Proceedings of the Nutrition Society (2004), **63**, 621–629 DOI:10.1079/PNS2004393
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A meeting of the Nutrition Society hosted by the Scottish Section with support from the British Society of Animal Science was held at the Royal Botanic Garden, Edinburgh on 19 April 2004

Symposium on 'Plants as animal foods: a case of catch 22?'

Antimicrobial properties of plant secondary metabolites

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Increasing awareness of hazards associated with the use of antibiotic and chemical feed additives has accelerated investigations into plants and their extracts as feed additives. The present review mainly discusses two classes of plant secondary compounds in this context, i.e. essential oils and saponins. The broader potential of plants and their extracts is illustrated by the progress of an EC Framework 5 project, 'Rumen-up'. Dietary inclusion of a commercial blend of essential oils causes markedly decreased NH_3 production from amino acids in rumen fluid taken from sheep and cattle. This effect is mediated partly by the effects on hyper-NH_3-producing bacteria and the protein- and starch-fermenting rumen bacterium, *Ruminobacter amylophilus*. Saponin-containing plants and their extracts suppress the bacteriolytic activity of rumen ciliate protozoa and thereby enhance total microbial protein flow from the rumen. The effects of some saponins are transient, because saponins are hydrolysed by bacteria to their corresponding sapogenin aglycones, which are much less toxic to protozoa. Saponins also have selective antibacterial effects that may prove useful in, for example, controlling starch digestion. The 'Rumen-up' project began with a targetted collection of European plants and their extracts, which partners have tested for their effects on rumen proteolysis, protozoa, methanogenesis and lactate production. A success rate of about 5% in terms of positive hits illustrates that plant secondary compounds, of which essential oils and saponins comprise a small proportion, have great potential as 'natural' manipulators of rumen fermentation to benefit the farmer and the environment in the future.

Essential oils: Manipulation: Ruminants: Saponins

Legislators in Europe have moved to prohibit the use of growth-promoting antibiotics in animal feeds from the end of 2005 (Chesson, 2004). This decision was based on public and political concerns that the heavy use of antibiotics in general can give rise to transmissible resistance factors that can compromise the potency of therapeutic antibiotics in man. Growth promotion was a clearly avoidable use. US legislators may soon follow suit. Whether many of the commonly-used growth promoters present such a threat is the subject of intense debate; nevertheless, livestock producers in many countries must face a future without antibiotic growth promoters. Problems may be more acute in pig and poultry production, but ruminants will also be affected given that existing and potential new strategies for manipulating rumen fermentation must avoid selective antimicrobials. There is an increasing consumer demand for organically-produced meat and milk, and organic farmers therefore face the same problems. Thus, there is increasing interest in exploiting natural products that have no similar public health hazard as feed additives to solve problems in animal nutrition and livestock production. The 'natural' products include probiotics, prebiotics, enzymes, organic acids and secondary plant metabolites or their nature-identical chemicals.

Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Some of these compounds are also toxic to animals, but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in man. The potential of essential oils and saponins as beneficial feed additives in ruminant production will be used here as an

Abbreviation: HAP, hyper-NH_3-producing.
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https://doi.org/10.1079/PNS2004393 Published online by Cambridge University Press

illustration of the potential benefits of plant compounds. The review also reports the progress of the EC research project 'Rumen-up', which investigates the broader potential of plants and their extracts.

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 the essence, its flavour or its antiseptic and/or preservative properties. Although commonly thought of as being derived from herbs and spices, they are present to some extent in many plants for their protective role against bacterial, fungal or insect attack. They comprise mainly monoterpenes, the cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives (Fig. 1).

There has been a resurgence of interest in essential oils because they 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. Oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureas* (including the clinically-problematic methicillin-resistant *S. aureus*), *Salmonella* sp. and *Vibrio parahemolyticus*; cinnamaldehyde is the main antibacterial component of the mixture (Chang *et al*. 2001). *E. coli* O157:H7 is inhibited by oregano oil (Elgayyar *et al*. 2001), peppermint oil (Imai *et al*. 2001) and essential oils from other herbs (Marino *et al*. 2001). *Helicobacter pylori* is highly sensitive to spearmint oil (Imai *et al*. 2001). Essential oils are potent against a wide range of oral bacteria (Shapiro *et al*. 1994), and they are used widely in antiseptic mouthwashes.

Effects of essential oils in ruminants

With this range of antimicrobial activity, it was considered logical to evaluate essential oils for possible beneficial selective effects against rumen micro-organisms. Essential oils were examined many years ago in rumen bacteria in relation to their contribution to poor palatability in some plant species (Oh *et al*. 1968). General inhibitory activity has been found across a range of plant materials, of which vinegar weed (*Trichostema lanceolatum*) is one of the most potent. Oh *et al*. (1967) have demonstrated that individual oils have different effects on mixed rumen bacteria. Monoterpene hydrocarbons are less toxic and sometimes stimulatory to microbial activity compared with the corresponding oxygenated compounds, the monoterpene alcohols and aldehydes (Oh *et al*. 1967). The sensitivity of rumen bacteria to the essential oils of big sagebrush (*Artemisia tridentata*) is the same in captive deer as it is in wild deer, which may indicate that rumen bacteria do not adapt to essential oils (Nagy & Tengerdy, 1968). Thus, essential oils are not necessarily toxic to rumen bacteria, and their effects may be expected to persist.

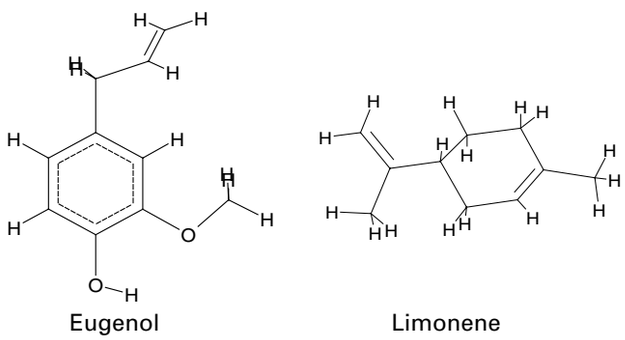

Fig. 1. Structures of two essential oil compounds.

Animal trials and microbiological experiments have been carried out with essential oils at the Rowett Research Institute in collaboration with the University of Reading, Reading, Berks., UK, the University of Wales (Aberystwyth), Aberystwyth, Ceredigion, UK and Crina SC, Gland, Switzerland. Some of these results have been published (McIntosh *et al*. 2000, 2003; McEwan *et al*. 2002*a*,*b*; Wallace *et al*. 2002; Newbold *et al*. 2004).

One trial was carried out with ruminally-fistulated sheep receiving a maintenance diet comprising (g/kg): 400 concentrate, 600 grass silage. Each sheep received daily 100 mg essential oils (Crina Ruminants, Akzo Nobel Surface Chemistry, St Albans, Herts., UK; a defined patented mixture of natural and nature-identical essential oil compounds that includes thymol, eugenol, vanillin and limonene on an organic carrier; Rossi, 1995) or the control diet in a 6-week Latin square design. Proteinase, peptidase and deaminase activities of rumen fluid were measured as described elsewhere (Floret *et al*. 1999). Nylon bag incubations were done according to Mehrez & Ørskov (1977).

The results show that essential oils have no influence on volatile fatty acids or NH_3 concentrations, on protozoal numbers or on microbial protein flow. The rate of degradation of soybean meal tends to be decreased at 8 and 16 h, but there is no effect on the breakdown of rapeseed meal. The breakdown sequence of protein to NH_3 was measured by assaying the rates of the individual reactions in rumen fluid *in vitro*. NH_3 formation is affected only at the last step, i.e. the deamination of amino acids (Fig. 2). Similar

		Control	Essential oils
Protein ↓ Oligopeptides	[^{14}C]casein breakdown (mg/mg microbial protein per h)	1·40	1·43
↓ Dipeptides	Ala_5 breakdown (nmol/mg protein per min)	2·62	2·71
↓ Amino acids	Ala_2 breakdown (nmol/mg protein per min)	1·19	1·06
↓ NH_3	Deaminase (nmol NH_3/mg protein per min)	204	155*

Fig. 2. Influence of dietary essential oils on different steps of the protein catabolic sequence in rumen fluid taken from sheep receiving a mixed grass hay–concentrate diet, with or without 110 mg essential oil mixture/d. Ala_5, Ala_2, peptides containing five and two alanine molecules respectively. (From McIntosh *et al*. 2000; Wallace *et al*. 2002.)

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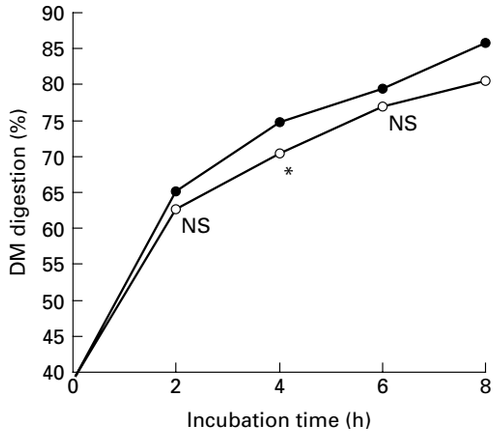

Fig. 3. Loss of DM from ground barley incubated in nylon bags in the sheep, using methods developed by Mehrez & Ørskov (1977), with (○) or without (●) dietary essential oils. Mean value was significantly different from that for ground barley incubated without dietary essential oils: * $P<0{\cdot}05$. Other values were not significant: at 2 h $P<0{\cdot}07$, at 6 h $P<0{\cdot}10$. (From NR McEwan, RJ Wallace and CJ Newbold, unpublished results.)

experiments in which cereals have been incubated in nylon bags in the rumen of sheep show that slower colonisation of the cereals takes place, resulting in significant decreases ($P<0{\cdot}05$) in rates of degradation caused by dietary essential oils (Fig. 3).

A similar experiment has been carried out with ruminally-fistulated dairy cows receiving a total mixed ration comprising grass silage, maize silage and concentrate. The effects of essential oils on the sequence of protein catabolism were assessed as described earlier. The results are similar, in that only the final step, which is the breakdown of amino acids to NH_3, is inhibited in cows receiving dietary essential oils. The deamination of amino acids was assessed further by including incubations to which monensin, the growth-promoting ionophore, had been added (Table 1). Again, dietary essential oils cause a decrease in the rate of NH_3 production. Monensin addition to the *in vitro* incubations causes a larger decrease in the deaminative activity of rumen fluid from both control and essential oil-supplemented cows; the decreased activity no longer shows a difference between the two groups, indicating that the species affected by dietary essential oils are also affected by monensin.

Table 1. Influence of dietary essential oils on the rate of breakdown of amino acids in bovine rumen fluid *in vitro*† (from McIntosh *et al.* 2003)

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

Mean value was significantly different from that for the control:
* $P<0{\cdot}05$.
†For details of procedures, see p. 623.
‡Treatment daily with 100 mg essential oils (Crina Ruminants, Akzo Nobel Surface Chemistry, St Albans, Herts., UK; a mixture of natural and nature-identical essential oil compounds that includes thymol, eugenol, vanillin and limonene on an organic carrier; Rossi, 1995).

Influence of essential oils on rumen micro-organisms

The influence of essential oils on rumen micro-organisms has been determined *in vitro* (McIntosh *et al.* 2003). Among the predominant species of rumen bacteria, only *Prevotella ruminicola*, *Clostridium sticklandii* and *Peptostreptococcus anaerobius* are prevented from growing at 40 μg essential oils/l. The last two species are so-called 'hyper-NH_3-producing' (HAP) bacteria (Russell *et al.* 1991). Furthermore, many species could adapt to grow at higher concentrations. Rumen ciliate protozoa are unaffected, and *Methanobrevibacter smithii*, a methanogenic archaeon related to those found in the rumen, is similarly unaffected. *Ruminobacter amylophilus* grows in the presence of 40 μg essential oils/l, but the oils greatly enhance its lysis in the stationary phase; thus, it may also be considered to be sensitive to essential oils. Rumen anaerobic fungi are sensitive to essential oils at concentrations similar to those for the most sensitive bacteria. Thus, fibre digestion may be decreased by essential oils under conditions in which the fungi are prevalent, such as high-fibre low-quality rations.

In another sheep trial the total HAP population has been enumerated, using the ability of the bacteria to grow on trypticase as the only source of C and N. The total viable count of bacteria is unaffected, but the numbers of HAP bacteria decrease by 77% in sheep receiving a low-protein diet (McEwan *et al.* 2000*a*; Wallace *et al.* 2002). These trials suggest that essential oils have no marked effect on protozoal numbers or activity.

Thus, the bacteria most sensitive to essential oils are the HAP species, *Prevotella* spp. and *R. amylophilus*. HAP bacteria have a high capability to generate NH_3 from amino acids and they are highly sensitive to monensin (Russell *et al.* 1991). They comprise only approximately 1% of the rumen bacterial population, however, and the fact that monensin inhibits only 32% of the total NH_3-forming activity suggests that they are not one of the main species involved in deamination, but 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 important. Essential oils have less effect on deamination than monensin, presumably because essential oils affect fewer bacterial species. *Prevotella* spp. are involved in all the steps of protein catabolism (Wallace *et al.* 1997). *R. amylophilus* is a highly-active starch and protein digester that proliferates on concentrate diets (Stewart *et al.* 1997). The effects of essential oils on the breakdown of barley starch are consistent with an effect on *R. amylophilus*. Thus, pure-culture results are consistent with observations that 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 presently available.

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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 of essential oils on the degradation of protein supplements may be mediated via *R. amylophilus*, 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. This factor 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. *Prevotella* spp. are important at all stages of protein degradation, but particularly at peptide breakdown. There is evidence, not shown, that essential oils have a direct effect on the metabolic activity of *Prevotella*. 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. The overall picture is summarized in Fig. 4.

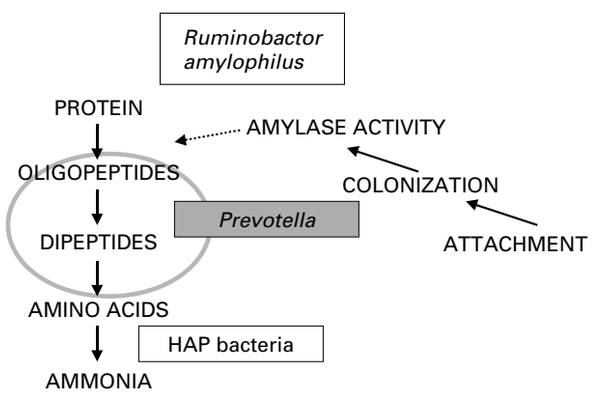

Fig. 4. A scheme representing the mode of action of essential oils in terms of the species affected and whether the response is adaptive (□) or acute (▨). HAP, hyper-ammonia-producing.

Saponins

Background

Saponins, like essential oils, cover a wide variety of chemical compounds and, also like essential oils, man has made use of their properties for centuries (Hostettmann & Marston, 1995). The word 'saponin' is derived from the Latin word *sapo* (soap) and traditionally saponin-containing plants have been utilized for washing. Chemically, saponins are high-molecular-weight glycosides in which sugars are linked to a triterpene or steroidal aglycone moiety (Fig. 5; Hostettmann & Marston, 1995).

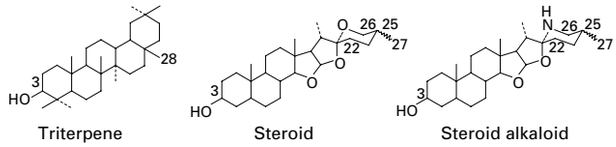

Fig. 5. Chemical structures of the three categories of sapogenins that are the parent compounds of the saponins. Saponins possess a variety of glycosyl groups covalently bound to the 3-OH group.

Effects of saponins in ruminants

Van Nevel & Demeyer (1990) have found no indication of any toxic effects or effects of sarsaponin on microbial growth or protein breakdown *in vitro*. In contrast, Lu *et al*. (1987) have reported that lucerne (*Medicago sativa*) saponins appear to suppress fermentation in continuous culture. Subsequent *in vivo* investigation (Lu & Jorgensen, 1987) has confirmed a general decrease in fermentative activity when lucerne saponins are supplied to the sheep rumen, of which decreased volatile fatty acid concentrations and decreased cellulose digestion are symptomatic. Importantly, Lu & Jorgensen (1987) have also noted large decreases in protozoal numbers in sheep receiving lucerne saponins. Goetsch & Owens (1985) have 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 & Coleman, 1992). A meta-analysis has recently demonstrated that the benefits of defaunation outweigh any disadvantages (Eugène *et al*. 2004). Anti-protozoal 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 rumen micro-organisms (Orpin, 1977; Bird & Leng, 1978; Bird *et al*. 1979; Eadie & Shand, 1981) or to the host (Lovelock *et al*. 1982). Lipids are toxic to protozoa (Newbold & Chamberlain, 1988; Matsumoto *et al*. 1991; Machmuller *et al*. 1998), and also to fibre digestion (Broudiscou *et al*. 1994). Thus, there has been, until now, no reliable safe on-farm method available for suppressing rumen protozoa.

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

Saponins and rumen micro-organisms

Dietary saponins are poorly absorbed, so their biological effects occur in the digestive tract (Cheeke, 1996). Although

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antimicrobial effects of saponins and saponin-containing plants are to be expected, based on the wealth of information from other biological systems, the earliest observations relating to rumen micro-organisms have come relatively recently from *in vitro* continuous-culture systems. Sarsaponin, from *Yucca schidigera*, has been observed to decrease protozoal numbers but not bacterial numbers in a 22 d semi-continuous system (Valdez *et al*. 1986), and in the presence of lucerne saponins the bacterial population is changed from a morphologically-diverse one to one in which fewer morphotypes are present (Lu *et al*. 1987). Not only can the saponins have an effect on rumen micro-organisms, but the micro-organisms can metabolise the saponins, thus introducing another factor to be considered in the application of saponins to ruminant nutrition.

Protozoa

Numerous studies have demonstrated that saponins and saponin-containing plants have toxic effects on protozoa. The toxicity of *Y. schidigera* extract towards protozoa *in vitro* has been observed as a fall either 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 results in all antiprotozoal activity being located in the butanol fraction, consistent with the active component being the saponins. Saponins from *Quillaja saponaria* and *Acacia auriculoformis* (Makkar *et al*. 1998) and foliage from *Sesbania sesban* (a multipurpose leguminous tree from sub-Saharan Africa; Newbold *et al*. 1997) are also antiprotozoal *in vitro*; the active component of *S. sesban* again being extractable in butanol.

In vivo powdered *Y. shidigera* decreases rumen protozoal numbers in heifers (Hristov *et al*. 1999). A decrease in protozoal numbers has been reported in the rumen of sheep infused with pure lucerne saponins (Lu & 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 in bacterial, membranes (Williams & Coleman, 1992). Thus, the sterol-binding capability of saponins (Hostettmann & Marston, 1995) most probably causes the destruction of protozoal cell membranes.

Bacteria and fungi

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

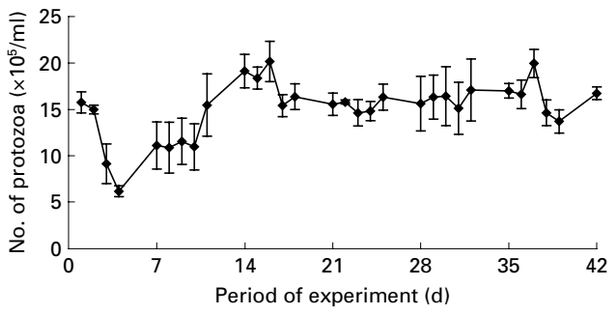

Fig. 6. Influence of *Sesbania sesban* on protozoal numbers in the sheep rumen. Values are means with their standard errors represented by vertical bars. (From Teferedegne *et al*. 1999.)

Experiments using pure cultures of rumen bacteria have indicated that there are also possible antibacterial effects of saponins. *Y. schidigera* extract abolishes growth of the fibre digester, *Butyrivibrio fibrisolvens*, and prolongs the lag phase of *Streptococcus bovis* (Wallace *et al*. 1994). Similar sensitivity of *S. bovis* to *Y. schidigera* extract has been found by Wang *et al*. (2000), who additionally found that cellulose digestion by *Ruminococcus* spp. and *Fibrobacter succinogenes* is inhibited.

A potentially very important observation is that the anaerobic rumen fungi, *Neocallimastix frontalis* and *Piromyces rhizinflata*, are highly sensitive to *Y. schidigera* saponins (Wang *et al*. 2000). Rumen fungi appear to fill an important niche in the digestion of recalcitrant plant fibres, because they cause physical as well as enzymic disruption of plant cell walls (Orpin & Joblin, 1997). Thus, it may well be advantageous to promote the bacterial detoxification of the saponins in animals receiving poor-quality forages or crop by-products.

Microbial adaptation and degradation of saponins

One of the problems encountered in the use of 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 has come from comparative studies between sheep in Ethiopia and sheep in the UK. Foliage from *S. sesban* inhibits protozoal activity *in vitro* in rumen fluid taken from sheep in the UK; similar inhibition does not occur in rumen fluid from Ethiopian sheep (Teferedegne *et al*. 1999). Protozoa that have been washed substantially free of bacteria respond in the same way in both places; thus, it appears that the bacterial population detoxifies *S. sesban* in the Ethiopian sheep but not the British sheep. It has been speculated that the Ethiopian sheep had probably been exposed to saponin-rich forage whereas the British sheep had not. The effectiveness of *S. sesban* in suppressing protozoa has been shown to be only transient in the UK sheep, presumably for the same reason (Newbold *et al*. 1997). Odenyo *et al*. (1997) have confirmed that dietary *S. sesban* has no effects in Ethiopian sheep, whereas other plants are more effective, suggesting that different saponins have different efficacies. Teferedegne *et al*. (1999) have demonstrated clearly the time dependence of the detoxification process (Fig. 6).

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The results also demonstrate that adaptation occurs in animals receiving dietary *S. sesban* (Fig. 6), such that the antiprotozoal component is destroyed more quickly than in control sheep.

Saponins are degraded in batch cultures of rumen fluid *in vitro* (Makkar & Becker, 1997), although the resultant sapogenins appear to be more resistant to degradation (Wang *et al*. 1998). Since it is the saponins, not the aglycone sapogenins, that are toxic to the protozoa (Teferedegne, 2000), it is most probably the cleavage of the sugar moieties from the sapogenins that detoxifies the saponins. *F. succinogenes* apparently deglycosylates the saponins from *Y. schidigera* (Wang *et al*. 2000).

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

Future research must address the issue of saponin 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 maximize their usefulness. For example, the antiprotozoal effects of *E. cyclocarpum* are much more persistent than those of *S. sesban*; the reasons are not presently understood (Teferedegne, 2000).

Saponins and ruminant production

There is no doubt, therefore, that saponins have selective effects on rumen micro-organisms that might be useful in livestock production. A safe persistent suppression of ciliate protozoa may have the widest application. Ciliate protozoa are primarily responsible for the substantial turnover of bacterial protein that occurs during fermentation (Wallace & McPherson, 1987; Ushida *et al*. 1991; Williams & Coleman, 1992). As a consequence, N retention is improved by defaunation, which has been amply demonstrated in many studies in which the protozoa are removed by chemical or physical means, or the animals have been isolated from birth and thus have not been colonized by protozoa (Williams & Coleman, 1997; Eugène *et al*. 2004). The argument in favour of defaunation also depends, however, on other factors. As protozoa are cellulolytic, there are implications for fibre breakdown of removing protozoa (Kayouli *et al*. 1984; Demeyer & Van Nevel, 1986). The protozoa are also proteolytic; thus, their removal would have consequences (Ushida *et al*. 1991). However, it is generally agreed that removing or suppressing protozoa would make the best use of nitrogenous resources, particularly with low-protein diets.

The effects of saponins on the bacterial population merit further examination. Wang *et al*. (2000) have suggested that *Y. schidigera* extract would be best used with high-grain diets, because of its suppressive effect on *S. bovis*, a starch-digesting lactate-producing Gram-positive species that is a major cause of rumen fermentation lapsing into lactic acidosis (Stewart *et al*. 1997). Caution may be required, however, with more fibrous diets. 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 in relation to fermentation and productivity. Lucerne saponins have been found to cause a decrease in the efficiency of microbial protein synthesis in sheep, because the growth of bacteria as well as that of protozoa is depressed (Lu & Jorgensen, 1987). A 36% fall in the efficiency of protein synthesis occurs in cattle receiving *Y. schidigera* extract (Goetsch & Owens, 1985). In contrast, inclusion of *E. cyclocarpum* increases the rate of body-weight gain in sheep by 24% (Leng *et al*. 1992) and 44% (Navas-Camacho *et al*. 1993), and wool growth by 27% (Leng *et al*. 1992), which has been attributed to a decrease in protozoal numbers. These differences imply, therefore, that the effects of saponins on ruminant nutrition are complex, and depend on diet and on the saponins involved. General observations with saponins that are typical effects of decreased protozoal numbers (Williams & Coleman, 1992) are that where changes in rumen fermentation characteristics occur the administration of saponins decreases NH_3 concentration (Lu & Jorgensen, 1987; Lu *et al*. 1987; Makkar *et al*. 1998) and where volatile fatty acids are affected saponins increase propionate concentration (Lu *et al*. 1987; Hristov *et al*. 1999). Saponin-containing *Y. shidigera* extract appears to have NH_3-binding properties (Headon *et al*. 1991). However, the reduction in rumen NH_3 concentrations when *Y. shidigera* extract is fed is most probably a result of suppression of ciliate protozoa (Wallace *et al*. 1994; Wang *et al*. 1998).

'Rumen-up'

The potential of a much wider range of plant materials in modifying rumen fermentation is being investigated in an EC-sponsored shared-cost action under Framework Programme 5, 'Rumen-up'. The aim of the overall project is to develop new plants or plant extracts as dietary supplements for ruminants to replace chemical additives and growth-promoting antibiotics. The plant materials, assembled from botanical and industrial collections, have been evaluated for their ability to prevent lactic acidosis and bloat, and to decrease pollution, by preventing CH_4 formation and decreasing N excretions. Bloat and acidosis are distressing disorders that result from a malfunction of microbial digestion in the rumen. CH_4, a potent greenhouse gas, and NH_3, which gives rise to urinary urea, derive from normal rumen fermentation. The intention of the project is to deliver plant-based sustainable solutions to these problems, thereby benefitting European biotechnological and agricultural industries, with the new plants increasing the diversity of crops used in agriculture.

The main objective to be completed within the first 12 months was to assemble a 500-sample collection of plants and plant extracts, together with brief descriptions of their traditional use, possible secondary compounds and an assurance of their cultivability in the EU. This objective has been achieved, and the collection will soon be posted on the project's website, www.rowett.ac.uk/rumen_up.

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The next broad objective was to screen all samples for their effectiveness in inhibiting rumen ciliate protozoa, rumen proteolysis, CH_4 formation, microbial protein synthesis, lactic acidosis and bloat. The samples were also to be investigated to ensure that potentially-useful samples have no detrimental effect on the other basic functions of the fermentation, such as fibre digestion and volatile fatty acid production. On the basis of this evaluation, which was completed at 18 months, and also an assessment of published information on traditional uses, known toxicity and palatability, a select number of samples has been taken forward for more detailed experimentation, with the aim of producing a short list of two samples that will be tested in production trials in the last 6 months of the project. Twenty-three samples have been taken forward for patent protection, fulfilling the criteria that they hit at least one of the target areas. Examples include some common plants, e.g. daisy (*Bellis perennis*), Japanese honeysuckle (*Lonicera japonica*) and lettuce (*Lactuca sativa*).

Several samples have recorded multiple hits: protozoa and CH_4, five samples; proteolysis and protozoa, two samples; protozoa and acidosis, three samples; CH_4 and acidosis, five samples. The connection between protozoa and CH_4 is known, because methanogenic archaea are known to attach to the surface of protozoa, and furthermore appear to live in the cytoplasm of the protozoa as intracellular commensal or symbiotic organisms (Finlay *et al*. 1994). Protozoa are proteolytic (Lockwood *et al*. 1988), which may explain the second connection. However, the relationship between protozoa and acidosis is usually thought to involve the protozoa protecting against acidosis by engulfing starch so that it is not susceptible to bacterial attack (Williams & Coleman, 1992), not the protozoa causing acidosis that seems to be the case in this situation. Why CH_4 and acidosis should be connected is unclear too, although both factors might result from an alteration in the stoichiometry of fermentation (Van Nevel & Demeyer, 1996).

Conclusions

Plants and their extracts have important potential as manipulators of rumen fermentation for productivity and health benefits. They have specific effects on members of the rumen microflora and fauna that can be beneficial to animal productivity and health.

Acknowledgements

The Rowett Research Institute receives most of its funding from the Scottish Executive Environment and Rural Affairs Department. Additional support was received from the Department for International Development and Akzo Nobel, Gland, Switzerland. 'Rumen-up' was a Framework 5 programme sponsored by the European Commission (QLK5-CT-2001–00992). Many scientific colleagues contributed to this work, including Neil McEwan, Freda McIntosh, Jamie Newbold, Rusmana Ningrat, Belete Teferedegne, Collette Walsh, Becky Graham, Klaus Becker, Harinder Makkar, Ellen Hoffmann, Natascha Selje, Stefan Meutzel, David Beever, Fergus Mould, Becky Morgan, Kirsty Kliem, Secundino López, Jesus Gonzalez, Miguel Fernandez, Cepta Duffy, Riccardo Losa and Marco Frehner.

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