The Effects of Dietary Saponins on Ruminal Methane Production and Fermentation Parameters in Sheep: A Meta Analysis

INTRODUCTION

Methane (CH₄) is a potent greenhouse gas. An often-reported global source is a by-product of enteric fermentation from ruminants, as they utilize low-quality fiber-rich feeds to produce meat, milk, fiber (wool), and a range of valuable co-products (e.g. leather and pharmaceuticals). Methane also represents a significant loss in digestible energy with up to 10% of a feed’s gross energy lost during its production (Johnson and Johnson, 1995). Numerous strategies have been proposed to lower CH₄ production in ruminants to reduce the environmental impact of production and enhance animal performance efficiency (Martin et al. 2010). There have been many chemical feed additives proposed to lower fermentation-induced CH₄ production in ruminants. However, they often either have toxic effects on the host, reduce significantly fiber digestibility in the rumen or only temporarily act to reduce CH₄, with temporal in-
Increases to previous levels observed as the rumen microbiota adapts (Liu et al. 2019). Other approaches proposed include the application of plant secondary metabolites such as saponins (Patra et al. 2017). Saponins are high molecular weight glycosides in which a triterpene or steroidal aglycone moiety is linked to one or more sugar chains, which can be found in a wide range of plants including Quillaja saponaria, Yucca schidigera, Sapindus rarak, and Sapindus saponaria (Wina et al. 2005). The number of sugars, the type of sugars, and the stereochemistry of aglycone may vary producing a diverse array of saponins. It has also been suggested that these compounds can mitigate CH4 emission by acting as rumen modifiers by lowering the number of protozoa and methanogenic archaea (Patra and Saxena, 2010). Experiments conducted in vivo and in vitro to examine effects of saponins on CH4 emissions in ruminants have varied in their response, possibly because of the diverse array of metabolites observed from different plant sources. In vitro studies outnumber in vivo studies with most conducted on sheep with a smaller number on other ruminants such as goats and cattle. A meta-analysis of effects of saponin-rich sources on CH4 emissions and rumen fermentation parameters based in vitro studies indicated the potential to lower ruminal CH4 emissions at the same time as altering ruminal VFA patterns with a reduction in acetate and an increase in propionate proportions (Jayanegara et al. 2014).

These authors also reported a reduction in the number of protozoa at higher levels of saponin intake (Jayanegara et al. 2014). In vivo experiments on sheep have revealed that supplementation with Sapindus saponaria fruits (Hess et al. 2004) or Yucca schidigera (Wang et al. 2009) reduced CH4 emissions in sheep. Contrary to these findings supplementation with saponin extract from alfalfa (Medicago sativa) root (Klita et al. 1996), Yucca schidigera (Śliwiński et al. 2002), Quillaja saponaria (Pen et al. 2007), and tea (Camelliaceae; Liu et al. 2019) were found to have no significant effect in lowering CH4 emission compared to a control group.

Furthermore, saponin extracted from Yucca schidigera and Quillaja saponaria (Pen et al. 2007), tea saponin (Yuan et al. 2007), and Yucca schidigera powder (Santoso et al. 2004) have been found to induce a significant reduction in CH4 per unit of dry matter intake (DMI) when compared to control group while Mao et al. (2010) reported a significant decrease in CH4/DMI for a group receiving tea saponin compared to the control group.

Given these contrasting findings in vivo, and the results of the in vitro studies’ meta-analysis indicating the potential of saponins to lower CH4 emissions, a similar meta-analysis is required to fully investigate the in vivo findings.

Since a meta-analysis can combine and statistically review results from different studies, and further investigate reasons of heterogeneity.

MATERIALS AND METHODS

Literature search and data collection

Literature searches were conducted through databases of ISI Web of Knowledge (http://wokinfo.com) and Google Scholar (http://scholar.google.com) for a period covering January 1990 through to March 2019. The keywords used to search relevant studies included: methane, saponin, and sheep. Several thousand hits were collected from Google Scholar and then the results were saved in order of relevance. After identifying the last relevant record, at least 100 records were saved and then the screening of papers stopped. To identify and collect further relevant papers, the references of the selected papers were evaluated using inter-library links or author correspondence with the aim of finding papers not available in the searched databases.

Inclusion and exclusion criteria

The process resulted in the identification of 149 articles in total. During the first step, we removed duplicate articles (n=48) and review papers (n=30) then identified and included only studies which specifically examined the effects of saponins on methane production, resulting in 49 papers. We then further included papers that contained experiments on sheep with a control group and a group that received saponin. We included studies that measured methane production in vivo while removing studies that measured this in vitro (n=33) or estimated methane production using equations (n=1). We excluded studies conducted in vivo to examine the effects of saponin on methane emission and production parameters in other animals (n=6). A list of the experiments included in the meta-analysis is depicted in Table 1.

Data extraction

The data extracted here included average CH4 production (g/day), CH4/DMI, ruminal pH, total VFA, acetate, propionate, butyrate, and acetate-to-propionate ratio. For papers that did not report CH4/DMI, the value was obtained by dividing CH4 by DMI. In cases where rumen fermentation parameters were reported temporally, the last reporting time was considered for calculations. We also extracted the standard error of the mean and the number of animals in saponin and control groups. Other data extracted included: author(s) name, year of publication, a method for measuring CH4 production, breed, and type and dose of saponin used.
RESULTS AND DISCUSSION

Database

Table 1 lists the studies collected and the data used in our meta-analysis. In general, 12 and 15 comparisons were made between control and saponin supplemented groups for CH4 production and CH4/DMI, respectively. The number of comparisons for rumen fermentation parameters including ruminal pH, the concentration of total VFA, acetate, propionate, butyrate, and acetate-to-propionate ratio was 15. The studies of Hess et al. (2004) and Mao et al. (2010) used lambs, Liu et al. (2019) conducted experiments on ewes whereas all remaining studies used wethers in their trials. The source of saponin varied across studies with Sapindus saponaria fruits in Hess et al. (2004), alfalfa root in Klita et al. (1996), tea in Yuan et al. (2007), Mao et al. (2010), and Liu et al. (2019), Yucca schidigera in Pen et al. (2007), Śliwiński et al. (2002), Wang et al. (2009), and Santos et al. (2004) and finally Quillaja saponaria was also used by Pen et al. (2007).

CH₄ production and CH₄/DMI

The effect size calculated based on a random model for CH₄ and CH₄/DMI is shown in Table 2, showing a decrease for CH₄ (P=0.062; Figure 1) and CH₄/DMI (P=0.001; Figure 2). Effect size reported as mean difference indicates that using saponin-rich sources reduced CH₄ production by 1.246 g/day and CH₄/DMI by 0.849 g/kg. No heterogeneity was observed for CH₄ production (P=0.142) nor CH₄/DMI (P=0.155) with the Egger test indicating the presence of publication bias for CH₄.

Rumen fermentation parameters

The effect size calculated from the random model together with heterogeneity for rumen fermentation parameters is reported in Table 3. No significant difference was observed between the effect sizes for pH, acetate, and butyrate concentration; while the effect size decreased for propionate concentration and tended to decrease for acetate-to-propionate ratio.

A low heterogeneity was observed for pH, acetate, propionate, and acetate-to-propionate ratio while the heterogeneity for VFA and butyrate was at a medium level. The Egger test results showed no publication bias for rumen fermentation parameters.

A meta-analysis involves the application of defined statistical methods to summarize multiple study results by combining results from these different studies and statistically summarizing the combined results. The current meta-analysis indicated the effectiveness of saponin sources in mitigating CH₄ production (as a trend) and CH₄/DMI in sheep.
### Table 1: Summary of papers used for meta-analysis

<table>
<thead>
<tr>
<th>Reference</th>
<th>NC</th>
<th>Animal</th>
<th>Breed</th>
<th>Saponin products</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hess et al. (2004)</td>
<td>3</td>
<td>Lamb</td>
<td>Swiss White Hill</td>
<td><em>S. saponaria</em> fruit</td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Klita et al. (1996)</td>
<td>3</td>
<td>Wether</td>
<td>Suffolk</td>
<td>Alfalfa root</td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Liu et al. (2019)</td>
<td>1</td>
<td>Ewe</td>
<td>Dorper × thin-tailed Han</td>
<td>Tea saponin</td>
<td>CH₄, CH₄/DMI, pH, Total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Mao et al. (2010)</td>
<td>1</td>
<td>Lamb</td>
<td>Huzhou</td>
<td>Tea saponin</td>
<td>CH₄, CH₄/DMI, pH, Total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Pen et al. (2007)</td>
<td>2</td>
<td>Wether</td>
<td>Cheviot</td>
<td><em>Q. saponaria</em>; <em>Y. schidigera</em></td>
<td>CH₄, CH₄/DMI, pH, Total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Santoso et al. (2004)</td>
<td>1</td>
<td>Wether</td>
<td>Cheviot</td>
<td><em>Y. schidigera</em></td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Śliwiński et al. (2002)</td>
<td>2</td>
<td>Wether</td>
<td>Swiss White Hill</td>
<td><em>Y. schidigera</em></td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Wang et al. (2009)</td>
<td>1</td>
<td>Wether</td>
<td>Mongolia</td>
<td><em>Y. schidigera</em></td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
<tr>
<td>Yuan et al. (2007)</td>
<td>1</td>
<td>-</td>
<td>Huzhou</td>
<td>Tea saponin</td>
<td>CH₄, CH₄/DMI, pH, total VFA, acetate, propionate, butyrate, acetate/propionate</td>
</tr>
</tbody>
</table>

1 NC: No. of comparisons.

### Table 2: Effect size and heterogeneity for the effect of saponin on CH₄ production and CH₄/DMI

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NC</th>
<th>Hedges², g</th>
<th>95% confidence intervals</th>
<th>P-value</th>
<th>Q⁴</th>
<th>P-value</th>
<th>I-squared⁴, %</th>
<th>MD⁵</th>
<th>95% confidence intervals</th>
<th>Egger</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄</td>
<td>12</td>
<td>-0.441</td>
<td>-0.904, 0.022</td>
<td>0.062</td>
<td>15.98</td>
<td>0.142</td>
<td>31.2</td>
<td>-1.246</td>
<td>-2.199, -0.293</td>
<td>0.018</td>
</tr>
<tr>
<td>CH₄/DMI</td>
<td>15</td>
<td>-0.671</td>
<td>-1.078, -0.265</td>
<td>0.001</td>
<td>19.26</td>
<td>0.155</td>
<td>27.3</td>
<td>-0.849</td>
<td>-1.304, -0.393</td>
<td>0.344</td>
</tr>
</tbody>
</table>

1 NC: No. of comparisons.
2 Standardized unitless effect size for differences between treatment and control groups.
3 Cochran’s Q-values to identify the presence of heterogeneity among studies.
4 Degree of heterogeneity among studies.
5 Mean difference.

### Figure 1: Forest plot of the effect size and 95% confidence interval of the effect of saponin on CH₄ production in sheep. The solid vertical line represents a mean difference of 0 or no effect. Points to the left of the line represent a decrease in CH₄ production and points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that study and reflects the relative weighting of the study to the overall effect size estimate. The larger the box, the greater the study contributes to the overall estimate. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size.
According to a classification of effect size proposed by Cohen (1988), a medium value for CH₄ production and CH₄/DMI was observed. These findings, agree with a previous meta-analysis of in vitro studies (Jayanegara et al. 2014) where saponin-rich sources reduced CH₄ per unit substrate and per total gas produced. However, although the meta-analysis reports a trend for a reduction in CH₄ production several studies reported that using such saponin sources as *Quillaja saponaria* (Pen et al. 2007) and *Yucca schidigera* (Śliwiński et al. 2002; Pen et al. 2007) did not result in a significant reduction, although numerically reductions were observed, which contributed to the meta-analysis result with no heterogeneity.

In contrast, Wang et al. (2009) reported a significant reduction in methane production as a result of using *Yucca schidigera* extract.

For CH₄/DMI, Yuan et al. (2007) and Mao et al. (2010) reported that tea saponin lowers CH₄/DMI while other studies reported no significant change in CH₄/DMI compared to the control group as a result of using saponin sources (Santoso et al. 2004; Pen et al. 2007; Liu et al. 2019), although numerical reductions were observed, which likewise to CH₄ production, explains the current result with no heterogeneity. It has been suggested that saponins reduce CH₄ production by reducing protozoa and methanogenic archaea in the rumen (Patra and Saxena, 2009). In the same vein, Jayanegara et al. (2014) reported in their meta-analysis that...

### Table 3: Effect size and heterogeneity for the effect of saponin on rumen fermentation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NC¹</th>
<th>Hedges², g</th>
<th>95% confidence intervals</th>
<th>P-value</th>
<th>Q³</th>
<th>P-value</th>
<th>I-squared⁴, %</th>
<th>Egger</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>15</td>
<td>-0.135</td>
<td>-0.456, 0.187</td>
<td>0.412</td>
<td>11.38</td>
<td>0.656</td>
<td>0</td>
<td>0.972</td>
</tr>
<tr>
<td>Total VFA</td>
<td>15</td>
<td>0.389</td>
<td>-0.086, 0.865</td>
<td>0.108</td>
<td>26.59</td>
<td>0.022</td>
<td>47.4</td>
<td>0.513</td>
</tr>
<tr>
<td>Acetate</td>
<td>15</td>
<td>-0.095</td>
<td>-0.416, 0.227</td>
<td>0.564</td>
<td>11.36</td>
<td>0.657</td>
<td>0</td>
<td>0.966</td>
</tr>
<tr>
<td>Propionate</td>
<td>15</td>
<td>0.430</td>
<td>0.098, 0.762</td>
<td>0.011</td>
<td>14.20</td>
<td>0.435</td>
<td>1.4</td>
<td>0.238</td>
</tr>
<tr>
<td>Butyrate</td>
<td>15</td>
<td>0.326</td>
<td>-0.134, 0.787</td>
<td>0.165</td>
<td>25.34</td>
<td>0.037</td>
<td>44.7</td>
<td>0.165</td>
</tr>
<tr>
<td>Acetate/propionate</td>
<td>15</td>
<td>-0.333</td>
<td>-0.674, 0.009</td>
<td>0.057</td>
<td>15.15</td>
<td>0.368</td>
<td>7.6</td>
<td>0.393</td>
</tr>
</tbody>
</table>

¹ No. of comparisons.  
² Standardized unitless effect size for differences between treatment and control groups.  
³ Cochran’s Q-values to identify the presence of heterogeneity among studies.  
⁴ Degree of heterogeneity among studies.

**Figure 2:** Forest plot of the effect size and 95% confidence interval of the effect of saponin on CH₄/DMI in sheep. The solid vertical line represents a mean difference of 0 or no effect. Points to the left of the line represent a decrease in CH₄/DMI and point to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that study and reflects the relative weighting of the study to the overall effect size estimate. The larger the box, the greater the study contributes to the overall estimate. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effect size.
the number of protozoa significantly dropped at higher levels of saponin. Since dihydrogen is the key element in ruminal methanogenesis, a reduced number of protozoa, as a producer of dihydrogen, can lead to lower CH₄ production (Morgavi et al. 2010). Protozoa’s sensitivity to saponins may be attributed to saponins binding with their membrane sterols (Wina et al. 2005).

As noted earlier, given insufficient and lack of uniform data, our meta-analysis did not include an assessment of the number of protozoa. However, Mao et al. (2010) reported a significant drop in protozoa number as a result of supplementation with tea saponin while other studies did not observe a significant change in this number (Klita et al. 1996; Śliwiński et al. 2002; Pen et al. 2007; Liu et al. 2019).

The meta-analysis reported no saponin supplementation effect on ruminal pH, driven by the vast majority of papers in the analysis (Klita et al. 1996; Śliwiński et al. 2002; Hess et al. 2004; Santosio et al. 2004; Pen et al. 2007; Wang et al. 2009; Liu et al. 2019). However, Yuan et al. (2007) and Mao et al. (2010) reported lower ruminal pH as a result of saponin supplementation which was driven by an increased ruminal VFA concentration (Mao et al. 2010). Our meta-analysis indicated that adding saponin sources did not significantly change acetate concentration, in line with most studies except for Yuan et al. (2007). Where as a significant increase was found in effect size for propionate. This increase in propionate concentration was reported by Liu et al. (2019) while other studies found a numerical increase in propionate concentration with no significant difference from the control group. These discrepancies appear to be related to the chemical structure and dosage of saponins, diet composition, microbial community, and adaptation of microbiota to saponins (Patra and Saxena, 2009).

An expected drop in the acetate-to-propionate ratio was observed while the effect size for this ratio was at a medium level and tended to decrease. Wina et al. (2005) suggested that the main effect of saponin on VFA was an increase in propionate proportion and a drop in acetate-to-propionate ratio. They also suggested that this increase in propionate proportion is attributable to lower concentrations of acetate and butyrate since they are among the main products of protozoa fermentation, which is suppressed by saponins (Wina et al. 2005). Our meta-analysis showed no significant change in effect size for butyrate although its value was positive. Furthermore, changes in concentrations of acetate and propionate can effectively mitigate CH₄ production since whilst acetate formation is a hydrogen source in the rumen propionate formation is a hydrogen sink, reducing dihydrogen substrate for methanogenesis (Moss et al. 2000).

It should be noted that the type of experimental design can influence experimental results. Most studies have used a cross over design which seems to be unsuited for studying the impacts of saponin on CH₄ production and rumen fermentation parameters, particularly because saponins may influence the balance of the rumen microbial community.

**CONCLUSION**

The findings of the present meta-analysis indicate that CH₄ production tends to decrease and CH₄DMI is significantly reduced through supplementation of saponin-rich sources in sheep. Besides, supplementation with saponin-rich sources leads to a significant increase in propionate concentration while the acetate-to-propionate ratio tends to decrease, with no significant change observed in ruminal pH and the total concentration of VFA, acetate, and butyrate.

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**REFERENCES**


