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Polyethylene glycol is more effective than surfactants to enhance digestion and production in sheep fed mulga (*Acacia aneura*) under pen and paddock conditions

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Abstract. Chemicals that interfere with the formation of tannin–protein complexes were evaluated as dietary additives for mulga-fed sheep in pens and under paddock conditions. Condensed tannins (CT) in mulga inhibit protein digestion, and the use of chemicals to precipitate CT or dissociate CT–protein complexes may improve production from sheep consuming a mulga diet. In a digestion study with mulga-fed sheep in pens, provision of polyethylene glycol (PEG) at a rate of 6 g/day significantly ($P < 0{\cdot}05$) improved nitrogen (N), phosphorus (P), and sulfur (S) balance, and apparent N, P, dry matter (DM), and organic matter digestibility, and the rate of liveweight gain. Addition of the surfactants SDS or alkanate 3SL3 to the diet of mulga-fed sheep did not improve N balance or digestion; however, apparent digestibility of P, and P and S balance, were significantly improved by SDS. Teric PE64, a compound structurally similar to PEG, significantly improved S balance, but not DM intake or N balance. For sheep consuming a predominantly mulga diet under paddock conditions, provision of PEG at a rate of 12 g/day significantly improved clean wool growth and liveweight gain compared with unsupplemented sheep, by 9% and 100%, respectively ($0{\cdot}809$ *v.* $0{\cdot}745$ mg/cm^2·day, and 44 *v.* 22 g/day). The studies demonstrated that although surfactants can affect mulga digestion, using PEG to precipitate CT is more effective to improve mulga digestion and animal production than the use of surfactants. However, the wool and liveweight production responses achieved with PEG were not sufficient to justify its wide-scale use for mulga-fed sheep. Consequently, alternative methods should be sought to reduce the negative effects of mulga CT on sheep production.

Additional keywords: wool, protein, condensed tannin.

Introduction

Mulga (*Acacia aneura*) is one of the most widely exploited fodder tree legumes in agriculture in Australia. During drought, mulga leaves have been used as a readily available source of protein for sheep for more than 100 years. Although mulga appears to contain adequate levels of the essential nutrients required to support liveweight gain, it is poorly digested (Harvey 1952) and sheep production responses have been recorded with nitrogen, phosphorus, and sulfur (NPS) supplements, and protein supplements during periods of prolonged feeding (McMeniman and Little 1974; Niven and McMeniman 1983).

Condensed tannins (CT) act as anti-nutritive compounds by precipitating dietary proteins, reducing the activity of digestive enzymes, and inhibiting the growth and function of rumen micro-organisms (Swain 1979; Brooker *et al.* 1994). The high concentration of CT in mulga contributes to the characteristic poor digestibility of mulga by sheep (Gartner and Hurwood 1976; Pritchard *et al.* 1992), and supplements that reduce

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the effects of these CT on digestion have since been sought to increase the nutritive value of mulga. NPS supplements partially alleviate nutrient imbalances. Moreover, addition of polyethylene glycol (PEG) to the diet, a compound that precipitates CT (Jones and Mangan 1977) and facilitates an increase in the efficiency of protein digestion, significantly improves wool growth and liveweight by a greater magnitude than NPS supplements alone (Pritchard *et al.* 1992). However, based on the results of pen studies, Pritchard *et al.* (1992) predicted that PEG may be too expensive to be added to the diet of sheep under paddock conditions.

Cheaper compounds that can inhibit the anti-nutritive effects of CT could be valuable to the grazing industry. Surfactants offer one such alternative. In the digestive tract of insects, surfactants protect against the effects of dietary CT (Martin and Martin 1984). Furthermore, they are used routinely in the laboratory to solubilise and dissociate pre-formed CT–protein complexes (Hagerman and Butler 1978; Fliedel and Kobrehel 1985; Martin *et al.* 1985). These chemicals may be useful if they can be used as dietary additives to improve digestion of mulga protein by sheep.

The aim of the present study was to determine if compounds that interfere with the formation of CT–protein complexes, by either precipitating CT or dissociating CT–protein complexes, could be used to increase the productivity of mulga-fed sheep. In addition, the potential to use PEG as a cost-effective supplement was further evaluated for mulga-fed sheep under paddock conditions.

Materials and methods

Digestion study

Thirty Merino wethers (2 years old) were placed in individual pens, and freshly harvested mulga leaves were offered *ad libitum* each morning. In addition, each sheep received a daily mineral supplement (NPS). NPS was prepared by dissolving 326 g urea (46% N), 1500 g monophos (25% P), and 1150 g sulfate of ammonia (20% N, 24% S) in 5 L of water. The sheep received 20 mL/day of NPS orally, which provided 1·5 g N, 1·5 g P, and 1·1 g S per day. Dry matter (DM) intake during the first 10 days of mulga feeding was used to stratify the sheep, and they were randomly allocated to 5 treatment groups, each containing 6 sheep. Integral to this procedure, 4 sheep within each group were allocated for subsequent use in a 10-day metabolism study. The treatments commenced on Day 11 and were (*i*) NPS, (*ii*) NPS plus 6 mL/day of SDS (active ingredient sodium decyl sulfate, Albright and Wilson Australia Limited), (*iii*) NPS plus 6 mL/day of alkanate (3SL3, active ingredient sodium lauryl diethoxysulfate, ICI Australia Limited), (*iv*) NPS plus 6 g/day of Teric (PE64, active ingredient polypropylene glycol ethoxylate, ICI Australia Limited), and (*v*) NPS plus 6 g/day of PEG (4000, ICI Australia Limited). In preparation for administration to sheep, SDS, alkanate, and Teric were prepared as 30% v/v concentrations in water, and PEG was prepared as a 30% w/v solution. This enabled the correct quantity of each compound to be delivered via a daily 20-mL oral drench. Liveweight was recorded weekly throughout the 47-day treatment period. Wool growth for the treatment period was measured by clipping wool from midside patches (Morley *et al.* 1955). Sheep initially were clipped 7 days after treatments began, and wool from the clipped patch was subsequently harvested the day after treatments concluded.

Twenty days after treatments commenced, the 4 sheep from each group identified during the initial stratification procedure were placed in metabolism cages for 10 days. Urine was collected into plastic vessels containing 10 mL concentrated HCl, and faeces was collected using faecal collection harnesses. The quantities of faeces and urine excreted by each sheep were measured daily, and subsamples representing 10% of daily excretion were frozen and stored for subsequent N analysis. For each sheep, the samples were bulked across the collection period and submitted for analysis. Samples of mulga fed to the sheep, and residues remaining in feed bins the following morning, were collected and stored at −20°C for analysis. After collections were concluded, the sheep were returned to individual pens for the remainder of the treatment period.

Paddock study

Merino wethers (140), aged 18–24 months, were shorn and weighed. They were treated with a broad-spectrum anthelmintic (Valbazen, Smith, Kline and French Laboratories, Australia) and a lousicide (Clout-S, Coopers Animal Health) in accordance with manufacturer's recommendations. Liveweight was used to rank the sheep, and they were randomly allocated to 4 groups of 35. Each group was allocated 1 of 2 treatments: (*i*) NPS (Groups 1 and 3), or (*ii*) NPS plus 12 g/day of PEG (Groups 2 and 4). Six paddocks (each 60·8 ha) containing dense stands of mulga, and located at the Mulga Master Site, Charleville (Burrows and Beale 1970), were given a productivity ranking according to records of wool production and liveweight change, and the highest and lowest ranking paddocks were discarded. The 4 remaining paddocks were then paired, and treatment Groups 1 and 2 were allocated to the 2 highest ranking paddocks (paddocks A and B, respectively), and Groups 3 and 4 to the remaining 2 paddocks (paddocks C and D, respectively). The pairing of paddocks C and D thus served as a replicate for paddocks A and B. Paddocks were paired in this manner to minimise bias in the results due to differences in the productivity of each paddock within each replicate. To minimise paddock bias further in the results, groups of sheep within each replicate were swapped between their 2 paddocks every 21 days.

When treatments commenced, pasture in the understorey was negligible in all paddocks, thus sheep were reliant on mulga for their entire diet. Branches were lopped from mulga trees within each paddock 3 times each week, and sufficient branches were lopped so that an *ad libitum* supply of leaves was maintained. NPS was provided for the sheep in the form of a nutrient block. The supplement was designed to deliver 1·5 g N, 1·5 g P, and 1·1 g S/sheep·day through consumption of 25 g of the block. The blocks were placed under cover and adjacent to water troughs, and replaced as consumed. Every fourth day, 1600 g of PEG 4000 flakes were placed in covered feed troughs. This was sufficient to provide 12 g/head·day. For the first 18 days of the treatment period, 100 g of molasses was used as a bait to entice consumption of PEG. Thereafter, 1000 g of coarse salt was provided with PEG to maintain PEG intake. Integral to the initial stratification

procedure, 15 sheep from each treatment group were allocated to measure clean wool growth for the treatment period, by clipping wool from midside patches. Sheep initially were clipped 1 week after treatments commenced, and wool from the clipped patches was subsequently harvested 14 weeks and 24 weeks later. Liveweight was recorded weekly, and the treatments were continued for 25 weeks. In each paddock, a water trough was enclosed in a small yard fitted with self-trapping gates. When the gates were set, the sheep were trapped in the yard whilst drinking.

Chemical analyses

N content of air-dried mulga feed and residues, wet faeces, and urine was determined using the Kjeldahl technique (Faichney and White 1983). S was determined on pressure-powdered samples of feed, residues, faeces, and filtered urine using X-ray fluorescence with an ARL 848 spectrometer. P in feed, residues, faeces, and urine was measured by a colorimetric method, following ashing at 550°C for 2 h and HCl digestion for feed, residues, and faeces, or following 3 acid digestion (HNO_3, $HClO_4$, and H_2SO_4) for urine (AOAC 1980). Organic matter (OM) was determined in feed, residues, and faeces following ashing at 600°C for 1 h. CT in mulga leaves was determined in methanol extracts of fresh material by the vanillin hydrochloric acid method (Burns 1963), and results are expressed as catechin equivalents. N, P, S, OM, and CT concentrations were only determined in samples collected during the digestion study, and all results are expressed on a DM basis. Weight of clean wool was determined after hexane scouring and overnight oven drying at 80°C.

Statistical analyses

The digestion study was analysed as a completely randomised design, and the liveweight study was a balanced factorial design. Both studies were subjected to analysis of variance or analysis of covariance where pre-treatment values were measured, using the statistical package (BALF) developed by the Queensland Department of Primary Industries.

Results

Digestion study

Crude protein and CT concentrations in mulga during the digestion study were 122 and 76 g/kg, respectively. Neither DM intake nor clean wool growth differed significantly among treatment groups (Table 1). The rate of liveweight gain recorded for sheep that received PEG was significantly ($P < 0{\cdot}05$) different from the liveweight loss for sheep provided with SDS, alkanate, and Teric, and sheep provided with NPS only. DM intakes did not differ significantly for the 10-day collection period (Table 2) compared across the entire 47-day treatment period (Table 1). Compared with sheep that received NPS only, PEG was the only compound that significantly improved apparent N digestibility and balance from mulga (Table 2). Apparent P digestibility and balance were significantly improved by both PEG and SDS. Negative P balances

Table 1. Dry matter intake (DMI), clean wool growth (CWG), and liveweight change (LWC) for mulga-fed sheep provided with a mineral supplement (NPS), and SDS, alkanate 3SL3, Teric PE64, or PEG 4000 in the digestion study

Within rows, means followed by the same letter are not significantly different at $P = 0{\cdot}05$

	NPS	NPS plus:				s.e.m.
		SDS	Alkanate	Teric	PEG	
DMI (g/day)	604	556	667	712	750	49·1
DMI ($g/kg^{0{\cdot}75}$·day)	46·4	41·6	53·0	54·7	56·4	4·50
CWG (mg/cm^2·day)	0·47	0·39	0·45	0·49	0·55	0·042
LWC (g/day)	−33a	−28a	−33a	−8a	51b	17·8

Table 2. Nitrogen (N), phosphorus (P), and sulfur (S) balances and apparent digestibilities, dry matter (DM) and organic matter (OM) digestibilities, and dry matter intake (DMI) during the 10-day collection period for mulga-fed sheep provided with a mineral supplement (NPS), and SDS, alkanate 3SL3, Teric PE64, or PEG 4000 in the digestion study

Within rows, means followed by the same letter are not significantly different at $P = 0{\cdot}05$

	NPS	NPS plus:				s.e.m.
		SDS	Alkanate	Teric	PEG	
N balance (g/day)	1·38a	2·74ab	2·04a	2·92ab	4·36b	0·575
P balance (mg/day)	−817a	74b	−761a	−647a	57b	142·5
S balance (mg/day)	272a	672c	432ab	565bc	760c	61·5
N digestibility (g/kg)	506a	520a	510a	533a	609b	18·1
P digestibility (g/kg)	−692a	115b	−478a	−452a	275b	129·4
S digestibility (g/kg)	578	635	588	590	645	18·7
DM digestibility (g/kg)	472a	523ab	488a	487a	569b	19·4
OM digestibility (g/kg)	488a	539ab	498a	495a	575b	19·2
DMI (g/day)	610	677	679	735	747	57·8

Table 3. Clean wool growth (CWG) and rate of liveweight change (LWC) for sheep provided with a mineral supplement (NPS) with or without 12 g/day polyethylene glycol (PEG) in the paddock study

Within rows, means followed by different lower case letters are significantly different at $P = 0{\cdot}05$. Within columns, means followed by the same upper case letter are not significantly different at $P = 0{\cdot}05$

	NPS	NPS plus PEG	s.e.m.
	CWG ($mg/cm^2 \cdot day$)		
Period 1 (Weeks 1–15)	0·745aA	0·809bA	0·0222
Period 2 (Weeks 16–25)	1·061B	1·075B	0·0229
	LWC (g/day)		
Period 1 (Weeks 1–15)	22aA	44b	3·8
Period 2 (Weeks 16–25)	70aB	46b	4·2

reflect excretion of P at levels exceeding intake. S balance was significantly improved by PEG, Teric, and SDS. Apparent S digestibility did not differ significantly between treatments. DM and OM digestibilities were significantly improved by PEG compared with the NPS supplement only.

Paddock study

During the first 15 weeks of the paddock study (Period 1), 313 mm of rain was recorded, 198 mm of which fell during Weeks 13–15. As a consequence of widespread pasture germination in the paddocks following the rain during Weeks 13–15, the study was divided into 2 measurement periods: (*i*) Weeks 1–15 (Period 1), and (*ii*) Weeks 16–25 (Period 2). The germination of pasture species, and the likelihood of a change in diet from predominantly mulga to a mixed diet of mulga and pasture, had the potential to confound interpretation of the results. To minimise such confounding effects, the study was divided into 2 periods so that the effect of a likely change in diet on the activity of PEG could be quantified. A total of 63 mm was recorded during Period 2. Consumption of the NPS blocks averaged 14 g/head·day for Period 1, and 17 g/head·day for Period 2. Consequently, average intakes of N, P, and S per animal were 0·84, 0·84, and 0·62 g/day, respectively, for Period 1, and 1·02, 1·02, and 0·75 g/day, respectively, for Period 2. Prior to the commencement of treatments, mean liveweights of sheep were 30·1 and 30·5±0·74 kg for the PEG group and the group without PEG, respectively. During Period 1, sheep that received PEG grew significantly ($P < 0{\cdot}05$) more clean wool per unit area, and gained weight at a significantly faster rate, than sheep without PEG (Table 3). At the end of Period 1, sheep that received PEG weighed significantly more than those that did not receive PEG (34·8 *v.* 32·5±0·38 kg). Wool growth did not differ significantly between treatments during Period 2. Sheep that received PEG gained weight at a significantly lower rate than those without PEG during Period 2, and liveweights were similar for both treatment groups by the end of this period (37·5 *v.* 38·0±0·35 kg). For both treatment groups, clean wool growth was significantly greater during Period 2 than Period 1. Sheep that did not receive PEG gained weight at a significantly faster rate during Period 2 than in Period 1; however, rate of liveweight gain did not differ significantly between periods for sheep that received PEG.

Discussion

In these studies, PEG was superior to the other compounds to enhance N digestion, liveweight, and clean wool growth in mulga-fed sheep. PEG readily forms complexes with CT (Jones and Mangan 1977), and Pritchard *et al.* (1992) established that a maximum of 77% of mulga CT can be precipitated by PEG. This maximum occurs at a PEG to CT ratio of 0·7:1 w/w. Therefore, in the digestion study, 44 g of the 57 g of CT consumed by the sheep that received PEG were free to be precipitated by PEG, and 31 g of PEG would have been necessary to precipitate fully all of the CT. As only 6 g of PEG was administered to the sheep, 80% of the reactive CT consumed were free to react with dietary protein and inhibit mulga digestion. Consequently, a production response of higher magnitude than that observed in the digestion study could have been expected if more PEG had been administered to the sheep. The improvements in apparent digestibilities of N and P, and N, P, and S balance, achieved with PEG suggest the digestion of mulga was improved. Unlike the earlier study of Eady *et al.* (1989) in which clean wool growth was significantly improved with 6 g/day of PEG, clean wool growth was unaffected by the same amount of PEG in the current digestion study. Since our sheep were stratified on DM intake and not wool growth, this result was not unexpected.

High concentrations of CT inhibit digestion of the carbohydrates in *Lotus* sp. by sheep (Barry *et al.* 1986); however, OM digestibility was significantly increased upon treating *Lotus* with PEG. Although

further detailed study is required to confirm the effects of mulga CT on carbohydrate digestion, the significant improvements in DM and OM digestibility attributable to PEG in the digestion study suggest that mulga CT can inhibit carbohydrate digestion in a manner similar to CT in *Lotus*. In the digestion study, sheep responded to PEG in the diet with significant increases in P and S balance, by 0·9 and 0·5 g/day, respectively, compared with sheep supplemented with NPS alone. Combined with the 1·5 g of P and 1·1 g of S provided by the NPS supplement, the improved concentration of P and S provided to the rumen of the sheep that received PEG is likely to have aided microbial growth and activity, and contributed to the improvement in DM digestibility. Improvements in DM digestion and intake have been similarly attributed to the provision of additional P and S to mulga-fed sheep in previous studies (Hoey *et al*. 1976; McMeniman 1976).

Proteins in plant samples that contain high concentrations of CT can be solubilised using detergents (Fliedel and Kobrehel 1985), and SDS is used routinely in the laboratory for its ability to dissociate CT–protein complexes (Hagerman and Butler 1978; Martin *et al*. 1985). Both SDS and alkanate were used in the present study in an attempt to exploit their ability as surfactants to dissociate CT–protein complexes. As neither SDS nor alkanate improved DM intake or N digestion, they appeared to be of little benefit at these rates to improve animal production. However, the significant improvements in S and P balance, and the apparent digestibility of P associated with SDS, were indicative that SDS can affect mulga digestion and warrants further study. The concentration of surfactants in the rumen may be reduced by absorption onto plant material (Wright and Curtis 1976). A reduction in the activity of the surfactant, caused by absorption, may mean that a higher dose of surfactant than that used in the digestion study is necessary to produce an effect on animal production. In a subsequent experiment (S. M. Miller, unpubl. data), sheep provided with 12 mL/day SDS lost appetite within 5 days of treatments commencing. This is consistent with observations that 10 g of alkanate decreases DM intake (Burggraaf and Leng 1980), and 20 mL of alkanate administered on 3 consecutive days can cause sheep death (Bird and Leng 1984). Consequently, these results suggest that if these surfactants are effective at reducing the anti-nutritive effects of mulga CT, then they become toxic to sheep at the concentrations at which they are effective.

PEG is constructed of linked ethylene glycol units that form hydrophobic bonds with CT (Oh *et al*. 1980). The propylene glycol units that are combined to form Teric (ICI 1988) have chemical properties similar to ethylene glycol, to such an extent that the compounds may be substituted for one another (Merck 1989). Consequently, we expected Teric to have a capacity to precipitate CT. In addition, Teric is used as an agricultural and industrial surfactant. Therefore, we considered that the surfactant properties combined with the ability to precipitate CT could make Teric an effective alternative to PEG. The digestion study results clearly demonstrated that at the same dosage, Teric was not as effective as PEG to improve N digestion and liveweight, and unlike PEG, Teric had no effect on P digestion and balance. This suggests that the capacity of Teric to reduce the effects of mulga CT is less than that of PEG, and that Teric's combined surfactant properties and the ability to precipitate CT were not as effective as the precipitating ability of PEG.

The digestion study demonstrated that PEG was the most effective chemical to reduce the effects of CT on mulga digestion. Consequently, to evaluate further its potential value in a practical mulga-feeding system, it was tested under grazing conditions. A higher rate of administration (12 g/day) was selected for this study, since the effects on clean wool growth with 6 g/day had not been consistent (Table 2 *v*. Eady *et al*. 1989), whilst 12 g/day had effected consistent responses (Pritchard *et al*. 1988, 1992). The significant improvements in clean wool growth (9%) and liveweight gain (100%) during the first measurement period for sheep provided with PEG compared with the control group demonstrated that PEG can be successfully used to improve production from sheep consuming a predominantly mulga diet under paddock conditions. Rainfall during the last 3 weeks of Period 1, and continuing rain throughout Period 2, stimulated the rapid growth of pasture in the understorey. As a consequence, the quantity of mulga left uneaten on the lopped branches increased, and the requirement to lop branches decreased. Clean wool growth increased significantly by 33–42% in both PEG and the control groups during this period, indicating that the quality of the diet consumed had improved markedly. This suggested that pasture was substituted in the diet, thus supplying the higher concentration of nutrients required to support the higher rate of wool growth. A shift to a pasture-based diet would have resulted in a marked reduction in the intake of mulga CT, and a reduction in the anti-nutritive effects of CT on digestion and animal production. The failure of PEG to effect a response on either clean wool growth or liveweight during Period 2 supports this conclusion. The significant difference in liveweight gain between the groups during Period 2 was unexpected, but since the sheep were young, compensatory growth could be considered in the NPS group as more pasture was con-

sumed, and the nutritional value of the diet improved. This contention was supported by the results showing both groups of sheep commencing the study at similar liveweights, sheep given PEG weighing significantly more than the control group at the end of the first measurement period, and the difference in liveweight being eliminated by the end of the study.

Conclusions

These studies suggest that reducing the effects of CT on the digestion of mulga is best achieved by precipitating CT with PEG rather than by dissociating CT–protein complexes with the surfactants tested. Furthermore, they reinforce previous observations that CT are a major limiting factor in the utilisation of mulga leaves as a feed, and that PEG is a potentially valuable compound to improve animal production from mulga. The liveweight advantage observed in sheep given PEG could be expected to improve sheep survival during long-term mulga feeding, and combined with the improvement in wool production, significant financial returns could be gained. In the short term, however, these benefits would not be sufficient to reduce the price differential between the cost of the compound and the financial returns gained from improved animal production. Unless PEG becomes cheaper in the future, it will be necessary to seek alternative solutions to the problems associated with mulga CT. The recent use of rumen micro-organisms from animals adapted to diets containing high concentrations of CT (Miller *et al*. 1995) may provide one such option to improve sheep production from mulga.

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