Examining the interactive effects of soil fertility, added nutrients, water (rainfall), season and age of regrowth would be beneficial, as all of these factors affect nutritive value of *A. saligna.*

Research needs to be conducted to determine if CP, OMD, total extractable phenolics, CT and PPC affect the DMI of *A. saligna*, and subsequent animal production responses.

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.

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.

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