Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria

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Samples and extracts of foliage from African multipurpose trees were screened for their effects on rumen protozoa and bacteria with a view to predicting their safety as feed supplements and for identifying species with potential antiprotozoal activity. The species tested were *Acacia aneura, Chamaecytisus palmensis, Brachychiton populneum, Flindersia maculosa, Sesbania sesban, Leucaena leucocephala* and *Vernonia amyedalina*. Antimicrobial effects were mild except for *S. sesban*, which was highly toxic to rumen protozoa *in vitro*, and *A. aneura*, which was toxic to rumen bacteria. The antiprotozoal factor in *S. sesban* was apparently associated with the fraction of the plant containing saponins. When *S. sesban* was fed to sheep, protozoal numbers fell by 60 % after 4 d, but the population recovered after a further 10 d. *In vitro* experiments demonstrated that washed protozoa from later times were no more resistant to *S. sesban* than on initial exposure, suggesting that other micro-organisms, probably the bacteria, adapted to detoxify the antiprotozoal agent. Thus *S. sesban* may be useful in suppressing protozoa and thereby improving protein flow from the rumen, but only if the bacterial metabolism of the antiprotozoal factor can be avoided.

Rumen: Protozoa: Defaunation

N metabolism in the rumen affects both the efficiency of ruminant production and the environmental impact of excreta from ruminant livestock production. Inefficient N retention by rumen micro-organisms is compensated in production terms by feeding excessive amounts of dietary protein to the animal to meet required output levels. This leads directly to the excretion of N-rich wastes. Microbial protein synthesized in the rumen accounts for between 50 and 90 % of the protein entering the small intestine in ruminants, and most microbial protein is in the form of bacteria (Beever & Siddons, 1986). However, microbial protein turnover in the rumen may result in the net microbial protein flow being less than half of the total amount synthesized (Nolan & Stachiw, 1979). The turnover could be due partly to autolysis of bacteria or lysis of bacteria by bacteriophages (Hoogenraad *et al.* 1967; Hoogenraad & Hird, 1970) or mycoplasmas (Robinson & Hungate, 1973). However, *in vitro* studies suggest that the engulfment and subsequent digestion of bacteria by ciliate protozoa is quantitatively the most important cause of bacterial protein turnover in the rumen (Wallace & McPherson, 1987). There is ample evidence that removing ciliate protozoa from, or defaunating, the rumen avoids the cycle of bacterial protein breakdown and resynthesis in the rumen and thereby increases the flow of protein to the animal (Lindsay & Hogan, 1972; Williams & Coleman, 1992). Thus defaunation would be

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expected to lessen the dependence on protein supplementation under high-production conditions. It would also be beneficial under conditions where the quantity of protein absorbed from the post-ruminal gut limits the productivity of the animal, which occurs frequently in animals receiving low-quality tropical forages.

Many antiprotozoal agents have been tried experimentally, but none has passed into routine use because of toxicity problems, either to the rest of the rumen microbial population or to the host animal (Williams & Coleman, 1992). It has been suggested that naturally-occurring plant metabolites might be toxic to rumen protozoa, and some preliminary studies on the use of tropical plants as defaunating agents have been reported (Navas-Camacho et al. 1994; Thalib et al. 1995). Plants produce a large number of chemicals, arbitrarily categorized as primary or secondary metabolites, which are essentially defence mechanisms for the plant against animal and insect predators and microbial infection (Feeny, 1970; Desphande et al. 1986). These include alkaloids, non-protein amino acids, mycotoxins, terpenoids, steroids, lectins and protease inhibitors (Cheeke & Shull, 1985; Lowry, 1990; D'Mello, 1992). Some phenolic compounds are toxic to rumen bacteria (Akin, 1982; Chesson et al. 1982; Borneman et al. 1986; Varel & Jung, 1986; Martin & Akin, 1988), rumen fungi (Akin & Rigsby, 1987) and rumen protozoa (Akin, 1982). Many tropical and subtropical browse species, some of which have the potential to be multipurpose trees, fulfilling functions of shelter, fuel, soil conditioning and feedstuffs for livestock, contain high levels of polyphenolics and also insoluble condensed tannins (proanthocyanidins) that bind to neutral-detergent fibre (Reed, 1986; Woodward & Reed, 1989; Reed et al. 1990; El Hassan, 1994).

The aim of the work described here was to determine if the foliage from several multipurpose trees contained factors that were antiprotozoal and therefore potentially able to defaunate the rumen either partially or completely without having a detrimental effect on the bacterial population. Foliage from Sesbania sesban was found to contain an antiprotozoal factor that may be useful in suppressing rumen protozoa and hence enhancing rumen productivity.

METHODS

Multipurpose trees

Foliage from Acacia aneura, Brachychiton populneum, Chamaecytisus palmensis, Flindersia maculosa, Leucaena leucocephala, Sesbania sesban (International Livestock Centre for Africa (ILCA) categorization no. 15036) and Vernonia amyedalina was collected at ILCA (now the International Livestock Research Institute), Debre Zeit Station, Ethiopia, between November 1991 and February 1992. All multipurpose-tree foliages (MPT) were sun-dried. In later in vivo experiments S. sesban (ILCA no. 10865), collected at Debre Zeit during 1995, was used. The foliage was ground to pass a 1 mm screen for use in experiments in vitro.

Antiprotozoal effects of foliage from multipurpose trees

Protozoal activity was measured by the breakdown of [^{14}C]leucine-labelled Selenomonas ruminantium in vitro as described by Wallace & McPherson (1987) and Wallace & Newbold (1991). Rumen fluid was obtained 2 h after feeding from four mature rumen-cannulated sheep fed on 1·4 kg/d of a mixed diet of grass hay, barley, molasses, white fishmeal and a mineral and vitamin mixture (500, 299·5, 100, 91 and 9·5 g/kg DM respectively) in two equal meals. The hay was prepared from a mature mixed grass sward

consisting mainly of perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*). Rumen fluid was strained through two layers of muslin and pre-incubated at 39° with a mixture (40 g/l) of MPT with wheat straw (0, 15, 30 and 60 % MPT) for 2 h before adding *S. ruminantium*. Unlabelled L-leucine was included in all incubations at a final concentration of 5 mmol/l to prevent reincorporation of released [^{14}C]leucine.

Antibacterial effects of foliage from multipurpose trees

The effects of MPT on rumen bacteria were assessed by their influence *in vitro* on ATP pools of mixed bacteria prepared from rumen contents. Rumen contents were obtained 2 h after feeding from four mature rumen-cannulated sheep fed on 1·4 kg/d of the same diet as described earlier. The contents were strained through two layers of muslin and centrifuged (300 *g* for 10 min) to sediment protozoa. The supernatant fraction was incubated at 39° with the same mixtures of MPT and wheat straw as before. After 4 and 24 h, ATP was extracted with H_2SO_4 and measured using luciferase (*EC* 1.14.14.3; Wallace & West, 1982).

Incubations to determine both antiprotozoal and antibacterial effects were conducted in duplicate. Results were analysed by a one-way ANOVA. Each sheep was considered to be an experimental unit (*n* 4). Following a significant *F* test ($P < 0.05$), significant differences between means were determined by Student's *t* test (Snedecor & Cochran, 1976).

Characterization of the active component of Sesbania sesban

To determine the influence of removing tannins on the antiprotozoal activity of *S. sesban*, polyvinylpyrrolidone (PVP) was added to rumen fluid at a concentration of 20 g/l together with *S. sesban*, (20 g/l) and incubated with strained rumen fluid from four sheep, prepared as described earlier, for 1 h before adding labelled *S. ruminantium*. Saponins were extracted from *S. sesban*, in a procedure based on the methods of Headon *et al.* (1991) and Wall *et al.* (1952). An aqueous suspension of *S. sesban*, (33 g/l) was shaken with an equal volume of *n*-butanol for 30 min at room temperature, the butanol was removed and the extraction repeated with fresh *n*-butanol. The butanol layers were pooled and dried in a rotary evaporator. The extracted solid was resuspended in 1/15 of the original volume of water and its antiprotozoal activity was tested in rumen fluid at a concentration of 40 ml/l, equivalent to the addition of 20 g/l of the original *S. sesban*. The residual aqueous layer was taken to dryness at 80°, resuspended in water and tested for antiprotozoal activity at the same concentration as the butanol extract. Both fractions plus the original *S. sesban*, (20 g/l) were added to strained rumen fluid taken from four sheep prepared as described earlier 1 h before adding labelled *S. ruminantium*. Results are presented as means with their standard errors.

Effects of Sesbania sesban on rumen fermentation in vivo

Three mature rumen-cannulated sheep received 1 kg fresh weight/d of the same diet as described earlier, supplemented with 250 g *S. sesban*/d (in two equal meals). Rumen samples were withdrawn 0, 1, 2, 4 and 6 h after the morning feeding on 2 d before adding *S. sesban* to the diet and after 1, 2, 5, 12, 19 and 40 d of *S. sesban* supplementation. Rumen contents pH was measured using a pH electrode connected to a Russell 660 pH meter (Russell pH, Auchtermuchty, Scotland). Volatile fatty acids were determined by GLC as described by Stewart & Duncan (1985). NH_3 was measured by the phenol–hypochlorite

method of Whitehead *et al.* (1967). L-Lactic acid was determined by the automated method of Goodall & Byers (1978) using porcine L-lactate dehydrogenase (*EC* 1.1.1.2). Samples were also withdrawn 2 h after feeding on the same days to determine microbial numbers and activities. Total viable counts of bacteria were made in roll tubes on Hobson's medium 2, a medium containing rumen contents and lactate, glucose, maltose and cellobiose as C sources (Hobson, 1969). The medium was dispensed under O_2-free CO_2 into Hungate tubes sealed with butyl rubber stoppers (Bellco Glass, Vineland, NJ, USA). Roll tubes were incubated for 72 h at 39°. The numbers of cellulolytic bacteria capable of degrading filter paper were determined using the method of Mann (1968). The breakdown of bacterial protein was determined using [^{14}C]leucine-labelled *S. ruminantium* Z108 as described by Wallace & McPherson (1987). Protease activities were measured by the release of label from ^{14}C-labelled casein (Wallace, 1983). Peptidase activities were measured with alanine peptides as substrates and deaminase activity was determined with casein acid hydrolysate as substrate (Newbold *et al.* 1990). Microbial protein was determined with Folin reagent (Lowry *et al.* 1951). The DM loss of hay from nylon bags was determined by the method of Mehrez & Ørskov (1977). Samples were incubated in the rumen for 24 h and when withdrawn from the rumen were washed in a domestic washing machine in cold water for 18 min and then dried to constant weight. Samples were withdrawn daily, with the exception of weekends, to determine protozoal numbers. Counts of ciliate protozoa were carried out microscopically in a counting chamber (Newbold *et al.* 1987).

Data were analysed statistically using Genstat 5 (Genstat 5 Committee, 1987). Based on the observed variations in protozoal numbers (see Fig. 3), the trial was split into three periods; before *S. sesban* addition, immediately after addition (1, 2 and 5 d after addition) and longer-term effects (12, 19 and 40 d after addition). Sampling time was considered as a subplot. However, since the effect of time after feeding did not vary between the periods, mean values were analysed and are reported later.

The effect of Sesbania sesban *on isolated protozoa*

In a separate experiment, the effect of *S. sesban* on the activity of protozoa isolated from rumen contents before and after the host animal had received *S. sesban* in the diet for 11 d was measured to determine if protozoa became resistant to the antiprotozoal agent.

Three mature rumen-cannulated sheep received 1 kg fresh weight/d of the same diet as described earlier, supplemented with 250 g/d *S. sesban* (in two equal meals). Rumen samples were withdrawn 2 h after the morning feeding on 2 d before adding *S. sesban* to the diet and after 11 d of *S. sesban* addition. Protozoa were recovered from rumen contents by filtration as described by Williams & Coleman (1992), then resuspended in salts solution D of Coleman (1978) at approximately 1×10^5 cells/ml. Protozoa were pre-incubated with *S. sesban* (20 g/l) for 1 h before adding labelled *S. ruminantium* to determine bacterial breakdown as described earlier.

Values for bacterial breakdown by protozoa plus or minus *S. sesban* before and after *S. sesban* feeding were compared by Student's *t* test (Snedecor & Cochran, 1976).

RESULTS AND DISCUSSION

The breakdown of the rumen bacterium *S. ruminantium*, in the presence and absence of added MPT, was measured in rumen contents taken from sheep (Table 1). Each plant was tested on different days with different samples of rumen contents, each with a slightly different protozoal population, thus accounting for the variation in the rate of breakdown in the absence of MPT, which ranged from 7·3 to 13·3 % per h. All of the MPT caused a

Table 1. *Influence of foliage from African multipurpose trees (MPT) on breakdown of ^{14}C-labelled Selenomonas ruminantium in rumen contents in vitro**

(Means of samples from four sheep analysed in duplicate)

| MPT | Rate of degradation (% per h) at inclusion level† of (mg/ml): | | | | |
	0	6	12	24	SED (df 8)
Acacia aneura	8·6[a]	7·5[ab]	6·8[ab]	4·6[b]	1·80
Brachychiton populneum	11·7[a]	10·1[ab]	8·8[bc]	7·5[c]	0·96
Chamaecytisus palmensis	7·3[a]	6·4[ab]	5·6[bc]	4·3[c]	0·72
Flindersia maculosa	13·3[a]	12·7[a]	11·6[a]	9·4[b]	0·84
Leucaena leucocephala	7·8[a]	6·9[a]	6·3[a]	3·7[b]	0·90
Sesbania sesban	11·4[a]	4·7[b]	0·2[c]	0[c]	1·14
Vernonia amyedalina	8·6[a]	7·6[a]	6·2[a]	3·9[b]	1·03

[a,b,c] Mean values within a row not showing a common superscript letter were significantly different ($P < 0.05$).
* For details of procedures, see pp. 238–249.
† The balance was made up by wheat straw to give a total substrate concentration of 40 g/l.

progressive decrease in the observed rate of release of [^{14}C]leucine from *S. ruminantium*, most probably due to a physical interference with the predatory activity of the protozoa as well as a degree of toxicity. However, the effect with *S. sesban* was particularly pronounced, causing a 59 % decrease at 6 mg/ml and complete abolition of predatory activity at 24 mg/ml (Table 1).

The effects of MPT on the mixed bacterial population were investigated in protozoa-free rumen contents (PFRL). ATP was analysed in acid extracts as described by Wallace & West (1982) after 4 and 24 h incubation *in vitro* of a mixture of PFRL with MPT and wheat straw. ATP plays a central role in the conversion and utilization of energy released during cellular metabolism and can be used as a measurement of live microbial biomass (Wallace & West, 1982). The ATP pools of the different samples of PFRL without addition of MPT were variable and tended to decline between 4 and 24 h, presumably reflecting some cell death over the longer incubation (Table 2). The only evidence of toxicity was seen in the presence of *A. aneura*, *S. sesban* and *V. amyedalina* foliage, which significantly decreased the ATP pool during the 24 h incubation (Table 2). In contrast, *C. palmensis* increased ATP at 4 h, most probably because it contained fermentable substrate, giving rise to a greater bacterial biomass. The effects of MPT on bacterial ATP are indicative only, because they represent a balance between ATP increases caused by fermentable materials in the MPT and decreases resulting from toxicity. Nevertheless, the results do indicate that while *A. aneura* was toxic to rumen bacteria, *S. sesban* was selectively toxic towards protozoa.

All of the MPT contained phenolic compounds (El Hassan, 1994), so the possibility that tannins or phenolic compounds were responsible for the antiprotozoal effect of *S. sesban* was tested. Tannins are removed from solution by PEG or PVP and their toxicity is neutralized (Garrido *et al.* 1991). When PVP was added to the incubation containing *S. sesban* and ^{14}C-labelled *S. ruminantium*, the precipitation of tannins had no effect on the antiprotozoal property of *S. sesban* (Fig. 1). Thus it was concluded that tannins were not responsible for the observed antiprotozoal property of *S. sesban*. When an aqueous extract of *S. sesban* was extracted with *n*-butanol and the butanol extract and remaining aqueous phase were tested for their effects on the degradation of *S. ruminantium*, the antiprotozoal activity was removed to the butanol phase (Fig. 2). The remaining aqueous extract had no

Table 2. *Influence of foliage from multipurpose trees (MPT) on the ATP content of mixed rumen bacteria incubated* in vitro*

(Means of samples from four sheep analysed in duplicate)

| MPT | Incubation time (h) | ATP (mM) at inclusion level† of (mg/ml): | | | | SED (df 8) |
		0	6	12	24	
Acacia aneura	4	21·10	22·10	21·56	20·13	2·073
	24	9·95[a]	8·59[ab]	6·56[b]	3·66[c]	1·775
Brachychiton populneum	4	34·72	32·51	31·80	30·79	10·022
	24	28·20	25·79	25·70	23·82	7·739
Chamaecytisus palmensis	4	29·06[a]	30·39[a]	35·04[ab]	41·01[b]	4·551
	24	21·05	18·67	17·48	16·47	2·642
Flindersia maculosa	4	35·61	34·28	33·31	36·17	8·587
	24	25·24	21·18	20·35	17·18	3·778
Leucaena leucocephala	4	28·97	28·78	30·66	29·75	7·730
	24	23·93	20·57	20·25	16·31	4·629
Sesbania sesban	4	24·90	28·82	29·42	27·06	3·973
	24	27·08[a]	21·89[b]	20·43[b]	15·37[c]	2·143
Vernonia amyedalina	4	32·34	32·68	32·47	32·22	6·350
	24	26·93[a]	24·33[ab]	21·91[ab]	18·63[b]	2·609

[a,b,c] Mean values within a row not showing a common superscript letter were significantly different ($P < 0.05$).
* For details of procedures, see pp. 238–249.
† The balance was made up by wheat straw to give a total substrate concentration of 40 g/l.

effect (Fig. 2). Butanol extraction of plant material causes the removal of saponins (Wall *et al.* 1952; Headon *et al.* 1991) and the butanol extract is likely to have contained the majority of the saponins from the *S. sesban*. Purified saponins from *Quillaja* bark and *Saponaria* sp. have previously been shown to be toxic to rumen protozoa (Wallace *et al.* 1994), but less so than the extract from *S. sesban*, suggesting that it is a specific type of saponin or saponin-like substance in *S. sesban* that is responsible for its antiprotozoal effect. The observation that saponins are toxic to rumen protozoa is consistent with earlier studies *in vitro* (Valdez *et al.* 1986) and in sheep (Lu & Jorgensen, 1987). Recent results from Columbia indicate that the antiprotozoal effects of other plants, including *Sapindus saponaria*, are similarly due to saponins (Navas-Camacho *et al.* 1994). Other antiprotozoal factors in plants have been known for a long time. Eadie *et al.* (1956) showed that certain terpenes and other substances present in plant material had marked toxic properties toward rumen protozoa. Warner (1962) found that minor plant constituents such as terpenes or alkaloids may have specific effects on individual species of rumen micro-organisms. It is unlikely that terpenes or alkaloids would be extracted into butanol by the method used here. Akin (1982) observed that *p*-coumaric acid reduced the motility of entodinimorphid protozoa, but again it is unlikely that phenolic acids would be extracted from *S. sesban* into butanol. Thus the indications are that it is the saponin fraction of *S. sesban* that is toxic to rumen protozoa, although more work is required to identify the precise component.

Selective elimination of protozoa, if it were to persist, would enhance the flow of microbial protein from the rumen and improve the nutrition of the animal, thus the effect of feeding *S. sesban* on rumen fermentation was investigated in rumen-cannulated sheep. The *S. sesban* used for *in vivo* trials was a different batch from that used *in vitro*; however it was shown that the antiprotozoal activities *in vitro* in rumen contents from these sheep were the same (results not shown). Addition of *S. sesban* to the diet caused a dramatic decline in protozoal numbers, such that after 2 d supplementation numbers had declined by almost
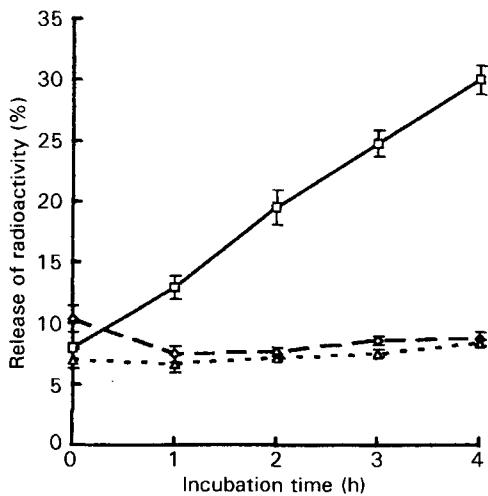
Fig. 1. Influence of removal of tannins from *Sesbania sesban* on breakdown of *Selenomonas ruminantium* by rumen protozoa. [^{14}C]leucine-labelled *S. ruminantium* was incubated in rumen contents alone (-□-), in the presence of *S. sesban* (-◇-), and in the presence of *S. sesban* and polyvinylpyrrolidone which removes tannins (-△-). Values are means for samples from four sheep analysed in duplicate, with their standard errors indicated by vertical bars.
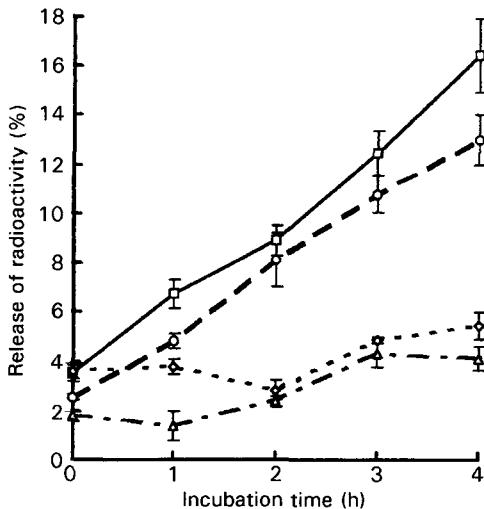

Fig. 2. Influence of butanol extraction of an aqueous extract of *Sesbania sesban* on its inhibition of the breakdown of *Selenomonas ruminantium* by rumen protozoa. [^{14}C]leucine-labelled *S. ruminantium* was incubated in rumen fluid alone (-□-), or in the presence of *S. sesban* (-◇-), an aqueous extract of *S. sesban* (-O-) or a butanol extract of *S. sesban* (-△-). Values are means for samples from four sheep analysed in duplicate, with their standard errors indicated by vertical bars.

60 % (Fig. 3). This decline in protozoa was associated with a 75 % reduction in the breakdown of *S. ruminantium* and 70 and 80 % increases in the total and cellulolytic bacterial populations respectively (Fig. 4), confirming the importance of protozoa in the breakdown of bacterial protein in the rumen (Wallace & McPherson, 1987). However, protozoal numbers recovered quickly and had returned to pre-supplementation levels by 9 d
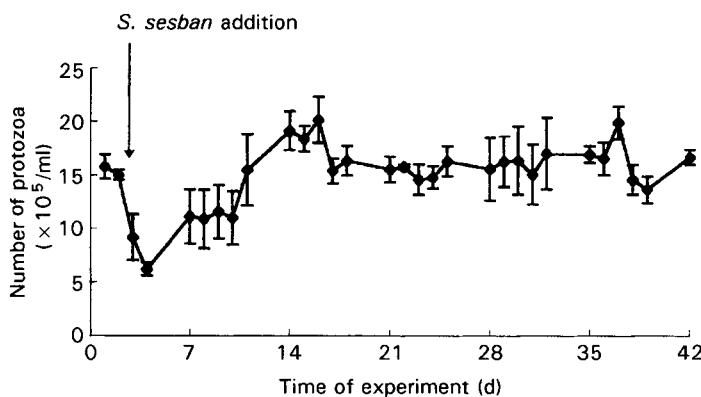
Fig. 3. Effect of including *Sesbania sesban* in the diet of sheep on the number of protozoa in the rumen of sheep fed on a mixed diet. Values are means for samples for three sheep, with their standard errors represented by vertical bars.

of *S. sesban* addition (Fig. 3); as protozoal numbers increased so did the breakdown of bacterial protein and the number of total and cellulolytic bacteria declined (Fig. 4). Thus *S. sesban* significantly reduced protozoal numbers and stimulated bacterial numbers only in the period immediately after its addition to the diet (Table 3). *S. sesban* addition was associated with an initial, non-significant, reduction in rumen NH_3 concentration (Table 3). This appeared to be a consequence of the decreased bacterial breakdown, as the *S. sesban* had no effect on either the proteolytic, peptidolytic or deaminative activity in rumen contents (Table 4). *S. sesban* addition caused an initial, but not prolonged, increase in total volatile fatty acid concentrations in rumen contents (Table 3). The removal of protozoa from the rumen is normally associated with a decrease in volatile fatty acid concentrations (Williams & Coleman, 1992), although in some experiments concentrations were higher after defaunation (Stern & Hinkson, 1974; Grummer *et al.* 1983). The decrease in butyrate concentrations following *S. sesban* addition (Table 3) is typical of many studies in which protozoa have been removed from the rumen (Williams & Coleman, 1992). *S. sesban* addition caused a prolonged increase in the molar proportion of acetate and a decrease in the proportion of branched-chain acids (Table 3). *S. sesban* stimulated propionate production at the expense of acetate when fed to sheep receiving teff straw (Bonsi *et al.* 1995), however there is apparently no published information on the effect of *S. sesban* addition to higher quality diets such as those used here.

Thus the effect of *S. sesban* on protozoal numbers in the rumen would appear to be transient. To investigate this further, the antiprotozoal activity of *S. sesban* was measured in protozoa isolated from the rumen of sheep before and after 11 d of *S. sesban* addition to the diet. Isolated protozoa were susceptible to the action of *S. sesban* irrespective of their prior exposure to *S. sesban* (Table 5). This suggests that it is not the protozoa *per se* that become resistant to the antiprotozoal compound and that another microbial population, probably the bacteria, adapts to become capable of degrading the antiprotozoal component. It is possible that this adaptation can be overcome by feeding the plant at intervals, as done by Thalib *et al.* (1995) with *Sapindus rarak*, rather than continuously as done here. Indications are that toxicity to the host animal will not be a problem (El Hassan, 1994; Bonsi *et al.* 1995), but more work is required.

Defaunation had been reported to increase microbial outflow (Leng *et al.* 1981) and increase the efficiency of feed utilization and hence growth rate of cattle on diets low in
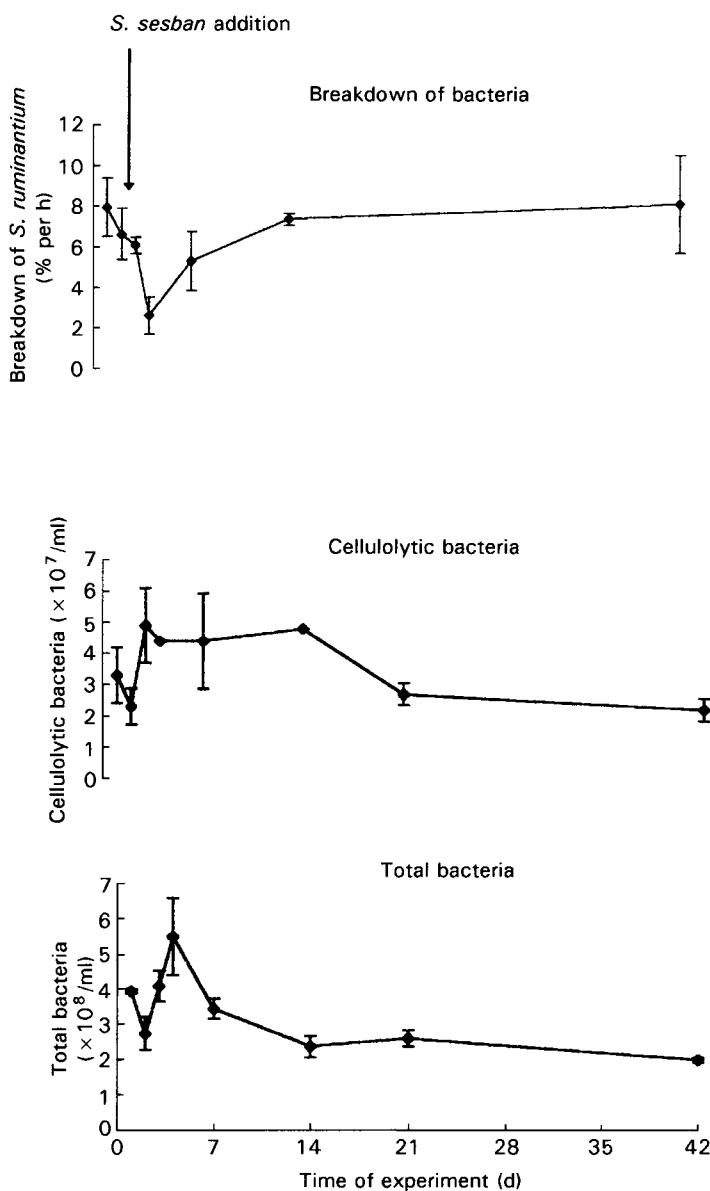
Fig. 4. Effect of including *Sesbania sesban* in the diet of sheep on the number of total viable and cellulolytic bacteria in the rumen and the breakdown of *Selenomonas ruminantium* incubated in rumen fluid *in vitro*. Values are means for samples for three sheep, with their standard errors represented by vertical bars.

bypass protein (Bird & Leng 1979). Similarly, the rate of wool growth was increased in defaunated lambs (Bird *et al.* 1979). Provided the loss of protozoa does not compromise the fibre breakdown, which can occur under some circumstances (Ushida *et al.* 1990), the removal of protozoa would be expected to have beneficial nutritional effects. Thus, if a feeding regimen can be formulated to minimize the effects of adaptation, the antiprotozoal effects of *S. sesban* may enhance its usefulness as a multipurpose crop, being not only a

Table 3. *Effect of including* Sesbania sesban *in the diet of sheep on fermentation and microbial numbers in the rumen of sheep fed on a mixed diet**

(Means of samples from three sheep analysed in duplicate; values for volatile fatty acids and ammonia are also means of samples taken at five times of day)

	Before S. sesban addition	Immediately after addition	Longer-term effect of S. sesban addition	SED (df 2)
pH	6·36[a]	6·47[b]	6·40[ab]	0·035
Total volatile fatty acids (mmol/l)	78·2[a]	97·8[b]	79·9[a]	7·69
Acetate (mmol/mol)	568[a]	584[ab]	608[b]	9·01
Propionate (mmol/mol)	215	234	198	17·2
Butyrate (mmol/mol)	111[a]	91[a]	131[b]	7·5
Branched-chain and longer acids (mmol/mol)	105[a]	91[a]	63[b]	8·9
NH_3-N(mg/l)	189[a]	172[a]	217[b]	9·0
Total bacteria ($\times 10^8$/ml)	3·23[a]	4·34[b]	2·31[a]	0·379
Cellulolytic bacteria ($\times 10^7$/ml)	2·34	4·73	2·66	0·958
Protozoa ($\times 10^5$/ml)	15·3[a]	8·9[b]	17·1[a]	1·59
DM loss (%) of hay from nylon bags in 24 h	44·8	45·2	47·3	0·81

[a,b] Mean values within a row not sharing a common superscript letter were significantly different ($P < 0.05$).
* For details of procedures, see pp. 238–240.

Table 4. *Effect of including* Sesbania sesban *in the diet of sheep on the breakdown of* Selenomonas ruminantium *and proteolytic, peptidolytic and deaminative activities in rumen contents from sheep fed on a mixed diet**

(Means of samples from three sheep analysed in duplicate)

	Before S. sesban addition	Immediately after addition	Longer-term effect of S. sesban addition	SED (df 2)
Proteolytic activity (mg [^{14}C]casein/h per mg protein)	0·695	0·720	0·735	0·1011
Peptidolytic activity (nmol Ala$_5$/h per mg protein)	14·8	17·3	15·5	2·36
Deaminase activity (nmol NH_3 produced/h per mg protein)	313	317	305	41·9
Breakdown of S. ruminantium (mg bacterial protein/mg protein per h)	7·30[a]	4·67[b]	7·76[a]	0·854

Ala$_5$, peptide of alanine units.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of procedures, see pp. 238–240.

Table 5. *Effect of* Sesbania sesban *on the breakdown of* Selenomonas ruminantium *by protozoa isolated from rumen contents of sheep fed with* S. sesban*

(Means of samples from three sheep analysed in duplicate)

	Rate of degradation (% per h)		SED (df 2)
	No S. sesban in vitro	+ S. sesban in vitro	
Protozoa isolated			
Before S. sesban addition	9·81[a]	4·15[b]	0·310
After 11 d S. sesban addition	10·42[a]	5·38[b]	0·561

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of procedures, see pp. 238–240.

good source of protein for ruminants but also a means of manipulating rumen fermentation for improved productivity. The effects may also be applicable to more-intensive systems if the active antiprotozoal component can be isolated and characterized fully.

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