Effects of saponin on enteric methane emission and nutrient digestibility of ruminants: An in vivo meta-analysis

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Abstract. Although saponin originated from plants has been used to control methanogenesis performed by archaea methanogens in the rumen, its effect has been inconsistent. The objective of this study was to evaluate the effect of dietary saponin on enteric methane emission and nutrient digestibility of ruminants across various studies by employing a meta-analysis method. A database was developed by integrating data from 17 studies that originated from 11 articles. Different ruminant species, i.e., both small and large ruminants were included in the database. Data on related in vitro studies were excluded. Papers should be based on in vivo studies only that simultaneously reported dietary saponin and methane emission. Treatments were divided into control (no saponin), low saponin (saponin level less than 0.5% DM) and high saponin (saponin level above 0.5% DM). The treatments were considered as fixed effects whereas various studies were considered as random effects in the statistical mixed model methodology. Results showed that dietary saponin decreased enteric methane emission of ruminants as compared to control (P<0.05), and this was true for both low and high levels of saponin. Interestingly, low level of dietary saponin increased dry matter digestibility, organic matter digestibility, neutral detergent fiber digestibility and acid detergent fiber digestibility in comparison to control (P<0.05). However, saponin at high administration level reduced these nutrient digestibility than that of control (P<0.05). In conclusion, saponin is effective to mitigate enteric methane emission from ruminants in vivo and enhances nutrient digestibility provided that the level use should not exceed 0.5% DM.

1. Introduction
Ruminants are among the contributors of methane accumulation in the atmosphere and contribute to global warming [1]. Such methane emission is also a form of energy loss from the animals which may account up to 14% loss from digestible energy intake [2]. Various dietary manipulations have been performed in order to mitigate enteric methane emission from ruminants, and these included the use of a high-quality forage [3], synthetic compounds [4] and natural plant secondary compounds [5]. Plant saponins are among the group of plant secondary compounds and they have been used as additives to mitigate methane emissions originated from enteric fermentation of ruminant animals. Most of the studies however were based on in vitro techniques, and only limited studies were directly conducted on the animals. Inconsistencies of saponin effects on methane emissions have been previously observed [6,7], which might be related to their levels in rations and the great structural diversity in nature. Furthermore, nutrient digestibility was varied by addition of plant saponins.
The present study was therefore aimed to assess the effects of saponin levels on methane emissions and nutrient digestibility of ruminants in vivo by integrating data from multiple studies. It was hypothesized that different saponin levels may elicit different responses regarding their particular effects on enteric methane emissions and nutrient digestibility.

2. Method

Reports on the use of saponins to mitigate in vivo methane emissions of ruminants were integrated in a database; both small and large ruminant species were included. Searching of literatures was performed by using the Scopus database with keywords “saponin” and “methane”. The main criteria for a paper to be included were: (1) studies were conducted in vivo, (2) saponin contents were reported, and (3) methane was directly measured, not estimated by any methods. Data originated from in vitro rumen fermentation experiments were excluded and already reported elsewhere [8]. Accordingly, a total of 11 articles comprised of 17 studies were obtained and used to construct the database. Apart from information on saponin sources, application levels and methane emissions, nutrient digestibility was also recorded, i.e., dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility. After collection, different units of measurements within a particular parameter were transformed into similar units in order to allow direct analysis. Various plant sources of saponins were tabulated, i.e., sapindus, yucca, quillaja, alfalfa and tea. Saponin levels (ranged from 0 to 4.0% DM) were classified into three groups, namely control (no saponins), low (0 < saponins ≤ 0.5% DM) and high (saponins > 0.5% DM) levels. Justification of 0.5% DM of saponins to separate the low and the high saponin groups was based on the average saponin levels across all studies included in the database.

The data obtained were subjected to a statistical meta-analysis based on mixed model methodology [9]. Accordingly, different studies were treated as random effects whereas categorical levels of saponin additions were considered as fixed effects. The following statistical model was used:

\[ Y_{ij} = \mu + s_i + \tau_j + s\tau_{ij} + e_{ij} \]

- \( Y_{ij} \) = Dependent variable
- \( \mu \) = Overall mean
- \( s_i \) = Random effect of the \( i \)th study
- \( \tau_j \) = Fixed effect of the \( j \)th level of factor \( \tau \)
- \( s\tau_{ij} \) = Random interaction between the \( i \)th study and the \( j \)th level of factor \( \tau \)
- \( e_{ij} \) = The unexplained residual error.

No weighting procedure was applied for different studies. When a variable showed significant difference at P<0.05 between various saponin levels, Duncan’s multiple range test was employed to compare the difference between means. All statistical analyses were performed with IBM SPSS Statistics version 20.

3. Results and discussions

Across all studies, methane emissions decreased significantly when saponins were added at both low and high levels (P<0.05) (figure 1), suggesting that the use of saponins for ruminants is beneficial in term of environmental conservation. The decrease of methane emissions due to saponin additions at low and high levels was 8.6% and 7.3% lower than that of control, respectively. There was no statistical difference between administration of low and high levels of saponins on enteric methane emissions. Saponins lower methane emissions through a direct inhibition on methanogen population in the rumen [10] and an adverse effect on the activity of methanogen [11]. Saponins also reduce a certain population of protozoa where part of the methanogens is living symbiotically with the fauna. Further, protozoa provide hydrogen as a substrate for methanogenesis [17]. Therefore, such decrease of protozoa population may lead to a decrease in methanogen population as well as methane emissions.
Figure 1. Influence of saponin additions at low (0 < saponins ≤ 0.5% DM) and high (saponins > 0.5% DM) levels on methane emissions (l/kg dry matter intake) from ruminants. Different letters above the bars show significantly different at P<0.05.

Low levels of saponins increased nutrient digestibility as compared to control (P<0.05) while, on the contrary, high levels decreased the nutrient digestibility (P<0.05). Such pattern was true DMD, OMD, NDFD and ADFD (table 1). In the case of CPD, addition of saponins at low levels did not significantly improved the digestibility parameter but it was significantly higher than that of high saponin additions (P<0.05). Apparently, saponins at low levels (e.g. less than 0.5% DM) are favorable in simultaneously mitigating enteric methane emissions and stimulating nutrient digestibility. When being added at high levels, saponins seem to cause an adverse effect on nutrient digestibility without any further reduction in methane emissions. Therefore, since there is no advantage of adding saponins at high levels, the addition is sufficient at low levels.

Table 1. Digestibility of nutrients on saponin additions at low (0 < saponins ≤ 0.5% DM) and high (saponins > 0.5% DM) levels

| Parameter  | N  | Control | Low saponin | High saponin | SEM |
|------------|----|---------|-------------|--------------|-----|
| DMD (g/kg) | 13 | 647b    | 663c        | 603a         | 13.7|
| OMD (g/kg) | 20 | 650b    | 701c        | 611a         | 17.2|
| CPD (g/kg) | 26 | 629ab   | 645b        | 620a         | 15.1|
| NDFD (g/kg)| 26 | 545b    | 583c        | 509a         | 19.5|
| ADFD (g/kg)| 21 | 486b    | 537c        | 454a         | 28.4|

DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility; N, number of data; SEM, standard error of the mean.

Different superscripts within the same row show significantly different at P<0.05.

4. Conclusion
Saponin is effective to mitigate enteric methane emission from ruminants in vivo and enhances nutrient digestibility provided that the level use should not exceed 0.5% DM.
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