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REVIEWS

The Impact of Saponins or Saponin-Containing Plant Materials on Ruminant Production—A Review

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Saponins are steroid or triterpene glycoside compounds found in a variety of plants. Some saponin-containing plants, mainly legumes, have been used as animal feed, but others are toxic. Several studies on the effect of saponins on ruminant production have also been reported. Some in vitro and in vivo experiments that demonstrate the beneficial effects of saponin such as defaunation of the rumen and manipulation of the end products of fermentation are described. Defaunation is the selective removal of protozoa from the rumen microbial ecosystem by a cell membrane cholesterol−saponin interaction, which causes cell rupture. Because protozoa in the rumen cause protein turnover by predating on bacteria, defaunation increases the nitrogen utilization of the ruminant and may lead to an increase in growth, milk, or wool production. The growth-promoting effect was evident in the high roughage diet suggesting that the application of saponins or saponin-containing plant materials may be beneficial for the subsistence farmers in developing countries. Saponins are deglycosylated by rumen microbes. Some sapogenins have been detected in the digestive tract of ruminants; however, the direct action of these compounds on the host animal is still unclear. No information on the effects of saponin on ruminant reproduction is available. There is an urgent need for a systematic evaluation of the most active structural components of the saponins, and their interaction with the microbial community, the host animal, and the diet. Along with these studies, the direct effects of saponins or their microbial degradation products on the host must be examined in order to get the full understanding of the metabolism and beneficial effects of saponins on animals.

Keywords: Saponin; ruminant; rumen

INTRODUCTION

Saponins are secondary compounds found in many plants. They form a stable foam in aqueous solutions such as soap, hence the name "saponin". Chemically, saponins as a group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives (**Figure 1A**,**B**, respectively). The main triterpene aglycone is a derivative of oleanane (**Figure 1C**). The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). Saponins that have one sugar molecule attached at the C3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C3 and one at C22, are called bidesmoside saponins. There are two main types of triterpenoid saponins: neutral, when a normal sugar is attached to sapogenin, and acidic, when the sugar moiety contains uronic acid or one or more carboxylic groups attached to the sapogenin (*1*). There have been several reviews in recent years about the implications or applications of saponins in animal systems or production (*2*−*5*). Most of them, however, deal with either specific sources or specific properties or biological effects of saponin on different animals. This review describes in more detail the effects of saponin or saponin-containing plants on rumen microbes, rumen fermentation, and the metabolism of saponins in ruminans and the effect of saponins as feed additives on ruminant production. The recent reports on the effects of saponin on ruminant are also included in this review. It is hoped that the information collected here will provide wider opportunities for the development and utilization of saponins to enhance ruminant production.

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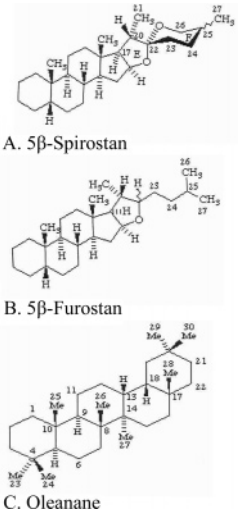

Figure 1. Basic structure of steroid (A and B) and triterpenoid saponin (C).

OCCURRENCE, DISTRIBUTION, AND ROLE IN PLANTS

Saponins are found in a large number of plants and some animals (such as the sea cucumber) (*6*−*8*). In plants, they occur in different parts such as root, tuber, bark, leaves, seed, and fruit. Triterpenoid saponins are found principally in dicotyledons while steroidal saponins occur in monocots. However, some plant species contain both triterpenoid and steroidal saponins. Avenacoside (steroidal), for example, occurs in oat leaves while avenacin (triterpenoid) is found in oat roots (*9*). Young leaves contain more saponins than mature leaves, but foliage saponins were found to be less haemolytic than root saponins (*2*). Soyasapogenols are mainly concentrated in the axis of the seeds rather than in the cotyledons and seed coat. In the seedlings, the root (radicle) contains the highest concentration of soyasapogenol A, while the plumule has the greatest amounts of soyasapogenol B (*10*).

Generally, saponins are found in tissues that are most vulnerable to fungal or bacterial attack or insect predation. Therefore, one of their roles is to act as a chemical barrier or shield in the plant defense system (*9*−*11*). Avenacin, for example, is a saponin found in large quantities in root tips, but only at low levels in the rest of the root system, and acts as a means of controlling the zoosporic fungus, *Gaeumannomyces graminis* (*11*). Alfalfa saponins are induced by insect attack and act as a deterrent to subsequent attacks. When alfalfa saponins were administered in the diet of larval *Spodoptera littoralis*, they caused prolongation of the larval and pupal stages, retarded growth, increased mortality, and decreased fecundity and fertility (*12*). Saponins also control rhizosphere bacteria in the soil (*13*).

SAPONINS OR SAPONIN-CONTAINING PLANTS FOR RUMINANT FEED

The present review will concentrate mainly on saponins in plants or forages eaten by ruminants or on saponins, which have been used as ruminant feed additives. **Table 1** lists some saponin-containing plants, which are used as forages. Many leguminous plants contain triterpenoid saponins, an exception being *Trigonella foenum-graecum*, which contains steroidal saponins. They are mainly found in leaves but also in seeds. Although only one major structure of saponin or sapogenin in these plants is mentioned (**Table 1**), it is actually found not as a single compound but as several compounds with different sugar moieties. For example, 29 saponins have been identified in alfalfa roots, leaves, and seeds with medicagenic acid as the major sapogenin. These saponins exert different biological activities due to differences in the sugar moiety, in the position of sugar attachment, and in the aglycone (*2*). Not all saponins have been isolated and identified in other forages. No information is available on the chemical structure of saponins in the tree species *Enterolobium cyclocarpum*, *Pithecellobium saman*, and *Moringa oleifera*. The presence of saponins in *Sesbania pachycarpa* was shown by thin-layer chromatography analysis without any further structural elucidation (*23*).

Table 2 describes saponin-containing plants, which have potential as feed additives. Usually the plants themselves are not used as animal feed; rather, the saponins are extracted from plant parts and used as feed additives. *Yucca schidigera* is a desert plant native to the southwestern United States and Mexico, while *Quillaja saponaria* (soapbark tree) originated from Chile (*3*). Yucca extract contains 4.4% of steroid saponins, which consist of 28 different structures of spirostanol and furastonal glycosides (*30*, *31*). *Q. saponaria* Molina extract contains 10% total saponins, and more than 20 different structures of triterpenoid saponin have been identified in the extract. Both Yucca and Quillaja saponins are commercially available products that have been used not only as feed additives but also for other purposes such as foaming agents in beverages and emulsifying agents in cosmetics (3).

Saponins have been identified in the fruit of several Sapindus species, which are found in different countries. *Sapindus saponaria* is distributed in Central and South America, and *Sapindus rarak* is native to South East Asia; *Sapindus emarginatus* is found throughout India and Thailand (*44*), *Sapindus drummonii* is found in North America, and *Sapindus delavayi* is found in China. Traditionally, these Sapindus fruits are used for washing clothes or hair and as herbal medicines. Recently, *S. saponaria* fruits (containing 12% saponins) have been evaluated as feed additives (*16*, *38*, *39*). The chemical structure of some identified saponins from *S. rarak* fruit pericarps is almost similar to those reported in *S. saponaria* fruit pericarps. Both species have monodesmoside triterpenoid saponins that possess hederagenin as the agylcone (*33*−*35*). Differences occur mainly in the sugar composition and arrangement. In *S. saponaria*, glucose is directly attached to hederagenin and rhamnose and arabinose are linked to the glucose (*33*, *34*). In *S. rarak*, arabinose is attached to hederagenin and rhamnose and xylose as sugar residues are attached to arabinose (*35*). Several reports showed that sugar composition and type of linkage are also directly related to the activity of the saponins (*36*−*38*). Chwalek et al. (*39*) demonstrated that the (1 → 4) linkage in hederagenin diglycosides had a higher hemolytic activity than the (1 → 6) linkage. Moreover, the β-linkage configuration in hederagenin diglycosides also showed a higher hemolytic activity than the α-linkage configuration. Jung et al. (*38*) showed that rhamnose contributed more cytotoxic activity than glucose in oleanane digylcosides isolated from *Akebia quinata*. The observed differences in the activity of saponins of closely related species may be partly due to differences in their chemical composition. No study on the correlation between hemolytic or cytotoxic activity and the antiprotozoal activity of saponin or on the chemical structure−antiprotozoal activity relationship has been carried out.

Tea saponin, isolated from tea seeds, is a new commercially source of saponin in China. Other saponins from tea leaves have antiallergic activity (*40*). The tea seed meal, which contains

Table 1. Saponin-Containing Forages Commonly Used as Livestock Feed

family and species	plant part	saponin or sapogenin name	ref
	Fabaceae		
Acacia auriculoformis	fruit	acaciaside	*14*
Albizia lebbeck	pods, bark	albiziasaponin	*15*
E. cyclocarpum	leaf, fruit	saponin content 3.9 mg/g	*16*
Gliricidia sepium	root, fruit	hederagenin	*17*, *18*
Glycine maxima (soybean)	seed	soyasapogenol	*10*
Lupinus spp. (lupin)	seed	soyasapogenol	*19*
Medicago sativa (alfalfa, lucerne)	leaf, root, seed	medicagenic (aglycone), soyasapogenol	*2*
Melilotus alba (white sweet clover)	leaf, flower, root	melitonin	*20*
Medicago hispida (burr clover)	leaf	hispidacin (soyasapogenol)	*6*
P. saman	fruit	saponin content 3.4 mg/g	*16*
Pueraria montana var. *lobata* (Kudzu)	root	kudzusaponins (soyasapogenol)	*21*
S. sesban	leaf, seed	glucuronide-oleanolic acid, stigmasta galactopyranoside	*22*
S. pachycarpa	leaf	saponin	*23*
Trifolium repens (ladino clover)	leaf	cloversaponins (soyasapogenol)	*24*
Trifolium pratense (red clover)	leaf	soyasapogenin	*25*
T. foenum-graecum (fenugreek)	leaf, seed	steroid saponin	*26*
	Moringaceae		
M. oleifera	leaf	80 g/kg diosgenin equivalent	*27*
	Poaceae		
Avena sativa (oat)	leaf, root, seed	avenacin	*28*
B. decumbens (signal grass)	leaf	dioscin, diosgenin, yamogenin	*29*

Table 2. Saponin-Containing Plants that Are Used as Feed Additives in Ruminant

family and species	plant part	saponin or sapogenin name	ref
		Rosaceae	
Q. saponaria	bark	quillaic acid	*30*, *31*
		Agavaceae	
Y. schidigera	trunk, root	sarsapogenin,gloriogenin, markogenin	*32*
		Sapindaceae	
S. saponaria	fruit	hederagenin (aglycone)	*33*, *34*
S. rarak	fruit	hederagenin (aglycone), mukurozi-saponin	*35*
		Theaceae	
C. sinensis	seed, leaf	theasaponin, camelliasaponins	*40*, *41*

saponins (*41*), is extensively used in aquaculture to eliminate unwanted fish and harmful insects in the fish and prawn ponds. Tea saponin, as a biological pesticide, could also be used as an insecticide and fungicide. The use of tea saponin as ruminant feed additives has been limited to in vitro evaluation (*45*, *46*).

EFFECTS OF SAPONINS ON RUMEN MICROORGANISMS

Protozoa. Rumen protozoa are divided into two genera, holotrichs and entodiniomorphs. Entodiniomorphs are more abundant, and the species *Entodinium* is the most predominant. Holotrichs will utilize soluble carbohydrates, while entodiniomorphs engulf starch grains and degrade them. *Diploplastron affine*, *Epidinium ecaudatum*, *Eremoplastron bovis*, *Eudiplodinium maggii*, and *Ophryoscolex caudatus* are protozoa that have been observed to engulf bacteria in vitro (*47*). For this reason, the presence of protozoa is undesirable in the rumen. They cause rapid intrarumen nitrogen cycling, and excess ammonia is excreted in the urine. Saponins kill or damage protozoa by forming complexes with sterols in the protozoal membrane surface. The membranes become impaired and eventually disintegrate (*4*). *Y. schidigera* saponin (0.1%) inhibited the motion of ciliate protozoa, the cilia of entodiniomorphs, and the contraction of holotrichs and decreased the rate of breakdown of [^{14}C] leucine-labeled *Selenomonas ruminantium*, which indicates the activity of protozoa to consume bacteria (*48*). The addition of *E. cyclocarpum* decreased the protozoal counts in the sheep rumen without changing the composition of protozoa community; *Entodinium* is the most predominant species (92%), followed by *Dasytricha* (3%), *Polyplastron* + *Eremoplastron* (2.2%), and *Isotricha* (2%) (*49*). A similar result occurred in a Holstein cow fed *E. cyclocarpum* where the predominant protozoa was *Entodinium* sp. (80%) followed by *Diplodinium* sp. (18%) and *Eudiplodinium* sp., and *Isotricha prostoma* were only 1% of the total protozoa (*50*). No other information about the composition of protozoa in the rumen of ruminants fed with other saponins or about the type of protozoa, which may have adapted to saponins, is available.

A compilation of in vitro and in vivo results on the effect of saponins or saponin containing plants on protozoa in the rumen is presented in **Table 3**. The effect on protozoa is described as the percent decrease of protozoal count (N) or percent decrease of protozoal activity (A), based on the amount of released [^{14}C] from labeled bacteria. There were 28 reports showing a reduction of protozoal numbers and eight reports showing a decreased protozoal activity in response to treatment with saponins; seven reports showed no effect of saponin on protozoa, but three reports showed an increase. The antiprotozoal effect of saponins or saponin-containing plants is mostly between in vitro experiments. However, in vitro results are not always consistent with results obtained in vivo so they should be viewed with caution.

There are more published data available on the utilization of Yucca saponin or sarsaponin as feed additives than other saponins (**Table 3**). Most of the in vitro experiments showed a negative effect of Yucca saponin on protozoa, but this effect of Yucca saponin in vivo varied. *E. cyclocarpum* leaves induced 100% inhibition on protozoa in the in vitro experiment (*53*), but the effect vanished after several days of feeding (*49*, *50*, *53*). None of the in vivo experiments using *E. cyclocarpum* could demonstrate a long-term antiprotozoal effect. The antiprotozoal activity of saponins is only transient. A similar situation occurred when feeding *Sesbania sesban* to sheep (*65*−

Table 3. Effect of Saponin-Containing Plants on Protozoa, Ammonia, and Propionate (%) Concentration in Rumen Contents in Vitro and in Vivo

plant	experiment	dosage	substrate/feed	effect on protozoa[a]	effect on ammonia	effect on propionate[b]	ref
A. auriculuformis	in vitro	1.2 mg/mL	hay or hay/concentrate	–46 to –63	–15	ND	*52*
C. sinensis	in vitro	0.4–1.2%	corn meal/grass meal	–43 to –73	–5 to –8	+40 to +51	*45*
E. cyclocarpum fruits	in vitro	100 mg/g	grass hay:barley straw *Arachis pintoi*	+54	no effect	–5[d]	*16*
E. cyclocarpum leaves	in vitro	1–10%	lucerne	–100	ND	ND	*53*
	in vitro	10 mg/L	no substrate	–91 (A)	ND	ND	*54*
	in vitro	0.5–10 mg/mL	no substrate	–20 to –95 (A)	ND	ND	*49*
	in vivo (cattle)	200 g/day	*Pennisteum clandestinum* and rice polishing	–25[c]	ND	ND	*50*
	in vivo (buffalo)	375 g/day	native grasses	–100[c]	ND	ND	*53*
	in vivo (sheep)	25–75 g/day	oaten chaff + 1% urea + lupin	no effect	ND	ND	*53*
	in vivo (sheep)	100 and 300 g/day	*Pennisetum* hay	+5	no effect	ND	*55*
	in vivo (sheep)	200 g/day	barley silage–barley grain–soybean meal	–35	no effect	+6[d]	*49*
	in vivo (sheep)	200 g/day	barley grain–barley grain–soybean meal	decrease[c]	ND	ND	*49*
Enterolobium timbouva leaves	in vitro	1–10%	lucerne	–100	ND	ND	*53*
M. sativa	in vitro	0.5–4%	cellulose and starch	ND	ND	+11 to +25	*56*
	in vivo (sheep)	2–4%	concentrate:roughage	–34 and –66	–29 and –37	no effect	*57*
P. dodecandra fruits	in vitro	10 mg/L	no substrate	–85 (A)	ND	ND	*54*
P. saman fruits	in vitro	100 mg/g	grass hay:barley straw *Arachis pintoi*	+54	+17	–5[d]	*16*
Q. saponaria	in vitro	1.2 mg/mL	hay or hay/concentrate	–38 to –54	–12 to –15	no effect	*52*
Q. saponaria	in vitro	0.1–0.4%	casein	–8	0 to +11	no effect	*58*
S. saman leaves	in vitro	10 mg/L	no substrate	–85 (A)	ND	ND	*54*
	in vitro	0.25–4 mg/mL	*Pennisetum* hay–wheat bran	–11 to –49	–8 to –16	ND	*60*
S. rarak extract (fruit)	in vivo (sheep)	0.07% BW	*Pennisetum*–concentrate	–57	–28	ND	*61*
	in vivo (sheep)	0.24–0.72 g/kg BM	*Pennisetum*–wheat bran	–32 to –79	–12 to –18	+8 to +19	*62*
S. saponaria pericarp	in vivo (sheep)	25–50 g/day	corn meal–fish meal	–7.5 to –15	0 to –40	+5 to +8[d]	*63*
S. saponaria fruit	in vitro	100 mg/g diet	grass hay:barley straw *Arachis pintoi:*	–54	no effect	+4[d]	*16*
	in vitro	80 mg/g diet	*Brachiaria dictyoneura*–*A. pintoi* or *Cratylia argentea*	–26 to –31	no effect	0 to +8	*64*
	in vivo (sheep)	8 g/kg $W^{0.75}$ (intrarumenly)	*B. dictyoneura* hay hay–*C. argentea*	+67	no effect	+17	*42*
	in vivo (sheep)	5 g/kg $W^{0.75}$	*C. argentea:B. dictyoneura*	–41 to –64	no effect	no effect	*43*
S. pachycarpa leaves	in vitro	10–60% (w/w)	barley straw	decrease	ND	ND	*23*
S. sesban leaves	in vitro	10–100 mg/mL	lucerne	no effect	ND	ND	*53*
	in vitro	6 and 24 mg/mL	wheat straw	–58 to –100 (A)	ND	ND	*65*
	in vitro	10 mg/L	no substrate	–98 (A)	ND	ND	*54*
	in vivo (sheep)	250 g/day	grass hay:barley:molases:fishmeal	–60 (A)[c]	no effect	no effect	*65*
S. sesban leaves	in vivo (sheep)	300 g/day	maize stover (basal diet)	no effect	ND	ND	*66*
	in vivo (sheep)	200 g/day	sululta hay–wheat bran	no effect	+75[c]	+31[c]	*67*
	in vivo (sheep)	200 g/day	grass hay–barley/fishmeal	decrease (A)	ND	ND	*67*
Y. schidigera (commercial)	in vitro	1 and 10 mg/mL	no substrate	–22 and –100 (A)	0 and –6	ND	*48*
	in vitro	1–100 mg/kg DM	grass silage and hay	no effect	no effect	no effect	*68*
	in vivo (cows)	125 mg/kg diet	hay–corn–cottonseed	ND	no effect	no effect	*69*
	in vitro	200 mg/L	hay	ND	no effect	no effect	*70*
	in vitro (sheep)	5–30 g/day	soybean hull:alfalfa:corn:oat	–18[e]	ND	ND	*71*
Y. schidigera (butanol fraction)	in vitro	1.2 mg/mL	hay and hay:concentrate	–24 and –49	–30	ND	*52*
	in vitro	0.5 mg/mL	alfalfa hay:concentrate	–70	–54[c]	no effect	*51*
	in vitro	10 mg/mL	barley grain or alfalfa hay	ND	–70 to –40	–26 to –50 (AP)	*72*
	in vitro	15 and 225 μg smilagenin/mL	barley grain	ND	–30 aND +70	–2 and –23 (AP)	*72*
Y. schidigera plant (commercial)	in vivo (heifer)	20–60 g/day	alfalfa hay:barley grain (39:61)	–20 to –32	no effect	+17 to +18	*73*
	in vitro	1–4 mg/mL	casein	no effect	–5 to –27	no effect	*58*
sarsaponin	in vitro	1.2–3.2 g/L	potato starch	–6 to –30	–21 to –50	+11 to +29	*74*
		1.2–3.2 g/L	corn starch	–13 to –32	–20 to –39	+5 to +14	
		1.2–3.2 g/L	hay:concentrate (6:4)	–18 to –43	–18 to –38	+10 to +37	
	in vitro	33–77 mg/kg	corn:alfalfa:soybean meal	–8 to –19	no effect	ND	*75*
	in vivo (cows)	77 mg/kg	concentrate:sorghum silage (55:45)	ND	no effect	no effect	*75*
	in vivo (sheep)	0.01 and 0.25%	hay–barley	no effect	–56 to –59	no effect	*76*

[a] Effect on protozoal counts (%), A = protozoal activity measured by ^{15}N released from labeled bacteria. [b] Effect on molar proportion of propionate (%), AP = acetate-to-propionate ratio. [c] Effects occurred only temporarily. [d] Effects were not significant; ND, no data. [e] Effect was significant at the highest level.

67). Protozoal counts decreased by 60% after 4 days, but the population recovered after a further 10 days of feeding *S. sesban*.

Saponin from *S. saponaria* fruit also showed a negative effect on protozoa in the Rusitec system (*64*) and depressed protozoal numbers in the rumen of Swiss White Hill sheep (*43*) but not in that of African sheep (*42*). This variability may be due to an adaptation of the microbial population to saponin, the previous experience of the animal to saponin, or both (*4*, *42*). A consistent antiprotozoal effect in vitro (*59*, *60*) and *in* vivo (*61*, *62*) was found when incorporating *S. rarak* extract into the diet. This effect may be due to the different type of saponins in *S. rarak* as compared to other plants. Further study to isolate and identify all saponin compounds in *S. rarak* and relate those compounds to the antiprotozoal activity is required.

Several other saponin-containing plants such as *Acacia auriculuformis*, *Camellia sinensis*, *Phytolacca dodecandra*, *Samanea saman*, and *Sesbania pacycharpa* exerted considerably high antiprotozoal activity in the in vitro rumen fermentation (*23*, *46*, *52*, *54*). The persistence of the antiprotozoal activity of these plants when fed to animals needs further evaluation.

Methanogens. Studies on the effect of saponins on methanogenic archaea and their products have attracted a lot of attention lately because of the potential for improving the environment by decreasing the production of "greenhouse gases". However, these studies concentrated more on the measurement of methane emission than on the methanogens themselves. As some methanogens (10−20% of total) live in association with protozoa (*77*, *78*), it was expected that reducing protozoa would also reduce methanogens, thus decreasing methane production. The addition of Yucca extract to a high roughage diet or to a mixed diet containing hay and barley grain did not decrease methane emission in the Rusitec system (*68*, *76*). However, decreased methane emissions in an in vitro system were obtained by adding sarsaponin, extracted from Yucca, to a starch diet and to a mixed diet (*74*). Suppression of methane emission was also achieved by the supplementation of *S. saponaria* fruit in the Rusitec system (*16*) or by addition of tea saponin to in vitro rumen fermentation.

Methane emission was also suppressed when sheep were fed *S. saponaria* fruit (*43*). However, the suppression of methanogenesis was not associated with decreased methanogen counts suggesting a suppression of activity per methanogen cell (*43*). Saponin in *S. rarak* extract may suppress methane emission in a similar way as saponin in *S. saponaria* did since it did not decrease the archaeal or methanogen RNA concentration either in vitro or in vivo (*59*). The availability of hydrogen to form methane may compete with the requirement of hydrogen to form propionate. So, if the concentration of propionate in the rumen increases in the presence of saponin, the methane emission would decrease. Further study is required to elucidate the mechanism of decreasing methane emission in vivo by saponin.

Fungi. Anaerobic fungi are important in the rumen for digesting fiber, but they only comprise a small proportion of the total mass of the rumen microflora. There is little information on the effect of saponins on rumen fungi. In pure culture, Wang et al. (*79*) demonstrated that the fungi, *Neocallimastix frontalis* and *Pyromyces rhizinflata*, are very sensitive to saponin from *Y. schidigera*, and even at a low concentration of the saponin (2.25 μg/mL), the growth of both fungi was completely inhibited. However, Muetzel et al. (*23*), using a membrane hybridization technique, showed that fungal concentration was not significantly reduced when increasing levels of saponin-containing *S. pachycarpa* were included in an in vitro fermentation system. In our experiment, we observed that *S. rarak* extract decreased fungal RNA concentration in the rumen liquor in an in vitro fermentation.

The fungal RNA concentration was decreased by addition of *S. rarak* extract in the in vitro rumen fermentation (*60*) but not in the rumen of sheep when fed for 3 months (*80*). The fungal population was significantly higher when sheep were fed with 25−50 g/day of *S. saponaria* for 30 days (*63*). An adaptation of fungi may occur during long-term feeding. Fungi produce certain carbohydrases (*81*), which may degrade saponin, but this also needs to be confirmed by further studies.

Bacteria. There are very few in vitro studies on the effects of saponins on specific rumen bacteria. Using pure culture, Wallace et al. (*48*) observed that the saponin fraction of *Y. schidigera* when added at a concentration of 1% to the medium stimulated the growth of *Prevotella ruminicola*, did not affect the growth of *S. ruminantium*, suppressed the growth of *Streptococcus bovis* by prolonging the lag phase, and completely inhibited the growth of *Butyrivibrio fibrisolvens*. The same fraction at much lower concentrations (0−250 μg/mL) in pure culture depressed the growth of the noncellulolytic bacteria *S. bovis*, *Prevotella bryantii* B14 (formerly *P. ruminicola*), and *Ruminobacter amylophilus* after 14 h of exposure (*79*). *Fibrobacter succinogenes* was unaffected, but *Ruminococcus albus* and *Ruminococcus flavefaciens* were virtually unable to digest cellulose in the presence of Yucca saponins. Wang et al. (*79*) concluded that Yucca saponin negatively affected the Gram-positive bacteria more than the Gram-negative bacteria. Muetzel et al. (*23*) also found that *S. pachycarpa* did not affect *F. succinogenes* (Gram-negative). The concentration of RNA from *Fibrobacter* sp. remained constant and was not affected by *S. rarak* extract either in vitro or in vivo.

Using a rumen simulation technique (Rusitec), the number of cellulolytic bacteria was decreased by 30% when 0.5 mg/mL Yucca extract was added to alfalfa hay (*51*). In a subsequent experiment, Wang et al. (*79*) demonstrated that cellulolytic bacteria are more susceptible to Yucca extract than amylolytic bacteria. Another study in pure culture showed that the growth of cellulolytic bacteria was slightly decreased by *S. rarak* extract (*59*).

In an in vivo study, Diaz et al. (*63*) observed a significant increase in cellulolytic and total bacteria in the rumens of sheep fed with *S. saponaria* fruit. A similar observation was made by Thalib et al. (*61*) who reported that total cellulolytic bacteria increased when sheep were fed with a methanol extract of *S. rarak*.

RUMEN MICROBIAL ADAPTATION TO SAPONINS

Although many studies show that saponins depress protozoal count, some show that the antiprotozoal effect is only transient. Newbold et al. (*65*) found that after day 9 of feeding *S. sesban*, protozoa counts in the rumen increased and reached the same level as the control. Ivan et al. (*49*) also observed a rapid increase in the protozoal population in the rumen of sheep after day 14 of daily feeding with *E. cyclocarpum*. To avoid rapid microbial adaptation to saponin, Newbold et al. (*61*) suggested that saponins should be fed intermittently. In our experiment, however, protozoa did not recover to normal population by daily feeding of saponins from *S. rarak* for 3 months suggesting no adaptation to saponin (*62*, *80*). Wina et al. (*80*) observed a negative effect on *Ruminococci* and *Chytridiomycetes* (fungi) with short-term feeding of *S. rarak* extract, but this effect disappeared after long-term feeding, indicating that there may be an adaptation of those microbes to *S. rarak* saponins. The

mechanism of adaptation of rumen microbes to saponin still needs to be clarified. Microbes may develop the ability to degrade saponins rapidly so that the antiprotozoal activity of the saponins is lost. The higher glycosidase activity, produced by *Ruminococci* after several days of to yucca saponins, perhaps may be part of the adaptation processes (79). An increase in the thickness of their cell wall was observed when *P. bryantii* in pure culture was adapted to Yucca saponins (*79*).

Previous exposure to saponin-containing plant material may help microbes to react quickly to the presence of saponins and minimize the antiprotozoal effect of saponin-containing plants (*67*). Species or breed differences or the animals' environment may contribute to the ability of rumen microbes to reduce the antiprotozoal activity of saponins. Ethiopian sheep may have developed a microbial population in the rumen capable of degrading antiprotozoal agents but not Scottish sheep (*67*). It is difficult to identify the exact mechanism of adaptation of rumimant microbes to saponin.

EFFECTS ON RUMEN FERMENTATION

Ammonia. Ammonia in the rumen is produced from both feed degradation and microbial lysis; some is absorbed through rumen wall, and the remainder is then directly utilized by microbes, which derive 50−80% of their N requirement from the ruminal ammonia-N pool (*82*). The decline in rumen ammonia after feeding could be due to a reduced substrate degradation or to the utilization of the ammonia by bacteria.

Table 3 presents the effects of saponins on ammonia released in the rumen. Out of 51 reports, 14 showed no effect and 17 showed a negative effect of saponins on rumen ammonia concentration. The extent of reduction of rumen ammonia concentration due to the addition of *Y. schidigera* was not consistent in different reports. In some in vitro and in vivo experiments, there was a slight or significant reduction in rumen ammonia concentration, but in other experiments using the same product of Yucca, it did not change (*48*, *69*, *71*, *72*, *76*). Observed decreases in rumen ammonia concentration may have been an indirect result of the decreased protozoal counts caused by the added saponin. Fewer protozoa would mean less predation and lysis of bacteria, hence, less release of the products of protein breakdown. Reduction in ammonia concentration may also be due to the fewer protozoa in the rumen since protozoa contributed some 10−40% of the total rumen nitrogen (*83*). Addition of saponin often did not decrease rumen ammonia concentration as saponin did not inhibit feed protein degradation in the in vitro rumen (*84*).

Short Chain Fatty Acid (SCFA). The major effects of saponins on rumen fermentation were a shift of SCFA pattern towards an increased proportion of propionate and a decreased acetate-to-propionate ratio. The higher propionate proportion may be the result of lower acetate and butyrate, which are both major fermentation end products of protozoa. Therefore, when the number of prozotoa was depressed by saponin, a significant increase in the proportion of propionate would be expected. Ten out of 12 results of increased propionate are related to the decrease of protozoa (**Table 3**). A high level of propionate can result in an increase in the efficiency of utilization of the end products of rumen fermentation by the ruminant (*85*). In **Table 3**, two results show a decrease of the acetate−propionate ratio, meaning that the molar proportion of propionate increased. However, there are 12 results that show no effect on propionate production in the rumen. It seems that the effects of saponins on SCFA composition vary according to the diet and the application level.

Total SCFA production in the in vitro fermentation did not change when supplemented with Quillaja saponin (*86*), Yucca extract (*51*, *68*), *S. saponaria* fruit (*64*), or *S. pachycarpa* (*23*). However, Lila et al. (*74*) found an increase in total SCFA in vitro with sarsaponin supplementation.

SCFA production did not change in in vivo experiments when Yucca extract was given to cows (*69*) or heifers (*73*). When sheep were fed *S. saponaria*, SCFA production was similar among treatments (*42*, *43*). *S. sesban* supplementation of sheep caused an initial, but not prolonged, increase in total SCFA (*65*).

Microbial Protein Synthesis. If saponins kill or inactivate protozoa, then there will be a lower predation of bacteria by protozoa, which will result in a larger bacterial population and a slower protein turnover in the rumen leading to an increase in bacterial N flow to the duodenum. Makkar and Becker (*86*) found that the efficiency of in vitro microbial protein synthesis (EMPS) was linearly increased by the addition of Quillaja saponins (0.4−1.2 mg/mL) to a hay substrate. On further purification of the saponins, Makkar et al. (*52*) also observed that saponins from Quillaja, Yucca, and *Acacia auriculiformis* fruit increased microbial biomass, ^{15}N incorporation, and EMPS. They concluded that saponins partition the nutrients in such a manner that a higher proportion of the digested substrate goes into the formation of microbial mass and a lower proportion to SCFA and gas. Liu et al. (*45*) showed that an increase in microbial protein synthesis of 49% occurred in the presence of 0.8% of tea saponins in an in vitro fermentation. Wang et al. (*72*), however, showed that microbial protein synthesis increased at a low level of Yucca saponin (15 μg/mL) but was decreased by higher concentrations (75 μg/mL). Other in vitro studies using the Rusitec system did not show any significant effect of Yucca saponin (100 mg sarsaponin/kg) (*68*) or of sapindus saponins (*16*) on microbial protein synthesis.

Various results on microbial protein synthesis have also been reported in in vivo experiments. Abreu et al. (*42*) and Hess et al. (*43*) found an increase in duodenal flow of microbial N when sheep were fed *S. saponaria* fruit. However, Hristov et al. (*73*) could not demonstrate a significant effect of Yucca saponin on microbial protein flow to the intestine of heifers. There was an increase of microbial N supply, efficiency of microbial N supply, and fecal N excretion with increasing levels of *S. rarak* extract, but this increase was not significant (*87*). Different techniques to measure microbial protein synthesis may cause the various effects of saponin on the production of microbial protein. Moreover, the different concentration of saponin added to the diet, the type of saponin, and the different composition of diet may influence the production of microbial protein. A more thorough study about type of saponin and dietary feed composition interaction on microbial protein synthesis is required.

Enzyme Activity. Because Yucca extract affected different species of rumen bacteria to different extents, it may alter the activities of those species important in protein digestion and metabolism (*51*). Protease activity was increased while deaminase and peptidase activities were unaffected by the addition of Yucca extract to a mixed diet (alfalfa hay:barley, 1:1 w/w) in the Rusitec system. Yucca extract did not depress the activity of deaminase or proteolytic activity in the cell-free rumen fluid of heifers (*73*). The addition of saponin-containing *S. sesban* leaves had no effect on proteolytic, peptidolytic, or deaminase activity in the rumen (*65*). This lack of effect of saponin on proteolytic activity suggests that saponin may not affect feed protein degradation in the rumen.

Supplementation with saponin-containing *S. pachycarpa* leaves did not affect carboxymethyl cellulase (CMCase) activity

in an artificial rumen (*23*). Yucca extract decreased CMCase, xylanase, and amylase activities in an artificial rumen (*58*). CMCase, xylanase, and amylase activitites in cell-free rumen fluid were not inhibited when Yucca was fed directly to steers at a level of 60 g/day (*73*). Sapindus extract depressed xylanase activity significantly at high concentrations in an artificial rumen (*60*) and in sheep rumen (*80*).

It seems that the decrease of xylanase or CMCase activity in the rumen is linked more closely to the decrease in protozoal numbers than to the decrease in fibrolytic microbes. Our experiment found a significant correlation between the protozoal counts and the xylanase activity (*80*), and this supports the fact that protozoa also excrete fibrolytic enzymes (*88*).

Rumen Digestibility. Makkar and Becker (*86*) reported that the presence of Quillaja saponins caused a significant reduction in the apparent and true digestibility of the substrate in an in vitro fermentation. Saponins from *S. rarak* extract (SE) also depressed the apparent and true digestibility of the substrate in vitro in a dose-dependent manner (*80*). Hess et al. (*16*) observed a reduction of NDF, ADF, and cellulose degradation when the whole fruit of *S. saponaria* was added to a Rusitec in vitro fermentation system. The in vivo experiments also showed that saponins decreased rumen digestibility. *E. cyclocarpum* decreased in sacco DM digestibility (*55*). Alfalfa saponins impaired fiber digestion in the rumen (*57*). All of this decreased rumen fiber digestion may be due to the lower fibrolytic enzyme activity in the rumen due to saponins as described before.

SAPONIN DEGRADATION

Rumen Metabolism. The rumen is the first site of plant and saponin degradation by microbes. Makkar and Becker (*89*) showed that Quillaja saponin could be degraded by rumen microbes. The degradation rate was very slow initially but much more rapid after 6 h of in vitro incubation. Mathison et al. (*90*) showed that a slow degradation of saponins from alfalfa leaves occurred at 8 h of incubation. Wang et al. (*72*) showed that the type of substrate influenced the degradation of saponins in the artificial rumen since the concentration of soluble saponins declined more rapidly with an alfalfa diet than with a barley grain diet during the first 8 h of incubation. When saponins were introduced directly into the sheep rumen, they were rapidly hydrolyzed even at 1 h after dosing (*91*). *Butyrivibrio* strains were presumed to degrade alfalfa saponins; however, the isolated strain failed to degrade any saponin (*92*). The concentration of Yucca saponins was decreased when added to the pure culture of *F. succinogenes* (*79*). The degradation products of these saponins have not been investigated in detail, but it is suggested that quillaic acid is one of the degradation products of Quillaja saponin (*89*) and medicagenic acid that of alfalfa saponin. The formation of other derivative products of saponins was confirmed by several in vivo studies in which sheep were dosed intrarumenly with saponins (*91*, *93*, *94*). Saponins from *Y. schidigera* and *Narthecium ossifragum*, which have the same aglycone (sarsapogenin), were degraded in the rumen to sarsapogenin as the major degradation product and five other derivative products of sarsapogenin: smilagenin, episarsapogenin, epismilagenin, sarsasapogenone, and smilagenone (*93*, *94*). However, Meagher et al. (*91*) showed that saponins from an extract of *Costus speciosus* rhizomes were hydrolyzed not to its aglycone (diosgenin) as the major degradation product but to epismilagenin, the epimerized product of diosgenin. The formation of sapogenin and its several derivative products in the rumen indicates that several processes such as hydrolysis of saponin, epimerization, and hydrogenation of sapogenin occur in the rumen (*91*, *94*). Rumen microbes that epimerize or hydrogenate other compounds beside saponin have been identified (*95*, *96*) but not those for saponin.

Gastointestinal Metabolism. All sapogenins are transported further along the digestive tract and are excreted in the feces. The concentrations of various sapogenins, degraded products of saponins from *Y. schidigera* (*94*) or from *N. ossifragum* (*93*) or from *C. speciosus* rhizomes (*91*), were much lower in the duodenum, suggesting that they may have been absorbed in the duodenal region and transported to the liver via the portal vein (*91*, *93*, *94*). In the liver, free sapogenins may be conjugated with glucuronide and excreted into the bile. The appearance of salts of sapogenin glucuronide in the liver and in the bile has been reported in sheep suffering from photosensitization disease caused by saponins. None of the free form of sapogenin can be detected in the bile (*97*, *98*). After passing the duodenum, the concentration of all sapogenins increased again especially in the caecum and colon (*91*, *93*). Variation in the dry matter content could explain an increase in concentration of those sapogenins, but it may also indicate a continued metabolism of saponin by caecal baceria (*91*). There is no information so far on the specific cecal microorganisms in ruminant, which can degrade saponins. However, Hasegawa et al. (*99*) isolated a human intestinal bacteria, *Prevotella oris*, which can degrade ginseng saponins. Furthermore, Bae et al. (*100*) found that several human intestinal bacteria isolated from feces could hydrolyze ginseng saponins. *Eubacterium* sp., *Streptococcus* sp., *Bifidobacterium* sp., and *Fusarium* K-60 metabolized ginsenoside Rb1 and Rb2 to compound K by different routes. Gestetner et al. (*101*) reported that soybean saponin and sapogenins can be degraded in the cecum and colon of mice, rats, or chicks; however, they did not attempt to isolate the microbes.

Gestetner et al. (*101*) also found that the soybean saponin or its sapogenin was not absorbed into the blood of mice, rat, or chicks. They assumed that saponins pass intact through the small intestine of monogastric animals and are degraded by the cecal bacteria. Flåøyen and Wilkins (*93*) and Meagher et al. (*91*) could not detect any sapogenin in sheep blood or urine. The renal excretion route for free and conjugated sapogenins seemed to be of little importance (*93*). In contrast, Chen and Staba (*102*) were able to detect the sapogenin of ginsenosides in rabbit plasma and urine samples by gas chromatography. Cui et al. (*103*) also detected the same compounds in human urine by a gas chromatography−mass spectrometric method that could detect sapogenin at ng/mL levels in urine. Different opinions about the route of sapogenins in the body were suggested by some authors (*91*, *93*, *101*−*103*). Therefore, there is a need to study the role and fate of sapogenins after they are absorbed into the body more thoroughly to detect the excretion routes of these compounds in ruminants.

EFFECT ON TOTAL TRACT DIGESTIBILITY

As described previously, rumen fiber digestibility was decreased by saponins but this did not affect the total tract digestibility. *Y. schidigera* did not affect total tract digestibility of diets when it was fed to heifers (*73*) or sheep (*76*) or dairy cows (*104*). *S. rarak* saponins were also found not to affect total tract digestibility. Abreu et al. (*42*) found that saponin-containing *S. saponaria* fruit had no effect on total tract digestibility of organic matter (OM), nitrogen (N), or neutral detergent fiber (NDF). However, acid detergent fiber (ADF) digestibility of a legume−grass diet and NDF digestibility of a grass diet were decreased, suggesting a diet-dependent effect of saponin-containing *S. saponaria* fruit on fiber digestibility.

Further experiments using a similar legume−grass diet (*43*) showed a decrease in both total tract NDF and ADF digestibility with *S. saponaria* supplementation. Beside these negative effects, a positive effect of saponins on digestibility was reported with a silage diet fed to cattle and supplemented with Sarsaponin (*105*). Alfalfa saponins increased digestion in the small intestine and thus increased total tract digestibility especially of high concentrate diets (*57*). Thalib et al. (*61*) found a slight but significant increase in total tract organic matter digestibility of a rice straw diet when sheep were supplemented with *S. rarak* extract (47.5% for control vs 50.3% for supplemented treatment).

EFFECT ON BLOOD PARAMETERS

Most reports on blood parameters were concerned mainly with plasma ammonia or urea concentration. As Yucca extract has the ability to bind ammonia (*48*), it was expected that it would slowly release ammonia and thus affect the ammonia or urea concentration in the plasma. Yucca extract, however, did not change plasma ammonia or urea concentration in steers (*106*) or heifers (*73*) fed high roughage or high concentrate diets. Yucca extract also had no effect on the concentration of urea in the plasma or milk of dairy cows (*107*). Œliwiñski et al. (*104*) reported that Yucca saponin had no effect on hematocrit readings or hemoglobin concentration when fed to dairy cows at a level of 0.1 g/kg. The addition of *S. saponaria* fruit to a sheep diet decreased plasma urea suggesting that less ammonia was absorbed from the rumen (*42*, *43*). Neither hematocrit readings nor the activities of aspartate aminotransferase or glutamate dehydrogenase was affected by *S. saponaria* fruit (*43*) suggesting that no hemolytic effect of saponin occurred when it was fed to sheep and there was also no occurrence of liver disfunction.

EFFECTS ON GROWTH, WOOL, AND MILK PRODUCTION

Limited information is available on the effect of saponins or saponin-containing plants on animal growth. Yucca extract or powder is the main source of saponin used for growth experiments since it is commercially available. Addition of 30 mg/kg DM of sarsaponin (*Y. schidigera* extract) to a diet of hay: concentrate (1:1) did not increase the body mass gain of sheep (*76*). Supplementation of 150 mg/day of sarsaponin and 1% urea in corn silage improved the body mass gain of steers during the first 28 days of an experiment (*108*), but by the end of the trial (62 days), there was no significant treatment effect. Supplementation with *E. cyclocarpum* leaves increased the body mass gain of sheep fed oaten chaff from 93 (unsupplemented) to 115 g/day (supplemented) (*53*) and that of sheep fed *Pennisetum clandestinum* from 19.8 (unsupplemented) to 29.7 g/day (supplemented) (*55*). Sapindus extract added every 3 days to a rice straw diet improved the body mass gain of sheep from 44.8 (control) to 54.8 g/day (saponin addition) (*61*), and daily addition to a high roughage diet (65% roughage in the diet) enhanced the body mass gain growth of sheep from 36.9 (control) to 53.2 g/day (saponin addition) (*80*). Improvements in body mass may be associated with high roughage diets. Supplementation of a low roughage diet (90% concentrate) with 150 mg/kg Yucca powder did not improve the weight gain of male lambs (*109*). Even the addition of 250 mg /kg of Yucca extract to a mixed diet (45% hay and 50% rolled barley, 5% soybean meal) did not enhance the growth of steers (*106*). Although there was no significant increase in body mass gain, the inclusion of 40 mg Quillaja saponins/kg in a low roughage diet (30% hay) induced a higher response of the male lambs (315 g/day) than the females (239 g/day) (*110*).

There are very few studies on the effects of saponins on wool or milk production. Two reports showed that wool growth rate increased by 27 (*53*) and 47% (*55*) when sheep were supplemented with *E. cyclocarpum* suggesting that the feeding of *E. cyclocarpum* increased the amount of absorbed amino acids.

Three studies reported that the milk yield from moderate or high yielding cows (20−30 kg/day) was not affected by adding Yucca extract to diets containing 20% crude protein (*75*, *107*) or less than 10% crude protein (*104*). Furthermore, the percentage and yield of milk fat and the percentage and yield of total solids were unaffected by Yucca extract (*107*). Future research is required to determine the effects of dietary supplementation of saponins on milk production in low yielding cows.

EFFECTS ON RUMINANT REPRODUCTION

Because the chemical structure of saponins is close to the structure of some hormones, saponins could influence the activity or the level of several hormones (*111*−*113*). There is no information on the effect of saponin on hormone level in the ruminant. There was a report that Quillaja saponins stimulated the production of luteinizing hormone (LH) in fish (*113*). In this fish study, saponins also retarded egg production and caused sex inversion from female to male suggesting that saponins or their metabolites may be useful in controlling reproduction in aquaculture but may be harmful in ruminant. It is very important to see the effect of saponins on ruminant reproduction in more detail.

S. sesban induced necrosis of the seminiferous tubules and adversely affected spermatogenesis in sheep and goats (*114*). Woldemeskel et al. (*114*) suspected that saponin was the cause of this negative effect as Norton (*115*) had previously reported that saponin isolated from *S. sesban* seeds exhibited spermicidal and hemolytic activities. However, this needs to be proven since the saponins in the leaves may have a different structure and activity from those in the seeds.

TOXIC EFFECTS OF SAPONINS

Besides some beneficial effects on ruminants, some saponin-containing plants are toxic (**Table 4**). Saponin toxicity leads to photosensitization followed by liver and kidney degeneration in ruminants and gut problems such as gastroenteritis and diarrhea (*119*, *123*, *127*).

Brachiaria decumbens is one grass that was reported to cause photosensitization in animals. Interestingly, this only occurs in some areas, and even then, not all the animals in a flock or herd were affected. Therefore, *B. decumbens* is listed in **Table 1** as forage and not as a toxic plant.

Most of the toxic plants containing saponins in **Table 4** are found mainly in rangelands in the United States. Examples such as corn cockle, soapwort, cow cockle, and broomweed cause serious toxicity problems for grazing livestock. Alfombrilla (*Drymaria arenaroides*) is a weed in northern Mexico containing 2.8% saponins that is responsible for cattle losses with the potential to spread to the southwest United States (*116*). *Tribulus terrestris*, an annual weed common on semiarid rangelands in Australia, South Africa, the United States, and Iran also induces hepatogenous photosensitization (*117*, *118*).

When animals suffer from photosensitization, weeds containing saponins are usually the first suspects, although many toxic weeds or plants have yet to be analyzed for their saponin content. Most saponins that have been identified in the toxic plants are steroidal saponins.

Table 4. Saponin-Containing Plants That Are Toxic

family and species	plant part	saponin or sapogenin	toxicity	ref
		Poaceae		
Panicum dichotomiflorum (fall panicum)	leaf	dichotomin (diosgenin type)	photosensitization	*120*, *122*
Panicum schinzii (swamp panicum)	leaf	diosgenin saponin	photosensitization	*120*
Panicum coloratum (klein grass)	leaf	diosgenin saponin	photosensitization	*123*
Panicum virgatum (switch grass)	leaf	diosgenin	photosensitization	*124*
		Zygophyllaceae		
T. terrestris (puncture weed)	leaf	terrestrioside	photosensitization	*125*, *126*
		Chenopodiaceae		
Kochia scoparia (goosefoot)	leaf, fruit	sulfate saponins	liver and kidney degeneration	*123*
		Asteraceae		
Gutierrezia sarothrae (snake weed, broomweed)	leaf	saponin	gastoenteritis	*127*
		Liliaceae		
N. ossifragum	leaf, flower	sarsapogenin	liver degeneration	*128*
		Caryophyllaceae		
Agrostemma githago (corn cockle)	seed	githagenin		*122*
Drymaria arenariodes (alfombrilla)	leaf	saponin	death	*123*
Saponaria officinalis (bouncing bet, soapwort)	leaf	saponariosides	liver degeneration and death	*129*
		Fabaceae		
Sesbania drummondii (coffee bean, rattlebox)	leaf	saponin	hemorrhagic diarrhea	*123*
Sesbania punicea (red or purple sesbania)	leaf	saponin	liver and kidney degeneration	*123*
Sesbania vesicaria (annual bladderpod)	leaf	saponin	liver and kidney degeneration	*123*
		Sapotaceae		
Bassia latifolia	seed	mowrin	photosensitization	*127*
		Agavaceae		
Agave lecheguilla (lechuguilla)	leaf	hepatotoxic saponins	photosensitization, death	*123*
Nolina texana (beargrass, sacahuista)	fruit	hepatotoxic saponins	photosensitization	*123*
		Asteraceae		
Tetradymia glabrata (horsebrush)	flower	hepatotoxin saponin	photosensitization	*123*

Photosensitization is indicated by skin lesions caused by reaction of an exogenous compound in the blood with UV light, producing free radicals that react with tissue proteins. This is classified as a primary photosensitization. The affected animals develop photophobia and severe dermatitis. Secondary photosensitization is a result of liver damage or damage of bile ducts thus impeding the excretion of bile, causing chlorophyll metabolites such as phylloerythrin to circulate in the blood (*117*, *119*).

Photosensitization is associated with the appearance of biliary crystals in the bile and in the liver, which has been identified as the insoluble salts of steroidal sapogenin (*117*, *94*). Lithogenic (crystal-forming) compounds such as diosgenin and yamogenin saponins may cause photosensitization, whereas titogenin, neotigogenin, and gitogenin saponins are nonlithogenic compounds, which do not cause photosensitization. Feeding isolated saponin from *N. ossifragum* to sheep, however, failed to induce photosensitization unless the sheep were fed with large quantities of this saponin (*93*). It was suggested that mycotoxin (such as sporidesmin) may have synergistic effects with saponins and caused photosensitization (*120*). However, the fungi producing sporidesmin only grow on certain plants but not on *N. ossifragum*, which causes photosensitization (*121*). A more detailed study on the mechanism of saponins causing photosensitization needs to be pursued.

In conclusion, despite a considerable amount of research, it is not yet possible to clearly explain why some saponins induced beneficial effects while others caused toxicity. Saponins are present in the plants in various forms, which may result in having different activities. An understanding of the structure−activity relationship between saponins and antiprotozoal activity is required to recommend a certain saponin for defaunation purpose. Studies to find the precise chemical structures of these saponins are urgent so that optimal doses of saponins could be recommended to solve the problem of inefficient N retention in ruminants. Few studies indicated a growth-promoting effect of saponins, but it is still difficult to explain the mechanism of action of saponins or their metabolites that enhanced growth although some findings showed an increase in microbial protein synthesis. This growth-promoting effect was evident in the high roughage diet suggesting that the application of saponins or saponin-containing plant materials may be beneficial for the subsistence farmers in developing countries and also for the feed industry if the formulations of the saponin-containing diet and are optimized industrially.

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