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The Effects of Feeding *Acacia saligna* on Feed Intake, Nitrogen Balance and Rumen Metabolism in Sheep

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ABSTRACT : The aim of this study was to determine the feeding value to sheep of *Acacia saligna* grown under temperate conditions. Pen trials were undertaken to determine the effects of feeding *A. saligna*, which had been grown in a Mediterranean environment, on feed intake, nitrogen balance and rumen metabolism in sheep. Sheep were given *ad libitum* access to *A. saligna* with or without supplementation with PEG 4,000 or PEG 6,000. 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*. Dry matter intake was greater ($p<0.05$) in sheep supplemented with either PEG 4,000 or PEG 6,000 compared to the control. There was no difference, however, in intake between those supplemented with either PEG 4,000 or 6,000. Although animals were not weighed throughout the trial, a loss in body condition was obvious, in particular in the control group. Intake of N was greater ($p<0.05$) in sheep supplemented with either PEG 4,000 or PEG 6,000 than in the control. There was no difference in N intake between those supplemented with either PEG 4,000 or PEG 6,000. There were no significant differences in either the faecal or urinary N output between any of the treatment groups and all treatment groups were in negative N balance. Neither the average nor maximum pH of ruminal fluid of the control group was different to those supplemented with PEG. The minimum pH for the control group, however, was significantly higher ($p<0.05$) than for either of the PEG treatments. 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. For all dietary treatments ruminal ammonia levels were well below the threshold for maximal microbial growth. Feeding *A. saligna*, without PEG, had a definite defaunating effect on the rumen. For all dietary treatments ruminal ammonia levels were well below the threshold for maximal microbial growth. It was concluded that A. *saligna* was inadequate as the sole source of nutrients for sheep, even with the addition of PEG 4,000 or PEG 6,000. The anti-nutritional effects on the animals were largely attributed to the excessive biological activity of the phenolics in the *A. saligna* leaves. There is a need to determine other supplements that may be complimentary with PEG to enhance the nutritive value of *A. saligna* to maintain a minimum of animal maintenance. (**Key Words :** Acacia saligna, Sheep, PEG, Tannins, Protozoa)

INTRODUCTION

Browse species such as *Acacia saligna* 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. During dry periods forage trees remain green and maintain a relatively high CP content. Their foliage may be used as a protein and energy supplement when animals are given low quality roughage. The presence, however, of secondary plant compounds could present major constraints to their use. The primary antinutritional agent in *Acacia* species and many other browse species are condensed tannins (CT) (D'Mello, 1992; Karabulut et al., 2007).

Inhibition of digestibility can be attributed mostly to soluble CT binding proteins and digestive enzymes and antimicrobial characteristics. The formation of indigestible fibre bound CT and protein macro structures and increased lignification within the fodder prior to consumption also contribute to losses in fodder feed value. It is evident from a number of studies that where high tannin feed is the main source of fodder, detannification through complexation of soluble CT is beneficial in retention of digestive capacity and optimisation of nutrient availability to the ruminant. Polyethylene glycol (PEG) is a well-recognised detannification agent; with PEG 4,000 appearing to be the major form used in trials involving high tannin feed despite the fact that PEG 6,000 has been shown to be more effective, *in vitro* (Makkar et al., 1995).

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Table 1. Composition of *A. saligna* foliage

Dry matter (%)	35
Organic matter (%DM)	92.7
Metabolisable energy (kJ/kg DM)	5.1[1]
Crude protein (% DM)	11.4
Total extractable phenolics[2] (%DM)	9.45
Condensed tannins[3] (% DM)	2.69
Protein precipitating capacity (PPC, % DM)	0.044

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

Most research of *A. saligna* tends to involve plant material grown in arid/semi-arid regions (Degen et al., 2000). However, it is also known to grow prolifically in areas of higher rainfall and in climates ranging from cool to tropical, where plant stress levels are lower and potentially lower phenolic/CT levels may be produced (Lees et al., 1994). Little information is available on the performance of animals fed *A. saligna* grown in such environments. The aim of this study was to determine the effects of *A. saligna* grown under temperate conditions on rumen metabolism in sheep. In addition, the effect of partial detannification of *A. saligna* on its value as a source of nutrients for sheep was also investigated.

MATERIALS AND METHODS

The experiment was based on a Latin square design. Six mature Merino wethers, each fitted with a permanent rumen cannula were used. The animals were randomly allocated to 1 of 3 dietary treatments. Each experimental period was of 21 d duration, made up of 12 d for diet adaptation followed by 1 d of sampling of ruminal fluid and then 7 d of recording of feed intake, and faecal and urinary output.

Once a week, *A. saligna* was lopped from a 3 years old plantation and only foliage less than 12 months old was used. 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 may be described as sandy gravel. After harvest, material was stored at -18°C pending feeding.

The 3 dietary treatments were: (1) Control: *ad libitum* access to *A. saligna* (basal diet); (2) PEG 4,000: basal diet+25 g/d PEG 4,000 and (3) PEG 6,000: basal diet+25 g/d PEG 6,000. Daily feed intake was recorded throughout the trial.

Both PEG 4,000 and 6,000 were used to enable a comparison of the two, *in vivo*. 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 which 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.

Table 2. Intake and digestibility of *A. saligna* offered to sheep with or without a supplement of PEG 4,000 or PEG 6,000

	Treatment		
	Control	PEG 4,000	PEG 6,000
DMI (g/d)	187±57[a]	499±101[b]	463±88[b]
DMD (%)	31.3±7.9[a]	36.8±9.1[b]	37.8±7.7[b]
OMD (%)	30.4±7.8[a]	32.1±9.1[b]	33.0±8.3[c]

Values within rows with different superscripts are significantly different ($p<0.05$).

Samples of ruminal content were obtained via the rumen cannula, just prior to feeding and thereafter, at regular intervals for 24 h using a sampling suction probe. 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 M H_2SO_4. The samples were then stored at -18°C. 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 through 2 layers of stocking material to remove feed matter, and stored at room temperature for counting of protozoa.

Samples of *A. saligna* foliage and faeces were dried in a forced-air oven at 35°C until constant weight to determine DM contents. Where applicable, the weight of PEG was subtracted from the faecal weight in determining DM digestibility (DMD).

Proximate analysis was used to determine the ash, organic matter (OM) and crude protein (CP) contents of the feed and faecal samples. The Kjeldahl procedure (Tecator digestion block and distillation unit) was used to determine CP contents. Total extractable phenolics, CT and protein precipitation capacity (PPC) were analysed according to the methods recommended by the FAO/IAEA (2000).

Ruminal fluid was centrifuged (3,000 g for 10 min) and analysed (in duplicate) for ammonia concentrations. The procedure used an automated segmented flow Technicon instrument and was based on the modified Berthelot reaction (Searle, 1984).

Numbers of protozoa contained in 1 ml of rumen were counted using a 0.2 mm counting chamber (Fuchs-Rosenthal) at 10×10 magnification. Two fields were counted.

An analysis of variance of the results was carried out using Genstat®. The P level of 0.05 was used when testing for significance.

RESULTS

The nutrient analysis of *A. saligna* foliage is shown in Table 1. The DM intake (DMI) of *A. saligna* (Table 2) was

Table 3. Nitrogen balance in sheep offered *A. saligna* with or without a supplement of PEG 4,000 or PEG 6,000

N (g/d)	Treatment		
	Control	PEG 4,000	PEG 6,000
N intake	3.4±1.03[a]	9.1±1.83[b]	8.4±1.61[b]
Faecal N	4.8±0.86	7.1±2.63	6.3±1.76
Urine N	3.1±0.41	2.5±0.57	2.4±0.61
N balance	-4.5±0.52[a]	-0.5±1.00[b]	-0.3±0.87[b]

Values within rows with different superscripts are significantly different ($p<0.05$).

Table 4. Ammonia levels and pH of ruminal fluid in sheep offered *A. saligna* with or without a supplement of PEG 4,000 or PEG 6,000

	Control	PEG 4,000	PEG 6,000
Av. NH_3-N (mg/L)	3.52±0.50[a]	10.23±4.58[b]	9.27±3.82[b]
Min. NH_3-N (mg/L)	2.79±0.10	6.5±2.81	8.46±5.26
Max. NH_3-N (mg/L)	5.0±0.89[a]	13.5±6.28[b]	14.0±7.07[b]
Average pH	7.6±0.4	7.0±0.3	7.0±0.4
Minimum pH	7.4±0.5[a]	6.6±0.4[b]	6.6±0.4[b]
Maximum pH	7.8±0.3	7.4±0.2	7.5±0.3

Values within rows with different superscripts are significantly different ($p<0.05$).

greater ($p<0.05$) in sheep supplemented with either PEG 4,000 or PEG 6,000 compared to the control. There was no difference, however, in intake between those supplemented with either PEG 4,000 or 6,000. Although animals were not weighed throughout the trial, a loss in body condition was obvious, in particular in the control group.

There was no difference ($p>0.05$) in the DMD of *A. saligna* foliage between animals supplemented with PEG 4,000 or PEG 6,000 but both of these groups were greater ($p<0.01$) than the control group. There was a significant difference ($p<0.001$) in OM digestibility (OMD) between all treatments with PEG 6,000 being the greatest, followed by PEG 4,000 and the control group.

Intake of nitrogen (N) (Table 3) was greater ($p<0.05$) in sheep supplemented with either PEG 4,000 or PEG 6,000 than the control. There was no difference in N intake between those supplemented with either PEG 4,000 or PEG 6,000. There were no significant differences ($p>0.05$) in either the faecal or urinary N output between any of the treatment groups and all treatment groups were in negative N balance.

Neither the average nor maximum pH of ruminal fluid of the control group was different ($p>0.05$) to those supplemented with PEG (Table 4). The minimum pH for the control group, however, was significantly higher ($p<0.05$) than for either of the PEG treatments.

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.

Protozoa were present in abundance in ruminal fluid only until the animals had undergone the control treatment, after which there were virtually no protozoa present (Table 5).

Table 5. Rumen protozoal numbers ($\times 10^5$ per ml) and their relationship with the order of treatment

Sheep number	Trial period/treatment		
	1	2	3
S1 and S2	PEG 6,000	PEG 4,000	Control
	>0.6	>0.6	0
S3 and S4	PEG 4,000	Control	PEG 6,000
	>0.6	0	0
S5 and S6	Control	PEG 6,000	PEG 4,000
	0	0	0

DISCUSSION

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; 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. 105-132 g/kg (Abou El Nasr et al., 1996; Degen et al., 1997; Ben Salem et al., 1999).

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. (1995) and Degen et al. (1997), 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, possibly due to lower plant stress levels in a more rainfall abundant or temperature zone, such comparisons may not be truly indicative of protein precipitation capacity and thus only provide an indicative guide to browse feed value or antinutritional potential.

Feed intake, digestibility and palatability

The DMI of *A. saligna* by sheep 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 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. In Degen et al. (1997), however, 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 3 years old trees. The foliage from 'mature' trees in the trial of Degen et al. (1997) was also obtained from 3 years old trees that had been cut in each of the previous 3 years.

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

PEG, particularly that of molecular weight 4,000 (PEG 4,000) has been widely used in the studies of tannin-rich forages (Barahona et al., 1997; Ben Salem et al., 1999; Fujihara et al., 2005). *In vitro*, PEG 6000 has been found to have a superior capacity to bind with tannins than PEG of various other molecular weights, including 4,000 (Makkar et al., 1995). In this study the DMI of *A. saligna* was significantly improved, as were both the DMD and OMD, where either PEG 4,000 or 6,000 was administered. PEG 6,000 increased OMD to a greater extent than did PEG 4,000. 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 (Barry and Duncan, 1984; Silanikove et al., 1996; Degen et al., 1998; Rubanza et al., 2003).

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 initial nutrient 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. (1997) suggested that a lack of palatability may have contributed to the low DMI of *A. saligna* observed. In the present trial, however, 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 (Reed et al., 1990; Chriyaa et al., 1997; Degen et al., 1997), with palatability having a minor influence on DMI.

Body condition and N balance

The N intake of sheep dosed with PEG 4,000 or PEG 6,000 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 4,000 or 6,000. 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. 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 was also binding with endogenous proteins such as enzymes, as well as with gut microbial protein. Although not as high as the control, faecal N was also high for the PEG groups. This suggests that a higher rate of PEG might have had further benefits.

A reduced urinary N is often a mechanism by which animals compensate for the higher faecal N with increasing CT levels in the diet, as evident in work such as that of Harrison et al. (1973), Fasslet and Lascano (1995) 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). It is not understood why the urinary N (as a proportion of N intake) was much greater for the control animals in this instance and it is not supported by the very low ruminal ammonia concentrations. It is possible that a greater extent of protein catabolism may have contributed to their higher urinary N excretion.

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 (Reed et al., 1990; Ben Salem et al., 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 ruminal N concentration as well as increasing DM digestibility, hence the improved (although still inadequate) N balance.

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

Tannins bind to 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 4,000 and PEG 6,000). PEG has been shown to have positive effects on digestible N and N retention in other trials involving high tannin feeds (Barry and Duncan, 1984; Ben Salem et al., 1999; Ozjan and Sahin, 2006). The response to PEG, however, appears to depend on the tannin content of the diet. Leden et al. (2002) suggest PEG is detrimental to N retention where animals are fed diets containing low levels of tannins.

Ruminal ammonia concentration and pH

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 (Ebong, 1995; Ben Salem et al., 1997; 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 (Terrill et al., 1992; McNabb et al., 1993). 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. (1996). 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) 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.

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. Defaunation, however, 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 binding capacity of the CT.

The results of this trial indicate that *A. saligna*,

harvested from a 3 year old plantation grown in a Mediterranean climate. could not be used as a sole diet to maintain the weight of sheep. Although the inclusion of either PEG 4.000 or PEG 6.000 in the diet improved the utilisation of *A. saligna*, the animals remained in negative N balance and the diets were still considered sub maintenance. In addition, whilst PEG 6.000 resulted in higher OMD than PEG 4.000 the improvement was not sufficient to confer any advantage in animal production. Whilst the addition of PEG did assist in the maintenance of normal rumen function and provided a degree of protection against the antinutritional characteristics of this high phenolic browse species there is need to determine whether a greater dose of PEG might have resulted in further improvements in nutrient digestion and utilisation. A further area of research is investigating other supplements that may be complimentary with PEG to enhance the nutritive value of *A. saligna* to maintain a minimum of animal maintenance.

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