

# The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production

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The growing public concerns over chemical residues in animal-derived foods and threats of antibiotic-resistant bacteria have renewed interest in exploring safer alternatives to chemical feed additives in ruminant livestock. Various bioactive phytochemicals including saponins appear to be potential 'natural' alternatives to 'chemical' additives in modulating rumen fermentation favourably and animal performance. Saponins are a diverse group of glycosides present in many families of plants. The primary effect of saponins in the rumen appears to be to inhibit the protozoa (defaunation), which might increase the efficiency of microbial protein synthesis and protein flow to the duodenum. Furthermore, saponins may decrease methane production via defaunation and/or directly by decreasing the activities (i.e. rate of methanogenesis or expression of methane-producing genes) and numbers of methanogens. Saponins may also selectively affect specific rumen bacteria and fungi, which may alter the rumen metabolism beneficially or adversely. The ammonia-adsorption and modulation of digesta passage in the rumen by saponins have also been implicated in altering rumen metabolism, but their physiological responses are likely to be negligible compared with microbiological effects. The effects of saponins on rumen fermentation have not been found to be consistent. These discrepancies appear to be related to the chemical structure and dosage of saponins, diet composition, microbial community and adaptation of microbiota to saponins. There is need for systematic research based on chemical structures of saponins, nutrient composition of diets and their effects on rumen microbial ecosystem to obtain consistent results. The present paper reviews and discusses the effects and mode of action of saponins on microbial community and fermentation in the rumen, and ruminant performance.

**Saponins: Mechanisms of action: Microbial ecology: Rumen fermentation: Ruminant production**

## Introduction

The term 'phytochemicals' is used to describe non-nutritive plant metabolites that are essential for the survival (i.e. protection against herbivores, pests and micro-organisms) and proper functioning of growth and reproduction in plants<sup>(1)</sup>. In recent years, there has been increasing interest among nutritionists and microbiologists to exploit bioactive phytochemicals such as saponins, condensed tannins and essential oils for improving rumen metabolism, and increasing the efficiency of ruminant production<sup>(2–7)</sup>. In particular, bioactive phytochemicals with selective antimicrobial properties can be of interest in animal nutrition<sup>(3,8)</sup>. The impetus behind much of the research of using phytochemicals as feed additives has grown from the necessity of substituting chemical feed additives in animal

agriculture because of growing concerns over chemical additives in terms of residues in animal products and the development of bacterial resistance to antibiotics<sup>(9)</sup>. This trend is likely to be continued due to growing demands for organic livestock products. Research is also increasingly being directed at avoiding undesirable impacts of ruminant production on the environment such as contributions to the greenhouse effect, and N and P pollution with the use of safer alternatives to chemical feed additives.

Saponins are a group of plant secondary compounds that have shown the possibility to modify rumen fermentation and enhance animal production<sup>(2–5,10–13)</sup>. Saponins of *Yucca schidigera* and *Quillaja saponaria* have been categorised as 'generally recognised as safe' for human consumption by the US Food and Drug Administration<sup>(14)</sup>.

**Abbreviations:** ppm, parts per million; VFA, volatile fatty acid.

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In recent years, a number of excellent reviews have been published on the potential use of saponins as rumen modifiers<sup>(2,3,5)</sup>. However, the mechanisms of action of saponins and discrepancies of results among studies on rumen microbiota and fermentation have not been discussed in details in those reviews. The present review aims to provide deeper insights into the impacts of saponins on the rumen microbial community, how this community can influence rumen metabolism and fermentation, and how this might ultimately lead to improvements in ruminant performance. The recent investigations on the effects of saponins on ruminants are also reported in the present review.

### Chemistry of saponins

The uses of saponins in traditional folk medicines and as a substitute for soap have been known for a long time. They occur in a wide variety of plants and also in some marine animals. Chemically, saponins are a group of high-molecular-weight glycosides in which saccharide chain units (1–8 residues) are linked to a triterpene (triterpene saponins) or steroidal (steroid saponins) aglycone moiety, i.e. sapogenin (Fig. 1). Triterpene saponins are more widely distributed in nature than steroidal types<sup>(15)</sup>. The steroidal moiety is in most cases in the furostanol or spirostanol form<sup>(15)</sup>. The saccharide chains are commonly attached at the C<sub>3</sub> position (monodesmosidic), but some sapogenins contain two saccharide chains (bidesmosidic) attached at the C<sub>3</sub> and C<sub>17</sub> (via C<sub>28</sub>) position<sup>(16)</sup>. Spirostanol is usually monodesmosidic and furostanol bidesmosidic in nature<sup>(15)</sup>. Most of the saponins are monodesmosidic or bidesmosidic. However, a lucerne and an *Acacia auriculiformis* saponin

are tridesmosidic<sup>(16)</sup>. Spirostanol saponins are found mainly in the seeds, roots and bulbs of the plants, whereas furostanol saponins are present in the assimilatory parts of the plants<sup>(15)</sup>. The number of sugars, the type of sugars (for example, glucose, galactose, xylose, glucuronic acid and rhamnose) and the stereochemistry of the sapogenin moiety with functional groups (–OH, –COOH, –CH<sub>3</sub>) vary greatly, producing a diverse group of metabolites in this compound class. The chemical structures of triterpene and steroidal saponins are described in detail elsewhere<sup>(15,16)</sup>.

### Effects of saponins on rumen microbial population

#### *Ciliate protozoa*

A number of studies have demonstrated that pure saponins and saponin-containing plants or extracts have inhibitory effects on protozoa (Table 1)<sup>(17–43)</sup>. One of the earliest observations was made by Valdez *et al.*<sup>(29)</sup> who showed that a commercial sarsaponin (steroidal spirostanol saponin) extracted from the *Yucca schidigera* plant decreased the protozoal population with a small increase in bacterial numbers in continuous culture systems. In many subsequent studies, the extracts of *Y. schidigera* have been shown to suppress the growth of rumen protozoa *in vitro*<sup>(44)</sup> without affecting DM disappearance and other rumen microbial populations<sup>(45)</sup>. The saponin-containing butanol extract of *Y. schidigera* has been shown to be responsible for all the plant's anti-protozoal activity<sup>(44,46)</sup>. Protozoal activity, as measured by the breakdown of [<sup>14</sup>C]leucine-labelled *Selenomonas ruminantium* in rumen fluid incubated *in vitro*, was stopped by the addition of 1% *Y. schidigera* extract<sup>(44)</sup>. A number of *in vivo* studies have also confirmed the anti-protozoal effects of *Yucca* saponins<sup>(43,47)</sup>.

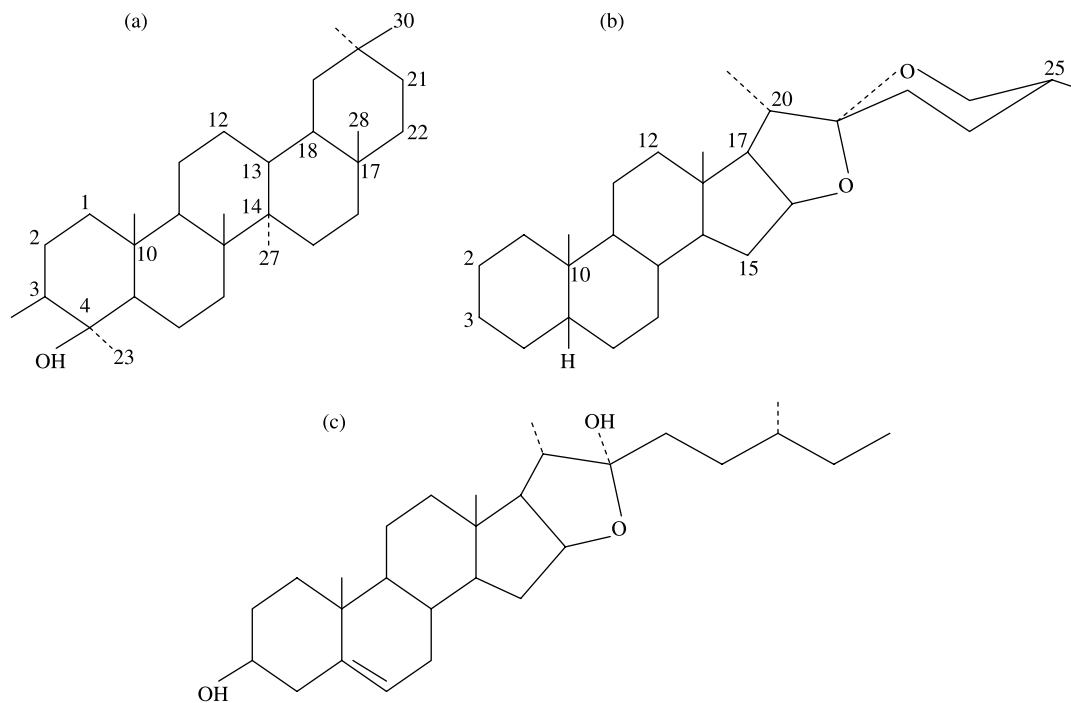


Fig. 1. Chemical structures of sapogenins: triterpenoid, for example, oleanane (a); steroids, for example, spirostanol (b) and furostanol (c).

**Table 1.** Effects of saponins or saponin-containing plants on protozoal numbers and rumen fermentation *in vivo* and performance of animals

Saponin type*	Animal	Dosage (g/kg DM)†	Feed (F:C)	Effect‡										Reference
				Prot	NH <sub>3</sub>	TVFA	A:P	Dig	MPS	Met	FI	ADG	MY	
<i>Biophytum petersianum</i>	Goats	13 mg/kg BW	70:30	↓	↓	↓	↓	=	=	×	×	×	×	Santoso <i>et al.</i> (2007) <sup>(19)</sup>
<i>B. petersianum</i>	Goats	26 mg/kg BW	70:30	↓	↓	=	↓	↓	↑	×	×	×	×	Santoso <i>et al.</i> (2007) <sup>(19)</sup>
<i>B. petersianum</i>	Goats	19.5 mg/kg BW	70:30	↓	↓	↓	↓	=	↑	×	×	×	×	Santoso <i>et al.</i> (2007) <sup>(19)</sup>
<i>Enterolobium cyclocarpum</i>	Sheep	25, 75 g/d	88:12	↓	×	×	×	×	×	×	×	↑	×	Leng <i>et al.</i> (1992) <sup>(20)</sup>
<i>E. cyclocarpum</i>	Sheep	100 g/d	100:0	↑	↓	×	×	= §	×	×	↑	↑	×	Navas-Camacho <i>et al.</i> (1993) <sup>(21)</sup>
<i>E. cyclocarpum</i>	Sheep	300 g/d	100:0	↓	↓	×	×	↓ §	×	×	=	↑	×	Navas-Camacho <i>et al.</i> (1993) <sup>(21)</sup>
<i>E. cyclocarpum</i>	Sheep	200 g/d	60:40	↓	=	=	=	×	×	×	×	×	×	Ivan <i>et al.</i> (2004) <sup>(22)</sup>
Lucerne (27.8%)	Sheep	10	100:0	↓	×	=	=	=	×	=	=	=	×	Klita <i>et al.</i> (1996) <sup>(17)</sup>
Lucerne (27.8%)	Sheep	20 to 40	100:0	↓	×	=	=	↓	×	=	=	×	×	Klita <i>et al.</i> (1996) <sup>(17)</sup>
Lucerne saponins	Sheep	20	60:40	↓	↓	=	↓	=	×	=	=	×	×	Lu & Jorgensen (1987) <sup>(18)</sup>
Lucerne saponins	Sheep	40	60:40	↓	↓	=	↓	=	×	↓	=	×	×	Lu & Jorgensen (1987) <sup>(18)</sup>
Lucerne saponins	Sheep	20	40:60	↓	=	↓	=	↑	×	=	=	×	×	Lu & Jorgensen (1987) <sup>(18)</sup>
Lucerne saponins	Sheep	40	40:60	↓	↓	↓	↓	↑	×	=	=	×	×	Lu & Jorgensen (1987) <sup>(18)</sup>
QS extract (10%)	Heifers	8	91:9	=	=	=	=	↑	=	×	=	×	×	Baah <i>et al.</i> (2007) <sup>(23)</sup>
QS extract (5.7–8.1%)	Sheep	13.5	60:40	=	↓	↓	=	↑	=	=	=	×	×	Pen <i>et al.</i> (2007) <sup>(24)</sup>
QS plant (3%)	Dairy cows	10	51:49	=	=	=	=	=	×	=	↑	=	=	Holtshausen <i>et al.</i> (2009) <sup>(25)</sup>
<i>Sapindus saponaria</i> fruit	Sheep	8 g/kg BW <sup>0.75</sup>	100:0	↑	=	=	↓	↓	↑	×	↑	×	×	Abreu <i>et al.</i> (2004) <sup>(26)</sup>
<i>S. saponaria</i> fruit (12%)	Sheep	5 g/kg BW <sup>0.75</sup>	67:33	↓	=	↑	↓	↓	×	↓	=	↑	×	Hess <i>et al.</i> (2004) <sup>(27)</sup>
<i>S. saponaria</i> pericarp	Sheep	25 g/d	72:28	↓	=	×	=	×	×	×	=	×	×	Diaz <i>et al.</i> (1993) <sup>(28)</sup>
<i>S. saponaria</i> pericarp	Sheep	50 g/d	72:28	↓	×	×	↓	×	×	×	=	×	×	Diaz <i>et al.</i> (1993) <sup>(28)</sup>
Sarsaponins	Dairy cows	0.077	40:60	×	=	=	=	=	×	×	=	=	=	Valdez <i>et al.</i> (1986) <sup>(29)</sup>
Sarsaponins	Dairy cows	0.2, 0.41, 0.62	36:64	=	↓	=	=	×	×	×	=	=	=	Singer <i>et al.</i> (2008) <sup>(30)</sup>
Sarsaponins	Calves	0.02	54:46	×	×	×	×	×	×	×	=	↑ ¶	×	Mader & Brumm (1987) <sup>(31)</sup>
Sarsaponins	Sheep	0.002, 0.03	50:50	=	↓	=	=	=	×	=	=	×	×	Sliwinski <i>et al.</i> (2002) <sup>(32)</sup>
Sarsaponins	Dairy cows	0.044	67:33	×	×	×	×	×	=	×	=	×	=	Goetsch & Owens (1985) <sup>(33)</sup>
YS	Steers	0.43	92:8	×	×	=	×	×	↑	=	=	↑	×	Zinn <i>et al.</i> (1998) <sup>(34)</sup>
YS (4.4%)	Wether	0.24	85:15	×	↓	=	↓	×	↑	×	=	×	×	Santoso <i>et al.</i> (2006) <sup>(35)</sup>
YS extract	Dairy cows	0.38	60:40	×	=	×	×	×	×	×	=	×	=	Wilson <i>et al.</i> (1998) <sup>(36)</sup>
YS extract	Dairy cows	0.38	51:49	×	=	×	×	×	×	×	=	×	=	Wilson <i>et al.</i> (1998) <sup>(36)</sup>
YS extract	Dairy cows	1.46	63:37	×	×	×	×	×	×	×	=	×	=	Lovett <i>et al.</i> (2006) <sup>(37)</sup>
YS extract	Dairy cows	3.1	63:37	×	×	×	×	×	×	×	↓	×	=	Lovett <i>et al.</i> (2006) <sup>(37)</sup>
YS extract	Steers	1.25	63:37	↓	×	↓	=	=	×	×	=	×	=	Lovett <i>et al.</i> (2006) <sup>(37)</sup>
YS extract	Steers	2.56	63:37	↓	×	↓	=	=	×	×	=	×	=	Lovett <i>et al.</i> (2006) <sup>(37)</sup>
YS extract	Sheep	0.13	75:25	×	↓	↑	↓	=	×	↓	=	×	×	Wang <i>et al.</i> (2009) <sup>(38)</sup>
YS extract	Sheep	0.12	70:30	×	↓	=	=	=	=	↓	=	×	×	Santoso <i>et al.</i> (2004) <sup>(39)</sup>
YS extract	Dairy cows	0.07	48:52	×	×	×	×	×	=	×	↓	×	×	Wu <i>et al.</i> (1994) <sup>(40)</sup>
YS extract	Dairy cows	0.06	48:52; urea added	×	×	×	×	=	=	×	↑	×	×	Wu <i>et al.</i> (1994) <sup>(40)</sup>
YS extract	Dairy cows	0.03 to 0.12	38:62	×	=	=	=	×	×	×	=	×	×	Wu <i>et al.</i> (1994) <sup>(40)</sup>
YS extract	Steers	0.075	92:8	×	=	=	=	×	×	×	=	×	×	Hussain & Cleeke (1995) <sup>(41)</sup>
YS extract	Steers	0.075	96:4	×	=	=	=	×	×	×	=	×	×	Hussain & Cleeke (1995) <sup>(41)</sup>
YS extract	Steers	0.075	45:55	×	=	=	=	×	×	×	=	=	×	Hussain & Cleeke (1995) <sup>(41)</sup>
YS extract	Steers	0.075	48:52	×	=	=	=	×	×	×	=	=	×	Hussain & Cleeke (1995) <sup>(41)</sup>
YS extract (10%)	Dairy cows	2.8	40:60	=	=	=	=	=	×	×	=	×	=	Benchaar <i>et al.</i> (2008) <sup>(42)</sup>
YS extract (9.6–11.7%)	Sheep	13.8	60:40	=	↓	↓	=	=	=	=	=	×	×	Pen <i>et al.</i> (2007) <sup>(24)</sup>
YS plant (4.4%)	Heifers	1.96	61:39	↓	=	=	↓	=	=	×	=	×	×	Hristov <i>et al.</i> (1999) <sup>(43)</sup>
YS plant (4.4%)	Heifers	5.83	61:39	↓	=	=	↓	=	=	×	=	×	×	Hristov <i>et al.</i> (1999) <sup>(43)</sup>
YS plant (6%)	Dairy cows	10	51:49	=	↓	=	=	=	×	=	↑	=	=	Holtshausen <i>et al.</i> (2009) <sup>(25)</sup>

F:C, forage:concentrate ratio in diet; Prot, protozoal numbers; NH<sub>3</sub>, ammonia concentration; TVFA, total volatile fatty acid concentration; A:P, acetate:propionate ratio; Dig, digestibility of fibre components; MPS, efficiency of microbial protein synthesis; Met, methane; FI, DM intake; ADG, average daily gain; MY, milk yield; BW, body weight; ↓, decrease; ×, not reported; =, no effect; ↑, increase; QS, *Quillaja saponaria*; YS, *Yucca schidigera*.

\* Values in parentheses are the saponin content (%) in extracts or plants.

† Dose is given as g/kg diet unless otherwise stated, and was calculated as g/kg from feed intake and inclusion of saponin products whenever possible.

‡ Effect is relative to control.

§ DM digestibility.

|| Wool growth also.

¶ ADG was greater at 0 to 28 d of feeding, after which there was no effect.

Probably all types of saponins inhibit protozoal activity depending upon the concentrations in the diet. Lucerne saponins (triterpene saponins), isolated by ethanol extraction and partial acid hydrolysis, caused a decrease in protozoal numbers in the rumen of sheep by 34 and 66 % at high concentrations of 2 and 4 % of dietary DM, respectively<sup>(18)</sup>. A methanol extract of *Sapindus rarak* fruit also depressed the protozoal population in the rumen of sheep by 57 %, which was attributed to the presence of saponins in the extract<sup>(48)</sup>. Saponin-like material in the organic phase of a butanol–water mixture of *Sesbania sesban* foliage (containing oleanane-type triterpene saponins), a leguminous tree, was found to be inhibitory to protozoal activity<sup>(49)</sup>. Similarly, the saponins from *Q. saponaria* and *Acacia auriculiformis*<sup>(46)</sup>, theasaponins<sup>(50,51)</sup> and *Sapindus saponaria*<sup>(52)</sup> have also shown an anti-protozoal activity *in vitro*. Monforte-Briceno *et al.*<sup>(53)</sup> also reported defaunating effects of saponins present in *Enterolobium cyclocarpum* and *Acacia angustissima*. However, there is some evidence that saponins do not influence protozoal numbers *in vitro* (44 to 176 mg *Yucca* saponins and 100 to 400 mg *Quillaja* saponins per litre medium<sup>(47)</sup>; 1 to 100 mg/kg DM substrate<sup>(32)</sup>), and in sheep (1180 to 1477 mg *Yucca* or *Quillaja* saponins/kg DM intake)<sup>(24)</sup>, dairy cows (60 g *Yucca* extract (10 % saponins) per d with feed intake of 21.8 kg, i.e. 275 mg *Yucca* saponins/kg DM intake)<sup>(42)</sup> and heifers (8 g *Quillaja* extract (10 % saponins) per kg DM intake, i.e. 800 mg *Quillaja* saponins/kg DM intake)<sup>(23)</sup>. The inefficacy of the saponins to cause protozoal inhibition in these studies is not clearly known. The lack of anti-protozoal effect of the saponins might involve the use of low doses of saponins in some studies, and an adaptation of rumen microbes to saponins in other studies.

The methods of extraction of saponins from plant materials may show different anti-protozoal activity. For example, Patra *et al.*<sup>(10)</sup> noted that a methanol extract of pods of *Acacia concinna* exhibited stronger activity against protozoa than water and ethanol extracts. Similarly, Agarwal *et al.*<sup>(54)</sup> noted that methanol, ethanol and water extracts of berries of *Sapindus mukorossi* inhibited protozoal numbers in an *in vitro* gas production system and methanol extract was more active than water and ethanol extracts. The dose-dependent effect of the saponins on protozoa has clearly been observed in many studies<sup>(10,19,54,55)</sup>. The oral administration of saponins from the plant *Biophytum petersianum* decreased protozoal numbers in goats by 35 and 40 % at the dose of 13 and 19.5 mg/kg body weight, respectively, beyond which protozoal counts did not further reduce<sup>(19)</sup>. Similarly, Pen *et al.*<sup>(55)</sup> reported that commercial extracts of *Y. schidigera* (9 % saponins; monodesmosidal steroid) and *Q. saponaria* (6 % saponins; bidesmosidal triterpene) decreased protozoal numbers linearly with increasing doses of both the extracts (0, 2, 4 and 6 ml/l of *in vitro* medium). From the studies of Santoso *et al.*<sup>(19)</sup> and Pen *et al.*<sup>(55)</sup>, it could be noted that the inhibition of protozoal counts plateaued at a certain dose specific for saponin type (for example, decreased protozoal numbers of 53 % for *Yucca*, 29 % for *Quillaja* and 40 % for *Biophytum petersianum*), beyond which there was no appreciable inhibition of protozoa. Therefore, it appears that

all of the protozoal species are not equally susceptible to the toxic effect of saponins, indicating that the range of activity of saponins might be dependent upon the type of saponins and protozoal species. Because the concentrations of saponins in *Yucca* (9 % saponins) and *Quillaja* (6 % saponins) extracts were different in the study of Pen *et al.*<sup>(55)</sup>, after adjusting the concentration of saponins (i.e. 4 ml *Yucca* equivalent to 6 ml *Quillaja* extract) in the medium, it was noted that *Yucca* extract (53 % reduction of total protozoa compared with control) strongly inhibited the protozoal growth compared with *Quillaja* extract (29 % reduction of total protozoa compared with control). It could be tested whether a mixture of the saponins of different types might show a synergistic anti-protozoal activity. It has been noted that the degree of toxicity of saponins to rumen protozoa greatly differs in different accessions of the same plant, for example, *Sesbania sesban*<sup>(56)</sup>. This would suggest the presence of different types and/or concentrations of saponins in different accessions of plants. Additionally, the effects of saponins on ruminal protozoa could be diet-dependent. For example, Hess *et al.*<sup>(57)</sup> reported that protozoa numbers were decreased by saponin-containing *Sapindus saponaria* fruits only when added to a diet supplemented with a high-quality legume (*Arachis pintoi*), but not with *Cratylia argentea*, a medium-quality legume. Conversely, anti-protozoal effects of lucerne saponins did not show a diet × saponin interaction, although inhibition was greater for concentrate- (75 %) than roughage- (47 %) based diets at 4 % saponin inclusion<sup>(18)</sup>. The composition of the diet has a strong influence on the protozoal community<sup>(58)</sup>. Therefore, a diet-dependent effect might arise from the selectivity of saponins to protozoal species. However, no information is available on the effect of saponins on the species of rumen protozoa. Ivan *et al.*<sup>(22)</sup> reported that saponins of *Enterolobium cyclocarpum* reduced the protozoal numbers without changing the protozoal generic composition in sheep. Benchaar *et al.*<sup>(42)</sup> observed no effect of saponins on total protozoal counts as well as generic composition.

#### Bacteria and fungi

Relatively few studies have addressed the effects of saponins on pure cultures of rumen micro-organisms. Wallace *et al.*<sup>(44)</sup> showed that *Y. schidigera* extract (1 %, v/v) inhibited the growth of pure cultures of *Butyrivibrio fibrisolvens* and *Streptococcus bovis*, while the growth of *Prevotella ruminicola* was stimulated and the growth of *Selenomonas ruminantium* was unaffected by *Yucca* extract. In another experiment, *Yucca* saponins inhibited the growth of *Streptococcus bovis*, *Prevotella bryantii* and *Ruminobacter amylophilus* by causing the alteration of the cell wall of the bacteria, while enhancing the growth of *Selenomonas ruminantium*<sup>(59)</sup>. Among the primary cellulolytic bacteria, the activity of *Fibrobacter succinogenes* was unaffected, but the cellulose-digesting ability of *Ruminococcus albus* and *Ruminococcus flavefaciens* was markedly reduced by *Yucca* saponins<sup>(59)</sup>. These studies clearly suggest species-dependent effects of saponins on rumen bacteria, which might offer a selective manipulation of rumen metabolism. For example, saponins included in

high concentrate-based diets may reduce the incidence of acidosis by inhibiting the growth of *Streptococcus bovis*, the numbers of which normally rapidly increase with concentrate-based diets, fermenting the soluble starch to lactate<sup>(60)</sup>. The reason for the growth-promoting effect of saponins on some pure cultures of bacteria is unclear. However, it is possible that low doses of saponins might increase the permeability of the cell membrane in a controlled manner, thus allowing the increased absorption of nutrients into the bacterial cells<sup>(61)</sup>. In studies with mixed rumen microbial population, saponin-containing plants increased bacterial numbers, presumably as a consequence of inhibition of protozoal numbers<sup>(27–29,48,49)</sup>. The study of Hess *et al.*<sup>(27)</sup> showed that supplementation with fruits of *Sapindus saponaria*, which supplied crude saponins at 0.6 g/kg body weight<sup>(75)</sup>, increased total bacterial numbers, and decreased total ciliate protozoa count in lambs fed basal diets of grass hay or grass and legume mixture (1:2 or 2:1) along with concentrate (1:3). Cellulolytic bacterial counts have been shown to increase due to the feeding of *Enterolobium cyclocarpum* containing saponins<sup>(28)</sup> and an extract of *Sapindus rarak*<sup>(48)</sup>. In a species-level study of cellulolytic bacteria, Muetzel *et al.*<sup>(62)</sup> found that the addition of saponin-containing *Sesbania pachycarpa* leaves did not affect the growth of *F. succinogenes* and *Ruminococcus flavefaciens*, but inhibited the growth of *Ruminococcus albus in vitro*. Another type of saponin, theasaponin (an oleanene-type triterpene) markedly (79%) decreased the population density of rumen fungi; however, the numbers of *F. succinogenes* increased (41%) and *Ruminococcus flavefaciens* were not affected by theasaponins at a concentration of 400 mg/l culture medium as quantified by real-time PCR<sup>(51)</sup>.

Ruminal fungi, *Neocallimastix frontalis* and *Piromyces rhizinflata*, were also sensitive to *Y. schidigera* saponins<sup>(59)</sup>. However, fungal numbers increased when sheep were fed with diets containing *Sapindus saponaria*<sup>(28)</sup> or *Enterolobium cyclocarpum*<sup>(21)</sup>. Wina *et al.*<sup>(63)</sup> noted that at low concentrations of *Sapindus rarak* saponins had a positive effect on *Chytridiomycetes*, a rumen fungus, during the long-term feeding, while no effect was observed with higher concentrations in sheep. These studies illustrate that a positive effect of saponins on rumen fungi might be obtained perhaps at low doses. Wina *et al.*<sup>(64)</sup> investigated the effect of saponins from a methanol extract of *Sapindus rarak* at concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml to an elephant grass and wheat bran (7:3, w/w) diet *in vitro*. The microbial biomass gradually increased with increasing doses of extract from 102.8 mg/g substrate incubated (control) to 147.3 mg/g substrate incubated at 4 mg/ml and the total protozoal counts decreased. Studies with membrane hybridisation (a molecular method to quantify rRNA of micro-organisms) also showed no eukaryotic RNA in the presence of the extract at concentrations greater than 1 mg/ml. The bacterial RNA concentration increased only at 1 mg/ml compared with the control. There was no effect on *Fibrobacter* spp.; however, there was a negative effect on *Ruminococcus albus* and *Ruminococcus flavefaciens* at 2 and 4 mg/ml of extract. The growth of *Chytridiomycetes* was reduced at the highest concentration. The *in vivo* trials with sheep and goats fed

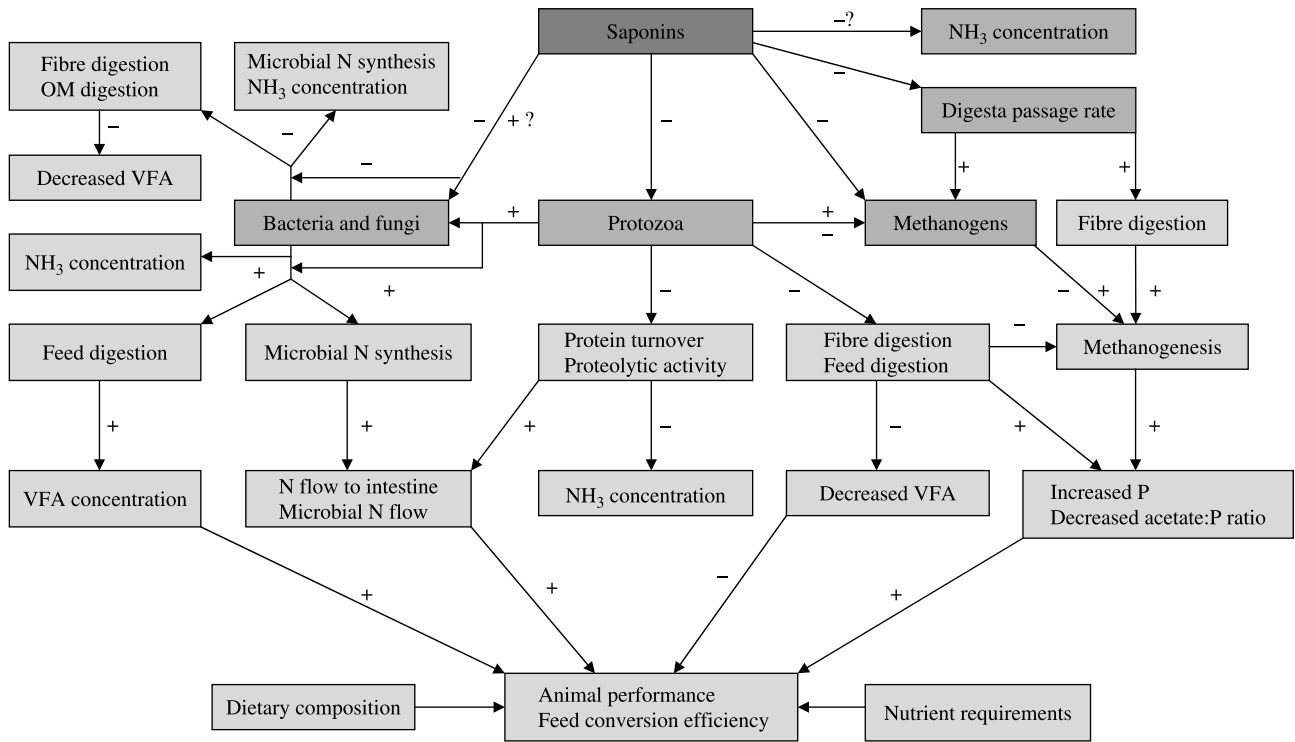
a saponin extract of *Sapindus rarak* up to 0.72 and 0.60 g/kg body weight, respectively, also confirmed that *Fibrobacter* spp. were not affected by the *Sapindus rarak* extract, while *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Chytridiomycetes* showed a short-term negative effect to the application of saponins, but were not affected when this extract was fed for a longer period (105 d)<sup>(63)</sup>. From the studies of Wang *et al.*<sup>(59)</sup>, Muetzel *et al.*<sup>(62)</sup>, Wina *et al.*<sup>(63,64)</sup> and Guo *et al.*<sup>(51)</sup>, it is clear that *F. succinogenes* is relatively resistant to saponins compared with other cellulolytic bacterial species. The presence of 2-aminoethylphosphonic acid in its cell wall may enhance the membrane stability of *Fibrobacter* spp.<sup>(65)</sup>, which might explain the insensitivity of saponins to this bacterium. 2-Aminoethylphosphonic acid covalently linked to membrane polymers renders the membrane resistant to enzymic hydrolysis, which may promote the longevity of the organisms<sup>(65,66)</sup>.

#### Rumen archaea

There has been little experimentation demonstrating the direct effect of saponins on rumen archaea, but the direct effect cannot be ruled out. The studies on pure cultures of rumen methanogens are scant. The reduction of methane production by *Sapindus saponaria* was observed in a defaunated state without any change in methanogen counts, indicating that saponins may directly influence the activity of methanogens<sup>(67)</sup>. Guo *et al.*<sup>(51)</sup> noted that tea saponins (400 mg/l) had no effect on the growth and expression of the methyl coenzyme M reductase subunit A gene (*mcrA*) of *Methanobrevibacter ruminantium*. In mixed ruminal cultures, tea saponins also did not affect total archaeal numbers, but the activity of the *mcrA* gene decreased markedly (76%) and methane production lowered by 8%<sup>(51)</sup>. Saponins from *Sapindus rarak* decreased the methanogen RNA concentration at a dose of 4 mg/ml of *in vitro* medium, while lower concentrations (<4 mg/ml) had no effect on methanogens<sup>(64)</sup>. The study of Wina *et al.*<sup>(64)</sup> did not confirm that a decrease in methanogen RNA concentration is due to direct inhibition by saponins, but did suggest it may also result from the inhibition of protozoa activity. However, it has also been reported that saponins of *Sapindus saponaria* fruits increased the methanogen population in sheep although methane production reduced<sup>(27)</sup>. The reason for the increase in the methanogen population was not clear in this study.

#### Explaining the effects of saponins on micro-organisms

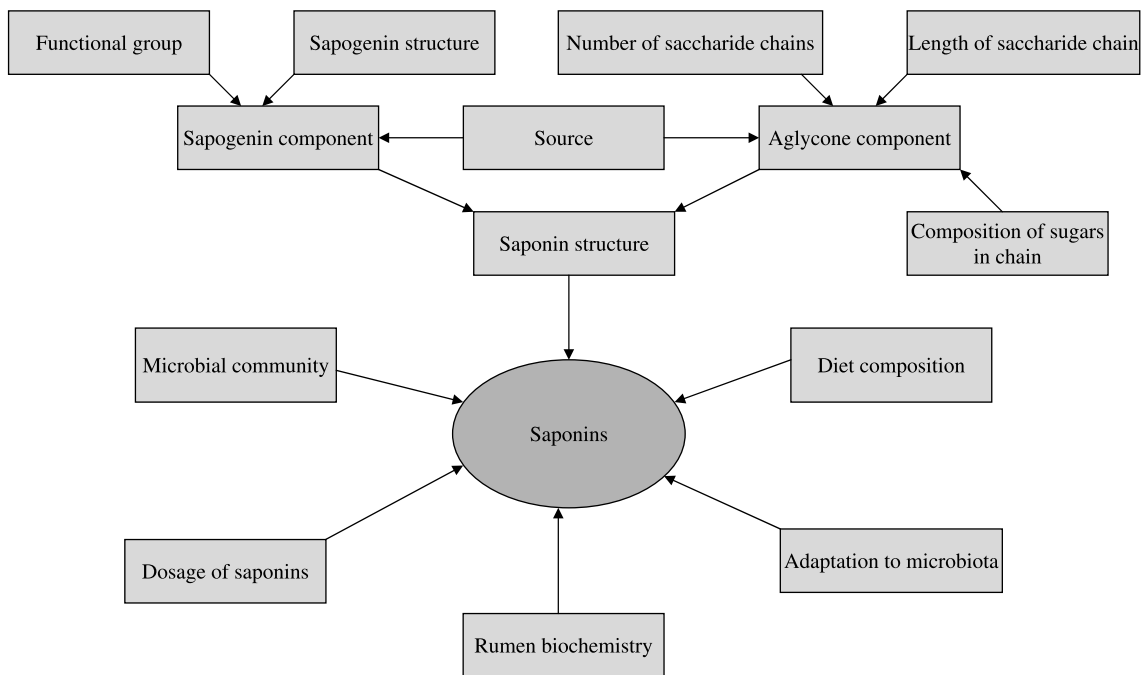
A schematic illustration of effects of saponins on rumen microbes and fermentation is presented in Fig. 2. The modifications of rumen fermentation by saponins are mediated via a direct influence on the composition and the activity of rumen protozoa, bacteria, fungi and archaea. The general mode of action of saponins on micro-organisms is their interaction with the sterol moiety<sup>(15)</sup>, which is present in the membrane of protozoa<sup>(58)</sup>. Thus, an anti-protozoal activity of saponins may be due to the destruction of protozoal cell membranes, causing leaking of cell contents. It has been reported that the main sterols present in entodiniomorphs are stigmastanol, campestanol



**Fig. 2.** A schematic presentation of the proposed effects of saponins on rumen microbes and fermentation. Primary effects modify the composition of rumen microbes and secondary effects modify the rumen fermentation. +, Increase; -, decrease; OM, organic matter; VFA, volatile fatty acids; P, propionate.

and cholestanol, whereas the major sterol in holotrichs is cholestanol<sup>(68)</sup>. The different composition of sterols in rumen protozoa may relate to the different sensitivity of protozoa to saponins. Several factors such as saponin

structure, dose level, adaptation process, diet composition and microbial community structure influence the effect of saponins on the rumen microbial population (Fig. 3). The structures of saponins and their varying proportions



**Fig. 3.** A schematic presentation of factors affecting the effects of saponins on rumen microbes.

from different sources of the same plant material may elicit different responses on rumen metabolism. Also the method of saponin estimation in the extract or plant may differ<sup>(12)</sup>, which may explain the differences in response of saponins despite similar dose levels<sup>(25)</sup>. For example, Hostettmann & Marston<sup>(15)</sup> discussed that spectrophotometric methods are very sensitive, but not suitable for estimation of saponins in crude plant extracts, since colour reactions are not specific, and coloured products may form with phytosterols and flavonoids extracted with saponins. Klita *et al.*<sup>(17)</sup> mentioned that concentrations of saponins determined by HPLC procedures are commonly lower than those determined by biological procedures (for example, haemolytic micro-method). Additionally, saponins may not be completely extracted from vegetable materials<sup>(15)</sup>.

The responses of rumen bacteria and fungi to saponins may be mediated through defaunation and/or direct effects. An increase in the number of bacteria and fungi due to the addition of saponins has been observed in some studies, which is believed to be as a consequence of reduced engulfment of these micro-organisms by protozoa<sup>(58)</sup>. The microbial growth in the rumen is also affected by the presence of saponins, which may decrease the bacterial and fungal numbers. Therefore, the observed effect of saponins on rumen microbes is the balance between anti-protozoal effects (hence increased microbial growth) and direct antibacterial effects of saponins. The antibacterial effects of saponins may be mediated by disrupting the cell membranes, rather than simply altering the surface tension of the extracellular medium<sup>(5,61)</sup>. Both Gram-positive and Gram-negative bacteria are sensitive<sup>(69)</sup>, although their sensitivity depends upon the structure of the aglycone moiety of saponins<sup>(70)</sup> and the fatty acid composition of cell membranes of target bacteria. For instance, Wallace *et al.*<sup>(44)</sup> concluded from their study that Gram-positive bacteria were more susceptible compared with Gram-negative bacteria in the presence of *Yucca* saponins. Later, Wang *et al.*<sup>(59)</sup> reported that *Yucca* saponins inhibited pure cultures of rumen cellulolytic bacteria and fungi, but their effects on amylolytic bacteria varied among species. Similarly, Gram-negative bacteria were negatively affected by saponins extracted from the leaves of *Gymnema sylvestre* and *Eclipta prostrata*<sup>(71)</sup>, whereas the effect of medicagenic acid, a lucerne saponin, was more pronounced against Gram-positive bacteria<sup>(70)</sup>. Although the addition of sugar units decreases the antimicrobial properties<sup>(70)</sup>, the sugar moiety may not be important for activity in the rumen, as the side chain of sugars might be rapidly degraded by the mixed rumen microbial cultures<sup>(72)</sup>. From this perspective, the sapogenin structure–activity relationship of saponins with greater anti-protozoal properties and weak antibacterial (for example, rumen cellulolytic bacterial populations) and fungal activity could be targeted for rumen micro-organisms for improvement of fermentation. Further, Li *et al.*<sup>(73)</sup> demonstrated that tea saponins (triterpenoid type) showed better antimicrobial activity at low pH *in vitro*. This indicates that the effect of saponins may be modulated by the pH in the rumen depending upon the concentrate- and roughage-based diets. The method of preparation and purification might also give rise to different responses to saponins. For example, Sen *et al.*<sup>(61)</sup> noted that *Quillaja*

saponins from different commercial sources showed maximum effects on growth of *Escherichia coli* at different concentrations of saponins in the medium.

The major mechanism suggested for the antifungal activity of saponins is their interaction with sterols<sup>(11)</sup>, followed by pore formation and loss of membrane integrity. Some saponins may show a specific inhibitory activity to fungi but not to bacteria. For example, saponins isolated from *Allium minutiflorum* exhibited an antifungal activity depending upon the concentration and type of saponins, but did not show an appreciable antibacterial activity in the non-rumen bacteria<sup>(74)</sup>. From the perspective of structure–activity relationships, saponins with the triterpene or spirostanol moiety generally demonstrate stronger antifungal activities, whereas furostanol saponins with bidesmosidic nature show little to no bacteriostatic and fungicidal effects<sup>(75)</sup>. The antifungal activity of these steroidal saponins is also associated with their individual steroidal sapogenins as well as the number and structure of monosaccharide units in their sugar chains. It has been demonstrated that diosgenin saponins with triglycosides were active against *Candida albicans* and *Candida glabrata*, while the monoglycoside and diglycoside did not show any activity<sup>(76)</sup>.

#### Microbial adaptation and metabolism of saponins

One of the challenges of using saponins or saponin-containing plants is that the anti-protozoal activity is transient<sup>(49,56)</sup>. After feeding of *Sesbania sesban* for 10 d<sup>(49)</sup> and *Enterolobium cyclocarpum* for 14 d<sup>(22)</sup>, the anti-protozoal activity of saponins present in these plants was not observed in sheep. Teferedegne<sup>(77)</sup> also noted a time-dependent detoxification process of the saponins in ruminal fluid *in vitro*. The study of Newbold *et al.*<sup>(49)</sup> suggested that protozoa *per se* did not become resistant to these anti-protozoal compounds, but rather the rumen bacterial population degraded the saponins<sup>(49,56)</sup>. However, anti-protozoal activities of the saponins from *Yucca* fed for 17 d<sup>(43)</sup> and *Sapindus rarak* fed for 3 months<sup>(63)</sup> were observed in the presence of mixed rumen microbial populations in the rumen. The study of Wina *et al.*<sup>(63)</sup> showed that a negative effect of saponins from *Sapindus rarak* on *Ruminococcus albus*, *Ruminococcus flavofaciens* and *Chytridiomycetes* for a short period, but this effect disappeared after a long period of feeding. From the study of Wina *et al.*<sup>(63)</sup>, it was also apparent that protozoal populations were not affected by *Sapindus saponaria* saponins on day 13 in goats, probably due to detoxification of saponins by microbes. However, anti-protozoal properties of these saponins were persistent up to 3 months in sheep, indicating probable previous exposure of the goats to saponins.

The metabolism of saponins in the rumen involves deglycosylation<sup>(45,59)</sup> and structural changes of the aglycone nucleus<sup>(45)</sup>. There appears to be no evidence of cleavage of ring structures of steroidal or triterpene cores of saponins by rumen anaerobic or even non-rumen anaerobic and aerobic micro-organisms. Saponins are degraded in batch cultures of rumen fluid *in vitro*<sup>(72)</sup> to sapogenins, the aglycone remaining after the removal of the sugar moiety. Thereafter,

the resultant sapogenins undergo structural changes (oxidation and reduction) more slowly<sup>(45)</sup>. Wang *et al.*<sup>(59)</sup> suggested that *F. succinogenes* apparently deglycosylated the saponins from *Y. schidigera*. Further, an increase in the thickness of the cell wall of *Prevotella bryantii* was noted when these bacteria were grown in the presence of *Yucca* saponins, indicating an adaptation to saponins<sup>(59)</sup>. The observation made by Odenyo *et al.*<sup>(78)</sup> that *Sesbania sesban* introduced directly into the rumen remained toxic to protozoa, but not dietary *Sesbania sesban*, implies that chewing might have caused the detoxification, perhaps by salivary amylase, or that the larger particle size protected saponins from degradation.

Lucerne saponins were degraded by 96–98 % in the total digestive tract of sheep<sup>(79)</sup>. Similarly, white clover saponins were not detectable in the residue of white clover (*Trifolium repens*) incubated in the rumen by nylon bags for 24 h<sup>(80)</sup> and mowrin, saponins from mahua (*Bassia latifolia*) seeds, was degraded by 46 % after 24 h of incubation *in vitro*<sup>(81)</sup>. Makkar & Becker<sup>(72)</sup> observed that *Quillaja* saponins were degraded 100 % in an *in vitro* medium containing rumen fluid of cows after 24 h of incubation, while no degradation was observed up to 6 h of incubation. Meagher *et al.*<sup>(82)</sup> investigated the metabolism of diosgenin-derived saponins in the gastrointestinal tract of sheep. In the rumen, saponins underwent rapid hydrolysis to afford free sapogenins, and then part of these free sapogenins underwent oxidation and reduction into epismilagenin, smilagenone, smilagenin and tigogenin. In the duodenum, jejunum and ileum, these products were absorbed and then secreted through the bile as conjugated and free sapogenins. The epimerisation of sapogenins was continued in the caecum and colon. A similar phenomenon of metabolism of saponins from *Narhecium ossifragum* and *Y. schidigera* (both plants contain predominantly sarsapogenin and smilagenin aglycone moieties) was observed by Flaoyen & Wilkins<sup>(83)</sup> and Flaoyen *et al.*<sup>(84,85)</sup> in sheep. Collectively, these studies suggest that the rate and extent of degradation of different saponins in the rumen varies greatly, which indicates that some saponins might be effective for long periods.

## Effects of saponins on rumen fermentation

### Nitrogen metabolism

A number of studies have reported that saponins or saponin-containing plants decreased rumen ammonia N concentrations *in vitro*<sup>(25,44,50,86–88)</sup> and *in vivo*<sup>(24,39,41,43,46)</sup>. The effect of saponins in reducing rumen ammonia concentrations was more pronounced for *Yucca* extract compared with *Quillaja* extract<sup>(25,55)</sup>, probably due to strong anti-protozoal properties of *Yucca* compared with *Quillaja* saponins. However, saponins do not always reduce rumen ammonia concentrations. *Sapindus saponaria*<sup>(27)</sup> and *Yucca* extract<sup>(42)</sup> included in concentrate diets did not significantly reduce rumen ammonia concentrations, although it should be noted that the *Yucca* extract did not affect rumen protozoal numbers either. In studies with dairy cows, inclusion of sarsapogenins up to 77 parts per million (ppm) of diet<sup>(29,33)</sup>, 9 g/d<sup>(36)</sup>, 8 g/d<sup>(40)</sup> and 10 g of *Yucca* whole plant (equivalent to 0.6 g saponins/kg DM) or 10 g *Quillaja* whole

plant (equivalent to 0.3 g saponins/kg DM)<sup>(25)</sup> did not influence ammonia concentrations in the rumen, probably due to low concentrations of saponins inclusion in the diets. Valdez *et al.*<sup>(29)</sup> and Hess *et al.*<sup>(27)</sup> have reported that by decreasing protozoal numbers slightly, saponins increased bacterial numbers, which may explain why saponin treatments are not always associated with reduced rumen ammonia concentrations.

*Yucca*, *Quillaja* and *Acacia* saponins have been shown to increase the efficiency of microbial protein synthesis in an *in vitro* fermentation system containing hay as a substrate<sup>(46)</sup>. Saponins in higher concentrations generally have adverse effects on microbial protein synthesis subject to saponin types. *Yucca* saponins increased microbial protein synthesis at 15 mg/l medium, but decreased it at higher concentrations<sup>(86)</sup>, whereas tea saponins increased microbial protein synthesis up to a concentration of 267 mg/l<sup>(50)</sup>. The greater bacterial protein synthesis, which could be the result of the prevention of predation of bacteria by protozoa, may overwhelm the decreased protozoal growth, and thus overall microbial protein synthesis could increase due to the administration of saponins. Therefore, saponin-specific doses needed for anti-protozoal effects, but with minimum inhibitory effects on bacteria and fungi, need to be established. In an animal feeding trial, Santoso *et al.*<sup>(35)</sup> reported that daily supplementation of a basal diet consisting of timothy silage and a concentrate (85:15 on a DM basis) with 240 ppm of *Yucca* saponins reduced rumen ammonia N concentrations in sheep. The microbial N supply and efficiency of microbial N synthesis were greater in sheep fed with *Yucca* diets *v.* those fed with control diets. In another experiment, Santoso *et al.*<sup>(19)</sup> reported that oral administration of saponins (0, 13, 19.5 and 26 mg/kg body weight) from *Biophytum petersianum* linearly decreased ruminal ammonia N concentrations, urinary N and total N excretion, and increased retained N as a proportion of N digested, efficiency of microbial N synthesis and microbial N supply in goats fed a diet consisting of elephant grass silage and concentrate (70:30). Klita *et al.*<sup>(17)</sup> reported that total N flow to the duodenum increased in sheep fed a saponin extract of lucerne root up to 4 % of DM intake. However, in studies with cattle receiving *Y. schidigera* saponins, a decrease in the efficiency of microbial protein synthesis was observed, probably due to the suppression of bacterial and protozoal growth<sup>(33)</sup>. A diet-dependent response to saponins on bacterial protein synthesis has been clearly observed by Lu & Jorgensen<sup>(18)</sup>, who noted that efficiency of bacterial protein synthesis and bacterial protein flow to the duodenum were decreased by lucerne saponins in a roughage-based diet, but had no effect in a concentrate-based diet.

### Digestion of feeds and volatile fatty acid production

Mixed effects of saponins on feed digestion are reported in the literature. Saponins from *Sapindus saponaria*<sup>(57)</sup>, *Yucca* and *Quillaja*<sup>(25)</sup> plants reduced the digestibility of fibre components *in vitro*. Similarly, lucerne saponins (up to 4 % of DM intake) linearly decreased both ruminal and total tract digestibilities of organic matter, fibre components and digestibility in sheep fed a grass hay diet<sup>(17)</sup>. The xylanase



activity and neutral-detergent fibre digestibility decreased at higher levels of *Sapindus rarak* extract<sup>(64)</sup>. The digestibilities of organic matter and neutral-detergent fibre also reduced in the presence of *Sapindus saponaria* fruits<sup>(27)</sup> and extracts of *Sapindus mukorossi*<sup>(54)</sup>. In many other studies<sup>(21,24,27,37,38)</sup>, saponins have been reported not to influence nutrient digestibilities. A number of studies have reported increases in digestibilities of organic matter and fibre components. Goetsch & Owens<sup>(33)</sup> reported a significant increase in the digestibility of organic matter in cattle fed 44 ppm of *Yucca* saponins. Valdez *et al.*<sup>(29)</sup> recorded an increased digestibility of organic matter, together with a tendency toward an increased digestibility of acid-detergent fibre with increasing application levels of *Yucca* extract (up to 77 ppm). Pen *et al.*<sup>(24)</sup> reported an increase in neutral-detergent fibre digestibility by sheep supplemented with *Quillaja* saponins. Similarly, the total tract digestibilities of organic matter and fibre were increased by lucerne saponins in a concentrate-based diet for sheep<sup>(18)</sup>. Saponins might change the site of digestion in the digestive tract. For example, in the latter experiment, the degradability of cellulose in the rumen was depressed by added saponins, but the degradability of fibre fractions in the hindgut was greatly increased.

There are varying reports on the effects of saponins on ruminal volatile fatty acid (VFA) concentrations, as digestibility of feeds varied among the studies. No difference in total VFA concentrations with the supplementation of saponins<sup>(17,24,25,39,43,51,55)</sup> have been reported. However, reductions in VFA concentration due to supplementation of lucerne saponins at 4 % of DM intake<sup>(18)</sup> and *Yucca* extract at levels of 25 and 50 g/d to Holstein cows<sup>(37)</sup> have also been reported. Saponins in higher concentrations might adversely affect rumen fermentation. Gas and VFA production were greater at a *Yucca* saponins concentration of 75 mg/l compared with the control, but decreased at higher concentration<sup>(86)</sup>. The rate of gas production decreased in the presence of *Yucca* and *Quillaja* saponins, but increased in the presence of *Acacia* saponins when a mixture of hay and concentrate (70:30) was used as a substrate<sup>(46)</sup>. The authors<sup>(46)</sup> also reported that *Yucca*, *Quillaja* and *Acacia* saponins either decreased the rate of gas production significantly or had a tendency to decrease it depending upon the dose levels when hay was used as a substrate. The potential extent of gas production was either not affected (*Quillaja* saponins) or decreased (*Yucca* and *Acacia* saponins) in this study<sup>(46)</sup>. A number of studies reported that saponins from different plants increased propionate and decreased acetate proportions<sup>(25,39,41,43,51,54,55,87–90)</sup>. Patra *et al.*<sup>(10)</sup> reported that extracts of *Acacia concinna* containing saponins, which inhibited protozoa number to the extent of 80 %, did not affect total VFA productions, but increased propionate production, and there was a decrease in the acetate:propionate ratio. The molar proportion of acetate was lower in sheep receiving the *Yucca* diets compared with the control diet, and molar proportions of butyrate and iso-acids were higher<sup>(35)</sup>. Similarly, an increased molar proportion of propionate concentration in the rumen by the *Biophytum* extract that contains saponins has been reported<sup>(19)</sup>. These characteristics of VFA pattern are often noted in studies with

defaunated animals<sup>(58)</sup>. Conversely, Hussain & Cheeke<sup>(41)</sup> found a numerically lower propionate concentration *in vivo* with 75 ppm of *Yucca* saponins. However, Valdez *et al.*<sup>(29)</sup> and Wu *et al.*<sup>(40)</sup> did not observe a significant change in propionate concentration *in vivo* up to 77 and 125 ppm, respectively, of *Yucca* saponins. The responses of saponins on rumen VFA production are often diet dependent. The study of Wang *et al.*<sup>(86)</sup> clearly demonstrated that gas and total VFA productions from barley grain were increased by *Y. schidigera*, whereas they were reduced from lucerne hay. Kil *et al.*<sup>(91)</sup> found a significant increase in propionate concentration *in vitro* when supplying 125 ppm of *Yucca* saponins with a mixed forage–concentrate diet, but reported a marginal effect with a concentrate diet. Additionally, Cardozo *et al.*<sup>(92)</sup> showed that *Yucca* saponins at dose levels up to 30 mg/l increased the molar proportion of propionate and reduced that of acetate at pH of 5.5, whereas no effect was noted at pH 7.0, which indicated that effects of saponins on VFA profiles might be modulated by rumen pH.

#### Methane production

Methane produced during anaerobic fermentation in the rumen represents a feed energy loss and contributes to the greenhouse effect. Therefore, decreasing methane emission has been an important objective for ensuring the sustainability of ruminant production. Methane is produced normally during fermentation of feeds in the rumen by methanogenic archaea. The removal of protozoa can decrease methane production, as some populations of methanogens remain associated with protozoa as ectosymbionts and endosymbionts<sup>(57,93,94)</sup>. Theasaponin caused an increase in propionate production, while methane and isobutyrate production were decreased<sup>(89)</sup>.

In a study by Hess *et al.*<sup>(67)</sup>, *Sapindus saponaria* in Rusitec reduced methane production by 20 %, without affecting the methanogens relative to control and there was a 54 % reduction in protozoa counts. This was confirmed *in vivo* – daily methane release was decreased by *Sapindus saponaria* supplementation in lambs fed basal diets of grass hay or grass and legume mixture (1:2 or 2:1) along with concentrate<sup>(27)</sup>. Agarwal *et al.*<sup>(54)</sup> reported that the depression in methane production was 96, 39.4 and 20 % with ethanol, water and methanol extracts of *Sapindus mukorossi*, respectively, compared with controls. Tea saponins decreased *in vitro* methane production by 13 % at a concentration of 67 mg/l to 22 % at a concentration of 133 mg/l, whereas greater concentrations (200 and 267 mg/l) had no further inhibitory effect on methane production<sup>(50)</sup>. Methane production also decreased linearly with increasing concentrations of *Yucca* saponins in an *in vitro* medium containing different substrates such as potato starch, maize starch and hay-concentrate<sup>(87)</sup>. However, saponins from *Acacia concinna* extracts did not affect methane production or DM digestibility, although it reduced protozoa numbers<sup>(10)</sup>. Similarly, *Q. saponaria* extract (0.12, 0.24 and 0.36 g saponins per litre) did not influence *in vitro* methane production<sup>(55)</sup> whereas *Y. schidigera* extract (0, 0.18, 0.36 and 0.54 g saponins per litre)<sup>(55)</sup> and whole *Yucca* plant (0, 15, 30 and 45 g/kg DM equivalent to 0, 0.023, 0.046 and 0.069 g saponins

per litre)<sup>(25)</sup> decreased *in vitro* methane production, with increasing concentrations of saponins<sup>(55)</sup> accompanied by decreased protozoal numbers. Klita *et al.*<sup>(17)</sup> demonstrated that saponin extract (27.8 % saponins) from lucerne root did not affect methane production in sheep when administered intra-uminally at the rate of 0, 1, 2 and 4 % of DM intake although protozoal numbers decreased linearly with increasing concentrations of saponins.

It has been observed that the effect of *Sapindus saponaria* on methane was more pronounced in the defaunated (29 %) than faunated (14 %) rumen fluid, indicating that reduced methane production was not entirely due to associated depression in protozoal counts<sup>(67)</sup>. Again, Goel *et al.*<sup>(95)</sup> noted that *Sesbania sesban* saponins decreased methanogen populations by 78 % and the methane inhibition effect of *Sesbania sesban* was more pronounced in concentrate-based diets compared with roughage-based diets.

#### *Explaining the effects of saponins in modifying digestion and rumen fermentation*

The presence of protozoa in the rumen has been shown to stabilise rumen pH and decrease the redox potential of rumen digesta, which should indirectly stimulate the cellulolytic bacterial activity<sup>(96)</sup>. Furthermore, approximately one-fifth of fibre degradation is of protozoal origin<sup>(97)</sup>. However, the engulfment and digestion of bacteria, fungal zoospores and archaea by protozoa causes a considerable proportion of microbial protein turnover in the rumen, decreasing the efficiency of protein utilisation in ruminants<sup>(98)</sup>. It has been recognised that many of the negative effects of defaunation may disappear as the bacterial and fungal populations increase and occupy the niches previously filled by the protozoa<sup>(58)</sup>, which may increase the efficiency of microbial protein synthesis and protein flow to the duodenum and improvement of N retention in animals<sup>(19)</sup>. All of the studies that have reported anti-protozoal properties of saponins or plants containing saponins were shown to cause partial defaunation. Veira *et al.*<sup>(99)</sup> found that partially defaunated sheep (8.3 % of faunated sheep) were intermediate between observations in the completely defaunated and faunated sheep, and differences between partially and completely defaunated sheep were not significant. These findings suggest that responses of saponins to the efficiency of microbial protein synthesis and flow of amino acids to the intestine might be in between complete faunated and faunated animals depending upon the extent of the partial defaunation.

One of the pronounced effects of saponins reported in many of the studies is a decrease in ammonia concentration in the rumen, which may be primarily due to reduced bacterial lysis<sup>(98)</sup> as a consequence of anti-protozoal properties of saponins. The inhibition of protozoa may also directly depress the proteolytic and deaminative activities of protozoal origin. The reduced proteolysis and deamination may also be the result of inhibition of bacteria by saponins. Moreover, it is possible that direct inhibition of microbial urease by saponins may lead to reduced ammonia concentrations in the rumen<sup>(100)</sup>. Additionally, the glycofractions of saponins are known to bind ammonia when concentrations of ammonia are low and when a high dose of

saponins is added<sup>(44)</sup>. This might improve N efficiency by maintaining adequate rumen ammonia (trapping of rumen ammonia when ammonia concentrations are high after feeding and then slowly releasing it with the degradation of the glycofraction and desaturation of ammonia binding) for use during microbial protein synthesis when ammonia concentrations decrease<sup>(36,40)</sup>. However, it has been shown that this ammonia binding appears to be negligible at ammonia concentrations typical of the rumen<sup>(44)</sup>. Collectively, all of these factors may result in reduced ammonia concentrations in the rumen. Although the greater bacterial activities may increase proteolysis and deamination, the contribution to the total ammonia pool in the rumen from this process could be minor due to the inclusion of saponins in diets. Therefore, it is apparent that the variations in ammonia concentrations due to the addition of saponins are caused by the different contributions that protozoa and bacteria make in different studies.

The ligno-cellulosic feedstuffs are degraded in the rumen by the synergistic activities of the bacteria, protozoa and fungi, with bacteria and fungi contributing approximately 80 % of the degradative activity, and the protozoa only 20 %<sup>(97)</sup>. The most active fibrolytic bacteria *F. succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are generally considered as the primary organisms responsible for the degradation of plant cell walls in the rumen while *Butyrivibrio fibrisolvens*, *Clostridium loheadii* and *Clostridium longisporum* are some of the secondary fibrolytic bacteria<sup>(101)</sup>. Ruminal fungi produce a broad array of enzymes and generally degrade a wider range of substrates than do rumen bacteria<sup>(101,102)</sup>. Furthermore, ruminal fungi are able to degrade the most resistant plant cell wall polymers and the cellulases and xylanases produced by them are among the most active fibrolytic enzymes<sup>(103)</sup>. The ruminal protozoa also contribute to the degradation of plant cell wall polymers, but their contribution in fibre degradation is considered not as important as that of the bacteria and fungi<sup>(101,104)</sup>. Some studies have demonstrated that saponins decrease the passage rate of digesta from the rumen<sup>(18)</sup>, which may increase the ruminal degradation of feeds. However, physiological effects of saponins are usually overridden by microbiological effects in the rumen<sup>(18)</sup> because of comparatively greater effects of saponins on microbial populations. Consequently, the positive effects of saponins on the digestibility of feeds in some studies might be attributed to the increased bacterial populations, whereas negative effects reported in other studies are due to decreased hydrolytic enzyme activities from protozoa and/or bacteria and fungi when saponins affect these populations. The substrate-dependent effect of saponins on digestibility might arise due to the effects of saponins on specific bacterial populations<sup>(41)</sup>. Wang *et al.*<sup>(59,86)</sup> observed that supplementation with *Yucca* extracts might be beneficial to ruminants fed a high-grain diet. *Yucca* saponins were found to have a direct negative effect on cellulolytic bacteria while being harmless to amylolytic bacteria<sup>(59)</sup>. Additionally, Killeen *et al.*<sup>(105)</sup> proposed that a surfactant or flocculent action of saponins on the feed constituents that alters the rate of digestion may account for the substrate-dependent nature of the effect of saponins on rumen digestibilities of feeds.

The VFA concentration may generally follow the trend of feed digestion in the rumen. However, a part of the digested substrate may be partitioned to microbial mass, decreasing VFA concentrations<sup>(46)</sup>. Besides, saponins may affect intestinal cell permeability by forming complexes with sterol moieties in mucosal cell membranes<sup>(106)</sup>. It has also been reported that saponins may reduce active nutrient transport mechanisms and increase membrane permeability in the small intestine to facilitate uptake of materials to which the gut would normally be impermeable<sup>(106,107)</sup>. Moreover, saponins may reduce the transmural potential differences in the small intestine<sup>(108)</sup>. Thus, although there appears to be no study demonstrating the effects of saponins on absorption of VFA from the rumen, a reduction of VFA absorption would be possible, which may increase VFA concentrations in the rumen. It is well established that VFA in their undissociated forms are predominantly absorbed by passive transport mechanisms from the reticulo-rumen<sup>(109)</sup>. But transport of dissociated VFA is coupled to the operation of an active transport mediated via a non-selective, electroneutral anion exchange system<sup>(109)</sup>. Therefore, considering the predominant role of passive transport on absorption of VFA, the effect of saponins on VFA transport is likely to be small.

An increase in the proportion of propionate production and a decrease in the acetate:propionate ratio have been observed. These characteristics of VFA profile are often noted in studies with defaunated animals<sup>(58)</sup>. Acetate and butyrate are the major endproducts of fermentation in ruminal protozoa<sup>(58)</sup>. Hence, a decrease in protozoal numbers by saponins may result in an increase in the proportions of propionate and the propionate:acetate ratio. Besides, saponins sometime stimulate the growth of *Selenomonas ruminantium*<sup>(59)</sup>, which is predominantly responsible for propionate production from succinate metabolism<sup>(110)</sup>.

Several factors may determine the effect of saponins on methanogen populations and methane production, which are discussed below. The direct effect of saponins on methanogen populations has not been proved so far, but cannot be ruled out. Nevertheless, some studies have demonstrated that saponins may decrease the activity of methane-producing genes or rate of methane production per methanogenic cell<sup>(51,57)</sup>. Many methanogens are found in both etc- and endosymbiotic associations with protozoa, which are responsible for up to 37% of rumen methanogenesis<sup>(93)</sup>. It has been estimated that a single protozoan cell may contain  $10^3$ – $10^4$  methanogens before feeding, decreasing to one to ten methanogens after feeding<sup>(94)</sup>. Sharp *et al.*<sup>(111)</sup> reported that *Methanobacteriaceae* are the most abundant archaeal population both in the rumen fluid (89.3%) and in a protozoal fraction (99.2%), but it declined from 84 to 54% in fermenters with the loss of protozoa. *Methanomicrobiales* are the second most abundant archaeal family (12.1%) in the rumen and they were found to be predominantly free-living. The *Methanosarcinales* can be free-living or associated with protozoa. Hence, defaunation, which disrupts the symbiotic relationship, has been suggested as a method to reduce methane production in ruminants. Contrary to this, protozoa engulf rumen methanogens<sup>(94)</sup>. As a result, there may be an increase in methanogen numbers not associated with protozoa when

protozoa are washed out in the rumen. For instance, Sharp *et al.*<sup>(111)</sup> showed that the numbers of *Methanomicrobiales* increased from 9.6 to 26.3% to replace the methanogens lost via protozoal displacement, whereas *Methanosarcinales* remained constant in the fermenters. Additionally, methane release may also be affected by saponins as a result of a reduced rate of methanogenesis via diminished activity of the methane-producing *mcrA* gene without changing the total methanogen population<sup>(51)</sup>. Since saponins favour the production of an increased proportion of propionate, the channelling of hydrogen from methanogenesis to propionate production may cause lower methane production. In contrast, methane production may be increased because of greater digestion of feeds and fibre components, as reported in some studies on saponins<sup>(24,29,33)</sup>, probably resulting from increased bacterial and fungal populations. Besides, as there is an inverse relationship between digesta passage rate and methane production, probably due to an increased fibre degradation in the rumen, and saponins decrease the rate of digesta passage<sup>(17,18)</sup>, methane production may be increased as a result of an influence of saponins on passage rate. However, physiological effects of saponins appear to be insignificant compared with the microbiological effects<sup>(18)</sup>. The rate of digesta passage could be affected by saponins via changes in the motility of the digestive tract. Klita *et al.*<sup>(17)</sup> demonstrated that the introduction of lucerne saponins inhibited rumen motility in sheep, while increasing the intestinal motility. No mechanism of action has yet been determined for the inhibitory effects of saponins on rumen motility. Klita *et al.*<sup>(17)</sup> assumed that epithelial receptors in the luminal epithelium might be involved, which is more likely to be a result of interactions of saponins with the sterols in biological membranes. The mechanism of saponins on intestinal motility might be through the modulation of pace-making cells in the intestinal muscles that generate the rhythmic oscillations in the membrane potential and this modulation is mediated through non-selective cation channels and intracellular  $Ca^{2+}$  mobilisation in a protein kinase C-independent manner, as demonstrated with ginseng saponins in mice<sup>(112)</sup>.

### Effects of saponins on ruminant performance

The anti-protozoal effects of saponins would be expected to have mixed effects on ruminant productivity depending on the diets and the saponins involved. Although defaunation might decrease fibre degradation in the rumen<sup>(58)</sup>, it is generally agreed that removing or suppressing protozoa would result in increased ruminant performances<sup>(93)</sup>, particularly on a low-protein diet. The effect of defaunation depends on the balance between the energy and protein requirements of animals and nutrients supplied by diets. For example, defaunation is considered useful for animals with high protein requirements, but fed diets low in true protein, but not limited in energy<sup>(113,114)</sup>. Therefore, the effect of saponins is likely to be diet dependent. Inclusion of saponin-containing *Enterolobium cyclocarpum* foliage increased the body-weight gain from 93 to 115 g/d in sheep fed oat hay<sup>(20)</sup> and from 19.8 to 29.7 g/d in sheep fed *Pennisetum clandestinum*<sup>(21)</sup> and wool growth by 27%<sup>(20)</sup>. The improvement of the performances of animals in these

studies with the feeding of *Enterolobium cyclocarpum* might have been due to an increased absorption of amino acids from the intestine as a result of decreased protozoal numbers. Thalib *et al.* (115) also reported that *Sapindus* extract added every 3 d to a rice straw diet improved the body-weight gain of sheep from 44.8 to 54.8 g/d. An improvement in the feed efficiency and economics was noted when grazing zebu cattle were fed with *Yucca* extract along with a urea molasses mineral block (116). Supplementation of a maize silage-based diet with 150 mg sarsaponin per d and 1 % urea improved the body-weight gain of steers during the first 28 d of an experiment (31), but there were no significant long-term (62 d) treatment effects, probably due to adaptation to saponins. From the above reports, it appears that saponins may enhance the performance of ruminants on roughage-based diets. Conversely, supplementation of concentrate-based diets with saponins generally did not show an improvement in ruminant performance. For instance, body-weight gain and feed efficiency in steers were not affected when an 88 % concentrate diet containing 65 % maize was supplemented with sarsaponin from *Y. schidigera* (34). Similarly, supplementation of a diet containing 90 % concentrate with 150 mg/kg *Yucca* powder had no effect on the weight gain of male lambs (117). The addition of 250 mg/kg *Yucca* extract to a mixed diet (45 % hay and 50 % rolled barley, 5 % soyabean meal) in steers (41) and 30 mg/kg DM of sarsaponin (*Y. schidigera* extract) to a diet of hay–concentrate (1:1) in sheep (32) also did not increase the daily gain. The inclusion of 40 and 60 mg *Quillaja* saponins/kg in a 70 % concentrate diet did not improve body-weight gain (118). However, *Quillaja* saponins induced a higher response in the male lambs (256, 315 and 278 g/d for control, 40 and 60 g saponins/kg, respectively) than the females (244, 239 and 203 g/d for control, 40 and 60 g saponins/kg, respectively) (118), probably due to a greater supply of protein to male lambs, which have greater requirement of protein compared with female lambs.

There are reports that milk yields and the composition of milk from moderate- or high-yielding cows (20–30 kg/d) are not improved by inclusion of *Yucca* or *Quillaja* extract in diets containing 10 to 20 % crude protein (25,29,30,36,37,40,42). Even supplementation of diets containing less than 10 % protein with *Yucca* saponins (0.1 g/kg) did not affect milk production (119). In the experiments of Benchaar *et al.* (42), Singer *et al.* (30) and Holtshausen *et al.* (25), rumen fermentation and protozoa numbers were also not affected by saponin treatment, probably due to the low doses of saponins used. Therefore, the lack of effect of saponins on milk production in some studies might be due to the administration of low levels of saponins, and consequent inefficacy to elicit rumen metabolism response, while in other studies, nutrient requirements are probably met from the diets without saponin supplementation. The effects of *Yucca* and *Quillaja* saponins on feed consumption are inconsistent. Some studies (37,42,41) have reported a slight decrease from 2 to 6 %, and others (43) did not find any effect of *Y. schidigera* supplementation on feed consumption. Holtshausen *et al.* (25) reported that *Yucca* and *Quillaja* saponins increased DM intake, which resulted in a decrease of milk production efficiency. Contrary to the study of Holtshausen *et al.* (25), Lovett *et al.* (37) noted that *Yucca*

extract supplementation (25 and 50 g/d) decreased feed intakes in dairy cows without affecting milk yields, thus increasing the efficiency of converting feedstuff to milk and in this study protozoal numbers were decreased by *Yucca* extract feeding. It is important to identify the feeding condition and animals' nutrient requirements that would show a beneficial response of saponins on ruminant production.

## Conclusion

Plants or their extracts with high concentrations of saponins appear to have the potential to act as natural rumen manipulators. The primary effects of saponins are to modify the composition of rumen microbial populations, resulting in the modification of rumen fermentation. The most important effect of saponins in the rumen is to inhibit the ciliate protozoa, which might improve the efficiency of microbial protein synthesis by reducing microbial protein turnover, and protein flow to the duodenum. Saponins may inhibit methane production through defaunating actions and/or directly by decreasing the activities of methanogens such as the rate of methanogenesis or expression of methane-producing genes. Saponins may also selectively affect specific rumen bacteria and fungi, which may alter the rumen metabolism beneficially or adversely. The ammonia-adsorption and modulation of digesta passage in the rumen by saponins have also been implicated in altering the rumen metabolism, but their physiological responses are likely to be negligible compared with microbiological effects. The mechanisms and effects of saponins on the rumen microbial community and fermentation are interdependent depending upon the types and levels of saponins, composition of diets, microbial populations affected and adaptation of microbiota to saponins. Therefore, there is a need for comprehensive research based on the interactions among chemical structures of saponins, nutrient composition of diets and their effects on the rumen microbial ecosystem if a consistent beneficial effect of saponin inclusion in diets is ever to be achieved. It is important to identify the most bioactive saponins that will specifically inhibit protozoa and/or activities and numbers of methanogens and which may stimulate the beneficial bacteria and fungi. Although most saponins are considered quite safe when they are administered orally, certain kinds of saponins may exert toxic effects in the body, which must be tested *in vivo* in long-term experiments. The beneficial effects of saponins can be used extensively in various feeding systems if more active metabolites can be isolated and identified from plants or plant biotechnological tools can be employed to produce target bioactive principles.

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