

Saponin content of lucerne fodder and its effect on rumen fermentation and microbial population in crossbred bulls

P K MALIK¹ and K K SINGHAL²

National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 10 March 2007; Accepted: 4 October 2007

ABSTRACT

Among the 3 different cuts of lucerne fodder, highest saponin content (2.0% on DM basis) was recorded in second cut. Effect of lucerne fodder (second cut) or its saponin content i.e. 60 mg/kg DM was determined on DMI, rumen fermentation and microbial population in crossbred bulls. Six male crossbred (Holstein Friesian × Sahiwal) bulls were randomly divided into 2 groups of 3 each on the basis of their body weights. Group 1 was fed on TMR-1 consisting of wheat straw and concentrates mixture (60:40) while group 2 was fed on TMR-2 consisting of wheat straw, lucerne fodder (second cut) and concentrate (30:30:40). DM intake, $\text{NH}_3\text{-N}$ (mg/100ml) and TVFA (m mol/l) concentration was not affected significantly by the dietary supplementation of lucerne fodder at 30% level (saponin content 60 mg/kg DM). However, saponin supplementation decreased ($P<0.05$) molar proportion of the acetate with an increase ($P<0.05$) in propionate while butyrate remained uninfluenced. Population of total anaerobic and cellulolytic bacteria was numerically higher on feeding TMR-2 than on TMR-1, however, methanogenic bacteria remained unaffected by the saponins supplementation. Protozoal population in the rumen decreased significantly following the supplementation of lucerne fodder or saponins. Saponin content of various herbs or feed may be explored in details.

Key word: Lucerne fodder, Rumen fermentation, Rumen microbe, Saponins

Supplementation of crop residues based diets with green leguminous fodders is a traditional feeding practice in India due to their high palatability and improved nutrient utilization (Krebs *et al.* 1989, Das and Singh 1999). Lucerne (*Medicago sativa*) fodder is rich source of saponins and affected by cultivars, soil and agronomic practices etc. Supplementation of green forage to the wheat straw based diet increase DM intake, digestibility, improve rumen fermentation and of course the type of the microbes prevail in the rumen. Keeping these facts in mind, the study was carried out to estimate the saponin content of lucerne fodder at 3 different cuts and its supplementation having highest saponin content on rumen fermentation and microbial population in crossbred bulls.

MATERIALS AND METHODS

Saponin content of lucerne (*Medicago sativa*) fodder grown at institute farm was estimated at its first, second and third cuts as per Yosioka *et al.* (1974). On the basis of the highest saponin content measured in first phase of the study in all the 3 different cuts, lucerne fodder of second cut was

selected for the supplementation of total mixed ration (TMR) at 30% level based on wheat straw and concentrate mixture to investigate the effect of saponins rich lucerne fodder on rumen fermentation and rumen microbial population namely total, cellulolytic and methanogenic bacteria as well as protozoa. The level of supplementation at 30% was selected because in another *in vitro* study this level was found more promising and efficient to mitigate the methane production. For this purpose 6 male crossbred (Holstein Friesian × Sahiwal) bulls were randomly divided into 2 groups of 3 each on the basis of their body weights. Group 1 was fed on TMR-1 consisted wheat straw and concentrate mixture (60:40) while group 2 was fed on TMR-2 consisted wheat straw, lucerne fodder (second cut) and concentrate mixture (30:30:40). The roughage: concentrate ratio in both the groups was maintained at 60:40 and both the rations were isonitrogenous. Chemical composition of different ingredients used for the formulation of TMR-1 and TMR-2 are presented in Table 1. Feeding of the animals was done as per NRC (2001). After a preliminary feeding for 21 days, rumen liquor samples were collected for 3 consecutive days by inserting a flexible rubber pipe (about 1" diameter) into rumen via mouth, through which a polythene tube was introduced for sucking the rumen liquor sample with the help

Present address: ¹SRA, Division of Animal Nutrition, IVRI, Izatnagar 243 122.

²Principal Scientist, Dairy Cattle Nutrition Division.

Table 1. Chemical composition of feeds

Feed	DM	% on DM basis					
		OM	CP	EE	ADF	NDF	Ash
Wheat straw	90.04	93.18	3.00	1.12	51.80	76.20	6.19
Lucerne fodder (Second cut)	23.18	89.42	16.70	3.20	35.83	43.64	10.58
Concentrate mixture* (TMR-1)	94.90	92.30	25.7	3.92	14.23	39.59	7.70
Concentrate mixture** (TMR-2)	95.16	93.29	15.75	3.68	13.97	36.48	6.71

*Consisted maize grain 33%, groundnut cake 20.2%, mustard cake 12%, wheat bran 20%, deoiled rice bran 11%, urea 0.8%, mineral mixture 2% and common salt 1%.

**Consisted maize grain 24%, groundnut cake 15%, mustard cake 8%, wheat bran 42%, deoiled rice bran 8%, mineral mixture 2% and common salt 1%.

of plastic syringe. Strained rumen liquor was stored into an insulated container, to maintain the anaerobic condition. The pH of rumen liquor samples was checked with the help of digital pH meter and brought to the laboratory for the analysis of ammonical nitrogen (Conway 1957), TVFA (Barnett and Reid 1957) and molar proportions of individual fatty acids (Erwin *et al.* 1961) using gas liquid chromatography.

Total anaerobic bacteria, cellulolytic and methanogenic bacteria (log cfu/ml) as well as protozoal population (log cfu/ml) were counted in rumen liquor samples collected from both the groups. Total and cellulolytic bacteria in rumen liquor samples were enumerated as per Hungate (1957); methanogenic bacteria in the samples were enumerated as per Ranade and Gadre (1988) using roll tube technique. Roll tube was incubated in an upright position at $39^{\circ}\pm 0.5^{\circ}\text{C}$ for 2 weeks for the enumeration of total and cellulolytic bacteria counts, 3 weeks for the enumeration of the methanogenic bacteria, and colonies developed on the agar film of the tube were enumerated at the end of the incubation period. Protozoal counts in the rumen liquor samples were enumerated with the help of microscope. The data thus obtained were subjected for the statistical analysis as per the method of Snedecor and Cochran (1986).

RESULTS AND DISCUSSION

Saponin content

Saponin content (on DM basis) varied between 0.92 ± 0.05 to $2.0\pm 0.05\%$ at 3 different cuts with an average of $1.36\pm 0.43\%$ (Table 2) in lucerne fodder. Saponin content was lower at the first cut and increased at second cut, however, at third cut, it decreased (lowest) dramatically almost to the level of first cut. The variation in saponin content among the 3 cuts was significant ($P<0.05$) however, the variation between first and third cut in terms of saponins content was not significant. Lower saponin content of lucerne fodder in the present study as compared to the values reported by Applebaum *et al.* (1969), Fenwick and Oakenfull (1983) may be attributed to the difference in cultivar and the agronomic

Table 2. Total saponin content of berseem and lucerne fodders (on% DM basis) at different cuttings

Cutting	Saponin (%)	Range
First cut	$1.17^a \pm 0.04$	1.16–1.19
Second cut	$2.00^b \pm 0.05$	1.99–2.01
Third cut	$0.92^a \pm 0.05$	0.92–0.93
Average	1.36 ± 0.43	

Values bearing different superscripts in a column differ significantly ($P<0.05$).

practices, followed for the production of lucerne fodder (Livingston *et al.* 1979).

DM intake

Dry matter intake in crossbred bulls did not vary (Table 3) significantly on the feeding TMR-2 (5.63 ± 0.16 kg/d) and TMR-1 (5.45 ± 0.10 kg/d). Higher DMI through TMR-2 may be attributed to the higher palatability and nutritional value of lucerne fodder than wheat straw. DMI on feeding TMR-1 and TMR-2 was 2.64 ± 0.09 and 2.79 ± 0.12 kg/100 kg BW/d, respectively. These values indicated that the dry matter intake on uniform body weight basis was similar and saponin content of lucerne fodder did not affect the intake. Saponin content of TMR-2 was calculated on the basis of its level in lucerne fodder (second cut, Table 2) and value was 0.60% of DM. Therefore, saponins intake in crossbred bulls fed TMR-2 was 33.78 g/day and this level did not affect feed intake.

Rumen fermentation

The pH value of the rumen fluid was higher on feeding TMR-1 (6.55 ± 0.14) than on TMR-2 (6.38 ± 0.15), however, the difference between the dietary treatments was not significant, showing that lucerne fodder or its saponin content did not affect rumen pH. Similar observations were recorded following the dietary incorporation of saponin through *Sapindus saponaria* (Abreu *et al.* 2004) or green alfalfa

fodder (Ngwa *et al.* 2001) in sheep. However, contrary to present observations Klita *et al.* (1996) reported a significant decrease ($P<0.01$) in rumen pH after intra ruminal administration of saponins of alfalfa root in sheep. Lower rumen ammonia N concentration was observed on incorporation of lucerne in the total mixed ration than the values reported by Grobner *et al.* (1982) and Hristov *et al.* (1999) following the dietary supplementation of Yucca saponins or sarsaponin in heifers. It may be attributed to the glyco component of the saponins, which bind ammonia N in rumen. Nonsignificant reduction of ammonia N concentration in present study, contrary to the above reports, may be attributed to the lower level of dietary saponins i.e. 0.60%. Nonsignificant variation between the dietary treatments for average TVFA concentration indicated that TVFA concentration in rumen fluid was not influenced by the dietary lucerne or its saponin content. Our results were corroborated with the findings of Abreu *et al.* (2004) and Lila *et al.* (2005). However, acetate proportion was decreased significantly ($P<0.05$) by 5–7 units with a simultaneous and significant increase ($P<0.05$) in propionate proportion by 2–3 units in the rumen fluid following the incorporation of lucerne fodder at 30% level in the total mixed ration, while proportion of butyrate remained unaffected by the supplementation. This change in rumen fermentation may be attributed to the saponin content of lucerne fodder as its saponins favour the propionic acid production. A/P ratio was also decreased by the supplementation of saponins rich lucerne fodder. Similar observations were recorded following the dietary incorporation of *Sapindus saponaria* (Diaz *et al.* 1993) and *Yucca schdigera* powder (Hristov *et al.* 1999) in the ration of ruminants. Wang *et al.* (2000) also reported a decrease in acetate with an increase in propionate production following the dietary incorporation of leguminous fodder or pure saponins.

Rumen microbes

Average total bacterial counts (log cfu/ml SRL) were 8.82 ± 0.10 and 9.78 ± 0.12 (Table 3) in crossbred bulls fed on TMR-1 and TMR-2, which showed that the population of total bacteria was increased 10.88% on the feeding of TMR-2 as compared to TMR-1, however, variation between the treatments was not significant. Similar to these observations, Lila *et al.* (2005) and Makkar and Becker (1997) also reported increase in bacterial population following the dietary incorporation of saponins or saponins rich feed to the control diet. The increase in the bacterial population could be attributed to the decreased protozoal counts in the study, which is predatory on bacteria. Rejil (2005) reported slightly increased bacterial counts from 25.33 to 27.83 ($\times 10^9$ /ml) following the incorporation of raw and roasted fenugreek seeds powder (source of saponins) to control diet. Cellulolytic bacteria counts (log cfu/ml SRL) on feeding TMR-1 and TMR-2 was 4.92 ± 0.17 and 5.18 ± 0.10 (Table 3), respectively.

Table 3. Effect of saponins rich lucerne fodder supplementation on rumen fermentation and microbial population in crossbred bulls

Attributes	TMR-1	TMR-2	Level of significance (at 5%)
Body Wt. (kg)	206.80 $\pm$ 4.4	202.00 $\pm$ 3.4	NS
DMI kg/d	5.45 $\pm$ 0.10	5.63 $\pm$ 0.16	NS
DMI kg/100 kg BW	2.64 $\pm$ 0.09	2.79 $\pm$ 0.12	NS
DMI g/kgW ^{0.75} BW	100.1 $\pm$ 0.49	105.0 $\pm$ 0.56	NS
pH	6.55 $\pm$ 0.14	6.38 $\pm$ 0.15	NS
NH ₃ -N (mg/100 ml)	30.84 $\pm$ 1.49	28.96 $\pm$ 1.18	NS
TVFA (m mol/l)	106.56 $\pm$ 1.02	107.25 $\pm$ 0.89	NS
Acetate (%)	74.90 ^b $\pm$ 0.49	69.54 ^a $\pm$ 0.68	1.03
Propionate (%)	15.93 ^a $\pm$ 0.40	17.67 ^b $\pm$ 0.26	0.97
A/P ratio	4.70:1 ^b	3.93:1 ^a	0.42
Total bacteria (log cfu/ml)	8.82 $\pm$ 0.10	9.78 $\pm$ 0.12	NS
Cellulolytic bacteria (log cfu/ml)	4.92 $\pm$ 0.17	5.18 $\pm$ 0.10	NS
Methanogens (log cfu/ml)	1.72 $\pm$ 0.14	1.71 $\pm$ 0.15	NS
Protozoa (log cfu/ml)	5.28 ^a $\pm$ 0.25	4.29 ^b $\pm$ 0.29	0.74

Values bearing different superscripts in a row differ significantly ($P<0.05$).

Though, the cellulolytic bacteria counts were numerically higher on feeding TMR-2 but data revealed a nonsignificant effect of supplementation of 60 mg saponins/kg DM intake through lucerne fodder. Contrary to these observations, Lila *et al.* (2005) reported a nonsignificant decrease in cellulolytic bacteria counts *in vitro*. Ningrant *et al.* (2002) and Wina *et al.* (2005) reported from their *in vitro* studies that the fibrolytic microbes responded differently, with no effect on *Fibrobacter* species up to a level of 4 mg saponin/ml and a negative effect on *Ruminococcus albus* and *Ruminococcus flavifacens* at levels higher than 1 mg/ml. In the present study, possibly the level of saponins in rumen fluid was far lower than the value reported by Ningrant *et al.* (2002) and Wina *et al.* (2005). Average methanogenic bacterial counts (log cfu/ml SRL) was 1.72 ± 0.14 and 1.71 ± 0.15 (Table 3) on feeding TMR-1 and TMR-2, respectively, which revealed that the supplementation of saponins through lucerne fodder @ 60 mg/kg DM did not affect the methanogenic bacteria significantly. Results of the study indicated that the supplementation of lucerne fodder at 30% level or its saponins content 60mg/kg DM did not affect methanogens significantly because saponins did not have any direct effect on methanogens and it caused reduction in protozoal numbers and thereby reduction in methane emission similar results were reported by Ningrant *et al.* (2002) and Wina *et al.* (2005).

The reduction in protozoal population following the feeding of TMR-2 was 18.75% as compared to the control treatment and the variation between treatments was significant ($P<0.05$). Significant reduction in protozoal population in rumen may be attributed to the saponin content of lucerne fodder as Lu and Jorgenson (1987) and Lila *et al.*

(2005) reported a similar reduction in protozoal population due to the dietary saponins. Less reduction in protozoal population in present study as compared to the others was possibly due to the low level of saponins intake. The reduction in protozoal population in present study was manifested in the increased proportion of propionic acid in the rumen fluid fed on TMR-2.

It can be inferred that the saponins content of lucerne fodder was higher at second cut as compared to other cuts and supplementation of total mixed ration (R:C 60:40) with second cut lucerne fodder at 30% level or its saponin content did not affect feed intake, $\text{NH}_3\text{-N}$ and TVFA concentration and bacterial population including that of methanogens in rumen. However, protozoal population was reduced significantly and propionate proportion in the rumen was increased significantly following the supplementation of lucerne or its saponins content.

REFERENCES

- Abreu A, Arulla J E, Lascano C E Diaz T E, Kreuzers M and Hess H D. 2004. Effect of *Sapindus saponaria* fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. *Journal of Animal Science* **82**: 1392-1400.
- Applebaum S W, Marco S and Brik Y. 1969. Saponin as possible factor of resistance of legume seeds to the attack of insects. *Journal of Agriculture and Food Chemistry* **17**: 622.
- Barnett A J G and Reid R L. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. The volatile fatty acid production from fresh grass. *Journal of Agricultural Science, Cambridge* **48**: 315.
- Conway E J. 1957. *Microdiffusion Analysis and Volumetric Error*. 5th edn. Crosson Lockwood and Sons Ltd., London.
- Das A and Singh G P. 1999. Effect of different proportions of berseem and wheat straw on *in vitro* dry matter digestibility and total gas production. *Indian Journal of Animal Nutrition* **16**(1): 60-64.
- Diaz A, Avendon O M and Escobar A. 1993. Evaluation of *Sapindus saponaria* as a defaunating agent and its effect on different ruminal digestion parameters. *Livestock Research and Rural Development* **5**: 1-10.
- Erwin E S, Moero G A and Emery E M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *Journal of Dairy Science* **44**: 1768.
- Fenwick D E and Oakenfull D. 1983. Saponin content of food plants and some prepared foods. *Journal of Science and Food Agriculture* **34**: 186-191.
- Grobner M A, Johnson D E, Goodall S R and Benz D A. 1982. Sarsaponin effects on *in vitro* continuous flow fermentation of a high grain diet. *Journal of Animal Science* **55**: 491-97.
- Hristov A N, Mc Allister T A, Van Herk F H, Cheng K J, Newbold C J and Cheeke P R. 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. *Journal of Animal Science* **77**: 2554-63.
- Hungate R E. 1957. Microorganisms in rumen of cattle fed on constant ration. *Canadian Journal of Microbiology* **3**: 289.
- Klita P T, Mathison G, Fenton T W and Hardin R T. 1996. Effect of alfalfa root saponins on digestive function in sheep. *Journal of Animal Science* **74**: 1144-56.
- Krebs G, Leng R A and Nolan J V. 1989. Effect of bacterial kinetics in the rumen of eliminating rumen protozoa or supplementing with soybean meal or urea in sheep on a low protein fibrous feed. *The Role of Protozoa and Fungi in Ruminant Digestion*. pp. 199-210. (Eds.) Nolan J V, Leng R A and Demeyer D I. Armidale Australia.
- Lila Z A, Nazimuddin M, Kanda S, Kurihara M and Itabashi H. 2005. Sarsaponin effects on ruminal fermentation and microbes, methane production, digestibility and blood metabolites in steers. *Asian Australasian Journal of Animal Science* **18**(12): 1746-51.
- Livingston A L, Knuckles B E, Edwards R H, Fremery D, Millar R E and Kohler G E. 1979. Distribution of saponin in alfalfa protein recovery system. *Journal of Agriculture and Food Chemistry* **27**: 362-65.
- Lu C D and Jorgensen N A. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. *Journal of Nutrition* **117**: 919-27.
- Makkar H P S and Becker K. 1997. Degradation of saponin by mixed cultures of rumen microbes. *Letter in Applied Microbiology* **25**: 243-45.
- Ngwa A T, Sahlai I V and Bonsi M L K. 2001. The effect of feeding of pods of multipurpose tree on the degradability of dry matter and cell wall constituents of maize stover and alfalfa incubated in the rumen of sheep. *Journal of Science Food and Agriculture* **81**(13): 1235-43.
- National Research Council. 2001. *Nutrient Requirements of Beef Cattle*. 7th revised edn. National Academy of Sciences, Washington DC.
- Ningrant R W S, Gransworthy P C and Newbold C J. 2002. Saponin fractions in *Sapindus rarak*: Effects on rumen microbes. *Reproductive Nutrition and Development* **42** (Suppl.1): 82.
- Ranade D R and Gadre R V. 1988. *Microbiological Aspects of Anaerobic Digestion, Laboratory Manual*. Published by Maharashtra Association for the Cultivation of Sci., Research Institute, Pune, pp. 67.
- Rejil M C. 2005. 'Mitigation of methane emission from ruminants by dietary supplementation of fenugreek seeds.' M.V.Sc. Thesis submitted to NDRI Deemed University, Karnal (Haryana).
- Snedecor G W and Cochran W G. 1986. *Statistical Methods*. Oxford and IBH Publishing Co., Calcutta, India.
- Wang Y, Mc Allister T A, Yanke L S and Cheeke P R. 2000. Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. *Journal of Applied Microbiology* **88**: 887-96.
- Wina E, Muetzel Z, Hoffman E, Makkar H P S and Becker K. 2005. Saponin containing methane extract of *S. Rarak* affect microbial fermentation, microbial activities and microbial community structure *in vitro*. *Animal Feed Science and Technology* **121**: 159-74.
- Yosioka et al. 1974. Estimation of saponins. *Tetrahedron* **30**: 707.