Effects of Fractions Containing Saponins from *Yucca schidigera*, *Quillaja saponaria*, and *Acacia auriculoformis* on Rumen Fermentation

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Fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria*, and *Acacia auriculoformis* on incubation in an in vitro rumen fermentation system containing hay as a substrate increased the efficiency of microbial protein synthesis as judged by (i) a gravimetric approach, (ii) ^{15}N incorporation into microbial protein, and (iii) purine levels in microbes. The true degradability of hay at 24 h of incubation was either not affected or increased by saponins, and the gas production, which is a measure of short-chain fatty acids and fermentative gases, was lower or similar. *Yucca* and *Quillaja* saponins decreased the rate of gas production when hay or a mixture of hay and concentrate (70:30) was used as substrate, but *Acacia* saponins increased the rate of gas production from the hay plus concentrate mixture. The potential extent of gas production from both of the substrates was lower in the presence of *Acacia* saponins. *Yucca* saponins also decreased the potential extent of gas production, but the decrease was lower compared to *Acacia* saponins. Ammonia levels and protozoal counts at 24 h in an in vitro rumen fermentation system were also reduced by saponins; the decreases were as high as 30 and 63%, respectively.

Keywords: *Saponins; yucca; quillaja; Acacia; rumen fermentation; microbial efficiency; defaunation*

INTRODUCTION

The beneficial effects of supplementing animal feed with extracts from *Yucca schidigera* and *Quillaja saponaria* on the performance and health of various livestock species are well documented (Mader and Brumm, 1987; Anthony et al., 1994; Cheeke, 1996; Cline et al., 1996). It has been suggested that one of the reasons for the beneficial effects of *Y. schidigera* and *Q. saponaria* extracts is that these influence microbial fermentation (Van Nevel and Demeyer, 1990; Wallace et al., 1994; Cheeke, 1996; Makkar and Becker, 1996; Killeen et al., 1998). The beneficial effects have also been attributed to binding of ammonia to saponins and glyco components present in these extracts (Anthony et al., 1994; Hussain and Cheeke, 1995; Hussain et al., 1996). The in vivo inhibition of microbial urease activity by *Y. schidigera* extracts, once thought to be the main mechanism for the in vivo effects of the extract, now appears untenable on the basis of studies in vitro (Killeen et al., 1994). The extracts of these two plants have been used for various food applications (Price et al., 1987). These have also been categorized under GRAS (generally recognized as safe) for human consumption by the U.S. Federal Drug Administration (Fenwick et al., 1992). Due to this status, these plant extracts have lately received the attention of several groups. Besides their traditional use as foaming agents in carbonated beverages and as flavor enhancers in foods, various novel applications have recently been proposed: (i) as adjuvants for human vaccines (presently used in veterinary vaccine) (Rouhi, 1995); (ii) to decrease the ammonia level in the atmosphere (Crober, 1991); (iii) to suppress or stimulate microbial growth (Price et al., 1987; Wallace et al., 1994; Rouhi, 1995; Sen et al., 1998); (iv) to increase binding of ammonia during urea ammoniation of straw and binding of ammonia in the soil (Makkar et al., 1998); and (v) to reduce odors from cattle manure in dairy barns (Giesy et al., 1992). The *Y. schidigera* and *Q. saponaria* plants are rich in saponins (Price et al., 1987). The plant *Acacia auriculoformis* is widely distributed throughout India and produces large amounts of fruits, which give copius froth when shaken with water. Some saponins of *A. auriculoformis* fruits have been characterized and are triterpenoid in nature (Mahato, 1996). This study presents in vitro effects of saponin fractions from extracts of *Y. schidigera* and *Q. saponaria* plants and *A. auriculoformis* fruits on various rumen fermentation parameters such as rate and potential extent of gas production, true degradability, microbial efficiency, ammonia level, and protozoal count.

MATERIALS AND METHODS

Y. schidigera, available commercially in a powder form under the commercial name DK Sarsaponin 30 (Desert King), was extracted with distilled water. Distilled water (250 mL) was added to 30 g of the powder, stirred for ~3 h at room temperature using a magnetic stirrer, and then filtered. The filtrate was divided into two parts. One part was freeze-dried and designated *Yucca* aqueous extract. The second part was treated with *n*-butanol as described by Newbold et al. (1997) and Thilborg et al. (1993) to obtain saponin fractions. This fraction was designated *Yucca* saponins. The aqueous extracts of the *Q. saponaria* plant (Quiponin S; Nor-feed) and *A. auriculoformis* fruits (collected near Calcutta, India; air-dried and powdered) were also subjected to identical treatment to obtain *Quillaja* and *Acacia* saponins, respectively.

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Rate and Potential Extent of Gas Production. The samples (200 mg) of hay or a mixture of hay and concentrate (70:30% w/w) were incubated in triplicate in graduated syringes with 30 mL of the in vitro medium containing rumen liquor (Menke and Steingass, 1988) in the absence and presence of saponin fractions (0.6 and 1.2 mg/mL). At 2, 4, 6, 8, 10, 12, 24, 30, 36, 48, 54, 60, 72, and 96 h, gas values were recorded. The potential extent (*b*) and rate (*c*) of gas production were determined using a one-pool exponential model, $y = b(1 - e^{-ct})$, where *y* is the gas produced at time *t* (Blümmel et al., 1997a).

True Degradability and Microbial Mass. For determination of true digestibility, 500 mg of samples (hay) was incubated with 40 mL of buffered medium containing rumen fluid (Makkar et al., 1995a) in the absence and presence of fractions (0.6 mg/mL) containing saponins. True digestibility was determined at 24 h of incubation by treating the syringe contents with the neutral detergent solution to obtain truly undegradable residue. Truly digested substrate was the difference in weight between the sample taken for incubation and the truly undegradable residue obtained (Makkar et al., 1995a). The contents of a parallel set of syringes were subjected to high-speed centrifuge (20000*g*) for determination of apparent residue, and the microbial mass was quantified as the difference between apparent and true undegradable residue according to the method of Blümmel et al. (1997b,c).

For the above-mentioned studies, the rumen fluid was collected before the morning feeding from a cow fed a roughage diet.

Other Analyses. For measurement of ^{15}N incorporation, fermentation was carried out in the usual buffer containing 25% of the total ammonium carbonate consisting of highly enriched (96.5%) ^{15}N. The ^{15}N measurement and counting of protozoa were as mentioned earlier (Makkar et al., 1995a,b). Purines in the apparent residue, a measure of microbial mass, were measured according to the method of Balcells et al. (1992). Ammonia was quantified by using the phenol−hypochlorite method (Weatherburn, 1967).

Statistical Analysis. The significance of differences between means was compared using Duncan's multiple range test after ANOVA for one-way classified data with the aid of SGC (1991). A level of $P < 0.05$ was chosen as the minimum for statistical significance.

RESULTS AND DISCUSSION

Rate and Potential Extent of Gas Production. When hay was used as the substrate, *Yucca* and *Acacia* saponins decreased the potential extent of gas production significantly at both 0.6 and 1.2 mg/mL, whereas *Yucca* aqueous extract had no effect. On the other hand, *Quillaja* saponins had no effect at 0.6 mg/mL but increased the potential extent of gas production significantly at a level of 1.2 mg/mL. The rate of gas production decreased at a level of 0.6 mg/mL by *Quillaja* and *Acacia* saponins. At the higher level of 1.2 mg/mL, *Yucca* and *Quillaja* saponins decreased but *Acacia* saponins increased the rate of gas production (Table 1).

Using the hay/concentrate mixture as the substrate, the potential extent of gas production decreased significantly at both levels of *Yucca* and *Acacia* saponins. *Yucca* saponins had no effect on the rate of gas production at eitherboth level, although they decreased the potential extent of gas production significantly. On the other hand, *Acacia* saponins increased the rate of gas production. Only *Quillaja* saponins decreased the rate at both levels (Table 1).

It may be inferred from the above that all of the saponins either decreased the rate of gas production significantly or had a tendency to decrease it when hay was the substrate. Only *Acacia* saponins increased the rate of gas production when hay plus concentrate was used as the substrate. Similarly, all of the saponins studied either decreased the potential extent of gas production significantly or had no effect. Commercially available *Quillaja* saponins from Roth (Karlsruhe, Germany) also affected the rate (decrease) and potential extent of gas production (no effect) in a pattern similar to the butanol fraction of quillaja bark used in the present study (Makkar and Becker, 1996).

Table 1. Effect of Fractions Containing Saponins on Potential Extent of Gas Production (*b*) and Fractional Rate (*c*) at Which *b* Is Produced per Hour[a]

	c	*b* (mL)
Hay		
control	0.0685 ± 0.0032a	47.3 ± 0.29a
Yucca saponins (0.6 mg/mL)	0.0639 ± 0.0013abd	45.5 ± 0.15b
Yucca saponins (1.2 mg/mL)	0.0599 ± 0.0007bc	42.0 ± 0.27c
Yucca aq extract (0.6 mg/mL)	0.0661 ± 0.0052a	47.0 ± 0.39a
Yucca aq extract (1.2 mg/mL)	0.0651 ± 0.0054ad	46.3 ± 0.95ab
Quillaja saponins (0.6 mg/mL)	0.0589 ± 0.0025bc	46.5 ± 0.49ab
Quillaja saponins (1.2 mg/mL)	0.0576 ± 0.0007c	47.0 0.42a
Acacia saponins (0.6 mg/mL)	0.0609 ± 0.0019cd	39.1 ± 0.54d
Acacia saponins (1.2 mg/mL)	0.0682 ± 0.0019a	37.7 ± 0.55e
Hay + Concentrate (70:30)		
control	0.0713 ± 0.0006a	51.9 ± 0.89a
Yucca saponins (0.6 mg/mL)	0.0676 ± 0.0012a	47.8 ± 2.43b
Yucca saponins (1.2 mg/mL)	0.0662 ± 0.0012a	46.2 ± 0.96b
Yucca aq extract (0.6 mg/mL)	0.0687 ± 0.0043a	52.8 ± 0.75a
Yucca aq extract (1.2 mg/mL)	0.0687 ± 0.0016a	54.0 ± 0.70a
Quillaja saponins (0.6 mg/mL)	0.0631 ± 0.0011b	53.3 ± 0.97a
Quillaja saponins (1.2 mg/mL)	0.0622 ± 0.0005b	52.9 ± 1.18a
Acacia saponins (0.6 mg/mL)	0.0778 ± 0.0026c	45.7 ± 0.81b
Acacia saponins (1.2 mg/mL)	0.0901 ± 0.0028d	44.4 ± 0.56b

[a] Values are mean ± SE. Means followed by dissimilar letters in the same vertical column differ significantly at $P < 0.05$.

Efficiency of Microbial Protein Synthesis Using Hay as Substrate. Fractions containing saponins at a level of 0.6 mg/mL were used in this experiment. Addition of *Yucca* or *Acacia* saponins decreased the gas production at 24 h significantly, whereas the two fractions (*Yucca* aqueous extract and *Quillaja* saponins) had no effect (Table 2). On the basis of the gas production only, one would conclude that *Yucca* and *Acacia* saponins have an adverse effect on the digestion of hay, whereas the other two fractions do not have any effect. In the gas method, substrate truly degraded during fermentation leads to three products: (i) short-chain fatty acids (SCFA): (ii) fermentative gases; and (iii) microbial mass production (Blümmel et al., 1997b). The gas in the in vitro systems is only a measure of SCFA production and fermentative gases and does not necessarily reflect microbial mass production during the fermentation. The microbial mass quantified as the difference between apparent and truly undegradable residue (Blümmel et al., 1997b) is also presented in Table 2. The microbial mass was higher for *Yucca* and *Acacia* saponins for which the gas production was lower than for the control. For *Quillaja* saponins the gas production was similar to that of the control and higher microbial mass production was observed. In the presence of *Acacia* saponins, there was a shift in the partitioning of fermented substrate. The same amount of truly degraded substrate (300 mg for control and 298 mg for control plus *Acacia* saponins) was partitioned to SCFA and microbial mass to a different extent (Table 2). *Acacia* saponins decreased SCFA production and increased microbial mass production. On the other hand, *Quillaja* saponins increased the extent of truly degraded substrate from 300 to 323 mg without a change in gas production (95.3 versus 94.5 mL). In the presence of *Quillaja* saponins, the extra amount of truly

Table 2. Effect of Fractions (0.6 mg/mL) Containing Saponins on in Vitro Gas and Microbial Mass Production and Truly Degraded Substrate at 24 h of Fermentation[a]

	gas (mL)	microb mass (mg)	microb mass (μmol of purines)	N in app residue[b] (mg)	microb mass (mg of ^{15}N in apparent residue[b])	truly degraded substrate (mg)	efficiency of microb protein synthesis (μmol of purines/mL of gas)	efficiency of microb protein synthesis ^{15}N/mL of gas
control (hay)	95.3 ± 0.52ac	90	6.94 ± 0.21a	5.95 ± 0.14a	0.487 ± 0.008ac	300.0 ± 5.95a	0.0728	5.11
Yucca saponins	91.2 ± 0.52b	122.9	9.11 ± 0.20b	7.15 ± 0.35b	0.592 ± 0.026b	320.7 ± 4.1b	0.0998	6.49
Yucca aq extract	96.7 ± o.58a	105.8	7.20 ± 0.19a	5.36 ± 0.23a	0.454 ± 0.014a	320.4 ± 4.86b	0.0744	4.69
Quillaja saponins	94.5 ± 1.01c	119.2	7.99 ± 0.26c	7.32 ± 0.16b	0.538 ± 0.089bc	323.0 ± 0.58b	0.0845	5.69
Acacia saponins	83.5 ± 0.17d	121.0	8.38 ± 0.23c	7.59 ± 0.48b	0.528 ± 0.026c	297.6 ± 6.0a	0.1004	6.32

[a] Values are mean ± SE per syringe. Means followed by dissimilar letters in the same vertical column differ significantly at $P < 0.05$. [b] Apparent residue: undigested feed + microbial mass; obtained using high-speed centrifugation (20000*g*).

degraded subtrate was channeled toward higher microbial mass production without any change in gas production. These results suggest that there could be (i) the same amount of truly degraded substrate but different microbial mass and gas production as for *Acacia* saponins, (ii) the same volume of gas production but different microbial mass production depending on the extent of truly degraded substrate as for *Quillaja* saponins, and (iii) occurrence of phenomena i and ii together, as for the other two fractions.

The efficiency of microbial protein synthesis, expressed as milligrams of microbial mass produced per 100 mg of truly degraded substrate, was 30.0 for the control and 38.3, 33.0, 36.9, and 40.7 for *Yucca* saponins, *Yucca* aqueous extract, *Quillaja* saponins, and *Acacia* saponins, respectively. The efficiency of microbial protein synthesis is also generally expressed as milligrams of microbial mass production per millimoles of SCFA. The gas and SCFA production in the in vitro gas system are very closely (positively) associated stoichiometrically (Blümmel and Orskov, 1993; Makkar et al., 1995a). Therefore, the efficiency of microbial protein synthesis in the gas method can also be expressed as milligrams of microbial mass production per milliliter of gas, which was also higher in the presence of saponins. The results using purines as a marker for microbial protein or incorporation of ^{15}N into microbial protein were also similar (Table 2). Higher microbial mass production and higher efficiency of microbial protein synthesis are not reflected by measurement of gas alone in the in vitro gas method. In addition, rate and potential extent of gas production may not be considered as the rate and potential extent of feed degradation, but rather as rate and potential extent of SCFA production. At 0.6 mg/mL, there was no change in the potential extent of gas production by *Yucca* saponins, *Yucca* aqueous extract, and *Quillaja* saponins (Table 1), whereas truly digested substrate was higher with these three saponins (Table 2). The lower rate of gas production in the presence of saponins appears to be indicative of better synchronization of substrate degradation and ATP production with microbial mass production leading to higher efficiency of microbial protein synthesis. Higher ratios of microbial mass (determined gravimetrically) to purines or of microbial mass to ^{15}N values in the presence of saponins compared to their absence (Table 2) indicate change in the composition of microbes in the presence of saponins.

Ammonia Levels in the in Vitro Rumen Fermentation System. Ammonia levels were lower in the presence of fractions containing saponins (Table 3). Similar results have been reported by Wallace et al. (1994) and Hussain and Cheeke (1995). The observed ammonia levels represent a balance between the two processes: degradation of feed proteins and uptake of ammonia for the synthesis of microbial protein. Higher efficiency of microbial protein synthesis in the presence of fractions containing saponins (Table 2) suggests that one of the reasons for the lower ammonia levels could be the higher incorporation of ammonia, peptide, or amino acids into microbial protein. *Yucca* extract and *Quillaja* saponins have been shown to reduce proteolysis (Wallace et al., 1994; Makkar and Becker, 1996). The improved efficiency of utilization of nitrogen in the rumen was suggested to be due to binding of ammonia to yucca extract when the ruminal ammonia concentration is high and release of the bound ammonia when its concentration is low in the rumen, thus modulating diurnal fluctuations in ruminal ammonia concentrations to provide a continuously adequate amount of ammonia for microbial metabolism (Hussain and Cheeke, 1995). This would also spare energy otherwise needed for conversion of excessive ammonia to urea in the liver and its excretion. The defaunating effect of saponins (see below) could also contribute to the lower level of ammonia in the rumen fermentation system.

Table 3. Ammonia Levels in in Vitro Rumen Fermentation System at 24 h[a]

	ammonia level (mg of N/mL)	reduction in ammonia (%)
Hay		
control	0.261	
Yucca saponins (0.6 mg/mL)	0.219	16.1
Yucca saponins (1.2 mg/mL)	0.184	29.7
Yucca aq extract (0.6 mg/mL)	0.239	8.4
Yucca aq extract (1.2 mg/mL)	0.222	14.9
Quillaja saponins (0.6 mg/mL)	0.238	8.8
Quillaja saponins (1.2 mg/mL)	0.222	14.9
Acacia saponins (0.6 mg/mL)	0.247	5.4
Acacia saponins (1.2 mg/mL)	0.223	14.6
Hay + Concentrate (70:30)		
control	0.272	
Yucca saponins (1.2 mg/mL)	0.190	30.0
Yucca aq extract (1.2 mg/mL)	0.210	22.8
Quillaja saponins (1.2 mg/mL)	0.239	12.1
Acacia saponins (1.2 mg/mL)	0.231	15.1

[a] Values are average from two observations; the deviation of each value from the mean was not more than 5%.

Protozoal Count in the in Vitro Rumen Fermentation System. All of the fractions tested decreased protozoal count. The effect was highest with *Acacia* saponins, followed by *Quillaja* and *Yucca* saponins (Table 4). Purified saponins from *Saponaria* sp. and *Quillaja* bark and *Yucca* extract have been shown to have antiprotozoal activity (Wallace et al., 1994). A butanol fraction of *Sesbania sesban* containing saponins or saponin-like moieties has also been shown to have a toxic effect on rumen protozoa (Newbold et al., 1997).

Table 4. Protozoal Count in in Vitro Rumen Fermentation System at 24 h[a]

	protozoal count ($\times 10^6$)	reduction in protozoa (%)
Hay		
control	37	
Yucca saponins	28	24.3
Yucca aq extract	32	13.5
Quillaja saponins	23	37.8
Acacia saponins	20	45.9
Hay + Concentrate (70:30)		
control	57	
Yucca saponins	29	49.1
Yucca aq extract	52	8.8
Quillaja saponins	26	54.4
Acacia saponins	21	63.1

[a] Concentration of fractions was 1.2 mg/mL. Values are average from two incubation vessels (syringes); the deviation of each value from the mean was not more than 8%.

The antiprotozoal effect of *Sapindus saponaria* has also been attributed to saponins (Navas-Camacho et al., 1994). The detergent action of saponins kills rumen protozoa. The susceptibility of rumen protozoa and lack of susceptibility of rumen bacteria to saponins is probably explained by the presence of cholestrol in eukaryotic membranes (including protozoa) but not in prokaryotic bacterial cells (Klita et al., 1996). Antiprotozoal effects of saponins have been reported in a semicontinuous fermentation system and in vivo [see Wallace et al. (1994), Cheeke (1996), and Newbold et al. (1997)]. Defaunation has been shown to have several advantages for ruminants. Suppression or elimination of protozoa may enhance the flow of microbial protein from the rumen, increase the efficiency of feed utilization, and improve the nutrition of the animal, provided the loss of protozoa does not impair the fiber breakdown (Newbold et al., 1997). It may also benefit rumen fermentation as is evident from the lower ammonia values and higher efficiency of microbial protein synthesis (Tables 2 and 3). A higher propionate-to-acetate ratio has also been observed in the presence of a saponin-rich feed (Bonsi et al., 1995).

Conclusions. The substrate degraded leads to the formation of SCFA, fermentative gases, and microbial mass. Higher microbial mass and lower gas production in the in vitro fermentation system coupled with the same or higher true digestibility of substrate in the presence of saponins suggest that saponins partition the nutrients in such a manner that a higher proportion of the digested substrate goes to the formation of microbial mass and a lower proportion to SCFA and gases. The incorporation of saponins into ruminant diets, in particular roughage-based diets, might be advantageous as it would lead to a higher microbial yield and lower emission of environment-polluting gases (CO_2 and CH_4). *Yucca*, *Quillaja*, and *Acacia* saponins have potential as feed additive to manipulate rumen fermentation for higher animal productivity and/or reduce environmental pollution. The possible use of natural plant products as a productivity enhancer provides cheaper, safer, and more consumer-acceptable alternatives to synthetic compounds. The concentrations of saponins, especially of yucca saponins used in in vitro systems, are higher than the levels generally in use for agricultural applications. It would be interesting to investigate the importance in vivo of the mechanisms proposed in vitro and to exploit the beneficial effects of saponins observed in in vitro studies for achieving higher animal productivity by incorporating saponins in feeds at concentrations that are safe for the animal and economically viable.

The measurement of gas released on incubation of a feedstuff in in vitro gas methods is easy, and therefore these methods have been used widely to characterize feedstuffs and to study effects of plant defensive components. The measurement of gas alone is not adequate and can lead to misleading conclusions. Measurement of gas should be complimented with measurement of true digestibility of substrate and/or with measurement of microbial mass using internal or external markers.

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