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A review: Effects of saponin on ruminal fermentation, nutrient utilization, body weight and methane mitigation in ruminants

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Abstract
Saponins are plant secondary compounds found in shrubs and trees. While saponins derived from plants have been employed to regulate methanogenesis conducted by archaeal methanogens in the rumen, the outcomes have shown variability. Saponins divided into two groups based on their structure: triterpene and steroid glycosides. Saponins offer benefits like membrane-permeabilizing, immunostimulant, and hypocholesterolaemic properties, some saponin-containing plants can be toxic. In animal nutrition, saponins play both positive as well as negative roles. They can enhance ruminal fermentation by acting as defaunating agents, improving N-use efficiency, Reduces methane emission and potentially boosting animal performance by affecting nutrient utilization and their body weights. Moreover, studies have indicated that saponins can hinder protein digestion in the gut by interacting with cholesterol in the cell membrane, leading to cell rupture and selective elimination of ruminal protozoa. This mechanism is believed to enhance nitrogen utilization efficiency and potentially contribute to increase in animal performance. Saponin also possess antimicrobial activities and manipulating the microbial ecosystem. Overall, saponins have diverse effects, making their impact on animal health and performance complex. In conclusion, saponin proves efficacious in reducing *In vivo* enteric methane emissions from ruminants while concurrently improving nutrient digestibility, with an optimal utilization level not surpassing 0.5-1% of dry matter.

Keywords: Digestibility, fermentation, methane emission, ruminants, saponin

Introduction
The term "plant secondary metabolite" (PSM), coined by Albrecht Kessel (Mathes, 1980, as referenced by Hartmann, 2007) [9], refers to compounds found in plants beyond primary metabolic processes. These secondary metabolites are abundant in plants and often exhibit antimicrobial properties. They can be employed to control and suppress undesirable microbial growth in the rumen, effectively manipulating the microbial ecosystem for specific purposes, as highlighted by Agarwal *et al*. in 2006 [2]. Saponins are plant secondary compounds found in the foliage and fruits of tropical, sub-tropical shrubs and trees. They are named "saponins" because they can form stable foam in water, resembling soap. Saponins can be divided into two groups based on their structure: triterpene and steroid glycosides. These compounds play an indirect role in reducing protozoa and inducing changes in the rumen ecosystem [Finlay *et al.* (1994), Hess *et al.* (2003[a]), and Malik *et al*. (2016)] [4, 12, 25].
Saponins have been documented for their antiprotozoal activity in various studies [Kamra *et al.* (2008) [20], Sliwinsky *et al.* (2002), Hess *et al.* (2004) [10], Lovett *et al*. (2006) [23], and Agarwal *et al.* (2006)] [2]. The suppression or elimination of protozoa from the rumen, attributed to saponins, contributes to improved microbial protein flow, enhanced feed utilization efficiency, and elevated nutritional status in animals. Provided it does not compromise ruminal fiber degradation (Newbold *et al*., 1997) [31]. The impact of saponins on *In vitro* rumen fermentation and microbial composition is contingent on diet composition, as highlighted by Patra and Yu (2015) [32]. Numerous studies have explored the effects of incorporating saponin-rich plants into ruminant diet. Those investigations consistently demonstrate the potent antiprotozoal activity of saponins, suggesting their potential as an alternative to in-feed antibiotics or growth hormones for ruminants due to their defaunation properties.

In this comprehensive review, our objective is to offer enhanced insights into the impacts of saponins or saponin-containing plants on the ruminal microbiome and fermentation patterns. Additionally, we explore saponin metabolism and its effects on ruminant performance.

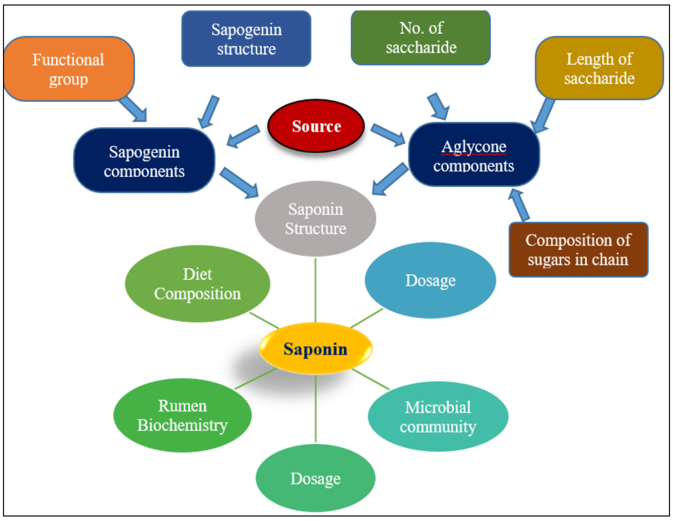

Fig 1: A schematic presentation of factors ffecting the effects of saponins on rumen

Rumen fermentation, digestibility, and methane emission in animals are influenced by several factors, including the species and breed of the animal, its size, physiological stage, dry matter intake, and digestibility. There are two broad techniques for manipulating the rumen: genetic manipulation and non-genetic manipulation. Genetic manipulation involves developing genetically engineered rumen microbes through gene transfer or manipulation techniques (Mhathung, 2015) [28]. Non-genetic manipulation involves dietary manipulation, the use of suitable chemicals, Direct-Fed Microbials (DFM), or different types of plant secondary metabolites. So, in present review, we aim to enhance our understanding of the impact of saponins or saponin-containing plants on the Ruminal microbiome, Nutrient utilization, Methane emission and fermentation patterns. Additionally, our focus extends to exploring saponins metabolism and elucidating the influence of saponins on the performance of ruminant animals.

Table 1: Some Sources of Saponin and their Saponin Contains

Sr. No.	Saponin Sources	Saponin Contains
1	Soapnut (*Sapindus mukorossi*)	10.1%
2	*Quillaja saponaria*	3%
3	*Yucca schidigera*	6%
4	*Sapindus saponaria*	120 mg/g
5	*Sapindus rarak*	14.6%
6	*Acacia concinna*	11.8%
7	*Yucca schidigera extract*	4.4%
8	*Agave Americana*	80 g/kg
9	*Enterolobium cyclocarpum*	19 mg/g
10	*Pithecellobium saman*	17 mg/g
11	*Camellia* seed shell	8%
12	Isolated alfalfa	1.8-3.6%)
13	Tea seed (*Camellia sinensis*)	15%
14	*Camellia sinensis*oil cake	5
15	*Tea saponin*- triterpenoid	60%

1. *In vitro* Effect of Saponin

1.1 *In vitro* nutrient digestibility

Poornachandra *et al.* (2019[b]) [35] studied comparatively with saponin as well as tannin source by using rumen liquor from the cannulated crossbred male cattle. Saponin as a soapnut added @ 5.1% in diet and observed higher decrease in IVDMD in individually supplemented soapnut compared to the control (57.9% *vs.* 60.6%). However, increasing the level of soapnut in combination resulted in a comparatively greater decrease in IVDMD. Rathod *et al.* (2024[b]) [38] studied with different levels of soapnut using HF cattle rumen liquior and reported IVDMD were significantly (p<0.05) increased @ 1 and 2% soapnut compared to control. Conversely lowest (p<0.05) IVDMD values were observed at the Soapnut concentrations of 4 and 8%. Holtshausen *et al.* (2009) [13] studied with two saponin sources, *Yucca schidigera* and *Quillaja saponaria* and used SRL of dairy cows, both added @ 15, 30, 45 g/kg DM) and reported reduction in IVDMD at 30 and 45g with both sources compared to the control (YS: 41.3% & 40.0% *vs.* QS: 39.4% & 37.1% *vs.* control: 44.5%). Hess *et al.* (2003[a]) [12] also studied on *Sapindus saponaria* @ 100mg/g DM by using SRL of fistulated brown Swiss cow and observed 12-15% degradation (p<0.05) of neutral detergent fiber. Thalib *et al.* (1996) [44] conducted an experiment by using rumen liquior of sheep fed *Sapindus rarak* fruit.

Table 2: *In vitro* Effect of Saponin on Digestibility of Nutrients

Saponin source	Saponin level	Effect on digestibility	References
Sapindus saponaria	100 mg/g diet DM	NDF degradation Reduced by 12-15%	Hess *et al.* (2003[a]) [12]
Tea saponin	0.2, 0.4 and 0.8 mg/ml	OMD reduc at 0.8 mg/ml	Hu *et al.* (2006)
Yucca schidigera, Quillaja saponaria	15, 30, 45 g/kg DM of each sources	Decreased significantly (p< 0.05)	Holtshausen *et al.* (2009) [13]
Tamarind seed husk & Soapnut	5.1% of diet (T:S ratio was 0:100, 75:25, 50:50,25:75)	Increasing soapnut level reduce DMD	Poornachndra *et al.* (2019[b]) [36]
Sapindus rarak fruit	0.5% of live weight	Significant increase in DMD% (50.56% *vs.* 48.17%)	Thalib *et al.* (1996) [44]
E. cyclocarpum and *P. saman*	@ 100 mg/g DM	Increased (p<0.05) OMD by 7-10%	Hess *et al.* (2003[a]) [12]
Acacia concinna pods	20 g/100 ml of solvent	IVDMD decreased significantly (p<0.05)	Patra *et al.* (2006) [33]
Soapnut	@ 1 and 2%	Significantly (p<0.05) increased	Rathod *et al.* (2024[b]) [38]

Their methanol-extracted *Sapindus rarak* fed @ 0.07% of live weight and reported significant increase in DMD% of *In vitro* incubation in the treatment groups (50.56% *vs.* 48.17%) compared to control.

Hu *et al.* (2006) also studied by using rumen liquior of sheep and they fed different levels of tea saponin (TS) @ 0, 0.2, 0.4, and 0.8 mg/ml in the *In vitro* rumen fluid. They reported organic matter digestibility decreased when TS added @ 0.8 mg/ml but, not differed with @ 0.2 mg/ml as compared (57.2 *vs.* 60.8 *vs.* 61.4%) to the control. Patra *et al.* (2006) [33] studied the *In vitro* effect of plant *Acacia concinna* (shikakai), extracts in the rumen liquor of buffalo. Three solvents extracted: water, ethanol (95%), and methanol (98%) at a concentration of 20 g/100 ml of solvent and extracts added @

0, 0.25 and 0.5 ml. The results showed that the *In vitro* DMD decreased significantly ($P<0.05$) with both extracts compared (0.446 *vs*. 0.453 *vs*. 0.467 g/g DM) to the control. While, Agarwal *et al*. (2006) [2] reported the *In vitro* degradability of wheat straw was adversely affected by @ 0.5 ml/30 soapnut extracts in water (9.32 *vs*. 28.54%), ethanol (14.67 *vs*. 28.02%), and methanol (6.51 *vs*. 30.8%) compared to control. Wang *et al*. (1998) [46] investigated the effects of *Yucca schidigera* extract (YSE) using the rumen simulation technique (RUSITEC). YSE was included in the diet at 0.5 mg/ml as a fed basis for 22 days. The results indicated a non-significant effect on the digestibility of dry matter and total gas production.

1.2 *In vitro* methane emission

Poornachandra *et al*. (2019[b]) [35] studied on saponin source by using rumen liquor from the cannulated crossbred male cattle. The basal diets were formulated @ 5.1% of the diet. The results reported that methane production in the test groups with higher saponin (soapnut) did not differ significantly compared to control group. Rathod *et al*. (2024[b]) [38] studied with different soapnut levels and observed reduction of *In vitro* methane gas 22.16, 15.79% with 1, 2% of soapnut in TMR. Holtshausen *et al.* (2009) [13], *In vitro* experiments were performed on dairy cows by feeding them saponin-containing *Yucca schidigera* and *Quillaja saponaria* at three different doses of 15, 30, and 45g/kg DM, showed that significantly decreased methane production ($P<0.05$) with both *Yucca schidigera* and *Quillaja saponaria* at all three dose levels compared to control. The methane production values for *Yucca schidigera* were 24.8, 22.9, and 20.1 mg/g DM, while for *Quillaja saponaria* they were 25.5, 24.0, and 23.8 mg/g DM, respectively, compared to the control value of 27.1 mg/g DM. Pen *et al.* (2006) [34] also investigated the effect of *Yucca schidigera* (YSE) and *Quillaja saponaria* (QSE) extracts on *In vitro* methane production. Added @ 0, 2, and 4 ml/l and reported significantly reduction of methane production up to 42% and 32%, with YSE and QSE, respectively. Hu *et al*. (2005) conducted the experiments to investigate the effect of tea saponin @ 0, 0.2, and 0.4 mg/ml during *In vitro* methanogenesis and showed that 0.2 and 0.4 mg/ml TS decreased methane emission by 12.5% or 14.0% respectively, compared to control.

Table 3: *In vitro* Effect of Saponin on Methane Emission

Saponin source	Saponin level	Methane reduction Result	References
Sapindus saponaria	100 mg/g diet DM	Reduced by 20%	Hess *et al.* (2003[a]) [12]
Tea saponin	0.2, 0.4 and 0.8 mg/ml	8.5, 15.0 and 22.7%	Hu *et al.* (2006)
Yucca schidigera, Quillaja saponaria	15, 30, 45 g/kg DM of each sources	Decreased significantly (P < 0.05)	Holtshausen *et al.* (2009) [13]
Tamarind seed husk & Soapnut	5.1% of diet (T:S ratio was 0:100, 75:25, 50:50,25:75)	12,19.4,7,14	Poornachndra *et al.* (2019b) [36]
Acacia concinna pods	20 g/100 ml of solvent	C *vs* T; 40, 41 *vs.* 31 ml/g DM	Patra *et al*. (2006) [33]
Sapindus mukorossi	20 g/100 ml of solvent	96, 20, and 22.7% with ethanol, water, and methanol extracts	Agarwal *et al.* (2006) [2]
Yucca schidigera	@ 2 ml per liter solvent	Methane reduction (50 *vs.* 110 ml)	Takahashi *et al.* (2000) [43]
Yucca schidigera and *Quillaja saponaria* extracts	0, 2, and 4 ml/l	Reduced methane production up to 42% and 32%,	Pen *et al*. (2006) [34]
Soapnut	@ 1 and 2%	Lowers methane production (2.81, 3.04ml/100mg DDM)	Rathod *et al*. (2024[b]) [38]

Patra *et al*. (2006) [33], studied on *In vitro* methanogenesis of feed using buffalo rumen liquor. *Acacia concinna* pods extracts were prepared using three solvents: water, ethanol (95/100 ml), and methanol (98/100 ml) at a concentration of 20 g/100 ml of solvent. The results showed that gas production was significantly higher with the extracts of *A. concinna* compared (40, 41 *vs*. 31 ml/g DM) to the control group. Hu *et al*. (2006) studied with tea saponin @ 0.2, 0.4 and 0.8 mg/ml in rumen fluid and reported that the addition of TS significantly reduced methane production compared to control. After 24 hours of incubation, the inclusion of 0.2, 0.4, and 0.8 mg/ml of TS reduced methane emission by 8.5%, 15.0%, and 22.7%, respectively. The researchers concluded that TS was an effective methane inhibitor, particularly when included at a concentration of 0.4 mg/ml during *In vitro* rumen fermentation. Agarwal *et al.* (2006) [2] studied *In vitro* methanogenesis and indicated that decrease in methane gas production was 96, 20, and 22.7% with ethanol, water, and methanol extracts, respectively compared to control. Hess *et al.* (2003[a]) [12] studied *In vitro* effect of saponin-rich tropical fruit *Sapindus saponaria* and *E. cyclocarpum*, indicated that daily methane release was reduced by up to 20% with *S. saponaria*, but increased by 14% with *E. cyclocarpum* ($p<0.05$). Takahashi *et al.* (2000) [43] investigated the *In vitro* effects of *Yucca schidigera* extracts and resulted lower production of total gas (230 *vs*. 430 ml) and methane (50 *vs*. 110 ml) with *Yucca extract* compared to the control.

2. Effect of Saponin on Body Weights

Feeding animals with saponins has been shown to enhance growth performance in various experiments. This improvement is attributed to a rise in intestinal amino acid absorption resulting from a reduction in protozoal numbers (Patra *et al*., 2006) [33]. In the study of Rathod *et al.* (2024[a]) [38] with 1 and 2% of soapnut levels in cattle, reported average weight gain was significantly ($p<0.05$) higher in T_1 compared to T_2, and observed. While, treatment did not affect significantly the final body weight of HF Cross-bred cattle. Kumar *et al.* (2017) [22] studied with tea seed saponin on the growth parameters of eighteen male Gaddi kids. The results reported that the average daily gain and feed conversion ratio were improved ($p<0.05$) for the T_1 and T_2 groups compared to the control group. However, the final body weight did not differ significantly (P>0.05). Thalib *et al.* (1996) [44], Hu *et al.* (2006) and Meel *et al.* (2015) [27] reported improved body weigh in sheep, Boer goat and Rathi calves, respectively, by treatments of different saponin sources compared to the control group. Sultana *et al.* (2012) [42] and Holtshausen *et al.* (2009) [13] reported no significance difference (P>0.05) in the final live weight of Native bulls and Holstein dairy cows, respectively.

Table 4: Effect of Saponin on Body weights

Saponin source	Saponin level	Effect on BW	References
Sapindus rarak fruits	@ 0.07% of live weight.	Significantly higher 20.93 *vs.* 18.58 kg	Thalib *et al.* (1996) [44]
Tea saponin	@ 3 g/day	Higher average daily gain (94 g/day)	Hu *et al.* (2006)
S. mukorossi (soapnut)	@ 3% leaves with ration	Average daily gain (318.99 g *vs.* 275.83 g) were higher	Meel *et al.* (2015) [27]
saponin at different doses	@ 0, 36, and 54 mg/kg DM	NS	Aazami *et al.* (2013) [1]
soapnut	@ 1 and 2% of DM	Average weight gain (p<0.05) higher in T_1 compared to T_2,	Rathod *et al.* (2024[a]) [38]

3. Effect of Saponin on Feed Intake and Nutrient Intake

Poornachandra *et al.* (2019[a]) [35] conducted an experiment on adult cross-bred cattle to compare the effect of 5.1% phyto-source soapnut as individual or combined supplementation. Result reported that dry matter intake was not affected with the supplement of soapnut compared to the control. But, significantly (p<0.05) decreased in CP intake of saponin group compared (0.4 *vs.* 0.5 kg/d) to the control group. Rathod *et al.* (2024[a]) [38] studied with of soapnut levels in cattle. The use of soapnut @ 1% and 2% with TMR had no adverse effect on dry matter intake (kg/d and kg/100 kg b.wt.), crude protein intake, DCP% as well as DCP and TDN intake, though apparently the values were higher in T_1 followed by T_0 than in T_2 treatment. Kumar *et al.* (2017) [22], Gaddi kids were supplemented with tea seed and tea seed saponin extract at levels of 2.6 and 0.4% of DMI. They reported DMI were significantly higher (P<0.05) than the control group, However, the intake of CP, DCP in grams per day (g/d) and g/kg of metabolic weight (g/kg $W^{0.75}$), as well as TDN was not differed significantly. Hu *et al.* (2006) reported DMI was significantly higher in T_1 as compared to T_2 and C up to 30 days in growing Boer goats but, after 31-45 days difference non-significance.

Table 5: Effect of Saponin on Nutrient Intake

Saponin source	Saponin level	Effect on Intake	References
S. mukorossi leaves	3% Leaves	Intake of N was high but excreted N contains not differed	Meel *et al.* (2015) [27],
Yucca schidigera extract	0, 25, and 50 g/head/day	Significant reduction (p<0.05) in DMI	Lovett *et al.* (2006) [23]
Sapindus mukorossi	5.1%	Significantly (p<0.05) decreased in CP intake	Poornachandra *et al.* (2019[a]) [35]
Sopanut	1, 2%	NS	Rathod *et al.* (2024[a]) [38]

Sultana *et al.* (2012) [42], Meel *et al.* (2015) [27], and Jadhav *et al.* (2017) [17] who reported no adverse effects of saponin sources on nutrient intake. While Lovett *et al.* (2006) [23] reported reduction of CPI with adding of saponin sources YE in the diet of HF dairy cows. Nasri *et al.* (2011) [30] conducted an experiment on Barbarine lamb and results reported that DMI (g/kg $BW^{0.75}$), OMI (g/kg $BW^{0.75}$), CPI (g/kg $BW^{0.75}$), NDFI (g/kg $BW^{0.75}$) and water intake (ml/kg $BW^{0.75}$) were more or less similar among all the groups of lamb. Nasri and Salem (2012) [29] investigated the effect of oral administration of *Agave Americana* extract and *Quillaja saponaria* extracts in Barbarine female lambs. The effect of both saponins sources on DMI (g/kg $W^{0.75}$), OMI (g/kg $W^{0.75}$) and CPI (g/kg W0.75) was reprted non-significant.

4. Effect of Saponin on Digestibility of Nutrients

Rathod *et al.* (2024[a]) [38] studied by using 1 and 2% of soapnut levels with TMR in HF CB cattle. The study revealed that there was no adverse effects of Soapnut on digestibility of nutrients like DM, OM, EE, NFE, ADF, and Cellulose, though the values gradually decreased with 1% and 2% supplement over the control diet. While, digestibility of fibre fractions *i.e.*, CF, NDF and hemicelluloses were found to be decreased significantly and CP digestibility was insignificantly improved in T_1 over T_0 group. The reduction in digestibility observed may be due to defaunating effect of saponins (Jadhav *et al.*, 2016) [18]. The non-significance results of nutrient digestibility were in accordance with Nasri and Salem (2012) [29], Jacob *et al.* (2012) [16] and Aazami *et al.* (2013) [1].

Table 6: Effect of Saponin on Nutrient Digestibility

Saponin source	Saponin level	Result	References
Tamarind seed husk & Soapnut	5.1% of diet (T:S ratio was 0:100, 75:25, 50:50,25:75)	Increasing soapnut level reduce DMD	Poornachndra *et al.* (2019[b]) [36]
Isolated alfalfa	@ 0, 2, or 4% levels	Cellulose and hemicelluloses was increased (P<0.02)	Lu and Jorgensen (1987) [24]
Yucca extract	@4g/day per animal	Digestibility of ADF was reduced (35.9 *vs.* 41.4%)	Wu *et al.* (1994)
E. cyclocarpum fruits	@ 200mg/kg DM	CP was higher (P<0.05)	Hess *et al.* (2003[a]) [12]
Sapindus saponaria	80 mg per gram of diet.	Degradation of ADF and NDF	Hess *et al.* (2003[b]) [12]
Quillaja saponaria	120 mg saponins/kg DM	OM, DM, CP and NDF was statistically similar (P>0.05)	Nasri and Salem (2012) [29]
Quillaja saponaria extract	@ 0, 30, 60, and 90 mg/kg	Digestibility of NDF was significantly reduced (P<0.011)	Nasri *et al.* (2011) [30]
Sapindus rarak fruit	0.O7% of live weight	DMD significantly (P<0.05) (64.91 *vs.* 62.36%)	Thalib *et al.* (1996) [44]
Garlic, Soapnut, Harad and Ajwain	Ratio of 2:1:1:1 at 1% of DMI	CPD was significantly (P<0.05) higher (730.1 *vs.* 634.4)	Samal *et al.* (2016) [40]
Soapnut	1, 2%	NS effect on dige. of DM, OD, CP, EE, NFE ADF but, reduced (P<0.05) CF, NDF, hemicellulose	Rathod *et al.* (2024[a]) [38]

Poornachandra *et al.* (2019[a]) [35] observed the dry matter and organic matter digestibility among all the soapnut treatment groups were no significant. Samal *et al.* (2016) [40] reported that crude protein digestibility was significantly (p<0.05) higher in Mix-1 as compared to the control group. Kumar *et al.* (2017) [22] reported that digestibility of ether extract was significantly reduced ($p<0.05$) in the groups supplemented with saponin sources, while, Jadhav *et al.* (2017) [17] reported digestibility of DM, OM, EE, NDF, ADF and cellulose were reduced ($p<0.05$) by higher tea seed saponin compared to low and without saponin supplemented group.

Meel *et al.* (2015) [27] studied the effect of supplementation of *S. mukorossi* in Rathi calves. Results reported that digestibility of DM, OM, CP, EE, CF and NFE were higher significantly ($P<0.01$) and ADF was higher ($p<0.05$) in *S. mukorossi* supplemented group compared to the control. While NDF and hemicellulose digestibility not affected by treatments of 3% soapnut leaves. Sultana *et al.* (2012) [42] studied on the twelve bulls of the Native breed fed UMS with *S. mukorossi* (soapnut) herbal additive. Results reported their non-significant ($P>0.05$) difference for the digestibility of DM, OM and ADF due to herbal additives but, a significantly ($p<0.05$) low digestibility of CP in the presence of as compared to the control.

5. Effect of Saponin on Rumen Fermentation Parameters

Poornachandra *et al.* (2019[a]) [35] studied on cross-bred cattle by using the 5.1% soapnut as individual or combined supplementation and reported that ammonia nitrogen was reduced significantly ($p<0.05$) in soapnut treatments, while, significantly ($p<0.05$) greater TVFA in the soapnut group than the control group. Acetate and Propionate proportion did not observed significant changes in test group and the control group. Rathod (2023) [37] studied by using 1 and 2% of soapnut levels with TMR in cattle and resulted the pH of average rumen liquor did not differ significantly ($P>0.05$) in treatment groups. Their average concentration of TVFA in SRL was 11.47, 11.39, 12.13 mM/dl in T_1, T_2, T_3 groups respectively, ($P>0.05$). While, total-N and ammonia-N in T_1, T_2, T_3 were 62.35, 59.92, 61.97 mg/dl and 14.93, 14.76, 15.49 mg/dl, respectively, indicated more or less similar ($P>0.05$) among treatment groups. However, NPN (42.00, 45.73, 43.49 mg/dl), TCA precipitable N (37.15, 34.16, 35.09 mg/dl), and soluble nitrogen (25.23, 25.76, 26.88 mg/dl) in SRL also not differed ($P>0.05$) within T_1, T_2, T_3 group comparatively but, higher ($p<0.05$) concentration at 3h post-feeding.

5.1 Ruminal pH

Meel *et al*. (2015) [26] and Sultana *et al*. (2012) [42] resulted rumen liquor pH did not affects with soapnut leaves and fruits, respectively compared to the control group. Hess *et al*. (2003[a]) [12] and Wang *et al*. (2019) [45] reported no significant differences in rumen fluid pH, but, Agarwal *et al*. (2006) [2] reported a significantly lower pH value in the *In vitro* incubated medium compared to the control group.

5.2 Ruminal TVFA

Saponins impact volatile fatty acids (VFAs), the primary fermentation end products in the rumen. They form complexes with mucosal cell membrane sterols, altering intestinal cell permeability. This may reduce active nutrient transport mechanisms and enhance small intestine membrane permeability, facilitating the uptake of otherwise impermeable materials. While the effects of saponins on total and individual ruminal VFAs vary, some studies found no significant impact (Gunun *et al*., 2022, Widyarini *et al*., 2021, Holtshausen *et al*., 2009) [6, 47, 13]. Agarwal *et al.* (2006) [2], Sliwinski *et al.* (2002) [41] found no significant difference, while, Meel (2014) [26] and Wang *et al*. (2019) [45] reported increased TVFA value in calves when used saponin sources.

5.3 Total N, ammonia N and other N utilization

Sliwinski *et al.* (2002) [41], Nasri *et al.* (2011) [30], Mhathung (2015) [28], resulted total-N level did not differ, while, Meel (2014) [26] reported a highly significant effect on total nitrogen with the use of soapnut leaves supplied. Sultana *et al*. (2012) [42], Nasri *et al*. (2011) [30], and Mhathung (2015) [28] also reported that NH_3-N concentration did not differ, while, Hess *et al*. (2004) [10] reported a significantly increaseed ($p<0.01$) in the ammonia concentration with *Sapindus saponaria* @ 250 g/kg DM. Meel (2014) [26], Sliwinski *et al*. (2002) [41], Hess *et al*. (2003[b]) [12] did not find a significant effect on NPN levels, whereas, Mhathung (2015) [28] reported significantly higher NPN concentrations ($p<0.01$) in the incubation medium with *Glyricidia maculata* leaves. Sliwinski *et al*. (2002) [41] and Hess *et al*. (2003[b]) [12] not reported changes in soluble nitrogen but, Meel (2014) [26] reported a significant increase ($P<0.01$) in the concentration of soluble nitrogen in rumen liquor when reetha (*Sapindus mukorossi*) herbs were included at a rate of 3%.

6. Effect of Saponin on Methane Emission

Gas production, particularly methane, carbon dioxide, and hydrogen, is a key metric for assessing feed nutritive value. The presence of saponins or saponin-rich plants is anticipated to influence gas production by impacting nutrient digestion in the rumen. Poornachandra *et al.* (2019[a]) [35] conducted a study on 24 male adult cross breed (HF x Hallikar) cattles of 5-6 year old, to compare the effect of individual or combined supplementation of saponin source soapnut with tannin @ 5.1% in diet. The methane emission was estimated by SF_6 tracer technique at last nine day of feeding period and reported significant ($p<0.05$) reduction in enteric methane emission (g/day) with supplementation of soapnut combined with tannin source as compared to the control diet. Whereas, supplementation of soapnut significantly reduced ($p<0.05$) enteric methane emission (g) per kg intake of DM, OM or per kg intake of digestible DM, OM and NDF as compare to control group. Holtshausen *et al.* (2009) [13] conducted experiments to decrease enteric methane production in dairy cows by using saponin sources *Yucca schidigera* and *Quillaja saponaria*. Both saponin sources were added @ 10g/kg DM and Methane emission was measured on a subset of the animals using the SF_6 tracer technique. The result of methane production did not differ ($P>0.05$) as grams per day or g/kg of DMI among treatments. But, methane emission as measured by SF_6 technique was 13.7% lower than that measured in environmental chamber.

Hess *et al.* (2004) [10] studied on six white hill lambs investigate methanogenesis and nitrogen utilization in sheep receiving tropical grass hay and concentrate diets offered with *Sapindus saponaria* fruits @ 250g/kg DM. On last 2day of gas measurement, using detectors in respiratory unit an Oxymat 3 for oxygen, a Binos 1001 for carbon dioxide and methane detecion. They reported an average daily methane absolute and per kg $M^{0.75}$ was reduced ($p<0.01$) by proportionately 0.05 and 0.09, respectively when supplemented with *S. saponaria.* Jayanegara *et al.* (2020) [19] noted that the addition of saponins from *S. rarak* fruits @ 0.5, 1, 1.5 and 2 mg/ml medium to two diets (high-forage or high-concentrate) did not affect gas production. The effects of saponins on methanogenic archaea numbers and methane production are controlled by several factors (Guyader *et al.*, 2017) [8]. Saponins may reduce the activity of methaneproducing mcrA genes, methane production rates, and methanogenic archaea numbers and/or activity (Guo *et al.*, 2008, Hess *et al.*, 2003) [7, 12]. Goel and Makkar (2012) observed decreased CH_4 production (by 34–48%) with the addition of *Achyranthus aspara*, *Tribulus terrestris*, and *Albizia lebbeck* saponin extracts at 3, 6, or 9% dietary DM.

Conclusion

Saponins, found in plants or as extracts, offer benefits as feed or additives for ruminants. High saponin concentrations act as natural rumen manipulators, influencing microbial populations and potentially alteration of rumen metabolism. A significant effect is ruminal defaunation, suppressing ciliate protozoa and enhancing microbial protein synthesis efficiency. Saponins may also reduce methanogenesis by inhibiting ruminal methanogens. Their impact on ammonia adsorption and digesta passage alters rumen metabolism. Saponins improve nitrogen metabolism, addressing issues of poor nitrogen retention in ruminants. Effects depend on saponin types, levels, diet composition, and microbial adaptation. Identifying bioactive saponins targeting protozoa and methanogens is crucial. While most saponins are considered safe and beneficial, some types may be toxic. Saponin may be toxic at higher level to rumen microbes, since approx. 20% of fibre degradation is contributed by protozoa (Dijksha and Tamminga, 1995) [3]. Saponins are particularly effective in high-roughage diets, making them beneficial for smallholder livestock farmers in developing countries.

Conflict of Interest

Not available

Financial Support

Not available

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