Natural Products as Manipulators of Rumen Fermentation

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ABSTRACT : There is increasing interest in exploiting natural products as feed additives to solve problems in animal nutrition and livestock production. Essential oils and saponins are two types of plant secondary compounds that hold promise as natural feed additives for ruminants. This paper describes recent advances in research into these additives. The research has generally concentrated on protein metabolism. Dietary essential oils caused rates of NH_3 production from amino acids in ruminal fluid taken from sheep and cattle receiving the oils to decrease, yet proteinase and peptidase activities were unchanged. Hyper-ammonia-producing (HAP) bacteria were the most sensitive of ruminal bacteria to essential oils in pure culture. Essential oils also slowed colonisation and digestion of some feedstuffs. *Ruminobacter amylophilus* may be a key organism in mediating these effects. Saponin-containing plants and their extracts appear to be useful as a means of suppressing the bacteriolytic activity of rumen ciliate protozoa and thereby enhancing total microbial protein flow from the rumen. The effects of some saponins seems to be transient, which may stem from the hydrolysis of saponins to their corresponding sapogenin aglycones, which are much less toxic to protozoa. Saponins also have selective antibacterial effects which may prove useful in, for example, controlling starch digestion. These studies illustrate that plant secondary compounds, of which essential oils and saponins comprise a small proportion, have great potential as 'natural' manipulators of rumen fermentation, to the potential benefit of the farmer and the environment. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1458-1468)***

Key Words : Essential Oils, Manipulation, Ruminants, Saponins

INTRODUCTION

Consumers and health authorities, particularly in Europe, increasingly dictate that the use of chemical feed additives, including ionophores and antibiotics, should be phased out and, where possible, only natural products should be used in animal production. Thus, new methods must be found that will enable farmers to manipulate rumen fermentation to obtain benefits with their animals. Among these new methods, the great diversity of secondary metabolites occurring in plant species offers tremendous opportunities for development. This paper summarizes some recent work carried out in this area in the authors' and other laboratories, where the aims were (1) to determine the effects of essential oils on ruminal microorganisms, and (2) to investigate plants and plant extracts that suppress the growth of ciliate protozoa. The results have important implications for the use of natural materials as additives to ruminant feedstuffs.

ESSENTIAL OILS

Background

Essential oils are steam-volatile or organic-solvent extracts of plants, used traditionally by man for many centuries for the pleasant odour of their essence, their flavour, or their antiseptic and/or preservative properties. Although commonly thought of as being derived from herbs and spices, they are present to some degree in many plants for their protective role against bacterial, fungal or insect attack. They comprise mainly cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives (Figure 1).

As the power of antibiotics became apparent, research into the action of essential oils became less important. However, interest has shown a resurgence because essential oils are perceived to be natural alternatives to chemical biocides and, in some applications, antibiotics. Recently, for example, useful effects of essential oils have been demonstrated against pathogenic bacteria. Protection against food-borne pathogens is of particular interest, partly because of the recent appearance of the new, highly dangerous, verotoxigenic strains of *Escherichia coli*, organisms which are transmitted from the animal right through to the consumer. In recent years, for example, oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *E. coli*, *Enterococcus faecalis*, *Staphylococcus aureas* (including the clinically problematic methicillin-resistant *S. aureus*), *Salmonella* sp. and *Vibrio parahemolyticus* cinnamaldehyde was the main antibacterial component of the mixture (Chang et al., 2001). *E. coli* O157:H7 was inhibited by oregano oil (Elgayyar et al., 2001), peppermint oil (Imai et al., 2001) and essential oils from other herbs (Marino et al., 2001). Several aromatic plants, mainly *Eucalyptus* spp., had potentially useful medicinal effects against *Pseudomonas aeruginosa*, although the effectiveness of different plants could not be correlated with the content of any major constituent of the oils (Cimanga et al., 2002). *Helicobacter pylori* was highly sensitive to spearmint oil (Imai et al., 2001). Essential oils

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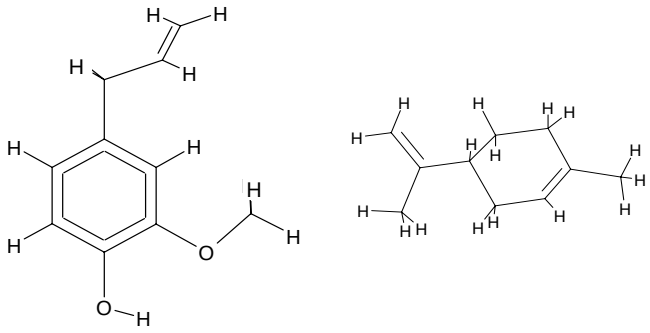

Figure 1. Structures of two typical essential oils.

are potent against a wide range of oral bacteria (Shapiro et al., 1994), and they are used widely in antiseptic mouthwashes.

Essential oils and ruminants

With this range of antimicrobial activity, it was logical to us and others to evaluate essential oils for possible beneficial selective effects against ruminal microorganisms. Essential oils were examined many years ago in ruminal bacteria, from the point of view of the oils contributing to poor palatability in some plant species (Oh et al., 1968). General inhibitory activity was found across a range of plant materials, of which vinegar weed was most potent. Oh et al. (1967) demonstrated that individual oils had different effects on mixed ruminal bacteria. Monoterpene hydrocarbons were less toxic and sometimes stimulatory to microbial activity compared to the corresponding oxygenated compounds, the monoterpene alcohols and aldehydes (Oh et al., 1967). The sensitivity of ruminal bacteria to essential oils of *Artemisia tridentata* (big sagebrush) was the same in captive deer as it was in wild deer, which was suggested to mean that ruminal bacteria did not adapt to essential oils (Nagy and Tengerdy, 1968). Thus, essential oils were not necessarily toxic to ruminal bacteria, and their effects might be expected to persist.

In order to determine the effects of essential oils on ruminal fermentation and ruminal microorganisms, we have undertaken several animal trials with essential oils, and also investigated their effects on individual rumen microbial species.

Our first trial was carried out with ruminally fistulated sheep receiving a maintenance diet comprising 40% concentrate and 60% grass silage. Each sheep received 100 mg per day essential oils (CRINA HC, Akzo Nobel Surface Chemistry, Hertfordshire, UK) or the control diet in a 6 week latin square design. Proteinase, peptidase and deaminase activities were measured as described elsewhere (Floret et al., 1999). Nylon bag incubations were done according to Mehrez and Ørskov (1977).

Essential oils had no influence on VFA or NH_3 concentrations, on protozoal numbers, or on microbial protein flow (not shown). The rate of degradation of soybean meal tended to be decreased at 8 and 16 h (Figure 2), but there was no effect on rapeseed meal breakdown (not shown). The breakdown sequence of protein to NH_3 was measured by assaying the rates of the individual reactions in ruminal fluid *in vitro*. Ammonia formation was affected only at the last step, namely the deamination of amino acids (Figure 3).

A similar experiment was carried out with ruminally fistulated dairy cows receiving a total mixed ration made up of grass silage, maize silage and concentrate. Effects of essential oils on the sequence of protein catabolism were assessed as before, and the results were similar, in that only the final step, which is the breakdown of amino acids to NH_3, was inhibited in cows receiving dietary essential oils (not shown). Several additional incubations were carried out in this experiment. In order to determine if the breakdown of different proteins was affected by essential oils in different ways because of their different structures, several pure proteins were labelled using ^{14}C-formaldehyde (Wallace 1983) and incubated in ruminal fluid *in vitro*. It

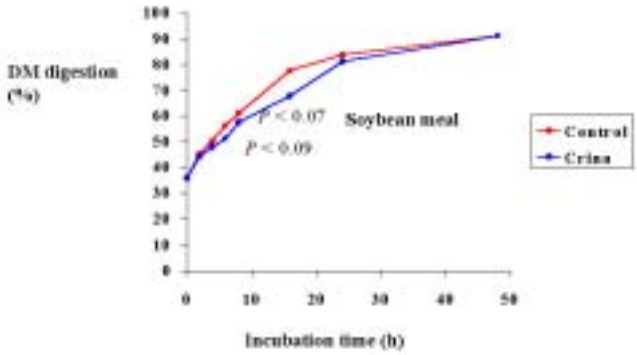

Figure 2. Influence of essential oils on the breakdown of soybean meal in the sheep rumen.

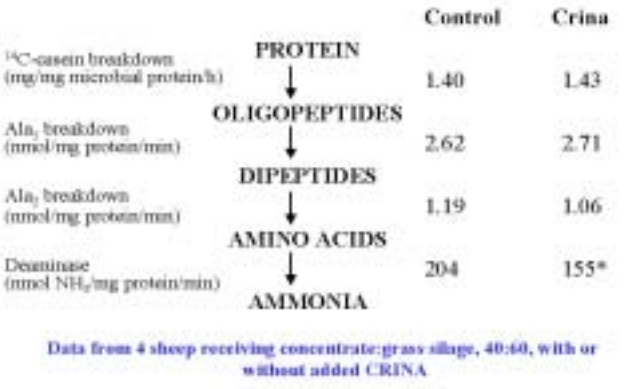

Figure 3. Influence of dietary essential oils on the catabolism of protein by ruminal microorganisms from the sheep rumen *in vitro*.

emerged that essential oils had no influence on the breakdown of the different proteins, whose rates of breakdown varied by two orders of magnitude (Table 1). Thus, it was concluded that essential oils had no influence on the first step of protein breakdown, namely proteolysis.

Peptidase activity was assessed by the breakdown of Ala_5 and Ala_2 as before, and also by the hydrolysis of Ala_2-*p*-nitroanilide and GlyArg-4-methoxynaphthylamide, synthetic substrates for the two main dipeptidyl peptidase activities in ruminal fluid (Wallace, 1999). Once again, neither activity was affected by the dietary treatment (Table 1). There seemed, therefore, to be no role for essential oils in controlling peptide metabolism in the rumen.

The final step, deamination of amino acids, was assessed further by including incubations to which monensin had been added (Table 2). Again, dietary essential oils caused a decrease in the rate of NH_3 production. Monensin addition to the *in vitro* incubations caused a larger decrease in deaminative activity of ruminal fluid from both control and essential oil-supplemented cows; the decreased activity no longer showed a difference between the two groups, indicating that the species affected by dietary essential oils were also affected by monensin.

In a second sheep trial, additional measurements were made in which the rate of breakdown of different protein meals was measured both *in vivo* and *in vitro* and the colonisation of feedstuffs incubated in nylon bags was assessed by attached enzyme activity (Silva et al., 1987). Of the protein meals tested, essential oils had a significant effect only on the breakdown of pea meal, the most rapidly degraded meal (Figure 4). There was no effect of a essential oils in the same animals receiving a high-protein diet. Bacterial proteinase and amylase activities associated with plant (pea, rapeseed, etc.) protein supplements tended to be lower in animals receiving essential oils, while corresponding activities associated with fishmeal were unaffected (not shown). Glutamate dehydrogenase activity, a measure of total microbial colonisation, associated with grass hay suspended in the rumen was decreased by essential oils (Figure 5), while colonisation of the less degradable fibrous substrates, grass silage and barley straw, was unaffected (Figure 5). Carboxymethylcellulase activity, a measure of the fibrolytic population, was unaffected (not shown). These data suggest that essential oils may suppress the colonisation and/or digestion of readily degraded

Table 1. Influence of dietary essential oils on the hydrolysis of different ^{14}C-labelled proteins and dipeptidyl peptidase substrates in bovine ruminal fluid *in vitro*

Protein	Degradation rate	
	Control	Crina
Proteinase activity (mg/mg microbial protein/h)		
Casein	0.46	0.49
Lactoglobulin	0.21	0.24
Hide powder azure	0.048	0.049
Albumin	0.033	0.035
Elastin congo red	0.0056	0.0054
Dipeptidyl peptidase activity (nmol/mg protein/min)		
Ala_2-pNA	1.64	1.68
GlyArg-MNA	0.36	0.40

Table 2. Influence of dietary essential oils on the rate of breakdown of amino acids in bovine ruminal fluid *in vitro*

	NH_3 production rate (nmol/mg microbial protein/h)	
	Control	Crina
No addition	410	372*
Monensin (5 μM)	280	287

* $p<0.05$.

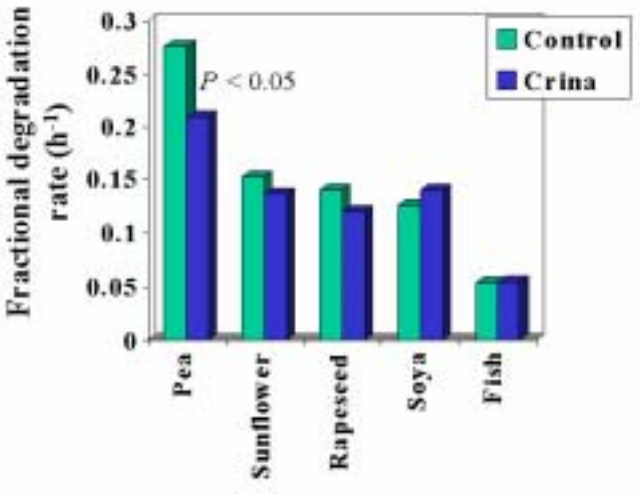

Figure 4. Influence of dietary essential oils on the catabolism of protein by ruminal microorganisms from the sheep rumen *in vitro*.

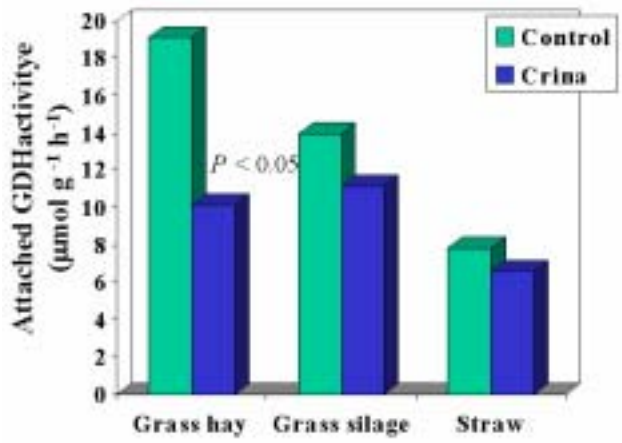

Figure 5. Influence of dietary essential oils on glutamate dehydrogenase activity extracted from feedstuffs incubated in the sheep rumen for 24 h.

substrates by amylolytic and proteolytic bacteria without affecting fibre digesters.

Essential oils and ruminal microorganisms

Many of the predominant species of ruminal bacteria were tested for growth in a range of concentrations of essential oils. *Butyrivibrio fibrisolvens*, *Clostridium aminophilum*, *Escherichia coli*, *Eubacterium ruminantium*, *Lachnospira multipara*, *Megasphaera elsdenii*, *Mitsuokella multiacidus*, *Prevotella albensis*, *Prevotella brevis*, *Prevotella bryantii*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Selenomonas ruminantium*, *Streptococcus bovis* and *Veillonella parvula* were insensitive to 40 ppm essential oils in medium M2 (Hobson, 1969). Only *Clostridium sticklandii*, *Prevotella ruminicola* and *Peptostreptococcus anaerobius* were prevented from growing at 40 ppm essential oils. *Methanobrevibacter smithii*, a methanogen related to those found in the rumen, was similarly unaffected. *Ruminobacter amylophilus* grew in the presence of 40 ppm essential oils, but the oils greatly enhanced its lysis in stationary phase, so it may be considered also to be sensitive to essential oils.

In the second sheep trial, the total and so-called 'hyper-ammonia-producing' (HAP) bacteria, which include *C. sticklandii* and *P. anaerobius* (Russell et al., 1991), were enumerated by their ability to grow on trypticase as sole source of C and N. The total viable count of bacteria was unaffected by essential oils, but the numbers of HAP bacteria decreased by 77% in sheep receiving a low-protein diet (Figure 6). In these trials, essential oils had no significant effect on protozoal numbers or activity (not shown).

Thus, the bacteria most sensitive to essential oils were HAP species, *Prevotella* spp. and *R. amylophilus.* HAP bacteria have a high capability to generate NH_3 from amino acids (Russell et al., 1991). They comprise only around 1% of the rumen bacterial population, however, and the fact that monensin inhibits only 32% of total NH_3-forming activity suggests that their role is not the main one in deamination,

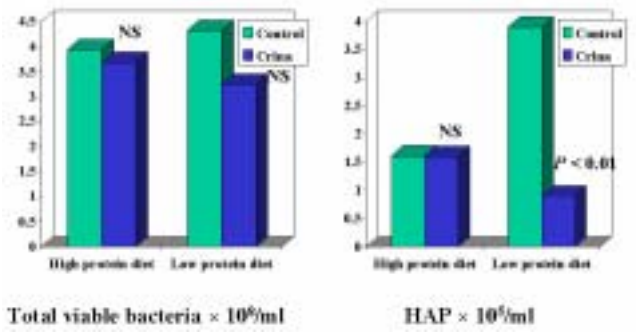

Figure 6. Viable counts in strained ruminal fluid from sheep receiving dietary essential oils.

and that other, monensin-insensitive bacteria are mainly responsible. Nevertheless, even a small decrease in the rate of NH_3 production may be beneficial nutritionally, so the suppression of these species would be expected to be nutritionally significant. Essential oils had less effect on deamination than monensin, presumably because essential oils affect fewer bacterial species. *Prevotella* spp. are involved in all of the steps of protein catabolism (Stewart et al., 1997). *R. amylophilus* is a highly active starch and protein digester which proliferates on concentrate diets (Stewart et al., 1997). Therefore, pure-culture results are consistent with observations which have been made *in vivo.* Whether these effects translate into improved productivity will depend on animal and dietary factors, of which little experimental evidence is as yet available.

A proposed mode of action for essential oils in the rumen

The effect of dietary essential oils on NH_3 production from amino acids, on HAP bacteria in pure culture, and on HAP numbers *in vivo* are all consistent with a primary effect of essential oils on HAP bacteria. As HAP species vary from diet to diet and perhaps geographically (Attwood et al., 1998; McSweeney et al., 1999), it may be important to look in more detail at the full range of HAP bacteria affected; it is also important to identify which of the multiple components of the commercial essential oils mixture is responsible for the effect.

The effects on proteolysis and the breakdown of protein supplements require more speculative interpretation. The effects of essential oils on the protein supplements may be mediated via *R. amylophilus*, but because *R. amylophilus* is amylolytic as well as proteolytic, it may be the loss of the amylolytic activity from the consortium digesting the supplement that affects the colonising microbial consortium more than the loss of the contribution of *R. amylophilus* to proteolysis. That would explain why the degradation of rapidly degraded starchy protein meals is affected by essential oils, but less rapidly degraded starchy meals or non-starchy protein supplements are unaffected. The effects of essential oils on *Prevotella* spp. were inconsistent in pure culture, and peptidase activity was unaffected in the mixed culture, suggesting that *Prevotella* spp. are not the most important target for the present formulation of essential oils.

The effects of essential oils on colonisation were, in contrast, very clear. The effect may stem from a decreased adhesion to readily digested solids; alternatively, the rate of development of solids-associated bacteria - once attachment has already occurred-may be inhibited. The exact cause is as yet unclear. Thus, essential oils may have several independent actions, depending perhaps on individual oils within the mixture, and they may be related to each other in their biochemical consequences (Figure 7).

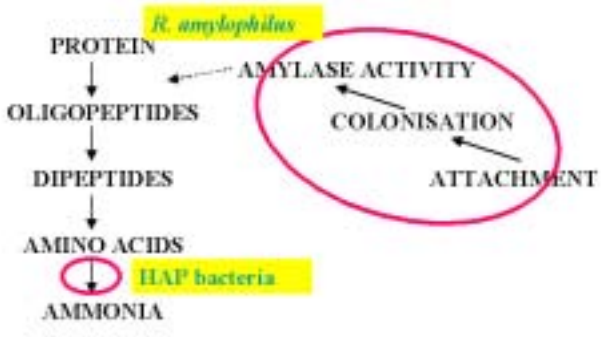

Figure 7. A proposed mode of action for essential oils in the rumen.

SAPONINS

Background

Saponins, like essential oils, cover a wide variety of chemical compounds and, also like essential oils, their properties have been used by man for centuries. The word 'saponin' is derived from the Latin word *sapo*, meaning soap, and traditionally saponins-containing plants have been used for washing. One example of such a saponin-containing plant which has multiple uses is *Phytolacca dodecandra*. The berries, locally called 'endod', have a long history of use in Ethiopia as a soap substitute. *P. dodecandra* is also used locally in Ethiopia as a traditional medicine to remove leeches in cattle. Moreover, Lemma (1970) showed that a preparation of *P. dodecandra* possessed molluscicidal properties, killing the intermediate host (snails), which causes Schistosomiasis in humans. In a subsequent snail control investigation with 'endod', *Schistosoma mansoni* infection rates were significantly reduced in humans, which was found to be due to molluscicidal saponins contained in *P. dodecandra* (Goll et al., 1983). Such multiple applications are typical of saponin-containing plants.

Chemically, saponins are high-molecular-weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety. Given the possible number of sugar moieties that could be added and the modifications that are possible in the ring structures, there is a huge number of possible structures that could be formed. The different classes of saponins are the triterpenes, the steroids and the steroid alkaloids, illustrated in Figure 8. For a full account of the chemistry and applications of saponins, the reader is directed to Hostettmann and Marston (1995).

Saponins and ruminants

Interest in saponins in relation to ruminants has taken two main forms, coming from two different directions. The first involved the assessment of saponins, as chemical modulators, for their effects on rumen fermentation -potential feed additives of unknown value - while the second started from the objective of finding natural materials which might be used to suppress the growth of rumen ciliate protozoa. What has happened is that the two approaches have merged to a significant extent, because one of the main effects of saponins on rumen fermentation is that they are toxic to protozoa.

In terms of investigating saponins for their effects on the whole fermentation and their nutritional implications, different results were obtained in different studies. Van Nevel and Demeyer's (1990) study of sarsaponin *in vitro* showed no indication of toxic effects or effects on microbial growth or protein breakdown. In contrast, Lu et al. (1987) discovered that alfalfa saponins appeared to suppress fermentation in continuous culture. Subsequent *in vivo* investigation (Lu and Jorgensen, 1987) confirmed a general decrease in fermentative activity when alfalfa saponins were supplied to the sheep rumen, of which decreased VFA concentrations and decreased cellulose digestion were symptomatic. Significantly, Lu and Jorgensen (1987) also noted large decreases in protozoal numbers in sheep receiving alfalfa saponins. Goetsch and Owens (1985) concluded that the benefits of sarsaponin would be diet-dependent, increasing the digestion of sorghum silage and other fibrous feeds but apparently decreasing digestion of cereal and protein meals.

Removal of rumen ciliate protozoa, or defaunation, has been an objective of rumen microbiologists for a generation. There are many consequences for the fermentation, and consequently for nutrition, that result from the removal of protozoa (Williams and Coleman, 1992). Antiprotozoal agents, such as surface-active agents, that have been investigated in attempts to apply defaunation at the farm level have been hampered by problems with toxicity, either to other ruminal microorganisms (Eadie and Shand, 1981; Orpin, 1977; Bird and Leng, 1978; Bird et al., 1979) or to the host (Lovelock et al., 1982). Lipids are toxic to protozoa (Machmuller et al., 1998; Matsumoto et al., 1991; Newbold and Chamberlain, 1988) but also to fibre digestion (Broudiscou et al., 1994). Thus, there is no reliable, safe method available to suppress ruminal protozoa.

Recently, some tropical plants were found to have the potential to be used as a safe possible means of suppressing or eliminating protozoa from the rumen (Diaz et al., 1994; Navas-Camacho et al., 1993; Newbold et al., 1997; Odenyo et al., 1997). These plants all had the characteristic that they were rich in saponins. Therefore, research on saponins and defaunating plants to some extent converged.

Saponins and ruminal microorganisms

Because dietary saponins are poorly absorbed, their biological effects occur in the digestive tract (Cheeke, 1996). Although antimicrobial effects of saponins and

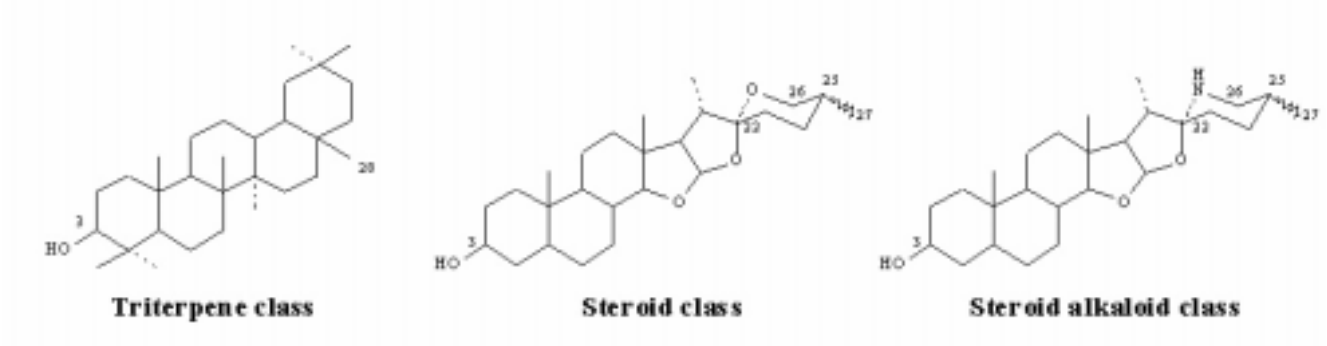

Figure 8. Chemical classes of saponins.

saponin-containing plants was to be expected from the wealth of information from other biological systems, among the earliest observations in ruminal microorganisms came relatively recently from *in vitro* continuous culture systems. Valdez et al. (1986) observed that sarsaponin, from *Yucca schidigera*, decreased protozoal numbers but not bacterial numbers in a 22 d semi-continuous system, and Lu et al. (1987) found that the bacterial population was changed in the presence of alfalfa saponins from a morphologically diverse one in controls to one in which fewer morphotypes were present in vessels receiving saponins. As well as the saponins having an effect on ruminal microorganisms, the microorganisms can metabolise the saponins, thus introducing another factor to be considered in the application of saponins to ruminant nutrition.

Protozoa

Numerous studies have now demonstrated that saponins and saponin-containing plants have toxic effects on protozoa. *In vitro*, toxicity of *Y. schidigera* extract towards protozoa has been noted from a fall in numbers in fermenters (Makkar et al., 1998; Wang et al., 1998) or in bacteriolytic activity (Wallace et al., 1994). Butanol extraction of the *Y. schidigera* extract resulted in all antiprotozoal activity being located in the butanol fraction, consistent with the active component being saponins. Saponins from *Quillaja saponaria* and *Acacia auriculoformis* (Makkar et al., 1998) and foliage from *Sesbania sesban* (Newbold et al., 1997) were also antiprotozoal *in vitro*, the *S. sesban* active component again being extractable in butanol.

In vivo, powdered *Y. shidigera* decreased rumen protozoal numbers in heifers (Hristov et al., 1999). A decrease in protozoal numbers was reported in the rumen of sheep infused with pure alfalfa saponins (Lu and Jorgensen, 1987) or fed saponin-containing plants, including *S. sesban* (Newbold et al., 1997; Odenyo et al., 1997) and *Enterolobium cyclocarpum* (Navas-Camacho et al., 1993).

The sensitivity of ciliate protozoa towards saponins may be explained by the presence of sterols in protozoal, but not bacterial membranes (Williams and Coleman, 1992). Thus, the sterol-binding capability of saponins (Hostettmann and Marston, 1995) most likely causes the destruction of protozoal cell membranes.

Bacteria and fungi

In mixed cultures and *in vivo*, saponins have been shown also to affect ruminal bacteria. Newbold et al. (1997) found that bacterial numbers increased when foliage from *S. sesban* was introduced into the diet, presumably as a consequence of the suppression of protozoal numbers. Valdez et al. (1986) found a similar trend with *Y. schidigera* extract. Steroidal saponins from *Y. schidigera* had no effect on total or cellulolytic bacterial counts in Rusitec; however, inoculating fluid from the fermenter into medium containing saponins decreased the viable count (Wang et al., 1998).

Pure cultures also indicate possible antibacterial effects of saponins. *Y. schidigera* extract abolished growth of the fibre digester, *Butyrivibrio fibrisolvens*, and prolonged the lag phase of *Streptococcus bovis* (Wallace et al., 1994). Similar sensitivity of *S. bovis* to *Y. schidigera* extract was found by Wang et al. (2000), who additionally found that cellulose digestion by *Ruminococcus* spp. and *Fibrobacter succinogenes* was inhibited.

A potentially very significant observation is that the anaerobic ruminal fungi, *Neocallimastix frontalis* and *Piromyces rhizinflata*, were highly sensitive to *Y. schidigera* saponins (Wang et al., 2000). Ruminal fungi appear to fill an important niche in the digestion of recalcitrant plant fibres, because they cause physical as well as enzymatic disruption of plant cell walls (Orpin and Joblin, 1997). In that case, it may well be advantageous to promote the bacterial detoxification of the saponins in animals receiving poor quality forages or crop byproducts.

Microbial adaptation and degradation of saponins

One of the problems we have encountered in saponin-containing plants is that there appears to be adaptation of the mixed microbial population of the rumen to saponins or

saponin-containing plants. The first indication came from comparative studies between sheep in Ethiopia and sheep in the UK. Foliage from *S. sesban*, a multipurpose leguminous tree from sub-Saharan Africa, inhibited protozoal activity *in vitro* in ruminal fluid taken from sheep in the UK; similar inhibition did not occur in ruminal fluid from Ethiopian sheep (Teferedegne et al., 1999). Protozoa which had been washed substantially free of bacteria responded in the same way in both places, so it was concluded that the bacterial population detoxified *S. sesban* in the Ethiopian sheep but not the British sheep. It was speculated that the Ethiopian sheep had probably been exposed to saponins-rich forage whereas the British sheep had not. The effectiveness of *S. sesban* in suppressing protozoa was only transient in the UK sheep, presumably for the same reason (Newbold et al., 1997). Odenyo et al. (1997) confirmed that dietary *S. sesban* had no effects in Ethiopian sheep, whereas other plants were more effective, suggesting that different saponins have different efficacy. Teferedegne (2000) demonstrated clearly the time dependence of the detoxification process (Figure 9). The results also demonstrate that adaptation occurs in animals receiving dietary *S. sesban*, such that the antiprotozoal component is destroyed more quickly than in control sheep (Figure 9).

Saponins are degraded in batch cultures of rumen fluid *in vitro* (Makkar and Becker, 1997) although, apparently, the resultant sapogenins are more resistant to degradation (Wang et al., 1998). Since it is the saponins, not the aglycone sapogenins, that are toxic to the protozoa (Figure 10), it is most likely the cleavage of the sugar moieties from the sapogenins which detoxify the saponins. *F. succinogenes* apparently deglycosylated the saponins from *Y. schidigera* (Wang et al., 2000).

A puzzling observation made by Odenyo et al. (1997) was that *S. sesban* which was introduced directly into the rumen remained toxic to protozoa, but dietary *S. sesban* was ineffective. This result implies either that chewing caused detoxification, perhaps by salivary amylase, or that the larger particle size protected saponins from degradation.

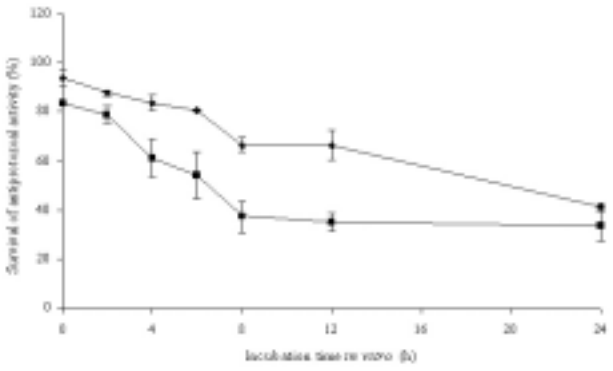

Figure 9. Destruction of antiprotozoal activity of *Sesbania sesban* during incubation in ruminal fluid *in vitro*.

Future research must address the issue of saponins breakdown, the influence of different bacterial species, the influence of saponin structure, and the role of the animal's own digestive system in order to maximise their usefulness. For example, the antiprotozoal effects of *E. cyclocarpum* were much more persistent than *S. sesban*, for reasons that are not presently understood (Teferedegne, 2000).

Saponins and ruminant production

There is no doubt, therefore, that saponins have selective effects on ruminal microorganisms that might be useful in livestock production. A safe, persistent suppression of ciliate protozoa may have widest application. Ciliate protozoa are primarily responsible for the substantial turnover of bacterial protein which occurs during fermentation (Ushida et al., 1991; Wallace and McPherson, 1987; Williams and Coleman, 1992). As a consequence, nitrogen retention is improved by defaunation, which has amply demonstrated in many studies where the protozoa were removed by chemical or physical means, or where the animals had been isolated from birth and thus had not been colonised by protozoa (Williams and Coleman, 1997). The argument in favour of defaunation depends on other factors as well, however. As some species of protozoa are cellulolytic, there are implications for fibre breakdown of removing protozoa (Demeyer and Van Nevel, 1986; Kayouli et al., 1984). Also, the protozoa are proteolytic, so there would be consequences there too (Ushida et al., 1991). However, it is generally agreed that removing or suppressing protozoa would make the best use of nitrogenous resources, particularly on low-protein diets.

Effects of saponins on the bacterial population merit further examination. Wang et al. (2000) suggested that *Y. schidigera* extract would be best used with high grain diets, because of its suppressive effect on *S. bovis* is a starch-digesting, lactate-producing Gram-positive species which is a major cause of rumen fermentation lapsing into lactic acidosis (Stewart et al., 1997). Caution may be required in more fibrous diets, however. The suppression of those bacteria involved in fibre digestion, as described earlier, could have serious consequences to overall digestion.

In animal feeding trials, there have been mixed observations concerning fermentation and productivity. Lu and Jorgensen (1987) found that alfalfa saponins caused a decrease in the efficiency of microbial protein synthesis in sheep, because the growth of bacteria as well as protozoa was depressed. A 36% fall in the efficiency of protein synthesis occurred in cattle receiving *Y. schidigera* extract (Goetsch and Owens, 1985). In contrast, inclusion of *E. cyclocarpum* increased the rate of body weight gain in sheep by 24% (Leng et al., 1992) and 44% (Navas-

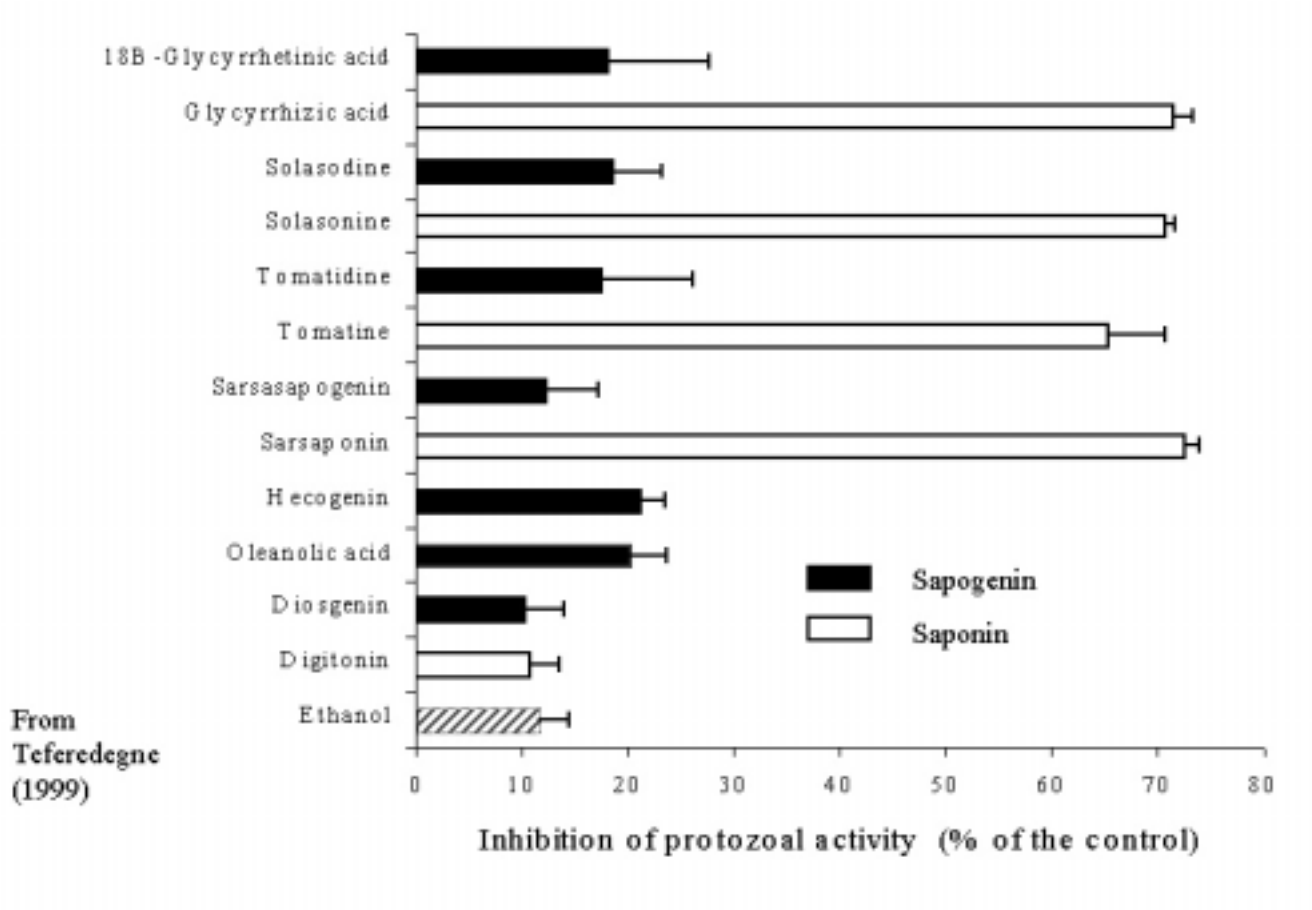

Figure 10. Effects of saponins and sapogenins (1 g/l) on bacteriolytic activity of rumen ciliate protozoa.

Camacho et al., 1993) and wool growth by 27% (Leng et al., 1992), which was attributed to a decrease in protozoal numbers. These differences therefore imply that the effects of saponins on ruminant nutrition are complex, and depend on diet, and the saponins involved. General observations with saponins, where changes in ruminal fermentation characteristics occur, that saponins administration decreases NH_3 concentration (Lu and Jorgensen, 1987; Lu et al., 1987; Makkar et al., 1998) and, where VFA are affected, increases propionate concentration (Lu et al., 1987; Hristov et al., 1999) are typical effects of decreased protozoal numbers (Williams and Coleman, 1992). Saponin-containing *Y. shidigera* extract appeared to have ammonia-binding properties (Headon et al., 1991). However, the reduction in rumen ammonia concentrations when *Y. shidigera* extract was fed is most likely due to suppression of ciliate protozoa (Wallace et al., 1994; Wang et al., 1998).

FUTURE PROSPECTS

Both categories of plant secondary compound featured in this article hold much potential for future development. If the precise chemical forms which exert the greatest potency and persistency can be identified, optimal chemical formulations or plant materials could be provided, leading to chemical or botanical solutions to problems of inefficient N retention in ruminants. Beneficiaries will be the feed industry, in cases where formulations are optimized industrially, and the most needy subsistence farmers who, by feeding small amounts of some plant materials as rumen manipulators rather than for their nutrient content, may enhance the production that they can obtain from limited resources.

Much of the emphasis with essential oils, saponins and the plants which contain these compounds has been directed in ruminants towards more efficient nitrogen retention. There are other objectives which should be addressed as well in order to maximise the usefulness of phytochemicals in ruminant nutrition. Antibacterial as well as antiprotozoal effects are worthy of future consideration. These include altering biohydrogenation, in order to decrease methane formation or to lower the saturated fatty acid content of rumen digesta and hence ruminant products; improving animal welfare, in terms of controlling bloat or lactic acidosis, which is also often a higher priority than increasing feed efficiency; finally, the control of pathogens

in the food chain must be an objective that could be achievable with phytochemicals or suitable plants, and one that would meet with the approval of consumers whose faith in ruminant products has been shaken in the last decade.

Essential oils and saponins are only a few of the plant secondary compounds that might be useful in a similar way. Among the polyphenolic compounds, tannins have received most attention in ruminant nutrition (Cheeke, 1998), because they bind to protein and may slow the degradation of those proteins which are degraded too rapidly in the rumen. Alkaloids, flavonoids, glycosides, amines and non-proteic amino acids also provide defensive functions in plants, and their potential needs to be examined. Indeed, arguably the best route would be not to anticipate what the effective chemical components might be, but instead to examine a wide range of plant species, favouring those which have been used, for example, as herbal remedies, but not excluding others which have no such traditional use. In these investigations, care will have to be taken that saponins or other natural additives do not damage the product. The plant materials must not damage the acceptability of milk or meat, by altering flavour or product quality. For example, saponins might suppress *B. fibrisolvens*, which is particularly important in the formation of the health-promoting conjugated linoleic acids (CLA) which appear in meat and milk (Newbold et al., 2001). They also suppress ciliate protozoa, in which most of the ruminal unsaturated fatty acids are found (Harfoot, 1981). Therefore, care would have to be taken that saponins would not change the fatty acid profile of the products while having other benefits elsewhere. Similar caution would apply to the introduction of any additive intended to manipulate ruminal fermentation. Above all, the safety of the additives to the animals themselves and to the consumer who eats the products must be beyond doubt.

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