

Review Article

<https://doi.org/10.20546/ijcmas.2020.906.394>

## Effect of Saponins Mitigation of Methane – A Review

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### ABSTRACT

#### Keywords

Herbal feed additives,  
Greenhouse gasses,  
Saponins, Total mixed ration,  
Methanogenesis

#### Article Info

Accepted:  
26 May 2020  
Available Online:  
10 June 2020

With technological advances made during 21<sup>st</sup> century we are facing a global threat in form of global warming which is leading to raised average earth temperature and melting of the ice caps from Polar Regions of Earth. Methane has much more GHG effect as compared to carbon dioxide. Ruminant livestock forms a major component among anthropogenic methane leading to increased GHG effect. To overcome this situation, the most promising way to mitigate enteric methane production is either through dietary manipulation by supplementing TMR with herbal feed additives containing saponins as an active principal. Saponins are amphipathic glycosides which can produce soap like foam when shaken in aqueous solution and thus can show positive or negative effects upon microbial activity to impact fermentation, protein degradation and methane production depending upon amount of doze given. There is a need for identifying feed additives with the potential to modify rumen fermentation for enhancing the efficiency of utilization of feed energy while decreasing rumen methanogenesis. However amount of saponins to be provided so as to increase nutrient digestibility needs further investigation as there is very fine line between positive and negative effects of saponins.

### Introduction

Methane forms major part of greenhouse gases and in recent year's greenhouse gases has become major concern as global warming is a ticking time bomb for our planet. Methane is a by-product of anaerobic fermentation and leads to approximately 2-12% of GE losses. Takahashi, 2011 estimated that livestock are the reason for approximately 15% of the total atmospheric methane. A decrease in methane production will not only help in reduction of environmental stress but will also improve

feed utilization efficiency of the animals (Yurtseven and Ozterk, 2009; Yurtseven *et al.*, 2009). A wide research has been carried on upon large number of techniques involving defaunation, manipulation of ruminal microflora using antibiotics, changing ruminal microflora genetically however nutritional techniques by feeding of herbal feed additives have shown most promising results (Bakshi and Wadhwa, 2009; Wadhwa and Bakshi, 2009 and Bakshi and Wadhwa, 2011). A numerous research on potential of plant secondary metabolites to manipulate ruminal micro flora has been conducted (Wallace *et*

*al.*, 2002). A large number of plant bioactive compounds have been identified which could which are composed of steroids, triterpenoids and steroid alkaloids (Wina *et al.*, 2005). Plants secondary metabolites (PSM), such as saponins, tannins, flavonoids, organosulphur compounds, and essential oils, have antimicrobial activities against several types of microorganisms (Patra 2012). There are various researches showing saponins having potential to modulate ruminal fermentation and increasing feed utilization efficiency in ruminants (Patra and Saxena, 2010, Wallace *et al.*, 1994, Hirstov *et al.*, 1999). In vitro studies carried by (Hu *et al.*, 2005, Lila *et al.*, 2003) and in vivo studies carried by (Yuan *et al.*, 2007) has shown promising reduction of ruminal ammonical-N and pH. However Lovett *et al.*, 2006 reported decreased, Holtshausen *et al.*, 2009 increased and Mao *et al.*, 2010 reported no effect upon feed intake on supplementation of saponins.

### **Methane production among ruminants**

Farmed ruminants form a very large portion of total anthropogenic methane emissions globally. Methanogens present in ruminal flora primarily uses Hydrogen [H] produced during anaerobic carbohydrate fermentation. Ruminal microflora can convert plant fiber unavailable for humans into microbial protein available for absorption for ruminants with biological value 70. Methane (CH<sub>4</sub>) acts as main sink for metabolic Hydrogen [(H)] produced during ruminal fermentation. Metabolism of carbohydrates by the fermentative microbiota of bacteria, protozoa and fungi reduces co-factors, which are re-oxidized mostly by transferring electrons to protons. Dihydrogen (H<sub>2</sub>) so formed is transferred to methanogenic Archaea, which utilize it to reduce carbon dioxide (CO<sub>2</sub>) to CH<sub>4</sub> (Russel *et al.*, 1997). Energy lost as CH<sub>4</sub> is an inefficiency in the conversion of digestible energy (DE) to metabolizable energy (ME), because CH<sub>4</sub> is formed from

organic matter (OM) digested and fermented in the rumen and hindgut (Hill *et al.*, 2016).

### **Saponins**

Saponins are naturally occurring surface-active glycosides, found in various plants in different forms and structures (Vincken *et al.*, 2007). A great many of studies have been conducted to investigate the effects of different saponins on rumen microbial fermentation characteristics and methanogenesis (Lila *et al.*, 2003; Wina *et al.*, 2005; Guo *et al.*, 2008; Pen *et al.*, 2008; Patra and Saxena 2009b and SzumacherStrabel and Cieslak, 2010). However, the results varied depending on the types, doses and structures of saponins, because saponins have different biological activities according to their aglycone portion and combined sugar body of the structure (Hassan *et al.*, 2010). The primary effect of saponins in the rumen appears to be to inhibit the protozoa (defaunation), which might increase the efficiency of microbial protein synthesis and protein flow to the duodenum. Furthermore, saponins may decrease methane production via defaunation and/or directly by decreasing the activities (i.e. rate of methanogenesis or expression of methane-producing genes) and numbers of methanogens. Saponins may also selectively affect specific rumen bacteria and fungi, which may alter the rumen metabolism beneficially or adversely. It was reported that saponins had the potential to suppress methanogenesis, reduce rumen protozoa and modify rumen fermentation. Sirohi *et al.*, (2014) concluded that saponin fraction of *Albizia lebeck* has antimethanogenic potential and has an ability to modulate the rumen fermentation parameters.

### **Effects of saponins on DMI and digestibility**

The effects of saponins on feed intake and digestibility are variable. By addition of

saponins in the diets of ruminants, feed intake decreased (Wu *et al.*, 1994, Lovett *et al.*, 2006), increased (Holtshausen *et al.*, 2009, Abreu *et al.*, 2004 and Aregheore, 2005), or remained unaffected (Klita *et al.*, 1996, Hess *et al.*, 2004, Singer *et al.*, 2008, Mao *et al.*, 2010). As far as digestibility is concerned, was not affected by saponin supplementation. Sliwinski *et al.*, (2002) and Lila *et al.*, (2005) showed that the apparent digestibility of the nutrients was not affected by saponin supplementation. However, some studies found that the apparent digestibility of OM, CP, and NDF decreased with the addition of saponin in the diets (Klita *et al.*, 1996 and Hess *et al.*, 2004). This was probably due to an increased flow of material to the duodenum by supplementing with saponin (Klita *et al.*, 1996).

Anantasook *et al.*, (2013) observed no negative effect was observed on feed intake when rain tree pod meal (RPM) and/ or palm oil (PO) was added to the diet of dairy cows. Furthermore, fiber digestibility and cellulolytic bacteria were increased with supplementation with PO. Supplementation of RPM lowered CP digestibility, NH<sub>3</sub>-N concentration, proteolytic bacteria and protozoa populations which resulted in greater microbial crude protein. In addition, efficiency of microbial N supply was increased in both RPM and RPO treatments. Elman *et al.*, (2013) indicated that there was increase in DMI and CPI among groups offered diet supplemented with varying levels of fenugreek seeds than that one offered control diet. These results agree with those reported by Petit *et al.*, (1993) and Abo El-Nor (1999) who found that (DM) Intake was increased when lactating buffaloes fed different levels of fenugreek seeds. Tomar *et al.*, (1996) found that the fenugreek seed stimulates feed intake in dairy cattle, resulting in a significant increase in milk production. Ismail (2000) who fed growing Barki lambs

fenugreek seeds and found that DM intake increased gradually as the level of fenugreek seed increased. The increased of DMI and CPI among groups fed diet supplemented with varying levels of fenugreek seeds than that one fed control diet, firstly may be due to the saponins content in fenugreek seeds, which increased feed intake. The boosting effect of fenugreek seeds supplementation might also be attributed to the fact that fenugreek seeds increase the appetite for food (Borca *et al.*, 2000). They showed that there was increased in nutrients digestibility of (DM, CP and OM) in supplemented groups than control. This results agree with (Abo El-Nor *et al.*, 2007) who found that there was an improvement in digestibility co-efficient of lactating buffaloes fed different levels of fenugreek seeds. The improvement in digestibility could be justified on the basis of that fenugreek seeds contain saponins which stimulate anaerobic fermentation of organic matter that improve efficiency of utilization of nutrients (Abo El-Nor *et al.*, 2007). In addition fenugreek seeds increased bacterial number in the rumen of lactating cows (Valdez *et al.*, 1986, AlAli *et al.*, 2005). The results revealed that NDF digestibility decreased in treated diet compared to control diets may be explained by Lu and Jorgensen (1987) who observed that saponins impaired fiber digestion in the rumen. Moreover, saponins may decrease cellulolytic bacteria rather than amylolytic bacteria (Wang *et al.*, 1998, Wang *et al.*, 2000).

Consistent with our findings, some earlier workers also did not find any effect of saponin supplementation on DMI (Nasri *et al.*, 2011). However, others reported improved DMI with saponin supplementation (Hu *et al.*, 2006). Kumar *et al.*, (2017), observed no such effect observed on feeding either tea seed or TSS (during short term or long term) on DMI to Gaddi goats. Saponins are bitter in taste, highly soluble in water and

at higher dose level may depress intake due to low palatability (Li and Powers 2015). It was reported, 5% increase in DMI when TSS was fed at the dose of 0.25% and 27% decrease in DMI when fed at 0.5% (Li and Powers 2015). However, we did not observe any negative effect on intake at the dose level studied.

There was no significant difference in digestibility of DM, OM, CP, NDF, and ADF during both short term as well as long term in Gaddi goats. However, during short term the EE digestibility was low ( $p < 0.05$ ) in T<sub>1</sub> (54.58%) and T<sub>2</sub> (54.68%) groups as compared to control (58.83%). Whether saponin had any role in reduction in EE digestibility during initial period is not clear. Similar to findings of Kumar *et al.*, (2017) no effect on digestibility due to feeding saponin containing diet in ruminants was reported by many workers (Nasri *et al.*, 2011).

The administration of 30, 60, or 90 mg of *Quillaja saponarie* per kg DMI had no effects on feed digestibility in Barbarine lambs and in another study (Aazami *et al.*, 2013) administration of 100 or 200 mg/kg DMI of Quillaza saponin also did not affect digestibility in Baluchi sheep. Contrary to our findings, supplementation of high doses of saponin decreased the digestibility of nutrients and linear decrease in apparent digestibilities of DM, OM, CP, and NDF with supplementation of saponin from *Biophytum petersianum* in goats, lucerne and *Sapindus saponins* in sheep were observed (Santoso *et al.*, 2007). In contrast, positive effect of saponins on digestibility was reported in cattle supplemented with sarsaponin (Goetsch and Owens 1985) and in sheep with *Sapindus rarak* saponin (Thalib *et al.*, 1996). So in some studies where there is substantial decrease in protozoal population and also where there is no compensatory increase in bacterial population there is reduction in digestibility.

### **Effects of saponins on rumen fermentation**

The effects of saponins on total ruminal VFA production and molar proportions are variable. Agarwal *et al.*, (2006) reported reduced acetate and increased propionate levels with addition of water, ethanol and methanol extracts of soapnut to buffalo rumen liquor, resulting in lower acetate: propionate ratio. TVFA concentration was significantly decreased when extracts (water, ethanol and methanol) of *A. indica* were added *in vitro*. There was also a decrease in acetate to propionate ratio with addition of *A. indica* and *A. concinna* (Patra *et al.*, 2006). Lila *et al.*, (2003) reported an increased ( $P < 0.05$ ) concentration of propionate and butyrate and decreased ( $P < 0.05$ ) acetate and acetate: propionate ratio. A decrease in molar proportion of acetate and an increase in butyrate were consistent with the results of the *in vivo* studies by Christopher and Neal (1987). Lila *et al.*, (2003) reported that saponin has a variable effect on ruminal propionate concentration. Other report indicated that propionate production was significantly increased (Grobner *et al.*, 1982), decreased (Hussain and Cheeke, 1995) or remained unaffected (Valdez *et al.*, 1986) on saponin supplementation. The variable effect of saponins supplementation may be due to its type, dose of saponin and composition of diet. The ruminal ammonia production is reduced with saponin supplementation. Grobner *et al.*, (1982) found a 15% reduction in ammonia concentration when saponins were included at 60 mg/kg in the incubation medium. The inclusion of yucca saponins in diet of sheep (Santoso *et al.*, 2004) or *in vitro* ruminal culture fermentation (Wallace *et al.*, 1994 and Wang *et al.*, 2000), the ruminal ammonia concentration was reduced significantly. Hristov *et al.*, (1999) also reported the decreased ( $P < 0.05$ ) ruminal ammonia concentration 2 h after YS dosing (at 20 or 60 g/d) relative to control but the acidity,

concentrations of reducing sugars, free amino acids, and peptides in the rumen remained unaffected ( $P>0.05$ ).

Szzechowiak *et al.*, (2013) used *Saponaria officinalis* (SO) or *Panax ginseng* (PG) saponins @ 1% on dry matter basis with high and low forage diets containing meadow hay and corn meal in 60:40 and 40:60, respectively and observed a significant decrease ( $P\leq 0.05$ ) in pH was observed in case of experimental group as compared to control for both HF and LF diet. A significant increase in acetate and propionate contents were observed for treatment in comparison to control leading to decreased A:P by 18% for SO and 51% for PG saponins in HF diets. Addition of PG increased ( $P\leq 0.05$ ) butyrate, iso-butyrate and iso-valerate concentrations. However in LF diet Supplementation with PG also increased the molar concentrations of propionate, butyrate, iso-butyrate and iso-valerate. VFA profile of rumen fluid incubated with PG lowered the acetate and valerate contents and the acetate to propionate ratio, by 22%, 57% and 45% respectively, when compared to the controls Though there was no effect on  $CH_4$  production in HF diet yet dietary supplementation of PG and SO resulted in 8% lower  $CH_4$  concentrations than in the control group in case of LF diet. Istiqomah (2011) studied the effect of waru leaf (*Hibiscus tiliaceus*) supplementation as saponin @5, 10, 15, and 20% saponin level added to feed substrate of Napier grass (*Pennisetum purpureum*). There was direct relationship in decrease in protozoal number and total gas production ( $P<0.05$ ) with increase of saponin source, however there was no effect on  $NH_3$ , VFA concentration and pH after 48 h fermentation were not affected by the treatment. VFA concentration increased by waru leaf up to 10% saponin level then decreased at level 15 and 20%. Although ratio of acetate to propionate (A/P) and non glucogenic ratio (NGR) decreased at 5, 10,

and 15% saponin level, but increased at level 20%. It could be concluded that waru leaf supplementation at 10% saponin level of feed was the optimum level which gave positive effect on rumen feed fermentation.

A study was conducted by Manatbay *et al.*, (2014) to investigate the effects of gynosaponin on *in vitro* methanogenesis and fermentative efficiency under different forage-concentrate ratios (F:C ratios). A 70:30 and 30:70 ratios of forage and concentrate were used with two levels of gynosaponins 0 and 16 mg. A decrease in acetate with increased propionate leading to decreased A:P ( $p<0.05$ ) was observed on addition of gynosaponins in case of high forage diets. However an increase in butyrate ( $p<0.05$ ) was observed in both the F:C ratios. Jayanegra (2014) analyzed 23 studies about saponin containing PSM and observed a curvilinear pattern between methane production and saponin supplementation. He observed, saponins added above 500 mg/g DM were effective in reducing methane production. On expression of  $CH_4$  as ml per 100 mL total gas produced, a linear decrease in  $CH_4$  ( $p<0.01$ ) was observed with increasing levels of the saponin-rich source. A decrease in total gas production (curvilinear;  $p<0.05$ ), OMD ( $p<0.01$ ) and rumenal  $NH_3$  concentration ( $p<0.1$ ) were observed on increasing saponin levels. Whereas SCFA production, the respective parameter increased linearly ( $p<0.01$ ) with increasing levels of saponin-rich source. A decrease in proportion of acetate (linear pattern;  $p<0.05$ ) and increase in propionate (linear pattern;  $p<0.01$ ) was observed which further led to decreased A:P. Log protozoal count decreased significantly ( $p<0.05$ ) at higher saponin levels.

Belanche *et al.*, (2016) evaluated ivy fruit saponins (IVY) as a natural feed supplements and observed that it had no nutritional value,

rather it had antimicrobial effects leading to decreased methane production due to decreased methanogens. Kang *et al.*, (2016) investigated the effects of *Momordica charantia* saponin (MCS) on ruminal fermentation of maize stover and abundance of selected microbial populations *in vitro*. Five levels of MCS supplements (0, 0.01, 0.06, 0.30, 0.60 mg/mL). The high dose of MCS increased the initial fractional rate of degradation at t-value = 0 (FRD<sub>0</sub>) and the fractional rate of gas production (*k*), but decreased the theoretical maximum of gas production (*V<sub>F</sub>*) and the half-life (*t*<sub>0.5</sub>) compared with the control. The NH<sub>3</sub>-N concentration reached the lowest concentration with 0.01 mg MCS/mL at 6 h. The MSC inclusion increased (*p*<0.01) the molar proportion of butyrate, isovalerate at 24 h and 48 h, and the molar proportion of acetate at 24 h, but then decreased (*p*<0.05) at 48 h. The molar proportion of valerate was increased (*p*<0.05) at 24 h. The acetate to propionate ratio (A/P; linear, *p*<0.01) was increased at 24 h, but reached the least value at the level of 0.30 mg/mL MCS. The MCS inclusion decreased (*p*<0.05) the molar proportion of propionate at 24 h and then increased it at 48 h. The concentration of total volatile fatty acid was decreased (*p*<0.01) at 24 h, but reached the greatest concentration at the level of 0.01 mg/mL and the least concentration at the level of 0.60 mg/mL.

Galindo *et al.*, (2016) used raw saponin extract of *Sapindus saponaria* on basal diet of star grass (*Cynodon nlemfuensis*) @ 0.6 %, 1.2 % and 1.8 % of the DM basis and observed no change in acetic acid on addition of saponins, however the propionic acid reduced at 1.2% inclusion level. A gradual decrease in valeric acid was observed with gradual increase in saponin level as 0.68; 0.62; 0.52 and 0.49 mmol.L<sup>-1</sup> for 0, 0.6, 1.2 and 1.8 % of saponin extract, respectively.

### **Effects of saponins on microbial population**

The effect of saponins on rumen bacteria is contradictory. Growth of cellulolytic bacteria has been decreased (Ningrat *et al.*, 2002), increased (Thalib *et al.*, 1995) or unaffected (Wina *et al.*, 2005) by the presence of *S. rarak* saponins. Goel *et al.*, (2008a) used saponin fraction from sesbania, cardus and knautia leaves and fenugreek seeds and found decreased protozoa (10-39%), fungal (20-60%), *Fibrobacter succinogenes* (21-45%) and *Ruminococcus flavefaciens*(23-40%) population. The relative abundance of Ruminococcus was little affected by tea saponins (TS), but Wang *et al.*, (2000) reported that the growth of *Ruminococcus* was negatively affected by saponins from Yucca. Saponins from *Sesbania pachycarpa* enhanced the growth of *Ruminococcus* (Muetzel *et al.*, 2003). The differences among these results may be due to the different types of saponins used. The supplementation of saponins, or saponin-rich feeds such as *Yucca schidigera* and *Quillaja saponaria*, also decreased ciliate protozoa population *in vitro* (Makkar *et al.*, 1998, Pen *et al.*, 2006) as well as *in vivo* (Valdez *et al.*, 1986). Protozoa were sensitive to saponins because their membrane sterols bind with saponins (Wina *et al.*, 2005). But contrary to these findings, Sliwinski *et al.*, (2002) did not observe any effect on protozoal numbers by saponin-rich products containing sarsaponin. In long term trial with sheep at the level of 0.24, 0.48, 0.72g *Sapindus rarak* saponins /kg body weight, protozoa numbers were decreased but not in short term trial of 7 days. Feeding of 60 g of *Y. schidigera* extract containing 10% saponins to dairy cattle had no antiprotozoal effect (Benchaar *et al.*, 2008). The inconsistencies of the effects of saponins on protozoa in some studies might be due to different doses of saponins used. Rain tree pod meal, which contains condensed tannins and saponins, has the potential to manipulate rumen

fermentation by depressing protozoa and methanogens thereby reducing methane production in *in vitro* (Anantasook and Wanapat, 2012).

Szzechowiak *et al.*, (2013) used RUSITEC to study the effect of *Saponaria officinalis* (SO) or *Panax ginseng* (PG) saponins supplemented high and low forage diets containing meadow hay and corn meal in 60:40 and 40:60, respectively. About 1% saponin was provided by SO and PG on dry matter basis so as to see their effect upon for rumen microbial fermentation and methane production. They observed a significant decrease ( $P \leq 0.05$ ) in number of protozoa by 50% and 72% without any effect on methane production was observed for both of the saponin sources in case of HF diet. However in case of LF diet both of saponin sources showed potential to decrease methane by 8%, however decrease in protozoal count was observed only in case of PG.

Manatbay *et al.*, (2014) observed by RT-PCR that F:C ratios significantly affected the abundances of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, total fungi and counts of protozoa ( $p < 0.05$ ), but no change was observed in mcr-A gene revealing that number of methanogens showed no significant change. It was also analyzed that there was a significant decrease in abundance of *F. succinogenes* whereas no significant change was observed for mcrA gene in case of high forage diets when treated with gynosaponins thus gynosaponin treatment under high-concentrate level did not affect the methanogenesis, fermentation characteristics and tested microbes.

Kang *et al.*, (2016) investigated the effects of *Momordica charantia* saponin (MCS) on ruminal fermentation of maize stover and abundance of selected microbial populations *in vitro*. Five levels of MCS

supplements (0, 0.01, 0.06, 0.30, 0.60 mg/mL) and revealed that the relative abundance of *Ruminococcus albus* was increased at 6 h and 24 h, and the relative abundance of *Fibrobacter succinogenes* was the lowest ( $p < 0.05$ ) at 0.60 mg/mL at 6 h and 24 h. The relative abundance of *Butyrivibrio fibrisolvens* and fungus reached the greatest value ( $p < 0.05$ ) at low doses of MCS inclusion and the least value ( $p < 0.05$ ) at 0.60 mg/mL at 24 h.

### Effects of saponins on methanogenesis

Plant material from *Y.schidigera*, which is rich in steroidal saponins, and *Quillaja saponaria* (triterpenoid type saponins) shown enhancement of N utilization, reduce methane production (Goel and Makkar, 2012). Rodriguez and Fondevila (2012) studied the effect of supplementation of saponin from *Enterobacterium cyclocarpum* on digestibility of *Pennisetum purpureum* and observed the reduction in methane production *in vitro*. Abatement of up to 50% in methane production with addition of saponin has been reported (Jouaney and Morgavi, 2007, Szumacher-Strabel and Cieslak, 2010).

A comparison of the effects on rumen fermentation of three saponin rich tropical fruits supplemented to forage-based diets was completed using a rumen simulation technique (Rusitec) by Hess *et al.*, (2003). The ruminal methanogens live in association with ciliate protozoa in the rumen. Saponins present in alfalfa (3-5%), *Yucca schidigera* (4%), *Quillajasaponaria* (10%), *Sesbania sesban*, *Sapindus saponaria* (Soap nut) and *Sapindus rarak* are supposed to have a potential to suppress or eliminate protozoa from rumen and hence reduced methane emission (Hess *et al.*, 2003 and Wallace *et al.*, 2002). Lila *et al.*, (2003, 2005) found that the numbers of protozoa in rumen liquor decreased from steer fed hay plus a

concentrate supplemented with saponin, and methane emission decreased approximately 12.7% *in vivo*, and 15–44% at 24 h fermentation *in vitro*. Agarwal *et al.*, (2006) observed reduced methane production *in vitro* with water, ethanol and methanol extracts of Soapnut (*Sapindus mukorossi*) with highest in ethanol extract (96%). Addition of tea saponin at 2, 4, 6 and 8 mg against 200 mg mixture *in vitro* decreased methane concentration by 13, 22, 25 and 26%, respectively (Wei-Lian *et al.*, 2005). Pen *et al.*, (2006) demonstrated that *Yucca schidigera* extract (YSE) decreased methane production in *in vitro* fermentation experiments using the ruminal fluid of Holstein cows whereas *Quillaja saponaria* extract (QSE) did not. Sliwinski *et al.*, (2002) comparing *Yucca schidigera* extract to *Castanea sativa* wood extract (CSE; containing hydrolysable tannins and lignan) in *in vitro* rumen models showed effects on methane production only at very high levels. Sliwinski *et al.*, (2002) further concluded that the effects on methanogenesis were inconsistent between *in vitro* and *in vivo* data. The inconsistency may be due to difference in basal diets, saponins concentration and adaptation period used.

Ivy fruit saponin decreased CH<sub>4</sub> production by 40%, modified the structure of the methanogen community, and decreased its diversity (Belanche *et al.*, 2016). Saponins from *Saponaria officinalis* decreased CH<sub>4</sub> and abundance of both methanogens and protozoa *in vitro* (Cieslak *et al.*, 2014). However, in other *in vitro* studies, *Quillaja* saponins at 0.6 g/L did not lower CH<sub>4</sub> production or methanogen abundance (Patra and Yu 2014), and *Yucca* and *Quillaja* saponins at 0.6 to 1.2 g/L even increased archaeal abundance (by 0.3–0.4 log), despite a decrease in protozoal abundance by *Quillaja* saponin (Patra *et al.*, 2012). Tea saponins (30 g/day) also did not lower CH<sub>4</sub> emission from steers or abundance of total methanogens but increased the

abundance of RCC methanogens and protozoa (Ramírez-Restrepo *et al.*, 2016). Galindo *et al.*, (2016) used raw saponin extract of *Sapindus saponaria* on basal diet of star grass (*Cynodon nlemfuensis*) @ 0.6 %, 1.2 % and 1.8 % of the DM basis and observed reduction in protozoal levels. PCR-RT used to determine microbial population showed no modification in *Fibrobacter succinogenes* while the values of *Ruminococcus albus* were 25.92; 26.72; 25.2 and 22.35 CT for the levels 0; 0.6; 1.2 and 1.8 %, respectively. Thus, the effects of saponins on methanogenesis and methanogen abundance are highly variable among studies.

### **Nitrogen utilization and Effect on microbial protein synthesis**

Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Firkins *et al.*, 2007). According to Anantasook *et al.*, (2013) the excretion of creatinine and allantoin concentrations in urine were not affected in any of the treatments ( $p>0.05$ ). In contrast, allantoin absorption and microbial crude protein (MCP) were increased by rain tree pod meal (RPM) supplementation. The MCP flow from the rumen as a result of allantoin absorption ranged from 284.6 to 339.3 g/d, respectively. Moreover, supplementation of RPM or RPO resulted in the greatest microbial protein synthesis in terms of quantity and efficiency. Similarly, Anantasook *et al.*, (2013) reported that efficiency of microbial protein synthesis was increased in dairy steers supplemented with RPM. Chanthakhoun *et al.*, (2011) also found that feeding *Phaseolus calcaratus* hay resulted in increasing efficiency of microbial protein synthesis in swamp buffalo. This could be due to effect of RPM reducing the protozoal population which contributed significantly to intra-ruminal cycling of

microbial N and reducing the EMPS (Jouany 1996). Therefore, reducing protozoa populations could improve dietary N utilization and increase MPS flow to the intestine (Wang *et al.*, 2012).

In few studies reported in literature, Chinese workers observed that supplementation with tea seed saponin (TSS) improved rumen fermentation, increased microbial N yield, decreased methane production (Wang *et al.*, 2012) and improved average daily gain (ADG) (Hu *et al.*, 2006). In contrast, supplementation of TSS at higher dose level decreased dry matter intake (DMI), digestibility and ADG (Li and Powers 2015, Jadhav 2014). So the effects of TSS supplementation are not uniform and highly variable depending upon the saponin dose level and type of diet (Patra and Saxena 2010).

Increased N retention during short term in TSS fed group and increased N retention in both the supplemented groups during long term indicates that TSS in the diet has some role in improved N retention (Kumar *et al.*, 2017). In tea seed (T<sub>1</sub>) fed group though the N retention was numerically higher during short term, however, only during long term the effect was significant indicating that tea seed supplementation needed more time for getting the beneficial effect as compared to TSS supplementation. Though, linear decrease in urinary N with saponin supplementation was reported earlier (Santoso *et al.*, 2007) however, we did not observe any effect of supplementation on urinary N.

In the study (Kumar *et al.*, 2017), the microbial protein supply was higher ( $p < 0.05$ ) for T<sub>1</sub> and T<sub>2</sub> during both the periods as compared with control. Similar to our findings, improvement in microbial N supply has been reported by many workers (Santoso *et al.*, 2007). Ciliated protozoa in the rumen

plays significant role in intraruminal cycling of microbial N and negatively affecting the efficiency of microbial protein synthesis, so reduction in protozoal populations invariably found in saponin supplementation could improve dietary N utilization and increase microbial protein flow to the intestine (Santoso *et al.*, 2007). Increased microbial biomass, <sup>15</sup>N incorporation and efficiency of microbial protein synthesis with the addition of saponins from *Quillaja*, *Yucca*, and *Acacia auriculiformis* fruit also reported in literature (Makkar *et al.*, 1998). Contrary to our findings, in a previous study (Nasri *et al.*, 2011) it was observed that the total excretion of urinary PD (allantoin, uric acid, and xanthine+ hypoxanthine) and microbial N supply were not affected by supplementation of *Quillaja saponaria* saponin extract (0, 30, 60, and 90 mg/kg DMI) in Barbarine lambs.

### **Effects of saponins on growth**

As being potential alternative to antibiotic growth promoters, many researchers tried saponins in the diets of growing animals. *Yucca schidigera* saponins supplementation resulted in decreased ruminal ammonia concentration, improved daily gain and feed efficiency in animals (Mader and Brumm 1987, Giesy *et al.*, 1992, Hussain and Cheeke 1995, Kawai *et al.*, 2000 and Mirza *et al.*, 2002). Growth of steers has been reported to improve when supplementing sarsaponin, a steroidal saponin extracted from *Yucca schidigera* (Zinn *et al.*, 1998). Mirza *et al.*, (2002) found highest average daily body weight gain (267 g/h/d vs 255g/h/d) in saponins fed group whereas Carcass traits between treatments were found to be statistically similar. Hu *et al.*, (2006) evaluate the effects of the TS addition on growth performance of growing Boer goats for 60 days. The animals received the same basal diets, and added TS at levels of 0 (C), 3 g (T<sub>1</sub>) and 6 g (T<sub>2</sub>) per day. The higher dry

matter intake (623 vs 537 or 534g/day), average daily gain (94 vs 69 or 71g/d) and feed efficiency (150 vs 127 or 131g/kg) in T1 group than the other two indicated that the TS could modify the ruminal fermentation and that proper doses of TS may have potential in improving the animal growth performance, whereas at high doses, it may have adverse effects on animal production.

Both TS and TSS supplementation improved ( $p < 0.05$ ) BW gain as compared to control. These results are in agreement with previous reports (Thalib *et al.*, 1996). ADG of goats recorded in this study was comparable to the performance of the breed for the age group. In a previous study (Hu *et al.*, 2006), it was observed that the goats supplemented with 3 g of tea saponin/d had higher ADG and FCR than those on 0 and 6 g of tea saponin/d. In sheep, feeding of *Sapindus rarak* saponin improved growth rate (Thalib *et al.*, 1996), in contrast, no effect on ADG due to saponin supplementation also reported (Sliwinski *et al.*, 2002). Contrary to these findings, decrease in ADG was also reported when saponin dose level was higher (Li and Powers, 2015, Jadhav 2014). It was postulated (Patra and Saxena 2010) that effect of saponin varied depending on type of diet, nature of saponin and dose level which may be the reason for the difference in the effects.

In conclusion, saponins were previously considered as anti-nutritional factors due to their astringent properties leading to decreased Crude Protein digestibility. However now they. However amount of saponins to be provided so as to increase nutrient digestibility needs further investigation as there is very fine line between positive and negative effects of saponins. Effects produced in rumen not only depend upon amount of saponins but also upon physiological status of animals leading to variable amount of methane inhibition.

Respiratory chambers being costly equipment making methane estimation difficult. Though saponins available in natural form are easily available yet industrial extracts of saponins are a costly affair to be used as agricultural by product in developing countries like India.

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**How to cite this article:**

Anant Simran Singh and Prabhjot Kaur. 2020. Effect of Saponins Mitigation of Methane – A Review. *Int.J.Curr.Microbiol.App.Sci*. 9(06): 3310-3324.  
doi: <https://doi.org/10.20546/ijcmas.2020.906.394>