Indian J. Anim. Nutr. 2024. 41 (2): 298-303
doi: 10.5958/2231-6744.2024.00038.4
Indian Journal of Animal Nutrition

Determining the Effect of Soapnut (*Sapindus mukorossi*) on *In Vitro* Methanogenesis and Dry Matter Degradation in Cross-Bred HF Cattle

S. V. Rathod*, P. R. Pandya, G. P. Mathukiya, K. K. Sorathiya, R. K. Dangi and B. R. Devalia
Animal Nutrition Research Station, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand- 388001, Gujarat.
Correspondence: drsunilvlsrtd147vetnutri@gmail.com

ABSTRACT

Soapnut fruits have high saponin content about 10% of fruits. An *in-vitro* studies were undertaken to explore the effects of different levels of Soapnut fruits on methanogenesis, total gas production, and dry matter degradation by using rumen liquor of cattle. Their nine treatments of three replicates each follows: Soapnut powder added at 0, 1, 2, up to 8% on DM basis with finely ground TMR in 100ml *in vitro* syringe. Among those treatments, reduction of methane gas was non significance (P>0.05), where reduction percent was recorded in S1, S2, S3, S5, S7, and S8 Soapnut supplementation *i.e*., 22.1, 15.8, 5.54, 14.4, 5.54 and 9.42% respectively, per 100 mg DDM compared to control S0 group. While methane gas production at S4 and S6 was at par as control S0 group. The total gas production during methane production was not differed (P>0.05) but, reduced up to 19.68, 13.64, 25.77, 15.18, and 22.73% respectively, in S1, S3, S5, S7, and S8 treatment groups compared to control. The DMD in S1 and S2 was increased up to 7.04 and 9.12% respectively, but other Soapnut treatment reduces DM degradation. Their highest DM degradation reduction observed in S4, S5, and S8 treatments *i.e.,* 8.94, 6.82, and 9.12% respectively, compared to the control S0 treatment. The IVDMD of Soapnut supplementation was reduced with increasing level of Soapnut supplementation.

KEYWORDS: Cattle, IVDMD, *In-vitro* Methane, Saponin, Soapnut.

Article received: 12 March 2024; Article accepted: 16 June 2024

INTRODUCTION

Soapnut (*Sapindus mukorossi*) is one of the valuable medicinal plants found mostly in the tropical and sub-tropical region of India. Previously, *in-vitro* studied in our department, with different levels of sea weed supplements, not reported any adverse effect on IVDMD as well as *In-vitro* Methane (Trivedi et al., 2020), SSF @ 4% with substrate feed reduces methane 38.4% and improved IVDMD (Patel, et. al., 2020), SSF improved result @ 5% in TMR *i.e*., IVDMD 12.9% and methane reduced 16.9% (Sherasia et al., 2017), and Mainly feed additives and recently plant secondary compounds had been tried to manipulate rumen fermentation, reduce methane production to improve animal productivity (Knapp et al., 2014).

The plant contains a large number of secondary metabolites that have antimicrobial activities and are used for undesirable microbial inhibition in the rumen for manipulation of the microbial ecosystem (Agarwal et al., 2006). Saponins are one of the plant's secondary compounds, present in foliage or many fruits of tropical and sub-tropical shrubs as well as trees. Saponins form stable foam in aqueous, as soap hence the name "saponins".

Soapnut fruits have high saponin content about ~ 10% of fruit (Poornachandra et al., 2019). Saponins from different sources resulted as manipulation in the microbial ecosystem of the rumen as microbial fermentation and inhibitory effect on the methanogenesis process (Jayanegara et al., 2014). Therefore, the present study was conducted to investigate the effects of Soapnut (*Sapindus mukorossi*) supplementation on dry matter degradation and methane gas production in HF cross-bred cattle by using *in-vitro* method.

MATERIALS AND METHODS

Test materials and location

The Soapnut purchased from market of Anand**.** The fruits of Soapnut (*Sapindus mukorossi*) broken along with seeds, grinded using 1mm sieves, and dried at 100±2 ^{0}C for 4h. The cereal and legume straw based TMR prepared (roughage: concentrate ratio 65: 35), *i.e*., 30% Jowar straw. 30% Moong straw, 19% De-oiled rice bran, 10% Groundnut cake, 10% sugarcane molasses, and 1% mineral mixtures, finely grinded (particle size < 1.0 mm) and dried. An *in vitro* experimental studies performed at Animal Nutrition Research Station, Anand.

Experimental design

A days before about 500 mg and 200 mg TMR samples was taken into 100ml oven dried *in vitro* glass syringes (3 replicates for each treatment groups; 27 tubes for IVDMD and 27 tubes for *in vitro* methane) for digestibility and methane production, respectively. Their Soapnut was supplemented as one control and eight treatment groups, at a rate of 0, 1, 2, 3, 4, 5, 6, 7 and 8% and termed as S0, S1, S2, S3, S4, S5 S6, S7, and S8 group, respectively as shown in below Table 1 (Each tubes numbers were marked and noted it treatment wise group of soapnut supplemented tubes).

Table 1. TMR with different levels of Soapnut treatment groups

Treatment groups	Soapnut supplementation		
	Percentages (%)	mg/tube, For DMD (500mg TMR)	mg/tube, For Methane (200mg TMR)
S0	0	0	0
S1	1	5	2
S2	2	10	4
S3	3	15	6
S4	4	20	8
S5	5	25	10
S6	6	30	12
S7	7	35	14
S8	8	40	16

Equipment and technique

The rumen liquor was collected before feeding and watering, from two cross-bred HF cattle fed of same TMR based diet used for *in vitro* studies, as per standards (ICAR, 2013) and collected rumen liquor using stomach tube against negative pressure created by a suction pump just 15 minutes before *in vitro* studies starts. The collected rumen liquor was immediately strained through four layered muslin cloth which was termed as Strained Rumen Liquor (SRL). The SRL was maintained at 39 ± 1°C and flushed with carbon dioxide (CO_2) gas. The required quantity of distilled water, micro and macro minerals solution, freshly prepared buffer (McDougall buffer) solution and resazurin (0.1%) was mixed in flask and kept in an incubator at 39 °C. The required volume of SRL was added with CO_2 which was flushed continuously in the medium. The required amount of rumen inoculum was measured and dispensed into the syringe through a silicone tube *i.e.,* 40ml for IVDMD and 30ml for *in-vitro* methane, and kept in a shaker water bath at 39°C. The *in vitro* digestibility and methane production was determined after 48h and 24h of incubation, respectively.

Table 2. Composition of media used for *in vitro* gas production technique

Particular	Quantity of different solutions and SRL	
Solution A (Micro minerals) (ml)	0.10	0.12
Solution B (Buffer solution) (ml)	190.23	237.78
Solution C (Macro minerals) (ml)	190.23	237.78
Resazurin solution (ml)	0.95	1.19
Reducing solution (ml)	38.05	47.56
Distilled water(ml)	380.45	475.57
Total media (ml)	800	1000
Rumen liquor (ml)	400	500
Total mixture (ml)	1200	1500

Measurement of methane gas

After 24hr of incubation fermentation was stopped by cooling the glass syringes and gas volume recorded. The 1ml gas samples were collected from syringe and directly injected into the Gas Chromatograph (GC) from each syringe minimum of two times and methane concentration was determined against the area of the standard methane sample in terms of CH_4%. The column temperature was maintained at 50^0C in which nitrogen was used as a carrier gas, with a low rate of 30ml/min. Calibration was completed using the standard methane gas and the temperature of the oven, injector, and detector was 100^0C, 120^0C, and 100^0C, respectively. Peak area was integrated using a Thermo Fisher Integrator (Johnson et al*.,* 1994).

Measurement of DM degradation

The IVDMD of basal TMR with Soapnut effect was determined by using Goering & Van Soest method. For *in vitro* digestibility after 48h of incubation syringes removed from a shaker bath and used for the next process. The contents of each syringe were filtered through a pre-weighed Gooch crucible, dried and weighed. The Gooch crucibles containing undigested residues was oven dry at 70 °C for 24 h, cooled in the desiccators and weighted for obtain dry matter as well as *in vitro* DMD (Menke et al., 1979).

Statistical Analysis

The data generated during the experiment was analyzed by using CRD (Complete Randomized Design). The experimental data collected during the study was analyzed using the one-way analysis of variance (ANOVA) method in SPSS16. To compare the treatment means, the Duncan Multiple Range Test was employed.

RESULTS AND DISCUSSION

In-Vitro Dry Matter Degradation (IVDMD)

Table 3. *In Vitro* Effect of Soapnut on DMD, methane gas, and total gas production.

Group	Soapnut%	Average IVDMD (%)	CH_4 (ml/100 mg DDM)	CH_4 ml /100mg DM	Total gas Production (ml)
S_0	0	54.8 ± 0.95^{bc}	3.61±0.06	2.09 ± 0.01	22.0±0.58
S_1	1	58.6 ± 1.45^{cd}	2.81±0.08	1.62 ± 0.05	17.6±1.45
S_2	2	59.8 ± 1.14^{d}	3.04±0.37	1.82 ± 0.22	21.3±2.73
S_3	3	51.6 ± 0.23^{ab}	3.41±0.11	1.76 ± 0.06	19.0±0.58
S_4	4	49.9 ± 0.64^{a}	3.54±0.31	1.76 ± 0.16	20.3±2.18
S_5	5	51.06 ± 0.18^{ab}	3.09±0.82	1.58 ± 0.42	16.3±2.96
S_6	6	53.4 ± 1.8^{ab}	3.64±0.19	1.76 ± 0.13	18.6±0.88
S_7	7	52.8 ± 1.00^{ab}	3.41±0.19	1.80 ± 0.9	20.6±1.76
S_8	8	49.8 ± 3.01^{a}	3.27±0.11	1.63 ± 0.05	17.00±0.57
F- Value		6.48	0.73	0.74	1.27
P-Value		0.00	0.67	0.66	0.32
CV%		4.59	17.4	17.5	20.6
CD @ 5%		4.25	NS		NS

Mean ± SE values with different superscripts (a, b, c, d) within column differ significantly ($p<0.05$).

The study aimed to examine the influence of various concentrations of Soapnut (ranging from 1% to 8%) on *in vitro* dry matter digestibility (IVDMD). The result outcomes are in Table 3. It demonstrated that IVDMD exhibited a significant increase ($P<0.05$) at the Soapnut levels of 1% and 2% compared to the other concentrations and the control group. Thalib et al. (1996) and Hess et al. (2003) both found agreement in their results raises digestibility when uses different saponin sources of S*apindaceae* family. Conversely, the lowest IVDMD values were observed at the Soapnut concentrations of 4% and 8%. Moreover, IVDMD at the Soapnut concentrations of 3%, 5%, 6%, 7%, and 0% showed a statistically significant decrease ($P<0.05$) in comparison to the remaining levels. The high saponin content in Soapnut has been associated with a reduction in dry matter digestibility (DMD) when high Soapnut level used. These varying results may variations in the composition of Soapnut samples. In line to the present results by Poornachandra et al. (2019) and Agarwal et al. (2006) reported a reduction in IVDMD when Soapnut was used and in contrast by Wang et al. (1998) and Hu et al. (2006) reported no effect on IVDMD with low levels saponin extracts.

There were non- significant reduction of *in vitro* methane gas ml per 100mg DDM and 100 mg DM. Reduction of CH_4 percent per 100 mg DDM (in comparison of control group) were 22.16, 15.79, 5.54, 14.40, 5.54, and 9.42 at 1, 2, 3, 5, 7, and 8 % Soapnut supplementation while no effect of 4 and 6% levels on *in vitro* methane gas in comparison of S_0 control, are shown in result Table 3. In consistent with the current findings by Agarwal et al. (2006), Holtshausen et al. (2009), Hu et al. (2005, 2006), Takahashi et al. (2000) reported reduced *in vitro* methane gas production with various saponin sources, While Poornachandra et al. (2019), Hess et al. (2003), and Patra et al. (2006) reported no effect of saponin sources on *in vitro* methane gas production. The total gas production during methane production was not differed ($P>0.05$) but, reduced up to 19.68, 13.64, 25.77, 15.18, and 22.73% respectively, in S1, S3, S5, S6, and S8 treatment group compared to control. The result of total gas production was in agreement of Wang et al. (1998).

CONCLUSION

The study concludes that the supplementation of Soapnut at low levels *i.e.,* 1 and 2% their increased ($P<0.05$) IVDMD, while with increasing Soapnut levels reduces *in vitro* DM degradation compared of control. The reduction of DMD may be due to high saponin contains of Soapnut. While *in vitro* methane gas was reduced up to 22.1 and 15.7 % at 1 and 2 % Soapnut levels, respectively. The methane gas reduction due to saponin contains of Soapnut and saponin have act as defaunating agent in rumen. So, we concluded that 1 and 2% are best for inhibit *in-vitro* methane production and higher IVDMD in cross-bred HF cattle, further *in-vivo* study is needed to determine best levels and it's impacts on lactating animals.

ACKNOWLEDGEMENT

We extend my sincere thanks to the Animal Nutrition Research Station, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Anand for providing research facilities and financial supports for this study.

REFERENCES

Agarwal, N., Kamra, D.N., Chaudhary, L.C. and Patra, A. K. 2006. Effect of *Sapindus mukorossi* extracts on in vitro methanogenesis and fermentation characteristics in buffalo rumen liquor. Journal of Applied Animal Research. 30 (1): 1-4.

Hess, H.D., Kreuzer, M., Diaz, T.E., Lascano, C.E., Carulla, J.E., Soliva, C.R. and Machmüller, A. 2003. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. Animal Feed Science and Technology. 109 (1-4): 79-94.

Holtshausen, L., Chaves, A.V., Beauchemin, K.A., McGinn, S.M., McAllister,T.A., Odongo, N.E., Cheeke, P. R. and Benchaar C. 2009. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. Journal of Dairy Science. 92 (6): 2809-2821.

Hu, W.L., Liu, J.X., Wu, Y.M., Guo, Y.Q. and Ye, J.A. 2006. Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Achieves of Animal Nutrition. 60 (1): 89-97.

Hu, W.L., Wu, Y.M., Liu, J.X., Guo, Y.Q. and Ye J.A. 2005. Tea saponins affect in vitro fermentation and methanogenesis in faunated and defaunated rumen fluid, Journal of Zhejiang University-Science B. 6: 787-792.

ICAR. 2013. Nutrient requirements of Animalscattle and buffalo. 3rd Edn. Indian Council of Agricultural Research, New Delhi

Jayanegara, A., Wina, E. and Takahashi, J. 2014. Meta-analysis on methane mitigating properties of saponin-rich sources in the Rumen: Influence of addition levels and plant sources. Asian-Australasian Journal of Animal Science. 27 (10): 1426-1435.

Johnson K.A., Huyler, M.T., Westberg, H.H., Lamb, B.K. and Zimmerman, P. 1994. Measurement of methane emission from ruminant livestock using SF_6 tracer technique. Environment Science and Technology. 28: 359-362.

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P. and Tricarico, J.M. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emission. Journal of Dairy Science. 97 (6): 3231-3261.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. The Journal of Agricultural Science. 93: 211-222.

Newbold, C.J., EIHassan, S.M., Wang, J., Oriega, M.E., and Wallace, R.J. 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. British Journal of Nutrition. 78: 237-249.

Patel, K.P., Katole, S.B., Pandya, P.R., Sorathia, K.K., and Patel S. 2020. Effect of Solid-state Fermentation Biomass Supplementation to Mixed Substrate on Digestibility and Methane Mitigation in vitro. Indian Journal of Animal Nutrition. 37 (2): 127-131.

Patra, A.K., Kamra, D.N. and Agarwal, N. 2006. Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Animal Feed Science and Technology. 128 (3-4): 276-291.

Poornachndra, K.T. Malik, P.K., Trivedi, S. Thirumalaisamy, G., Kolte, A.P., Dhali, A. and Bhatta, R. 2019. Effect of individual vs. combined supplementation of tamarind seed husk and Soapnut on methane production, feed fermentation and protozoal population in vitro. Approaches in Poultry, Dairy & Veterinary Science. 6 (4): 572-581.

Sherasia, P.L., Pandya, P.R., Parnerkar, S., Murty, S. and Devalia B.R. 2017. Influence of incorporating different levels of solid state fermented biomass in total mixed rations on in vitro digestibility and methane production. Indian Journal of Animal Nutrition. 34 (2): 145-149.

Takahashi, J., Miyagawa, T. Kojima, Y. and Umetsu, K. 2000. Effects of *Yucca schidigera* extracts, probiotic, monensin and L-cysteine on rumen methanogenesis. Asian-Australian Journal of Animal Science. 13: 499-501.

Thalib, A., Widiawati, Y., Hamid, H., Suherman, D. and Sabrani, M. 1996. The effects of saponin from Sapindus rarak fruit on rumen microbes and performance of sheep. Journal Ilmu Ternak dan Veterinary. 2 (1): 17-21.

Trivedi, S.K. Trivedi, M. M., Pandya, P.R., Bhavsar, M.Y. and Prajapati, D.T. 2020 Influence of Seaweed (*Sargassum johnstonii*) Supplementation on in vitro Digestibility and Methane Production. Indian Journal of Animal Nutrition. 37 (3): 296-298.

Wang, Y., McAllister, T.A., Newbold, C.J., Rode. L.M., Cheeke, P.R. and Chengand K.J. 1998. Effect of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (Rusitec). Animal Feed Science Technology. 74: 143-53.