Effect of *Sapindus mukorossi* as herbal feed additive for ruminants

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Abstract

Sapindus mukorossi, a herbal plant source of saponin, was tested for its effects on defaunation, ruminal ammonia production and subsequent impact on growth performance of cattle. Twelve bulls were equally divided into two groups: Control group fed *ad libitum* with urea-molasses straw supplemented with concentrate mixture (UMS) and Treated group fed with UMS and *S.mukorossi* herbal additive for growth performance test. Two rumen fistulated bulls about 400 kg live weight were used to determine the degradability of pericarps of *S. mukorrosi*. There was no significant difference in final live weight in the Control and Treated groups (160.7 kg vs 174.0 kg, respectively) or in average daily live weight gain (483.2g vs 612.0g/head, respectively). The pericarps of *S. mukorossi* fruits had defaunating effects but without hampering intake and digestibility of nutrients (especially of fiber) and N balance in cattle. Moreover, *Sapindus mukorossi* reduced the NH_3-N concentration by maintaining an acceptable range for optimum microbial fermentation in the rumen, which might assure the better utilization of N in lower gut. However, these effects did not produce any significant effect on growth of the animals.

Keywords: Herbal feed additive, saponin, cattle, growth.

Introduction

Manipulation of substrates in the rumen would increase the productivity of ruminant animals much dependent on available feed resources. Feeding antibiotics, anabolic steroids or other chemical growth promoters is one of the technologies long been used in livestock production system to promote meat and milk production, but recently, there has been a growing resistance among the general public against the use of such synthetic chemical products (Shearer, 1990). As consumers' acceptance shifts away from antimicrobial use in livestock production, alternatives are being explored for their antibiotic-like effects but without causing bacterial resistance (Doyle, 2001). Tropical plants normally contain high or medium level of content of secondary compounds such as saponin which have been shown to exert a specific effect against rumen protozoa while the rest of the rumen microbial-biomass remains unaltered (Wang *et al.*, 2000). Suppression or elimination of protozoa from the rumen may enhance the flow of microbial protein from the rumen, increase efficiency of feed utilization, and improve the nutritional status of the animal, provided that the loss of protozoa does not impair ruminal fiber degradation (Newbold *et al.*, 1997). The ultimate effect of feeding saponin containing plant materials is increased animal production as is observed

in the positive response to the inclusion feeding of antibiotics or other synthetic chemicals. Numerous studies have been conducted to determine the effects of feeding ruminants with saponin-rich plants (Klita *et al.*, 1996, Navas-Camacho *et al.*, 1993, Diaz *et al.*, 1994, Thalib *et al.*, 1995, Teferedegne *et al.*, 1999, Makkar *et al.*, 1998, Wang *et al.*, 2000). These results have indicated that the saponins have strong antiprotozoal activity and might serve as an alternative to 'in feed' antibiotics or growth hormone used for ruminants through its defaunating properties. The present study was conducted to evaluate the effects of feeding the fruits of *S.mukorossi* on growth performance of growing native bulls.

Materials and Methods

Animals and Treatments

The experiment was conducted using 12 bulls of native breed of average age of 1.5 years and 113.5 kg in live weight. Animals were randomly allocated to two groups in equal number: Control group which was fed *ad libitum* with urea-molasses straw (UMS) supplemented with concentrate mixture (UMS-C) and Treated group fed with UMS-C and *S.mukorossi* herbal additive. The experimental treatment in the Treated group was the addition of ground *Sapindus mukorossi*, a herbal additive, seed pericarps to the UMS-C diet.

Feeding of the Animals

All animals received *ad libitum* (at least 10% in excess of requirement) urea molasses straw, 3% urea, 15% molasses and 82% straw on DM basis as the basal diet named. They also received a concentrate mixture at the rate of 1% of their live weight. The concentrate allowance was divided into two halves and offered at 0800 and 1500 hours. The composition of concentrate mixture is shown in Table 1. The CP content of the UMS was 13.2%. The concentrate mixture was a mixture of 57% wheat bran, 14% khesheri bran, 12% till oil cake, 2% oyster shell, 1% fish meal, 0.5% salt and 0.5% DCP and contained 17.9% CP (Table 1). Concentrate mixture was adjusted every week according to the change in the body weight of the animal. The animals were weighed every week and UMS refusal was weighed every day to estimate daily intake throughout the experimental period of 98 days.

Management of Animals

At the onset of feeding trial, animals were dewormed with the required dose of Endex. The animals were reared in face-out stanchion barn for the whole experimental period except the period when they were transferred to metabolic crates. Animals were cleaned and washed prior to their morning meal daily.

Table 1. Composition of the experimental diet

Feeds	% Dry matter	Composition of feeds (% DM basis)			
		Ash	OM	CP	ADF
Urea molasses straw	56.5	17.2	82.8	13.2	44.4
Concentrate mixture	91.2	12.3	87.7	17.9	22.3

Digestibility trial

Twelve animals were transferred to metabolic crates for determining nutrient digestibility and balance. All animals were maintained individually to collect feces and urine. Feces were collected in the morning and sub-samples were taken every day from each animal. Urine was collected with 6N H_2SO_4 to keep pH value below 4 and was made into a constant volume of 15 L. Feed refusals were weighed every morning, and samples were taken for proximate analysis. UMS and concentrated sample were taken from the lot of feeds. During the collection period all samples were preserved at -20^0C.

Chemical analysis

All samples were taken out from the freezer and thawed. After thawing seven samples of feces and refusal (7 days) from an animal were mixed thoroughly, and a composite sample was taken for the determination of dry matter (DM), organic matter (OM) and crude protein (CP) on fresh basis. The other part of the sample was dried in an oven to determine acid detergent fibre (ADF) according to the method described by AOAC (1990).

Response of Sapindus Mukossi to rumen environment

Two rumen fistulated bulls about 400 kg live weight were used to determine the degradability and protozoal load of pericarps of *S. mukorrosi*. The experiment was continued for 15 days with 5 days as an adjustment period. About 2.0g of washed and dried sample of straw was weighed in a Dacron bag and was incubated in the rumen to determine degradability at 8, 16, 24, 48, and 96 hours of incubation. A strict time schedule was followed to incubate and withdraw of the nylon bags following the method of Ørskov *et al*. (1980). Rumen liqour samples were collected three times a day to determine pH, ammonia-N and protozoal concentration. The pH was immediately determined using a pH meter (WTW pH 530). The protozoa were also counted immediately. One ml of the rumen fluid was diluted with 9 ml of methyl green formaline- sodium chloride (MFS) to count total ciliated protozoa (ASC Great Northern Science Handbook. www.akscience.org.). For NH_4 –N analysis, sample was acidified with concentrate H_2SO_4 and stored at $-20\ ^0$C until they were analyzed.

Statistical Analysis

Data were analyzed using Independent sample t-test procedure of SPSS 11.1 (SPSS Inc. 2000) statistical package.

Results and Discussion

The daily average intake of UMS, concentrate and the total diet of the two groups of bulls is presented in Table 2. The use of *S.mukorossi* herbal additive had no significant effect on the intake of UMS (Table 2). The concentrate was supplied at the rate of 1% of live weight that resulted in a range of ratio of 29.8:70.2 to 28.5:71.5 with the *ad libitum* UMS intake. The average daily intake of UMS and total diet of Control and Treated groups of bulls was 3.30 and 3.70 kg/head, and 4.60 and 5.20 kg/head, respectively. Conversion of the total diet intake of % LW or $LW^{0.75}$ showed that it varied from 2.90 and 3.00% or 102.5 and 108.5 g, respectively.

There was no significant difference ($p>0.05$) in final live weight between Control and Treated groups (160.7 and 174.0 kg, respectively) and average daily live weight gain (483.2 and 612.0g/head, respectively). Nevertheless, the average extent of difference in daily live weight gain

was 128.8g/head, expressing the increase of daily rate of gain of 26.7%. This increase was quite considerable from the point of economy or in making the diet more efficient for the beef production. The feed conversion ratio for the Control and Treated groups were 9.70 and 8.70, respectively.

Nutrients utilization

Dietary daily total N intake did not vary significantly ($p>0.05$) between the Control and Treated diets (123.3 and 136.5 g/head, respectively) (Table 3). However the fecal nitrogen excretion varied significantly ($p<0.05$) between Control and Treated groups (55.4 and 73.7 g/d/head, respectively). The urinary nitrogen excretion was not significantly different. The total nitrogen excretion of 75.0 g/d/head in the Treated diet was significantly higher ($p<0.05$) than the Control diet (56.6 g/d). However, no significant ($p>0.05$) difference in nitrogen balance between the two groups (64.8 g/day/head for Control group and 61.8 g/day/head for Treated group). In the present study N balance remained unchanged after feeding of *S. mukorossi*. N utilization in terms of N absorption and retention was reported to be unaffected by feeding fruit peel of *Garcinia mangostana* to cattle (Ngamsaeng *et al.*, 2006b). The present results were in close agreement with the results reported by Wina *et al.* (2006) and Abreu *et al.* (2004). However, Hristov *et al.* (1999) and Lu and Jargensen (1987) found high level of alfalfa saponin to be strongly inhibitory of N digestion in rumen when a forage-based diet was fed. These researchers also reported a trend towards reduced N retention with increasing saponin level.

Table 2. Effect of *Sapindus mukorossi* fruit on feed intake and live weight changes of bulls

Variable		Diets		Significance	
		Control	*S. mukorossi*	SED	Level[1]
Initial live weight (kg)		113.0±12.95	114.0 ± 9.19	15.88	NS
Daily DMI, kg/head	UMS	3.3 ± 0.32	3.7 ± 0.32	0.46	NS
	Concentrate	1.3 ± 0.13	1.5 ± 0.11	0.17	NS
Daily Total diet DMI	kg	4.6 ± 0.42	5.2 ± 0.43	0.60	NS
	% LW	2.9 ± 0.16	3.0 ± 0.05	0.17	NS
	$LW^{0.75}$ (kg)	102.5 ± 5.97	108.5 ± 3.10	6.74	NS
UMS:Concentrate in diet		70.2:29.8	71.5:28.5	-	-
Final live weight (kg)		160.7± 14.59	174.0± 13.27	13.67	NS
Live weight gain (g/d)		483.2± 52.32	612 ± 47.67	70.79	NS
FCR (kg feed/kg gain)		9.7 ± 0.76	8.7 ± 0.30	0.82	NS

[1]NS not significant at $P < 0.05$

Table 3. Effect of *Sapindus mukorosi* fruit on nitrogen utilization in growing bulls

Variable	Diets		Significance	
	Control	*S.mukorossi*	SED	Level[1]
Total N intake (g/d)	123.3 ± 7.93	136.5 ± 8.89	11.91	NS
Faecal N excretion (g/d)	55.4 ± 2.20	73.7 ± 5.90	6.30	p<0.05
Urinary N excretion (g/d)	1.1 ± 0.29	1.0 ± 0.06	0.07	NS
Total N excretion (g/d)	56.6 ± 2.21	75.0 ± 5.9	6.34	p<0.05
N-balance (g/d)	64.8 ± 6.49	61.8 ± 5.68	8.63	NS

[1]NS not significant at P < 0.05

Table 4. Effect of *Sapindus mukorossi* fruit on degradability of straw and total Tract digestibility of the diet

Variable	Diets		Significance	
	Control	*S. mukorossi*	SED	Level[1]
(a) Digestibility *in sacco*				
i) Water-soluble fraction of straw (%)	5.29	5.29	-	-
ii) Rumen degradability of washed and dried straw (% DM at different hours)				
8	8.9 ± 0.29	8.7 ± 0.4	0.48	NS
16	15.2 ± 2.02	18.9± 1.68	2.63	NS
24	22.3± 1.73	24.6± 1.11	2.06	NS
48	33.5 ± 1.13	39.4± 1.37	1.77	P<0.01
72	43.0 ± 0.58	44.8± 0.81	0.995	NS
96	52.1 ± 0.92	53.2± 1.01	1.37	NS
Digestion rate of straw (c, %/h)	1.30	2.30	-	-
Extent of degradability of straw (a+b, %)	70.88	58.3	-	-
(b) Digestibility *in vivo* of the total diet				
Dry matter (DM)	50.4 ± 3.10	47.3± 1.72	3.55	NS
Crude Protein (CP)	54.0 ± 2.40	46.0± 2.80	3.72	P< .05
Acid Detergent Fibre (ADF)	64.0 ± 2.58	65.0± 1.64	3.06	NS
Organic matter (OM)	51.0 ± 2.90	51.0± 1.70	3.37	NS

[1]NS not significant at P < 0.05

Change in the rumen environment and rumen flora

The rumen environment in terms of cellulotic activity (indicated by straw degradability in the rumen at different hours) was better due to feeding *S.mukorossi* which gave a significantly higher (p<0.05) degradability of straw at 48 hour of incubation. The straw degradability at 16 and 24 hour was 18.9% and 24.6%, respectively in the Treated diet and 15.2% and 22.3%, respectively in the Control diet. This cumulative higher effect of *S.mukorossi* made the difference in straw degradability to be significantly higher at 48 hours of incubation. Nevertheless, these effects were minimized at longer periods of incubation

(44.8 and 43.0% at 72 hours and 53.2 and 52.1% at 96 hours) (Table 4). Increase in degradation of straw at early incubation hours helped better utilization of feeds in the rumen. However, no significant ($p>0.05$) difference was observed in the total tract digestibility of DM, OM, ADF of the diet due to feeding the herbal additive, except a significant ($p<0.05$) low digestibility of CP in the presence of *S. mukorossi* (Table 4).In the present study, reduction in ruminal protozoa could not hamper ADF digestibility. The effect of defaunation on fibre digestion *in vivo* was generally small or negligible. This suggests that the removal of rumen protozoa allows more bacteria to colonize the plant fibres, filling the niche formerly occupied by the protozoa, therefore, fibre digestion is not generally decreased (Hedayat *et al.*, 1993; Newbold *et al.*, 1989).

The rumen ammonia nitrogen and pH of the bulls fed the Control or *S.mukorossi* added diet varied from 218.0 to 257.0 mg/l and 6.63 to 6.86 in different hours of feeding, and the differences due to feeding the herbal additive were not significant ($p>0.05$), except a significantly lower concentration of the nitrogen at 7 hours of feeding in the bulls fed with *S.mukorossi*. In addition, at 2 hours of feeding the level was lower in the Treated than the Control diet (219.0 and 237.0 mg/l, respectively) (Table 5).

S.mukorossi feeding decreased the number of rumen ciliated protozoa significantly ($p<0.05$) both at 2 (2.30×10^4/ml and 5.3×10^4/ml) and 7 (2.6×10^4/ml and 5.4×10^4/ml,) hours of post feeding, and a significantly ($p<0.01$) lower concentration (1.50×10^4/ml) before morning feeding provided evidence that its effect on ciliated protozoa concentration continued even throughout the day (Table 5). The alleviation of the rumen ciliated protozoal concentration might have favoured better growth of the bulls fed with *S.mukorossi* diet containing saponin. Protozoa ingest bacterial cells and results in a lower bacterial protein production in the rumen, it increases when poor quality roughage based diets are fed to animals (Leng, 1990). Elimination or alleviation of rumen protozoa was found to increase animal production performance (Newbold *et al.*, 1997; Makkar *et al.*, 1998) and the response to production may be the result of inhibition of methanogens that are associated with protozoa, stimulation to propionate production, increase in bacterial nitrogen yield and inhibition of rumen protein degradation (Demeyer, 1988).

It was shown that tropical plants have the potentials for eliminating protozoa in the rumen (Diaz *et al.* 1994; Navas Camacho *et al.*, 1993; Newbold *et al.*1997; Odenyo *et al*., 1997).

From the above results and discussion it may be stated that *S.mukorossi* may be a good herbal feed additive for the growing beef cattle without any deleterious effect on health. Its non-significant response to growth of bulls was estimated to be 26.7%, and this is even higher than the average growth response of hormones used in the beef cattle in the feedlot system. Further research works are essential to compare its effects on beef production with that of the growth hormones. Moreover, easy and cheapest source of saponin containing tropical plants need to be searched, and technologies for their processing and manufacturing should be developed.

Table 5 . Effect of *Sapindus mukorosi* fruits on pH, protozoa number, ammonia-N in the rumen fluid of bulls

Variable	Diets		Significance	
	Control	*S. mukorossi*	SED	Level[1]
Ciliated protozoa ($\times 10^4$/ml)				
0	4.2±0.48	1.5±0.36	0.59	p<0.001
2	5.3±0.89	2.3±0.57	1.06	p<0.05
7	5.4±0.76	2.6±0.49	0.90	p<0.05
NH_3-N (mg/l)				
0	236.0±19.4	243.0±23.9	30.8	NS
2	237.0±16.5	219.0±11.6	20.2	NS
7	257.0±16.4	218.0±7.85	39.5	p<0.05
pH				
0	6.70±0.16	6.70±0.13	0.20	NS
2	6.77±0.09	6.86±0.07	0.11	NS
7	6.63±0.16	6.70±0.12	0.20	NS

[1]NS not significant at $P < 0.05$

References

Abreu, A., Carulla, J. E., Lascano, C. E., Diaz, T. E., Kreuzer, M. and Hess, H. D. 2004. Effects of *Sapindus saponaria* fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. J. Anim. Sci. 82: 1392-1400.

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemistry (15 Ed), Washington, D.C., U.S.A.

Demeyer, D. I. 1988. Effect of defaunation on rumen fibre digestion and digesta kinetics, In: The Role of Protozoa and Fungi in Ruminants Digestion, editors: J. V. Nolan, R. A. Leng and D. I. Demeyer, Proceedings of an international seminar held at the University of New England, Armidale, Australia p.171-179.

Diaz, A., Avendan, O. M. and Escobar, A. 1994. Evaluation of Sapindus saponaria as a defaunating agent and its effect on different ruminal digestion parameters. Livestock Res. Rural Develop. 5:1-10.

Doyle, M. E. 2001. Alternatives to antibiotics use for growth promotion in Animal Husbandry. University of Wisconsin-Madison 2001. Food Research Institute Briefings, pp. 1-17.

Hidayat, K., Hillman, C. J., Newbold, C. S. and Stewart, C. 1993. The contributions of bacteria and protozoa to forage fermentation *in vitro*, as determined by microbial gas production. Anim. Feed Sci. Technol., 42: 193-208.

Hristov, A. N., McAllister, T. A., Van Herk, F. H., Cheng, K. J., Newbold, C. J. and Cheeke, P. R. 1999. Effect of *Yucca schidigerea* on ruminal fermentation and nutrient digestion in heifers. J. Anim. Sci. 77: 2554-2563.

Klita, P.T., Mathison, G.W., Fenton, T.W. and Hardin, R.T. 1996. Effects of alfalfa root saponins on digestive function in sheep. J. Anim. Sci. 74: 1144–1156.

Leng, R.A. 1990. Factors affecting the utilization of poor-quality forages by ruminants particularly under tropical condition. Nutrition Research Reviews 3: 27-91.

Lu, C. D. and Jorgensen, N. A. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117: 919-927.

Makkar, H.P.S., Bl¨ummel, M. and Becker, K. 1998. Application of an in vitro Gas Method to Understand the Effect of Natural Plant Products on Availability and Partitioning of Nutrients. Occasional Publication no. 22. British Society of Animal Science, London, pp. 147–150.

Navas-Camacho, A., Laredo, M. A., Cuesta, A., Anzola, H. and Leo´n, J. C. 1993. Effect of supplementation with a tree legume forage on rumen function. Livestock Res. Rural Dev. 5: 58–71.

Newbold, C. J., Griffin, P. W. and Wallace, R. J. 1989. Interaction between rumen bacteria and ciliate protozoa in their attachment to barley straw. Lett. Appl. Microbiol., 8: 63-66.

Newbold, C. J., El Hassan, S.M., Wang, J., Ortega, M.E. and Wallace, R. J. 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Br. J. Nutr. 78, 237–249.

Ngamsaeng, A., Wanapat, M. and Khampa, S. 2006b. Effects of mangosteen peel (*Garcinic mangostana*) supplementation on rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake in cattle. Pakistan J. Nutr. 5(5): 445-452.

Odenyo A, P.O. Osuji and O. Karanfil. 1997. Effect of multipurpose tree (MPT) supplements on ruminant ciliate protozoa. Animal Feed Science and Technology, 67: 169-180.

Shearer, G. 1990. Testing for tissue residues and results of current programmes. Pig. Vet. Soc. Proc., 24: 80-87.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., and Jiang, Y. 2004 . Saponin from edible legumes: chemistry, processing and health benefits . J. Medicine Food., 7(1): 67-78.

Teferedegne, B., McIntosh, F., Osuji, P.O., Odenyo, A., Wallace, R. J., and Newbold, C. J. 1999. Influence of foliage from different accessions of the sub-tropical leguminous tree, *Sesbania sesban*, on ruminal protozoa in Ethiopian and Scottish sheep. Anim. Feed Sci. Technol. 78: 11–20.

Thalib, A., Widiawati, Y., Hamid, H., Suherman, D. and Sabrani, M. 1995. The effect of saponins from *Sapindus rarak* fruit on rumen microbes and host animal growth. Annales de Zootechnie 44, p. 161 (Abstr.).

Wang, Y. X., McAllister, T. A., Newbold, C. A., Cheeke, P. R. and Cheng, K. J. 1997. Effects of Yucca extract on fermentation and degradation of saponins in the Rusitec, *Proc. Western Section, Am. Soc. Anim. Sci.* 48 **(**1997), pp. 149–152.

Wang, Y., McAllister, T.A., Yanke, L.J., Xu, Z.J., Cheeke, P.R. and Cheng, K.-J., 2000. In vitro effects of steroidal saponins from *Yucca schidigera* extract on rumen microbial protein synthesis and ruminal fermentation. J. Sci. Food Agric. 80: 2114–2122.

Wina, E., Muetzel, S. and Becker, K. 2006. Effects of daily and interval feeding of *Sapindus rarak* saponins on protozoa, rumen fermentation parameters and digestibility in sheep. Asian-Aust. J. Anim. Sci. 199: 1580-1587.