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A review on effect of saponin on rumen microbiota and methane production

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Abstract
Saponins are plant glycosides possess a number of pharmaceutical and biochemical properties. They have the ability to alter ruminal microbial population both in *in vivo* and *in vitro* conditions. Saponins inhibits the methane formation during enteric fermentation of ruminants by altering the activity of methanogenic bacteria. They also affects the growth and number of several ruminal fibrolytic bacteria and fungi. In several studies it has been also observed that Saponin has no effect on mitigation of greenhouse gases. The inconsistence results on effect of Saponin on rumen ecosystem needs more studies for concrete outputs.

Keywords: methane, saponin, methanogenic bacteria, enteric fermentation

Introduction
Saponins are bioactive secondary metabolites synthesized by many different plant species, endophytic fungi and marine organisms. They are glycosides in nature. They derive their name from the Latin word "sapo" meaning soap, due to their surfactant properties, which allows forming stable soap-like foam when shaken in aqueous solution. They are large molecules and contain a hydrophobic part, composed of a triterpenoid (30 carbon atoms) or steroid backbone (27 carbon atoms with a 6-ring spirostane or a 5-ring furostane skeleton) and a hydrophobic part consisting of several saccharide residues linked to the hydrophobic scaffold through glycose bonds. Depending on the chemical nature of aglycone, saponins are of triterpenoid and steroid saponins. They have several pharmaceutical and biochemical properties like anti-rumen protozoal, anti-tumor, anti-insect, immunomodulating, hepatoprotective, hemolytic and anti-inflammatory activities. They also decrease the blood cholesterol level and may be used as adjuvant in vaccines. In addition, saponins are used in preparation of soaps, detergents, fire extinguishers, shampoos, beer and cosmetic. Many saponins that exhibit haemolytic activity have a bitter taste and are toxic to fish.

Saponin have been reported to have an antimicrobial activity to decrease methane emission, and alter rumen fermentation by inhibiting the ruminal protozoa and increasing ruminal microbial protein synthesis (Hu *et al.*, 2006; Guo *et al.*, 2008; Wang *et al.*, 2012) [12, 8, 35]. The reduction in methane emission along with increased ruminal microbial protein synthesis might be an indicator that the rumen fermentative environment is good for animal production (Wang *et al.*, 2012) [35]. Decreased methane emissions were reported in some studied (Wang *et al.*, 2012) [35], but several other researchers also found variable results. Some studies also predicted that the methane emissions was not affected (Guyader *et al.*, 2015; Ramírez-Restrepo *et al.*, 2016) [9, 25] while Mao *et al.* (2010) [18] found that the growth of lambs changed after feeding of saponin.

Biological Properties of Saponin
Saponin poses a number of physiochemical properties which are going to discussed below

Effects on rumen microorganisms
Protozoa
Nearly 300 species of protozoa have been discovered in ruminal ecosystem. (Williams *et al.* 1991) [37]. Ruminal protozoa are divided in two groups the “holotrichs” and “entodiniomorphs”. The holotrichs plays essential role in utilizing soluble sugars and because their growth rate is slower and they are larger in size than bacteria, the holotrichs help control the rate of carbohydrate fermentation when large quantities of soluble carbohydrates are present in the diet.

The entodiniomorphs plays role in starch digestion of whole starch granules. Several studies has been carried out *in vitro* and *in vivo* to predict Saponin effect on ruminal protozoa. In the studies of Hu *et al.* 2005; Guo *et al.* 2008 [8], In *in vitro* gas production tests using equivalent grass meal and corn meal (50:50, w/w) as a substrate, tea saponin (TS) significantly (P<0.05) decreased protozoa counts. After 24-h incubations, protozoal counts were reduced by 19, 25, 45 and 79% when the TS was added at 10, 20, 30 and 40 g/kg substrate, respectively. Wallace *et al.* (2002) [33] predicted that that saponins might kill or destroy protozoa by forming complexes with sterols in the protozoal membrane surface. The membrane may become impaired and eventually disintegrate. The studies of shows that effect of Saponin on protozoa is temporary because after several days of feeding results were not consistent. (Teferedegne *et al.* 1999; Ivan *et al.* 2004) [29, 13].

In the study of Koenig *et al.* (2007) [15] on Saponin observed that the protozoal numbers in sheep rumen markedly reduced 2h after feeding *Enterolobium cyclocarpum. Entodinium* protozoa were present in the rumen as the dominant protozoal species, *Diplodinium, Isotricha* or *Dasytricha* were also present in the *in vitro* and *in vivo* experiments. There was inconsistent defaunation effect of saponin between *in vitro* and *in vivo* experiments observed when using other saponin sources such as in Yucca and Quillaja saponins (Pen *et al.* 2006, 2007; Lovett *et al.* 2006; Baah *et al.* 2007) [23, 24, 16, 1].

The difference in results on ruminal protozoa of Saponin may be due to adaptation of microbes, Nutrient flow, dilution effect and source of Saponin in the *in vitro* and *in vivo* studies (Benchaar *et al.* 2008) [3].

Methanogens

It has been observed that Inclusion of saponin significantly reduces the methane production in faunated rumen fluid, but not in the defaunated rumen fluid, suggesting that inhibition of methanogenesis by tea saponins was primarily due to their anti-protozoal activity.

Formation of acetate and butyrate are usually accompanied by production of hydrogen and carbon dioxide, whereas propionate formation involves a net uptake of hydrogen, thus, defaunation decreases the hydrogen supply for methanogens in the rumen, leading to lower methane emission.

Several experiments have reported that saponins or plants rich in saponins decreases the methane production in the rumen both *in vitro* (Pen *et al.*, 2006, 2007; Holtshausen *et al.*, 2009) [23, 24, 11] and *in vivo* (Pen *et al.*, 2007; Santoso *et al.*, 2004; Wang *et al.*, 2009; Holtshausen *et al.*, 2009) [24, 26, 34, 11] experiments.

It has been found that the Methanogenic archaea are situated on the exterior surface of rumen ciliate protozoa (Vogels *et al.* 1980) [32] and as endosymbionts within the ciliates (Finlay *et al.* 1994) [60]. Protozoa also provide some advantage to methanogens by quenching oxygen through their oxygen-tolerant hydrogenosomes, or they simply provide a vehicle for retention of slower-growing methanogens in the rumen (Müller 1993; Zinder 1993) [41]. Because 10 to 20% of methanogens live in association with protozoa (Tokura *et al.* 1999) [30], it is expected that reducing protozoa would also reduce methanogens, thus decreasing methane production.

Goel *et al.* (2008) observed a weak association between protozoal suppression and methanogens and also reported that the Sesbania saponins were having more inhibitory effects on methanogens (78%) than the Fenugreek and Knautia saponins (22 and 21%), while the reductions in protozoal numbers were 36, 39 and 25%, respectively.

To separate the effect of TS on protozoa and methanogens, defaunated and faunated (refaunated) sheep were used in a recent study (Zhou *et al.* 2011) [40]. The abundance of methanogen mcrA genes relative to total bacterial 16S rRNA gene was reduced by defaunation (P<0.05), whereas additional TS had no effect on mcrA gene abundance in either refaunated or defaunated sheep. Therefore, tea saponin appeared to reduce methane production by inhibiting protozoa and presumably lowering methanogenic activity of protozoal associated methanogens.

Fungi

Karnati *et al.*(2009) observed with molecular technique and revealed that about 99% of protozoa-associated methanogens belong to the family of *Methanobacteriaceae* and 20 novel sequences which differed from sequences previously known for protozoa-associated methanogens were obtained from rumen samples of goat, sheep and cow (Morgavi *et al.* 2006) [19].

Protozoa metabolizes the carbohydrate to produce hydrogen which is used in methane formation.

The studies of Ushida *et al.* (1997) [31] showed that there is occurrence of interspecies hydrogen transfer between the rumen ciliate *Polyplastron multivesiculatum* and the methanogenic archebacterium, *Methanosarcina barkeri*. Hence, reducing protozoa will also reduce ruminal methanogens, resulted in reduced methane production.

The experiments of Bauchop (1979) [2] shows that anaerobic fungi comprise only a small proportion of the total mass of the rumen microflora, they plays an important role in the rumen for digesting fiber. Wang *et al.*, (2000) [36] observed that the anaerobic rumen fungi, Neocallimastix frontalis and Piromyces rhizinflata, are highly sensitive to Y. schidigera saponins. Rumen fungi appear to fill an important niche in the digestion of recalcitrant plant fibres, because they cause physical as well as enzymic disruption of plant cell walls (Orpin and Joblin, 1997) [22].

The saponin extracted from tea has been reported to decrease methanogens diversity (Zhou *et al.* 2010), without having any effect on the relative abundance of methanogens in sheep (Mao *et al.* 2010) [18].

Bacteria

Methane producing bacteria are associated with other rumen microbes through H_2 supply and utilization. Protozoa, fungi and some bacteria produces hydrogen as the one of the major end products of fermentation. Methanogens live by consuming H_2 in the rumen and have to compete with propionate producing microbes that consume H_2 to form propionate (Zinder 1993) [41]. Newbold *et al.*, (1997) [20] have observed in their experiments that the bacterial numbers increase when foliage from S. Sesban is introduced into the diet, presumably as a consequence of the suppression of protozoal numbers.

It has been observed that the Saponins also affects three major fibrolytic bacteria, *Ruminococcus albus, Ruminococcus flavefaciens* and *Fibrobacter succinogens* in the rumen. The studies of Wina *et al.* (2006) on *S. rarak* saponins shows that it reduced RNA concentrations of *Ruminococcus albus* and *R. flavefaciens* both in the *in vitro* fermentation and *in vivo* during short term feeding (6 days), but this effect was not occurred during a long term feeding trial on sheep (100

days).the above study also revealed that the RNA concentration of *Fibrobacter succinogenes* was unaffected in sheep rumen during short and long term feeding of saponin.
On the basis of Quantitative analysis by real time PCR showed that extract of *S. rarak* not affects *R. flavefaciens* and *F. succinogenes* population in the *in vitro* fermentation (Suharti *et al.* 2010). However, Goel at al. (2008) observed that sesbania saponin in the *in vitro* fermentation increased *F. succinogenes* (21–45%) and *Ruminococcus flavefaciens* (23–40%) populations measured by real time PCR. It has been also observe that in *in vitro* system Tea saponin did not affect the number of *R. flavefaciens* but increased that of *F. succinogenes* (Guo *et al.* 2008) [8]. Several *in vivo* also has been conducted to observe the effect of Saponin on different animals. Mao *et al.* (2010) [18] found no effect of tea saponin on relative abundance of *R. flavefaciens* and *F. succinogenes* while Zhou *et al.* (2010) reported a decrease of *F. succinogenes* but no effect on *R. flavefaciens, R. albus, and Butyrivibrio fibrisolvens.* The inconsistency in results still needs more studies to be conducted for concrete results.

Effect of Saponin on Methane emission

During enteric fermentation in ruminants leads Methane is produced which is a substantial loss of feed energy for the animals, and increased ecological problems through greenhouse gas emissions. Therefore, methane production mitigation has significant economic and environmental benefits.
Guo *et al.* (2008) [8] found that the relative quantity of methanogens to total bacteria increased slightly, while methane production decreased, indicating the lack of correlation between methane production and methanogens. While Soliva *et al.* (2003) [28] reported the apparent lack of correlation between methane release and counts of microbes involved in methanogenesis. Further A weak relationship between methanogenesis and the methanogen population expressed as a proportion of total anaerobes was observed by Nollet *et al.* (1998) *in vitro* and *in vivo* and by Goel *et al.* (2008) *in vitro*.

Effects of saponins in modifying of fiber digestion

The studies of Dijkstra *et al.* (1995) [5] predicts that the ligno-cellulosic feedstuffs are degraded in the rumen by the synergistic activities of the bacteria, protozoa and fungi, the degradative activity majorly contributed bacteria and fungi only and the protozoa contributes only 20%. The most active fibrolytic bacteria F. succinogenes, Ruminococcus albus and Ruminococcus flavefaciens are generally considered as the primary organisms responsible for the degradation of plant cell walls in the rumen while Butyrivibrio fibrisolvens, Clostridium locheadii and Clostridium longisporum are some of the secondary fibrolytic bacteria (Chesson *et al.*,1997) [4]. Forsberg *et al.* (2000) [7] observed that Ruminal fungi produces a huge array of enzymes which generally degrade a wider range of feedstuffs than do rumen bacteria. Furthermore, ruminal fungi are able to degrade the most resistant plant cell wall polymers and the cellulases and xylanases produced by them are among the most active fibrolytic enzymes. The ruminal protozoa also contribute to the degradation of plant cell wall polymers, but their contribution in fiber degradation is considered not as important as that of the bacteria and fungi. The studies of Lu *et al.* (1987) [17] have demonstrated that Saponin decreases the passage rate of digesta from the rumen, which may increase the ruminal degradation of feedstuffs. However, physiological effects of saponins are usually overridden by microbiological effects in the rumen (Lu *et al.*, 1987) [17] because of comparatively greater effects of saponins on microbial populations. Consequently, the positive effects of saponins on the digestibility of feeds in some studies might be attributed to the increased bacterial populations, whereas negative effects reported in other studies are due to decreased hydrolytic enzyme activities from protozoa and/or bacteria and fungi when saponins affect these populations. The effect of Saponin in *in vivo* studies has been listed in table No.1.

Table 1: Effects of saponin-rich plants or extracts on ruminal methanogenesis *in vivo*

Saponin-rich source and content	Animal and feeding level	Treatments	Methane reduction	Decrease in digestibility	Reference
YS extract (saponin content: 30%; source: micro-aid or Sevarin, Distributors Processing Inc., USA)	Lamb (1.16 kg/day)	Hay/concentrate (1:1)+2 mg saponin /kg DM Hay/concentrate (1:1)+30 mg saponins /kg DM	No effect	No effect	Sliwinski *et al.* (2002) [27]
SP dried fruits (saponin 12%)	Lamb (fed at 60 g DM per kg metabolic BW)	Grass hay+0.6 g/kg metabolic weight of crude saponin from fruits of SP Grass/CA (1:2)+0.6 g/kg metabolic weight of crude saponin from fruits of SP Grass/CA (2:1)+0.6 g/kg metabolic weight of crude saponin from fruits of SP	10.5% as L/d 5.7% as L/d No effect	5.3% in OMD 3.7% in OMD 3.6% in OMD	Hess *et al.* (2004) [10]
TS (triterpenoid saponins>60%,	Sheep (1 kg DM)	Hay/concentrate (3:2)+5 g/kg TS	8.7% as L/kg DMI	Not reported	Yuan *et al.* (2007) [39]
QS extract (saponin 5–7%) or YS extract (saponin 8–10%) source: Mitsuba Trading Co., Ltd. Tokyo, Japan)	Sheep (fed at 55 g DM per kg metabolic BW)	Concentrate and Italian ryegrass hay (2:3)+0.8–1.13 g QS extract/day or 1.31–1.64 g of Yucca saponins/day	No effect	No effect	Pen *et al.* (2007) [24]
TS (triterpenoid saponins >60%,	Lamb (at maintenance requirement for digestible energy)	60:40 Wild rye/concentrate + TS 3 g/day	27.2%	Not reported	Mao *et al.* (2010) [18]
TS (600 g triterpenoid saponins/kg DM), Zhejiang Orient Tea Development Co. Ltd. (Hangzhou, Zhejiang, China)	Sheep (at maintenance requirement for digestible energy)	60:40 Wild rye/concentrate + TS 3 g/day	10.6%	Not reported	Zhou *et al.* (2011) [40]

YS, Yucca schidigera; SP, Sapindus saponari; TS, Tea saponins; QS, Q. saponaria; DM, dry matter; DMI, dry matter intake; OMD, organic matter digested

Conclusions

The above discussion reveals that the Saponin can be used to mitigate methane production from the livestock. But still there is a big gap in the knowledge of this specific group of microorganisms which is a prerequisite to get success in developing a strategy to reduce enteric methane emission by the ruminants during enteric fermentation. When Saponin were used as rumen modifier there is shift in various rumen microbial populations. Since methanogens are the main targeted group of microbes their community structure is to be explored in detail. Different *in vivo* and *in vitro* studies having differences in results might be due to variations in dose, source of Saponin and type of animals used for trial.

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