Utilization of Saponins, a Plant Secondary Metabolite in Enteric Methane Mitigation and Rumen Modulation

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Authors' contributions

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Review Article

ABSTRACT

Methane (CH_4) emission originated from the livestock is a major concern globally as it is a potent greenhouse gas and also accounts for 3-10% loss of ingested feed energy for productive purposes. Interventions for CH_4 mitigation based on plant secondary metabolites (PSM) have been considered as safe, economical and effective strategies. Saponins, a multifunctional PSM, exhibited immense medicinal importance in traditional medicine system as well as in experimental biological systems. Last few decades witnessed a steady increase in interest towards application of saponins as rumen fermentation modulating agent as well a promising methane inhibitor. Saponins containing plants and their purified saponins have shown encouraging results in *in vitro* and *in vivo* experimental conditions. However, further studies are warranted to evaluate their toxicity profile, metabolism and mechanism of action at molecular level. This review focuses on the current status of role of saponins in ruminant nutrition.

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1. INTRODUCTION

Anthropogenic emission of methane (CH_4) from ruminants has a huge environmental impact. CH_4 has 21 times more global warming potential than CO_2 but half life of CH_4 is only 11 years in comparison to CO_2 thus makes CH_4 a right candidate for mitigation. Recent years witnessed an increasing interest in mitigating CH_4 emission and improving livestock performances by manipulating the rumen ecosystem. In this regard, plant secondary metabolites (PSM) have been considered as a viable option by animal nutritionist worldwide [1-3]. PSM are represented by alkaloids, glycosides, steroids, triterpenoids, phenolics, phenolic glycosides, quinolizidines, tannins, saponins, lignins, polysaccharides and essential oils [4,5].

The name 'saponin' is derived from the Latin word 'sapo' meaning soap. Saponins usually produces foam in water and therefore, also called foaming glycosides. These are amphiphilic and surface active compounds with detergent, wetting, emulsifying and foaming properties [6]. These are high molecular weight glycosidic compounds having a fat-soluble nucleus, the aglycone (either triterpenoid or steroids) and sugar side chains linked through ether or ester linkages to the aglycone [7]. Triterpenoid saponins naturally occur as saponin or free aglycone forms, while steroid saponins exclusively occur in saponins form. The chemical structure of saponins and its components is illustrated in Fig. 1. Depending upon the side chains and sugar moieties attached to aglycone skeletal, saponins can be categorized as monodesmoside, bidesmoside and tridesmoside [8].

Saponins are widely distributed in the plant kingdom and reported to occur in nearly 100 plant families. Saponins found applications in several food products and some medicinal formulations. Some traditional Asian folk medicines and dietary supplements are rich in saponins. Gubanov et al. [9] reported the existence of saponins in 754 species of central Asian plants. The soap bark tree (*Quillaja saponaria*), alfalfa (*Medicago sativa*), soapwort (*Saponaria officinalis*), mojave yucca (*Yucca schidigera*), gypsophila (*Gypsophila paniculata*), and sarsaparilla (*Smilax regelii*) are especially rich in saponins [7,10,11]. Plants such as siris, mahua, reetha and shikakai are also rich in saponins content. Triterpenoidal saponins occurs in higher amount in beans, peas, soybean, lucerne, tea, spinach, sugar beet, horse chestnut, and ginseng whereas, steroidal saponins are reported from yucca, oat, capsicum pepper, aubergine, tomato, alliums, asparagus, yam, fenugreek and ginseng [7]. Saponin content in different plants species varies widely and is influenced by age, physiology, environment as well as agronomic factors [12]. Saponins are synthesized by a common metabolic pathway similar to cholesterol and other steroids where squalene acts as common intermediary precursor [13]. The two major commercial sources of saponins are *Yucca schidigera*, which grows in the arid Mexican desert, and *Quillaja saponaria*, a tree that grows in arid areas of Chile. Micro-Aid® is commercially available animal feed additive manufactured from *Yucca schidigera* plant.

Apart from plants, marine invertebrates are also reported to synthesize triterpene glycosides. Takada et al. [14] isolated a triterpenoidal saponin nobiloside from the marine sponge *Erylus nobilis* with potent neuraminidase inhibitory activity. Van Dyck et al. [15] isolated saponins from sea cucumber *Holothuria atra, Holothuria leucospilota, Pearsonothuria graeffei* and *Actinopyga echinites*.

Table 1. Effect of different saponins extract and pure compounds on in vitro methanogenesis and rumen fermentation

Plant species	Test concentrations	Substrate/ feed	Methane reduction (%)	Rumen fermentation parameters	References
Knautia arvensis	10.2 and 20.4 g/kg	Hay: concentrate (50:50)	5.8 and 7.1	No significant effect on TVFA, A/P and methanogens	[16]
Quillaja saponaria	15, 30, 45 g/kg DM	Barley silage: concentrate (51:49)	5.33 4.43	IVDMD and A/P decreased; TVFA unaffected	[17]
Sapindus saponaria	100 g/kg DM	Meadow grass + *Arachis pintoi* hay + barley straw (56:22:11)	14.9	IVDMD and TVFA unaffected; protozoa decreased by 54%	[18]
	80 g/kg DM	Brachiaria grass	14.5	IVDMD and A/P decreased; TVFA unaffected	[19]
Trigonella foenum-graecum	14.8 and 30.4 g/kg DM	Hay: concentrate (50:50)	2.21 and 2.21	No significant effect on TVFA, A/P and methanogens	[16]
Camellia sinensis	10, 20, 30, 26.7. 53.3 g/kg DM	Grass hay: corn (50:50)	8.76- 27.8	IVDMD and protozoa decreased, TVFA and A/P unaffected	[20]
	26.7, 53.3, 106.7 g/kg DM	Grass hay: corn (50:50)	7.3 – 16.6	IVDMD and TVFA unaffected; A/P and protozoa decreased by 19-45%	[21]
Yucca schidigera	0.5 g/L	Lucerne hay: concentrate (50:50)	12.8	No significant effect	[22]
Quillaja saponaria	92.0, 276.4 g/kg DM	Oat hay: concentrate (50:50)	-	TVFA and A/P unaffected, protozoa decreased (34.4–41.3%)	[23]
	92.0, 184.0 g/kg DM			TVFA and A/P unaffected, protozoa decreased	[24]
Pithecellobium saman	200 g/kg DM	Meadow grass: *Arachis pintoi* hay: barley straw (56:22:11)	no effect	Digestibility and TVFA unaffected; protozoa increased by 54%	[18]
Enterolobium	2.84 g/l or 200 g/kg diet		no effect	IVDMD and TVFA unaffected;	

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				protozoa increased by 54%	
cyclocarpum					
Medicago sativa	0.001 and 0.02, and 0.1 g/kg DM of effective sarsaponin	Grass silage and hay: barley (77:23)	−5.16, 3.87 and 1.29	IVDMD, TVFA, A/P, total bacteria unaffected	[25]
Sesbania sesban	1.65 g/l or 174 g/kg substrate	Hay: concentrate (32:68)	11.9	50.5% reduction in protozoa	[16]
Acacia concinna	0.2 g/kg DM	Wheat straw: Concentrate (50:50)	3.8 and 18.6	TVFA and IVDMD unaffected, A/P and protozoa numbers decreased	[26]
Sapindus mukorossi	0.2 g/kg DM	Wheat flour: wheat straw (75:25)	22- 96	IVDMD, A/P and protozoa decreased (70-90%), TVFA unaffected	[27]
Sapindus saponaria	0.25, 0.5, 1, 2, 4 g/l	Elephant grass: wheat bran (70:30)	nd	Methanogens, A/P ratio decreased; TVFA increased; digestibility and protozoa decreased	[28]
Medicago sativa	1.2–3.2 g/l or 180–480 g/kg substrate	Corn starch	36.0–64.1	TVFA increased, A/P decreased, protozoal numbers decreased	[29]
Camellia sinensis	4 -12 g/kg DM	Corn meal : grass meal (20:60)	nd	TVFA and propionate increased, protozoa and ammonia decreased	[30]
Tribulus terrestris	0.30, 60, 80 g/l cultural media	Corn grain : Chinese wild rye (50:50)	23.43	TVFA, acetate and ammonia decreased, propionate and A/P increased, protozoa decreased	[31]

DM: dry matter; TVFA: Total volatile fatty acids; A/P: Acetate: propionate ratio; IVDMD: in vitro dry matter digestibility; nd: not described

Table 2. Effects of saponins on rumen microbial population, methanogenesis and rumen fermentation parameters in different ruminant animals

Saponin source	Animal host	Period (Days)	Dose tested	Substrate/ feed	Methane reduction (%)	Effects on rumen microbes and fermentation parameters	References
Agave americana	Lamb	60	120, 240, 360 mg/kg DM	Hay: concentrate (75:25)	nd	Protozoa decreased by 39-42%; growth rate increased	[32]
Camellia sinensis	Sheep	21	5 g/kg DM	Lucerne hay: concentrate (60:40)	8.71	No significant effect	[33]
	Lamb	60	4.1 g/kg DM	Wild rye: concentrate (60:40)	27.2	TVFA increased; A/P unaffected; protozoal and methanogen decreased	[1]
Ilex kudingcha	Goat	10	0, 400, 600, 800 mg/kg DM	Hay: concentrate	nd	No significant effect	[34]
Medicago sativa	Sheep	14	10.2, 20.4 g/kg DM	Hay : concentrate (50:50)	5.8- 7.1	TVFA, A/P, methanogens unaffected	[35]
Quillaja saponaria, Yucca schidigera, C. sinensis	Steers	22	0.25 – 1.5% DM	Corn: corn silage	No effect	nd	[36]
Q. saponaria	Sheep	18	13.5 g/kg of diet or 16.1 g/day	Ryegrass hay: concentrate (60:40)	21.7	TVFA decreased, digestibility, A/P, protozoa not affected	[37]
	Cattle	28	10 g/kg of DM	Barley silage: concentrate (51:49)	7	No significant effect	[17]
Sapindus saponaria	Sheep	21	5 g/kg body wt	Forage: concentrate (49.2–56 : 21)	7.8	Digestibility, A/P and protozoa decreased; TVFA and methanogens increased	[38]
Y. schidigera	Sheep	21	0.002 and 0.03 g/kg DM	Hay: barley-concentrate (50:50)	1.4 and −2.2	No significant effect	[25]

Sheep	15	0.12 g/kg DM	Orchard grass silage : concentrate (70:30)	6.7	No significant effect	[39]
Seep	18	13.8 g/kg DM	Ryegrass hay: concentrate (60:40)	15.6	TVFA decreased, digestibility, A/P, protozoa not affected	[37]
Sheep	15	0.13 g/kg DM	Mixed hay: concentrate (75:25)	13.7	Digestibility unaffected, A/P decreased, TVFA increased	[40]
Cow	28	10 g/kg of DM	Barley silage: concentrate (51:49)	2.5	No significant effect	[17]

DM: dry matter; TVFA: Total volatile fatty acids; A/P: Acetate: propionate ratio; nd: not described.

2. TYPES AND STRUCTURE OF SAPONINS

(i) Triterpenoid Saponins

These saponins are most widely distributed in the plant kingdom. These are present in Magnoliopsida families mainly *Primulaceae*, *Sapotaceae* and *Caryophyllaceae*. Triterpenoid saponins consist of a 30 carbon skeleton comprising mostly four or rarely five units. These saponins are structurally highly diverse. Pentacyclic triterpenoid saponins skeleton belongs to oleanane, ursane, lupane, hopane, germanicane and dammarane types (Fig. 1 and 2). Their chemical structure and properties have been extensively reviewed elsewhere [11,41,42].

ii) Steroid Saponins

Steroid saponins are less widely distributed than the triterpenoid types and usually found in members of families such as *Liliaceae, Dioscoreaceae* and *Agavaceae*. These have 27 carbons skeleton which consists of either 6-rings (spirostane) or a 5-rings (furostane; Fig. 1 and 2).
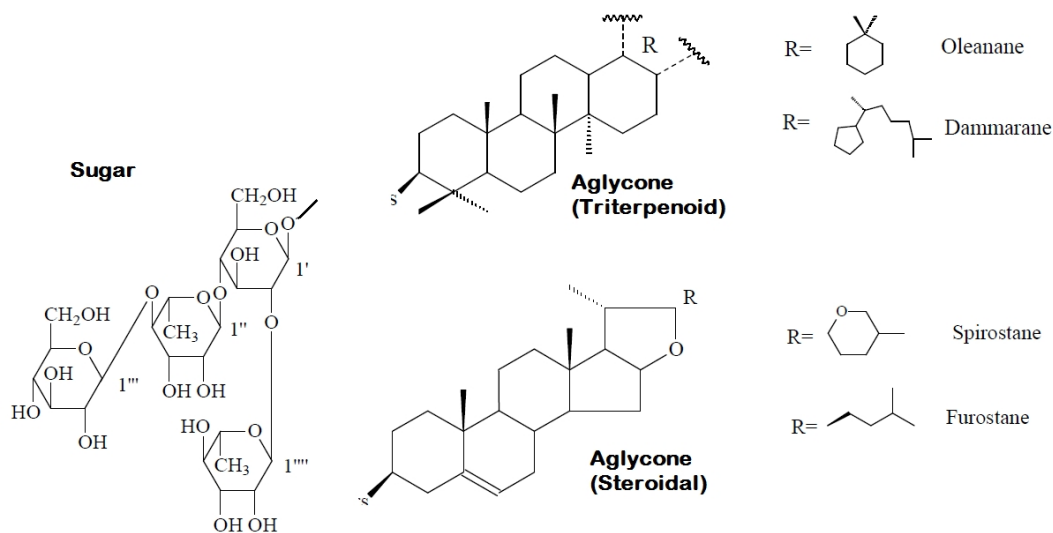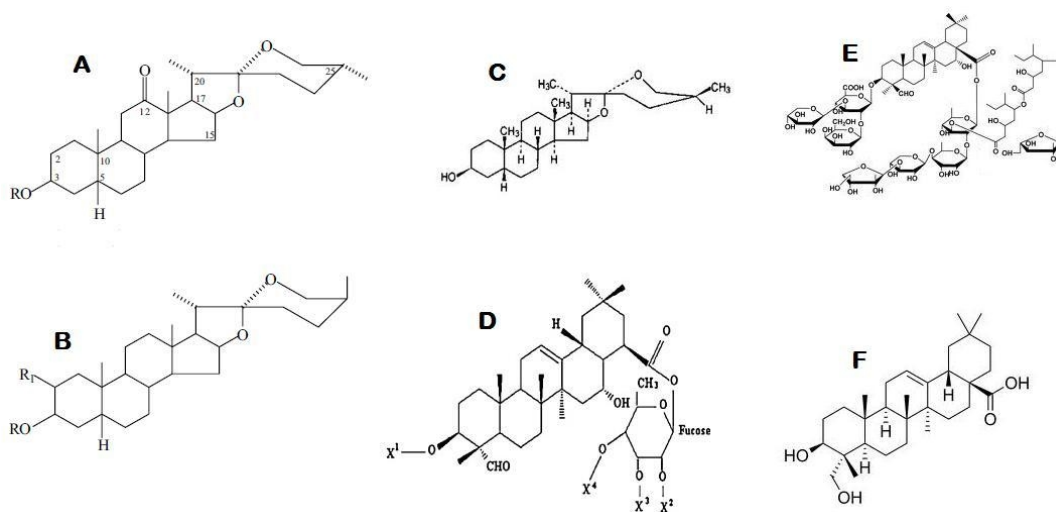

Fig. 1. Components and chemical structure of saponins

Fig. 2. Chemical structure of some common saponins of plant origin. (a, b) *Yucca schidigera* **saponins (c) sarsasapogenin (d) Quillaic acid (e) QS-21 from** *Quillaja saponaria* **(e) Hederagenin**

3. ISOLATION AND STRUCTURE ELUCIDATION TECHNIQUES

Due to their large size, non-volatile nature and complex structure, saponins isolation and identification is cumbersome, difficult and poses a serious challenge. In addition to conventional chromatographic methods, newer time-saving, environment friendly, efficient and solvent-economic methods have been developed and applied.

3.1 Thin Layer Chromatography (TLC)

TLC densitometric and colorimetric methods have been used for confirming the presence of saponins in plant crude extracts [42-45] as well as for their quantitative estimation. It involves use of colorants such as Ehrlich or vanillin reagent. However, interfering agents such as sterols and bile acids with hydroxyl group at C_3 may give a colour reaction with some reagents leading to false positive results. In this context, anisaldehyde-sulphuric acid-ethyl acetate reagent is considered to be superior as it gives color reaction only with steroidal sapogenins [46].

3.2 Gas Chromatography (GC)

Saponins, being polar and not volatile, are converted into volatile compounds by derivatization with acetyl, methyl or trimethylsilyl ethers prior to analysis [47,48]. The key step involved in GC is the hydrolysis of intact sapogenins to their aglycones components. The identification of derivatised aglycones can be performed by comparison of retention times of peaks in the sample GC spectrum with the retention times of appropriate standards. Best resolutions with relatively short retention times are obtained for trimethylsilyl derivatives.

3.3. High Performance Liquid Chromatography (HPLC)

HPLC is presently the most powerful and frequently used technique for qualitative and quantitative analysis of both aglycones and intact saponins [11,42,43,45,49]. In HPLC, separation of samples is performed on silica gel and C_{18} columns. Select carbohydrates, borate anion-exchange and hydroxyapatite supports have also find applications. Acetonitrile–water gradients are most commonly used for HPLC analysis of saponins. Carbohydrate and NH_2-modified columns were used for separation of glycoalkaloids and steroidal saponins [50,51]. Since saponins lack chromophore, their detection by HPLC is also cumbersome. Efficiency of HPLC can be improved by derivatization of saponins [52]. Apart from HPLC, new methods have evolved in recent years. Xu et al. [53] developed a MAE method coupled with HPLC-ESI-MS/MS system for rapid determination of saponins in *Pulsatilla turczaninovii*. This method was rapid, specific and reliable successfully applied to determine saponins in *P. turczaninovii*. This method reduces the extraction time and solvent consumption and increases the extraction efficiency.

4. Biological Activities of Saponins

Saponins exhibit a wide spectrum of biological activities which includes haemolytic, anti-inflammatory, anti-oedematous, antibacterial, antifungal, antiviral, insecticidal, anticancer, antitumor, cytotoxic, anthelmintic, molluscicidal, piscidal and immunomodulatory action (reviewed elsewhere by 7, [54-60]). In addition, saponins are reported to exhibit cholesterol-lowering action in animals [61] and human trials [62,63]. Waheed et al. [64] isolated a novel steroidal saponin glycoside from *Fagonia indica* that can induce cell-selective apoptosis or necrosis in cancer cells. Quillaja saponin showed anti-allergic and immunomodulatory effects in rat basophilic leukemia RBL-2H3 cells [65]. Ginseng and its ginsenoside constituents showed pronounced antioxidant, vasorelaxative, anti-inflammatory, anticancer, cholesterol lowering actions [66,67].

5. Saponins Metabolism in Ruminants

The decades old perception that saponins are not suitable for animal use has been changed in recent years following demonstration of their beneficial effects in different ruminant species under various dietary conditions. Saponins are poorly absorbed in intestine due to their large molecular mass (> 500 Da), high hydrogen-bonding capacity and high molecular flexibility. Due to this reason, most of their effects are manifested in the gut or rumen only. Most of the saponins and their degradation products are transported further, along the intestinal tract and finally excreted in the feces. However, there are reports that these may be absorbed in the duodenum and transported to the liver via the portal vein [68, 69]. Salts of the sapogenin glucuronide in the liver and bile have been reported from sheep suffering photosensitization [68,70]. Saponins undergo hydrolysis, epimerization and hydrogenation in the rumen mediated by microbial action [68,69]. Sarsapogenin of *Y. schidigera* and *Narthecium ossifragum* are degraded further to episarsapogenin, epismilagenin, sarsapogenone, smilagenin and smilagenone [68,71]. Quillaja saponin is metabolized to quillaic acid by rumen microbes after 6 h of in vitro incubation [72]. Animals have developed innate defense strategies to avoid excessive exposure to PSMs. These mechanisms include pre-systemic degradation by rumen microbes, intestinal barriers, hepatic biotransformation [73].

6. *In vitro* Effects of Saponins on Rumen Fermentation

In vitro effects of saponins or saponins-rich extracts on rumen microbes are presented in Table 1. *Y. schidigera* extracts were found to inhibit the growth of *Butyrivibrio fibrisolvens* [74], *Ruminococcus* spp. and *Fibrobacter succinogenes* [22] whereas stimulated the growth of *Prevotella ruminicola*. Similarly, *Y. schidigera* saponins exhibited inhibitory effect on Gram-positive amylolytic bacteria whereas Gram-negative species were unaffected or stimulated [75]. Goel et al. [76] determined the anti-methanogenic potential of total saponins of *Achyranthus aspara, Tribulus terrestris*, and *Albizia lebbeck* at 3, 6 and 9% (DM basis). *A. aspara* imparted highest methane reduction, whereas *A. lebbeck* was particularly effective against protozoa. In a recent report, Patra et al. [77] found that quillaja and yucca saponins can modulate ruminal microbial communities in a dose-dependent manner. Both the saponins are failed to alter the abundance of total bacteria, however, increased the archaeal abundance. Quillaja saponin decreased the abundance of *Ruminococcus flavefaciens* but not affected *Fibrobacter succinogenes* and *Prevotella*. In contrast, yucca saponin significantly increased the abundance of *R. flavefaciens, Prevotella* and F. succinogenes. However, both the saponins were not effective in mitigating methane emission. Patra et al. [77] suggested that saponins can improve the digestibility of the feed matter at low dose levels through stimulation of cellulolytic bacteria and other rumen bacteria. However at high doses, saponins cause defaunation and significantly affect ruminal fermentative activities.

Rumen fungi were found to be very sensitive to saponins. However, only limited studies have performed on rumen fungi. Goel et al. [16] observed 20-60% decrease in ruminal fungal population by saponin-rich fractions from *Carduus, Sesbania* and *Knautia* leaves and fenugreek seeds. Fenugreek was the most active against fungi. *Neocallimastix frontalis* and *Piromonas rhizinflata* were found to be highly sensitive to yucca saponins even at a low concentration of 2.25 μg ml^{-1} [75]. Similarly, Muetzel et al. [78] reported inhibitory effect of saponins on ruminal fungi with saponin-containing *Sesbania* leaves when included in the fermentation at >20% of the substrate. Triterpenoid saponin from *Camellia sinensis* exhibited 79% reduction in relative abundance of anaerobic fungal populations [79].

Saponins have been found to be most effective against protozoa among various rumen microbes and have the potential as defaunating agent [74,80,81]. Sarsaponin from *Y. schidigera* decreased protozoa numbers but not the bacterial number in 22 day semi-continuous system [82]. The protozoal populations were decreased significantly by quillaja saponin, but not by yucca saponins as reported by Patra et al. [77]. The in vitro effect of saponins on pure cultures of rumen methanogens have not been investigated extensively. Saponins have shown anti-methanogenic activity by reducing the numbers of protozoa harbouring methanogens as reported by Sharp et al. [83]. In another study, 78%, 22% and 21% reduction in methanogens population by *Sesbania sesban*, fenugreek and *Knautia* saponins, respectively was reported during in vitro fermentation containing cattle rumen liquor ([16]. Wina et al. [28] observed anti-methanogenic effect of saponins at a dose as high as at 4 mg/ml whereas protozoa are completely inhibited at 1 mg/ml. Some other studies also revealed the dose dependent effect of saponins against rumen methanogens. At 0.4 mg/ml dose level tea saponins failed to inhibit the growth of *Methanobrevibacter ruminantium* [79]. Kamra et al. [84] found saponins containing extracts of *Sapindus mukorossi* and *Yucca schidigera* to inhibit in vitro methanogenesis by more than 25% accompanied by a sharp decline in methanogen numbers and ciliate protozoa. Reduction of nearly 50% in CH_4 production with addition of saponins rich plant extracts has been reported by several investigators [85-88].

7. *In vivo* Studies with Saponins

Reduction in ruminal protozoa numbers is observed when saponins are fed to ruminants ([74,89,90], Table 2). However, a significant increase in cellulolytic and total bacteria in the rumens of sheep was observed when fed with *S. saponaria* fruit [91]. Similarly, Thalib et al. [92] who reported an increase in total cellulolytic bacteria in sheep fed with methanol extract of *S. rarak*. Administration of saponins to sheep every third day was effective in suppressing protozoa and reducing ruminal ammonia concentrations [92]. Sarsaponins from *Y. schidigera* and triterpenoidal saponins from *Q. saponaria* have been most extensively studied for their potential to reduce or inhibit CH_4 production in vivo. Administration of 5 g/kg of *S. saponaria* fruits to sheep for 21 days reduced CH_4 release by 6.5% [38] while the supplementation of *Y. schidigera* plant (6% saponins) for 28 days to dairy cows did not influence CH_4 production significantly [17]. It has been shown that saponins decreased the expression of genes involved in CH_4 synthesis in methanogens [18,79]. The anti-methanogenic activities of saponins are found to be dependent on type of solvents used for extraction of saponins and composition of diets. Saponins of *S. sesban* and fenugreek were more effective in animals fed concentrate based diets compared to those fed roughage-based diets [16]. Mechanistically, saponins form irreversible complexes with cholesterol, an integral component of protozoal cell membrane, thus leading cell lysis and death. However, saponins are hydrolyzed by ruminal bacteria leading to increased outflow of bacterial proteins from rumen [74,93].

8. Effect on Rumen Fermentation Parameters

Saponins also have variable effects on VFA production, but most studies indicate an increase in the proportion of propionate and a reduction in acetate, butyrate and branched chain VFA [87,94]. Further, the effects of saponins on VFA are pH and diet dependent where more pronounced the effects were seen at low pH [18, 29, 81]. Wang et al. [22] found enhanced breakdown of casein in continuous culture fermenters (RUSITEC) by extracts of *Y. schidigera*. In another study, NH_3-N concentration in the presence of *Y. schidigera*, *Q. saponaria* and *Acacia auriculoformis* saponins were 29.7%, 14.9% and 14.7% lower after 24 h incubation [93]. Yucca extract increased VFA levels when hay and straw were used as substrates [95]. Saponins derived from *Quillaja saponaria* reduced VFA levels whereas sarasaponin enhanced VFA levels. However *Acacia concinna*, *Enterolobium cyclocarpum* and tea saponins did not shown any appreciable effects on VFA levels [18,26,29,37,79]. Studies also revealed that the dietary incorporation of saponin containing extracts enhances propionate production in the rumen [28,29,81,96].

Inclusion of saponins in ruminant diets showed no adverse effects on feed intake [1,37,38,90,97]. Interestingly, increase in feed intake is observed following inclusion of saponins in diets of dairy cows [17] and sheep [98]. Few studies reported decrease in feed digestibility induced by addition of saponins [17,21,27,38,90,]. Dose-dependents effects of saponins on feed digestibility and methanogensis are reported. Santoso et al. [39] and Wang et al. [38] found that saponin extracts or saponin-containing plants did not alter digestibility, but decreased CH_4 production. At low dose of saponins exhibit anti-methanogenic effects without affecting digestibility, while at higher doses reported the reduction of both digestibility and methanogenesis [17,20].

9. Mechanism of Action of Saponins in Rumen Ecosystem

Saponins exhibited multifaceted effects on rumen fermentation and its microbial population which are manifested through different mechanisms. Saponins reduce methane production via inhibition of either protozoa or methanogens or both. These inhibited protozoa at relatively low concentrations whereas higher concentrations were required to kill or suppress methanogenic archaea as evident from several in vitro findings [28,35]. Their anti-protozoal action is manifested through interaction with cholesterol in the cell membrane leading to its disruption, breakdown, lysis and finally cell death. The intact saponin structure is suggested to be essential for anti-protozoal activity [22,72]. Anti-methanogenic activity of saponins is believed to occur by limiting hydrogen availability to methanogens and re-channeling of metabolic hydrogen from methane to propionate production in the rumen [28]. However, an entirely different mechanism is also suggested where saponins caused lesser fermentation of feed matter in the rumen which consequently resulted in more extensive fermentation in hindgut thus making conditions more favorable for acetogenesis through diversion of hydrogen from methanogenesis pathway [87,89,99]. The major mechanism suggested for the antifungal activity of saponins is their interaction with membrane sterols [87]. Biological action of saponins against eukaryotic cells is possibly due to their complexing with cholesterol in the lipid bilayer, formation of domains enriched with cholesterol-saponin complexes, and finally the lysis of cell membrane [100,101]. Saponins are also expected to interact with the lipid A part of LPS leading to increased permeability of bacterial cell wall [102]. Saponins form insoluble complexes with cholesterol and bile acids by sequestration in the intestine and also reduce blood glucose. Saponins administration in ruminants is reported to decrease plasma cholesterol and blood glucose levels [103,104].

10. Structure Activity Relationship and Chemisynthesis

PSM exhibit a remarkable structural diversity of chemical skeleton and scaffolds that could be utilized for chemical synthesis. In addition, computational chemical biology has the potential to offer new directions in finding suitable molecular entities in collections of natural products for new drug discovery. Combinatorial and multiple parallel synthesis methodologies have been developed for synthesizing new saponins derivatives [105]. Yu and Tao [106] synthesized dioscin andxiebai saponin I whereas Li et al. [107] prepared several stigmasterol saponins by semisynthesis. Similarly, Cheng et al. [108] synthesized β-hederin and hederacolchiside A1 triterpenoid saponins. Song et al. [109] and Ding et al. [110] generated saponin libraries of potent H5N1 virus entry inhibitors and performed further derivatization at aglycone and sugar moiety levels. SARs study revealed the critical importance of 3-O-b-chacotriosyl residue and the aglycone moiety for activity. Ding et al. [110] used Chlorogenin 3-O-b-chacotrioside as lead compound to design and synthesize a series of analogs with different sugar chains and aglycones. Presence of L-rhamnose units is reported to facilitate the cellular uptake of saponins due to interaction with lectins. Perez-Labrada et al. [111] implemented a linear glycosylation strategy to synthesize novel spirostan saponins with structural modifications at saccharidic and steroidal moieties including addition of β-D-glucopyranosides branched with a-L-rhamnopyranosyl residues at positions 4 and 6.

Industrial scale production of saponins is feasible and quite achievable. Wu et al. [112] developed a method for large scale production of akebia saponin D from Chinese herb *Dipsacus asper*. Using HPD-722 and ADS-7 macroporous absorption resins, akebia saponin D was successfully purified to the level of 95%.

2. CONCLUSION

By decreasing protozoal populations, saponins enhance propionate production, microbial biomass synthesis, microbial nitrogen flow and gluconuogenesis. This results in improvement in ruminant performance. Saponins are also found to be an effective and promising agent for CH_4 mitigation from livestock. Despite their multifunctional roles, there is a need for further evaluation to elucidate their exact mechanism of action, toxicity, effects at gene levels and dose-activity relationships. Studies are needed to determine the absorption, disposition, and pharmacokinetics profiles of different saponins to order to more accurately ascertain which types of saponins will be exerting maximal pharmacological effects in vivo. Further research is needed to expand our understanding of microbial modifications of aglycone moiety of saponins in the rumen and their transportation to different organs in animal host.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Mao HL, Wang JK, Zhou YY, Liu JX. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of Growing lambs. Livestock Sci. 2010;129:56-62.
2. Patra AK, Sexena J. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry. 2010;71:1198-1222.
3. Goel G, Makkar HP. Methane mitigation from ruminants using tannins and saponins. Trop Anim Health Prod. 2012;44:729-39.
4. Wallace RJ. Antimicrobial properties of plant secondary metabolites. Proc Nutr Soc. 2004;63:621–29.
5. Hartmann, T. From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 2007;68:2831–46.
6. Sarnthein-Graf C, La Mesa C. Association of saponins in water and water–gelatin mixtures. Thermochimica Acta 2004;418:79-84.
7. Sparg SG, Light ME, Van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol. 2004;94:219-43.
8. Guclu-Ustundag O, Mazza G. Saponins: properties, applications and processing. Cr Rev Food Sci Nutr. 2007;47:231–58.
9. Gubanov IA, Libizov NI, Gladkikh AS. Search for saponins containing plants in the flora of central Asia and south Kazkhstan. Farmatsiia 1970;19:23-31.
10. Vincken JP, Heng L, de Groot A, Gruppen H. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 2007;68:275-97.
11. Hill RA, Connolly JD. Triterpenoids. Nat Prod Rep. 2012;29:780-818.

12. Malik PK. Effect of dietary leguminous fodder on methane and nitrous oxide emission from ruminants. Ph.D. Thesis submitted to National Dairy Research Institute, Karnal, Haryana, India, 2007.
13. Pollier J, Moses T, Goossens A. Combinatorial biosynthesis in plants: a review on its potential and future exploitation. Nat Prod Rep. 2011;28:1897-1916.
14. Takada K, Nakao Y, Matsunaga S, van Soest RW, Fusetani N. Nobiloside, a new neuraminidase inhibitory triterpenoidal saponin from the marine sponge *Erylus nobilis*. J Nat Prod. 2002;65:411-13.
15. Van Dyck S, Gerbaux P, Flammang P. Qualitative and quantitative saponin contents in five sea cucumbers from the Indian ocean. Marine Drugs 2010;8:173-89.
16. Goel G, Makkar HPS, Becker K. Effects of *Sesbania sesban* and *Carduus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage- and concentrate-based feeds to methane. Anim Feed Sci Technol. 2008b;147:72–89.
17. Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA,Odongo NE, Cheeke PR, Benchaar C. Feeding saponin-containing Yucca schidigera and Quillaja saponaria to decrease enteric methane production in dairy cows. J Dairy Sci. 2009;92:2809-21.
18. Hess HD, Monsalve LM, Lascano CE, Carulla JE, Diaz TE, Kreuzer M. Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on in vitro ruminal nitrogen turnover and methanogenesis. Aust J Agric Res. 2003a;54:703-13.
19. Hess HD, Kreuzer M, Diaz TE, Lascano CE, Carulla JE, Solvia CR. Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated fluid. Anim Feed Sci Technol. 2003b; 109:79-94.
20. Hu W, Wu Y, Liu J, Guo Y, Ye J. Tea saponins affect in vitro fermentation and methanogenesis in faunated and defaunated rumen fluid. J Zhejiang Univ Sci B. 2005;6:787–92.
21. Hu W, Liu J, Wu Y, Guo Y, Ye J. Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Arch Anim Nutr 2006;60:89–97.
22. Wang Y, McAllister TA, Newbold CJ, Rode LM, Cheeke PR, Cheng KJ. Effect of *Yucca schidigera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (Rusitec). Anim Feed Sci Technol. 1998;74:143-53.
23. Pen B, Sar C, Mwenya B, Kuwaki K, Morikawa R, Takahashi J. Effects of Yucca schidigera and Quillaja saponaria extracts on in vitro ruminal fermentation and methane emission. Anim Feed Sci Technol. 2006;129:175-86.
24. Pen B, Sar C, Mwenya B, Takahashi J. Effects of *Quillaja saponaria* extract alone or in combination with *Yucca schidigera* extract on ruminal fermentation and methanogenesis in vitro. Anim Sci J. 2008; 79:193-199.
25. Sliwinski BJ, Kreuzer M, Wettstein HR, Machmuller A. Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins and associated emissions of nitrogen and methane. Arch Anim Nutr. 2002;56:379-92.
26. Patra AK, Kamra DN, Agarwal N. Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim Feed Sci Technol. 2006;128:276–91.
27. Agarwal N, Kamra DN, Chaudhary LC, Patra AK. Effect of *Sapindus mukorossi* extracts on in vitro methanogenesis and fermentation characteristics in buffalo rumen liquor. J Appl Anim Res. 2006;30:1-4.

28. Wina E, Muetzel S, Hoffmann E, Makkar HPS, Becker K. Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure in vitro. Anim Feed Sci Technol. 2005;121:159-74.

29. Lila ZA, Mohammed N, Kanda S, Kamada T, Itabashi H. Effect of sarsaponin on rumen fermentation with particular reference to methane production in vitro. J Dairy Sci. 2003;86:3330–36.

30. Liu JY, Yuan WZ, Ye J, Wu Y. Effect of tea (*Camellia sinensis*) saponin addition on rumen fermentation in vitro. In: Matching herbivore nutrition to ecosystems biodiversity. Tropical and subtropical agrosystems. Proceedings of the Sixth International Symposium on the Nutrition of Herbivore. Merida, Mexico; 2003.

31. Feng Z-H, Cao Y-F, Gao Y-X, Li Q-F, Li J-G. Effect of gross saponins of *Tribulus terrestris* on ruminal fermentation and methane production in vitro. J Anim Vet Adv. 2012;11:2121-25.

32. Nasri S, Salem HB. Effect of oral administration of *Agave americana* or *Quillaja saponaria* extracts on digestion and growth of Barbarine female lamb. Livestock Sci. 2012;147:59-65.

33. Yuan ZP, Zhang CM, Zhou L, Zou CX, Guo YQ, Li WT, Liu JX, Wu YM. Inhibition of methanogenesis by tea saponin and tea saponin plus disodium fumarate in sheep. J Anim Feed Sci. 2007;16(Suppl 2):560–65.

34. Zhou CS, Xiao WJ, Tan ZL, Salem AZM, Geng MM, Tang SX, Wang M, Han XF, Kang J. Effects of dietary supplementation of tea saponins (*Ilex kudingcha* C.J. Tseng) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats. J Anim Feed Sci. 2012;176:163-69.

35. Goel G, Makkar HPS, Becker K. Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. J Appl Microbiol. 2008a;105:770–77.

36. Li W, Powers W. Effects of saponins extracts on air emissions from steers. J Anim Sci. 2012;90:4001-13.

37. Pen B, Takaura K, Yamaguchi S, Asa R, Takahashi J. Effects of *Yucca schidigera* and *Quillaja saponaria* with or without b-1, 4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. Anim Feed Sci Technol. 2007;138:75-88.

38. Hess HD, Beuret RA, Lotscher M, Hindrichsen IK, Machmüller A, Carulla JE, Lascano CE, Kreuzer M. Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with *Sapindus saponaria* fruits and *Cratylia argentea* foliage. Anim Sci. 2004;79:177-89.

39. Santoso B, Mwenya B, Sar C, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H, Takahashi J. Effects of supplementing galactooligosaccharides, *Yucca schidigera* and nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. Livestock Prod Sci. 2004;91:209-17.

40. Wang CJ, Wang SP, Zhou H. Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. Anim Feed Sci Technol. 2009;148:157–66.

41. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry 1997;44:1185-1236.

42. Oleszek WA. Chromatographic determination of plant saponins. J Chromatogr A. 2002;967:147–62.

43. Oleszek W, Bialy Z. Chromatographic determination of plant saponins- an update (2002-2005). J Chromatogr A. 2006;1112:78-91.

44. Gauthier C, Legault J, Pichette A. Recent progress in the synthesis of naturally ocurring triterpenoid saponins. Mini Rev Org Chem. 2009;6:321–44.

45. Majinda RRT. Extraction and isolation of saponins. In: Methods in Molecular Biology, 864, 415-426, 2012.
46. Baccou JC, Lambert F, Sauvaire Y. Spectrophotometric method for the determination of total steroidal sapogenin. Analyst 1977;102:458-65.
47. Tava A, Oleszek W, Jurzysta M, Berardo N, Odoardi M. Alfalfa saponins and sapogenins: Isolation and quantification in two different cultivars. Phytochem Anal. 1993;4:269–74.
48. Ruiz RG, Price KR, Rose ME, Rhodes MJC, Fenwick GR. Determination of saponins in lupin seed (*Lupinus angustifolius*) using high-performance liquid chromatography: comparison with a gas chromatographic method. J Liq Chromatogr. 1995;18:2843–53.
49. Nowacka J, Oleszek W. Determination of alfalfa (*Medicago sativa*) saponins by high-performance liquid chromatography. J Agric Food Chem. 1994;42:727-30.
50. Xu CJ, Lin JT. Comparison of silica-, C18−, and NH2− HPLC columns for the separation of neutral steroid saponins from *Dioscorea* plants. J Liq Chromatogr. 1985;8:361-68.
51. Saito K, Horie M, Hoshino Y, Nose N, Nakazawa H. High-performance liquid chromatographic determination of glycoalkaloides in potato products. J Chromatogr A. 1990;508:141-47.
52. Oleszek W. Structural specificity of alfalfa (*Medicago sativa*) saponin haemolysis and its impacts on two haemolysis-based quantification methods. J Sci Food Agric. 1990;53:477-85.
53. Xu H, Shi X, Ji X, Du Y, Zhu H, Zhang L. A rapid method for simultaneous determination of triterpenoid saponins in *Pulsatilla turczaninovii* using microwave-assisted extraction and high performance liquid chromatography–tandem mass spectrometry. Food Chem. 2012;135:251-58.
54. Hostettmann K, Marston A. Saponins: chemistry and pharmacology of natural products, Cambridge University Press, Cambridge, 1-341, 1995.
55. Milgate J, Roberts DCK. The nutritional and biological significance of saponins. Nutr Res. 1995;15:1223-49.
56. Sirtori CR. Aescin: Pharmacology, pharmacokinetics and therapeutic profile. Pharmacol Res. 2001;44:183-93.
57. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: A Review. Br J Nutr. 2002;88:587-605.
58. Yu B, Sun J. Current synthesis of triterpene saponins. Chem Asian J. 2009;4:642-54.
59. Fuchs H, Bachran D, Panjideh H, Schellmann N, Weng A, Melzig MF, Sutherland M, Bachran C. Saponins as tool for improved targeted tumor therapies. Curr Drug Targets 2009;10:140-51.
60. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: a review. Phytochemistry Rev. 2010;9:425-74.
61. Matsuura M. Saponins in garlic as modifiers of the risk of cardiovascular disease. J Nutr. 2001;131:1000S-1005S.
62. Oakenfull DG, Sidhu GS. Could saponins be a useful treatment for hypercholesterolemia? Eur J Clin Nutr. 1990;44:79-88.
63. Kim SW, Park SK, Kang SI, Kang HC, Oh HJ, Bae CY, Bae DH. Hypocholesterolemic property of *Yucca schidigera* and *Quillaja saponaria* extracts in human body. Arch Pharm Res. 2003;26:1042–46.
64. Waheed A, Barker J, Barton SJ, Owen CP, Ahmed S, Carew MA. A novel steroidal saponin glycoside from *Fagonia indica* induces cell-selective apoptosis or necrosis in cancer cells. Eur J Pharma Sci. 2012;47:464-73.

65. Zhang T, Yang C, Rupa P, Jiang B, Mine Y. Inhibitory effects of Quillaja saponin on IgE-mediated degranulation of rat basophilic leukemia RBL-2H3 cells. J Funct Foods 2012;4:864-71.

66. Lu JM, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol. 2009;7:293-302.

67. Kim JH. Cardiovascular diseases and *Panax ginseng*: a review on molecular mechanisms and medical applications. J Ginseng Res. 2012;36:16-26.

68. Meagher LP, Smith BL, Wilkins AL. Metabolism of diosgenin-derived saponins: Implication for hepatogenous photosensitization diseases in ruminants. Anim Feed Sci Technol. 2001;91:157-70.

69. Flaoyen A, Wilkins AL, Sandvik M. Rumen metabolism in sheep of saponins from *Yucca schidigera*. Vet Res Commun. 2002;26:159-69.

70. Miles CO, Wilkins AL, Erasmus GL, Kellwerman TS. Photosensitivity in South Africa VIII. Ovine metabolism of *Tribulus terrestris* saponins during experimentally induced geeldikkop, Onderstepoort. J Vet Res. 1994;61:351-57.

71. Flaoyen A, Wilkins AL. Metabolism of saponins from *Narthecium ossifragnum*- a plant implicated in the etiology of alveld, a hepatogenous photosensitization of sheep. Vet Res Commun. 1997;21:335-45.

72. Makkar HPS, Becker K. Degradation of Quillaja saponins by mixed culture of rumen microbes. Lett Appl Microbiol. 1997;25:243-45.

73. Fink-Gremmels J. Defense mechanisms against toxic phytochemicals in the diet of domestic animals. Mol Nutr Food Res. 2010;54:249–58.

74. Wallace RJ, Arthaud L, Newbold CJ. Influence of *Yucca schidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl Environ Microbiol. 1994;60:1762-67.

75. Wang Y, McAllister TA, Yanke LJ, Cheeke PR. Effect of steroidal saponins from *Yucca schidigera* extract on ruminal microbes. J Appl Microbiol. 2000;88:887-96.

76. Goel NG, Sirohi SK, Dwivedi J. Estimation of total saponins and evaluate their effect on in vitro methanogenesis and rumen fermentation pattern in wheat straw based diet. J Adv Vet Res. 2012;2:120-26.

77. Patra AK, Stiverson J, Yu Z. Effects of quillaja and yucca saponins on communities and select populations of rumen bacteria and archaea, and fermentation in vitro. J Appl Microbiol. 2012;113:1329-40.

78. Muetzel S, Hoffmann EM, Becker K. Supplementation of barley straw with *Sesbania pacyhcarpa* leaves in vitro: Effects on fermentation variables and rumen microbial concentration structure quantified by ribosomal RNA-targeted probes. Br J Nutr. 2003;89:445-53.

79. Guo YQ, Liu JX, Lu Y, Zhu WY, Denman SE, McSweeney CS. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. Lett Appl Microbiol. 2008;47:421-26.

80. Newbold CJ, El Hassan SM, Wang JM, Ortega ME, Wallace RJ. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Br J Nutr. 1997;78:237-49.

81. Hristov AN, McAllister TA, Van Herk FH, Cheng KJ, Newbold CJ, Cheeke PR. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. J Anim Sci. 1999;77:2554–63.

82. Valdez FR, Bush LJ, Goetsch AL, Owens FN. Effect of steroidal sapogenins on rumen fermentation and on production of lactating dairy cows. J Dairy Sci. 1986;69:1568-75.

83. Sharp R, Ziemer CJ, Stern MD, Stahl DA. Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. FEMS Microb Ecol. 1998;26:71-78.

84. Kamra DN, Patra AK, Chatterjee PN, Kumar R, Agarwal N, Chaudhary LC. Effect of plant extracts on methanogenesis and microbial profile of the rumen of buffalo: a brief overview. Aust J Exp Agric. 2008;48:175-78.

85. Jouany J-P, Morgavi DP. Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. Animal 2007;1:1443-66.

86. Makkar HPS, Siddhuraju P, Becker K. Plant secondary metabolites. Methods in Molecular Biology 393, Humana Press, Totowa, New Jersey, pp.130, 2007.

87. Patra AK, Saxena J. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr Res Rev. 2009;22:204-19.

88. Szumacher-Strabel M, Cieslak A. Potential of phytofactors to mitigate rumen ammonia and methane production. J Anim Feed Sci. 2010;19:319-37.

89. Lu CD, Jorgensen NA. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J Nutr. 1987;117:919-27.

90. Klita PT, Mathison GW, Fenton TW, Hardin RT. Effects of alfalfa root saponins on digestive function in sheep. J Anim Sci. 1996;74:1144–56.

91. Diaz A, Avendano M, Escobar. Evaluations of *Sapindus saponaria* as a defaunating agent and its effects on different rumen digestion parameters. Livestock Res Rural Dev. 1993;5:1-6.

92. Thalib A, Widiawati Y, Hamid H, Suherman D, Sabrani M. The effects of saponin from *Sapindus rarak* fruit on rumen microbes and performance of sheep. Jurnal Ilmu Ternak dan Veteriner 1996;2:17-20.

93. Makkar HPS, Sen S, Blümmel M, Becker K. Effects of fractions containing saponins from *Yucca Schidigera, Quillaja saponaria* and *Acacia auriculoformis* on rumen fermentation. J Agric Food Chem. 1998;46:4324-28.

94. Castro-Montoya JM, Makkar HPS, Becker K. Chemical composition of rumen microbial fraction and fermentation parameters as affected by tannins and saponins using an in vitro rumen fermentation system. Can J Anim Sci. 2011;91:433-48.

95. Ryan JP, Quinn T, Mullally J, Leek BF. Effects of *Yucca schidigera* plant extract (De-Odorase) on the fermentation of hay, straw and concentrates by sheep's ruminal fluid in vitro and in vivo. Iranian Vet J. 2003;56:209-13.

96. Anamika. Effect of plant extracts on rumen fermentation and methane emission. M.V.Sc. Thesis, National Dairy Research Institute, Karnal, Haryana, India, 2007.

97. Singer MD, Robinson PH, Salem AZM, De Peters EJ. Impacts of rumen fluid modified by feeding *Yucca schidigera* to lactating dairy cows on in vitro gas production of 11 common dairy feedstuffs, as well as animal performance. Anim Feed Sci Technol. 2008;14:242-58.

98. Abreu A, Carulla JE, Lascano CE, Diaz TE, Kreuzer M, Hess HD. 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. 2004;82:1392-1400.

99. Varadyova Z, Zelenak I, Sirka P. In vitro study of the rumen and hindgut fermentation of fibrous materials (meadow hay, beech sawdust, wheat straw) in sheep. Anim Feed Sci Technol. 2000;83:127–38.

100. Gogelein H, Huby A. Interaction of saponin and digitonin with black lipid membranes and lipid monolayers. Biochimica et Biophysica Acta 1984;773:32–38.

101. Chwalek M, Lalun N, Bobichon H, Ple K, Voutquenne-Nazabadioko L. Structure-activity relationships of some hederagenin diglycosides: haemolysis, cytotoxicity and apoptosis induction. Biochimica et Biophysica Acta 2006;1760:1418–27.

102. Arabski M, Wasik S, Dworecki K, Kaca W. Laser interferometric and cultivation methods for measurement of colistin/ ampicilin and saponin interactions with smooth and rough of Proteus mirabilis lipopolysaccharides and cells. J Microbiol Meth. 2009;77:178-83.

103. Cheeke PR. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. J Anim Sci. 2000;77:1-10.

104. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from edible legumes: chemistry, processing, and health benefits. J Med Foods 2004;7:67-78.

105. Fecik RA, Frank KE, Gentry EJ, Mitscher LA, Shibata M. Use of combinatorial and multiple parallel synthesis methodologies for the development of anti-infective natural products. Pure Appl Chem. 1999;71:559-564.

106. Yu B, Tao HC. Glycosyl trifluoroacetimidates. 2. Synthesis of dioscin and xiebai saponin I. J Org Chem. 2002;67:9099-9102.

107. Li CH, Guo TT, Wang P, Guan HS, Li YX Semi-synthesis of several stigmasterol saponins. Chin J Chem. 2006;24:917-22

108. Cheng MS, Yan MC, Liu Y, Zheng LG, Liu J. Synthesis of β-hederin and hederacolchiside A1: triterpenoid saponins bearing a unique cytotoxicity inducing disaccharide moiety. Carbohydrate Res. 2006;341:60-67.

109. Song GP, Yang S, Zhang W, Cao YL, Wang P, Ding N, Zhang ZH, Guo Y, Li YX. Discovery of the first series of small molecule H5N1 entry inhibitors. J Med Chem. 2009;52:7368-71.

110. Ding N, Chen Q, Zhang W, Ren S, Guo Y, Li Y. Structure activity relationships of saponin derivatives: A series of entry inhibitors for highly pathogenic H5N1 influenza virus. Eur J Med Chem. 2012;53:316-26.

111. Perez-Labrada K, Brouard I, Estévez S, Marrero MT, Estévez F, Bermejo J, Rivera DG. New insights into the structure-cytotoxicity relationship of spirostan saponins and related glycosides. Bioorg Med Chem. 2012;20:2690-2700.

112. Wu Y, Ji D, Liu Y, Zhang C, Yang Z. Industrial-scale preparation of Akebia saponin D by a two-step macroporous resin column separation. Molecules 2012;17:7798-7809.