



Mini Review: The Strategic Approach of Saponin to Reducing Methane Emissions

Ghasaq Sami Mshary^{1*}, Cristina Castillo², Joaquin Hernandez³, Rodrigo Muiño⁴

¹ Department of Physiology, Chemistry and Pharmacology, College of veterinary medicine, AL-Muthanna University, Samawa, Iraq

² Cristina Castillo, Department de Patoloxia Animal, Facultad de Veterinaria de Lugo, Campus Terra-IBADER, Universidad de Santiago de Compostela, Spain

³ Joaquin Hernandez Department de Patoloxia Animal, Facultad de Veterinaria de Lugo, Campus Terra-IBADER, Universidad de Santiago de Compostela, Spain

⁴ Rodrigo Muiño, Department de Patoloxia Animal, Facultad de Veterinaria de Lugo, Campus Terra-IBADER, Universidad de Santiago de Compostela, Spain

ABSTRACT

Ruminant production is facing a critical period within global agriculture due to unique digestive systems allowing them to utilize low-quality fibre-rich feed, producing the potent greenhouse gas methane (CH₄) as a by-product. Methane is eliminated from the atmosphere by eructation. Saponins are secondary metabolites in plant seeds, leaves, bark, and roots. Some saponins were found from legumes and microbial populations, including *Quillaja saponaria*, *Yucca schidigera*, *Camellia sinensis* and *Samanea saman*. Saponin consists of a lipophilic triterpene or steroid aglycone attached to one or more hydrophilic sugar moieties. Their structural variety has appeared in their chemical and

biological properties. Numerous pharmacological aspects of saponin include antifungal, antiprotozoal, antimicrobial, and anti-methanogen effects. More factors are related to the rumen microbe's power to mitigate the antiprotozoal activity of saponins, such as breeds of animals and the environment. Tannin and saponin having plant species, such as alfalfa are widely utilized because of their beneficial matter and clearly can decrease methane production. The membrane-disrupting action of saponins illustrates their harmful influence on protozoa and the formation of complexes with sterols found in the protozoal cell wall, thereby causing cell lysis. This review aims to understand saponin structural features and focus on the prospect of being a useful resource for researchers in using saponin extract as a bioactive compound in ruminant diets to mitigate greenhouse gas emissions.

ARTICLE INFO

Article history:

Received: 29 June 2024

Accepted: 18 September 2024

Published: 31 December 2024

DOI: <https://doi.org/10.36326/kjvs/2024/v15i216715>

E-mail addresses:

ghassaq51@mu.edu.iq

ORCID: [0000-0002-8770-7468](https://orcid.org/0000-0002-8770-7468)

cristina.castillo@usc.es

ORCID: [0000-0002-2467-6406](https://orcid.org/0000-0002-2467-6406)

joaquin.hernandez@usc.es

ORCID: [0000-0002-2588-0089](https://orcid.org/0000-0002-2588-0089)

rodrigo.munio.otero@usc.es

ORCID: [0000-0002-9518-808X](https://orcid.org/0000-0002-9518-808X)

* Corresponding author

KEYWORDS: Plant phytochemicals, Feed additives, Protozoa, Fermentation, Ruminant.

P- ISSN: 2077-9798

E- ISSN: 2959-8478

INTRODUCTION

Globally, grazing animals such as goats, cattle, and sheep emit considerable amounts of greenhouse gases (GHG), which are nitride-nitrous-oxide (N_2O), carbon dioxide (CO_2), methane (CH_4), and chlorofluorocarbon (CFC) [1]. Methane is assembled in the rumen (80–95%), while another part is assembled in the large intestine (5–20%) [2]. The dietary manipulation process can reduce CH_4 emissions by approximately 40%-75% [3]. Some medicinal plants are anti-methanogen, effectively lowering ruminant intestinal methane emissions such as ionophores, saponin, and tannin [4,5]. Plant phytochemicals like flavonoids are qualified to make pleasant flavours, while several others, like tannins and saponins, have imperfect tastiness [6]. The botanical aspect of the plant in these mixtures varies from plant to plant, meandering from the leaves [7], roots, rhizome [8], seed, bark [9], or entire plant [10].

The attention of saponins is more elevated in immature leaves than mature leaves and is advanced in roots than in leafage [11]. The US Food and Drug Administration remarks that saponins are ‘Generally Recognized as Safe’ for human consumption [12]. Saponins ingested via the diet (vegetables, complements, or extracts) experience vast gastrointestinal metabolism, offering low absorption at the gastric and small intestine, thereby entitling them to get the large intestine where they are hydrolyzed through the colonic microbiota and, hence, are later absorbed [13]. Saponin is one of the supporters of global warming, and it has a chief role in many plants, especially legumes (soybeans) [14]. Plant saponins hold methane-reducing prospects, and some bases of saponins are more adequate than others in decreasing methane production due to their antiprotozoal effects [15]. The mechanism steps of saponins to decrease CH_4 production is through the detergent-like act inhibiting the integrity of methanogen archaea cell membranes [16]. Saponins control the ability to interfere with CH_4 production by directly influencing the formatting and activity of rumen protozoa, bacteria, and archaea by inhibiting the effects

of ruminal methanogens [17]. Several research studies have shown that saponins' antibacterial activity is linked to their connection to cell membranes [18]. The antiprotozoal effect of saponin is temporary [19].

Methane:

CH_4 reserves for 14% of GHG emitted into the atmosphere, and enteric CH_4 from ruminants reserves for 30% of this emission [5]. Methane is one of the greenhouse gases made from anthropogenic actions and livestock. It is second to the CO_2 contribution to climate change [20]. Methane is created as part of an inescapable and natural rumen fermentation product. Over the decades, scientists and investigators have tried multiple ways to repress ruminant methane emissions, such as production intensification, varying diet administration, diet, and rumen manipulation, so the preference for low- CH_4 -producing animals [21]. In ruminants, methanogen bacteria synthesized CH_4 gas in the rumen through a bioconversion track utilizing hydrogen and carbon dioxide as the principal precursors [22]. Reducing the CH_4 emission and improving feed efficiency utilization. Methane mitigation procedures can be broadly categorized into three principal classes: animal and feed management, diet formulation, and rumen manipulation [23].

Structure of Saponins:

The natural effects of saponins have chemical design and bioavailability [14]. The term saponins derives from their knowledge of initiating a steady foam in aqueous solutions such as soap. The saponins are classified into steroidal, triterpenoid, and alkaloid related to the core of the aglycone [24]. Steroid saponins are primarily found in the form of fur-stanol or spiro-stanol, where the carbohydrate element including glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid, bound to aglycone through glycosidic bonds [25] (Figure 1). There are two significant sorts of triterpenoid-saponins: the neutral type (normal sugar binds to sapogenin) and the acidic type (the sugar moiety consists of uronic acid, or one or more carboxylic groups bound to the sapogenin) [26]. The numeral and style

of the sugars and the chemistry of the moiety counter much, resulting in diverse classes of metabolites [11]. The saccharide chains are usually united to the C₃ place (mono-desmoid), but some sapogenins have two saccharide chains (bi-desmoid) related to the C₃ and C₁₇ (via C₂₈) places. Saponin can modulate ruminal fermentation and enhance animal production [27]. Considerable reflections have declared that a wide

multiplicity of saponins, which were obtained from restorative plants or species of agricultural matter, display distinct biological and pharmacological possessions [28,29]. Regardless, the feeding of saponins did not reveal toxicity indications, meaning that other aspects of chewing and salivary enzymes lead to protection from detoxification and degradation by the feed particle matrix [13].

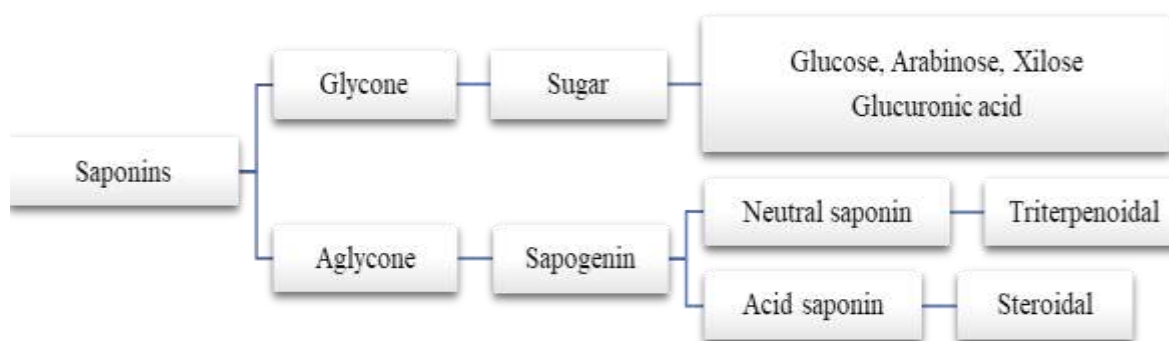


Figure 1. General classification of saponin. (Source : Nguyen *et al.*, 2020).

Saponins for Ruminant Livestock:

Methanogens and protozoa persist by competing with the propionate producers that employ H₂ [30]. Feed additive of saponin extract for ruminants can boost fermentation in the rumen and decrease methane gas emissions [31]. Saponin sources have variable products for microbial populations, such as *Quillaja saponaria*, *Yucca schidigera*, *Camellia sinensis*, *Samanea saman*, and *Sapindus rarak*, that virtually lower protozoa and bacterial populations [32]. Ku-Vera et al [33], revealed that protozoa with saponins lead to a change in cell permeability by rupturing and disintegrating the microorganism's cell wall. Likewise, Lambo et al. [5], found about an 18% drop in methanobacterial and ciliate-associated methanogens with the extracts of *Artemisia capillaris*, rich in allicin, and *Glycyrrhiza uralensis*, rich in saponin and alkaloid. The tea extracted from *Camelia seed* at a dose of 0.8% reduced the total number of

protozoa by about 60% and reduced CH₄ emissions by about 36% with a higher short-chain fatty acid production [34]. The management of saponin level advised is 0.3-3.1% of the whole percentage to enhance version and improve meal efficiency [35]. In addition, tea saponin altered volatile fatty acid (VFA) profiles by improving H₂-consuming VFA and decreasing H₂-producing VFA [36]. Tea saponin decreased methanogenesis via both tracks, first, the plant extract linearly decreased protozoa concentration, which provides extensive parts of H₂ to methanogenic archaea living or inside the protozoal cell through interspecies H₂ transfer [5]. In cattle, CH₄ production decreased by around 50.9% when saponin of *Samanea saman* 30% dry matter was added [51]. The tea saponin at 2-20 g at sheep to methane mitigation effect decreased from 8.8 to 47.9% [32, 38]. Saponin of *Enterolobium cyclocarpum* 0.3 g/kg dry matter of sheep reduced methane production by 36.6% [50]. See table 1.

Table 1.

The data on saponin sources was used to reduce enteric methane mitigation (Source: Lileikis et al., 2023).

Saponin sources	Dose	Type of animales	Effect on methane production	References
<i>Enterolobium cyclocarpum</i>	0.3g/ kg dry matter	Sheep	Reduced methane production in 36.6%	[50]
<i>Samanea saman</i>	0.3g/kg dry matter	Crossbred cattle	Reduced methane production about 50.9%	[51]
<i>Tea saponin</i>	20 g/kg dry matter	Sheep	Reduced methane production on 47.9%	[32]
<i>Tea saponin</i>	2 g/animal/day	Sheep	Reduced methane production at 8.8%	[38]

Effect of saponins on protozoa:

Cherdthong et al. [37] the protozoa decreased by more than 75% when cattle obtained crude saponin from pellets having *Delonix regia* seed meal. Liu et al. [38], carried out a study on how tea saponin reduces methanogenesis via an inhibitory effect on protozoa. However, that the tea saponins of *Camellia seeds* could lower the number of protozoa resembling the non-supplemented class. Liu et al. [38] and Kim et al. [39] reported a decreased ciliate protozoa number with the administration of saponin-containing *Aloe saponaria* and tea saponin daily. Numerous methanogens have been reported in ecto- and endosymbiotic relationships with protozoa [40]. The crude saponin concentration in *Sesbania grandiflora* pods was a little higher than that of further tropical plants founded on what encloses *rambutan peel* fruit [40], or *Delonix regia* seed meal [41].

Abdullah and Al-Galbi. [41] revealed that providing saponins from tea leaves at 180 mg/kg dry matter (DM) enhanced gain weight from 15–17.5 kg. Enhanced animal growth through feeding with saponins is a result of the increased intestinal absorption of amino acids due to decreased protozoal numbers.

Different plant secondary metabolites, such as saponin, have valuable effects, including boosting immune metabolism, modulating ruminal fermentation, and reducing the environmental influence of GHG emissions [42]. According to Yanza et al. [43], the doses of saponin up to 40 g/kg DM had no negative effect on feed intake, and the palatability of the diets was unchanged. Therefore, large ruminants have a more varied ability to accept the bitter taste of saponins than small ruminants [43].

Saponins and Tannins:

Tannins have proved beneficial and detrimental effects in inhibiting unwanted bacteria and protozoa [44]. But those microorganisms can also most likely adapt by changing their cell walls. Tannins can also lessen the amount of protein that breaks down in the rumen, although this advantage is diminished if they also make RUP less digestible in the intestinal tract. Tannins can directly or indirectly reduce the rumen's release of methane by blocking protozoa or methanogens [33]. The kind, source, and molecular weight of tannins as the animal's methanogenic community can affect the methane response to tannin feeding. Furthermore, it showed that other tropical legumes, like *Desmanthus spp*, further reduced intestinal CH₄ emissions in cattle grazing in tropical grasslands [36]. Thus, it may be possible to lower intestinal CH₄ output using hydrolyzable and condensed tannins, albeit animal production and intake may be affected. There has been conflicting research on the benefits of saponins and tannins [45]. Studies have examined the nutritional benefits of forages high in secondary plant chemicals, such as tannins, on animal productivity and their potential to prevent methanogen development [33].

Adding saponin-rich sources decreased ruminal methane emission, decreased acetate proportion, and increased propionate, according to research on the effects of saponin-rich sources on methane production and ruminal fermentation parameters conducted in vitro [48].

The danger of reduced rumen function and animal output has risen with tannins than with saponins, according to a comprehensive evaluation of the effects of saponins and tannins on CH₄ release in ruminants [46], primarily based on in vivo investigations. The feeding of saponins can result in varying reactions (such as inhibiting protozoa), and there is also a drop in DMI [44]. The study demonstrated that saponin-rich sources did not affect reducing the relevant CH₄ parameter when given at a dose of around 500 mg/g DM. At increased saponin levels, the protozoal count decreased. Tannin and saponin having

plant species, such as alfalfa and birdsfoot trefoil are widely utilized because of their healthy value and obviously can decrease methane production [52].

Other Medicinal Purposes:

Chen et al. [47] assessed the experimental importance of triterpene saponins to stimulate blood circulation in blood stasis syndrome employing strategy pharmacology-based techniques. Saponins are established to be valuable while they have more biomedical advantages that involve anti-bacterial, anti-fungal, antioxidant, anti-cancer, and antiviral effects [48]. The explored curative significance of saponins has developed the analysis of extracting saponins from extra plant species [49].

CONCLUSIONS

This review concluded that the use of saponin extract or crude in ruminants did not damage the nutritional value of ruminant diets. However, the temporary effect of saponins on rumen microbes is a restriction on their utilization. Saponins stay toxic to ruminal protozoa when found straight in the rumen due to their purity. Elevated concentrations of saponins have natural rumen manipulators that revise the formatting and fermentation of microbial cultures and change rumen metabolism. Saponin was confirmed to be a practical emission mitigation strategy. In future research, we must focus on more sources of saponin that may be added to feed or water to reduce CH₄ emissions from farmed ruminants.

REQUIREMENTS

1. Saponins can be utilized to manage internal parasites in ruminants.
2. Feeding with saponins are enhanced animal growth functioning.
3. Saponins impacted on rumen fermentation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank the Facultad de Veterinaria de Lugo, Campus Terra-IBADER, Universidad de Santiago de Compostela, Spain, for providing experimental facilities with constant for encouragement.

REFERENCES

- Canadell JG, Monteiro PM, Costa MH, Cotrim da Cunha L, Cox PM, Eliseev AV, Henson S, Ishii M, Jaccard S, Koven C, Lohila A. Intergovernmental Panel on Climate Change (IPCC). Global carbon and other biogeochemical cycles and feedback. In Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change 2023 (pp. 673-816). Cambridge University Press. Available at <https://oceanrep.geomar.de/id/eprint/54719>
- Hidayat T, Espinoza ME, Yan X, Theodoridou K, Peng Q, Feng B, Zhang W, He J, Yu P. The utilization of prairie-based blend pellet products combined with newly commercial phytochemicals (feed additives) to mitigate ruminant methane emission and improve animal performance. In Feed additives—recent trends in animal nutrition (working title) 2024 Mar 6. In TechOpen. Available at: <https://doi.org/10.5772/intechopen.114219>
- Haque MN. Dietary manipulation: a sustainable way to mitigate methane emissions from ruminants. Journal of animal science and technology. 2018 Dec; 60:1-0. Available at: <https://doi.org/10.1186/s40781-018-0175-7>
- Trotta RJ, Kreikemeier KK, Foote S, McLeod KR, Harmon DL. Influence of Anti-Coccidial Compounds and Phytogetic Saponin Extracts on In Vitro and In Vivo Ruminant Fermentation and Methane Production of Cattle. Animals. 2023 Jul 14;13(14):2308. Available at: <https://doi.org/10.3390/ani13142308>
- Lambo MT, Ma H, Liu R, Dai B, Zhang Y, Li Y. Mechanism, effectiveness, and the prospects of medicinal plants and their bioactive compounds in lowering ruminants' enteric methane emission. animal. 2024 Mar 15:101134. Available at: <https://doi.org/10.1016/j.animal.2024.101134>
- Ban C, Paengkoum S, Yang S, Tian X, Thongpea S, Purba RA, Paengkoum P. Feeding meat goat's mangosteen (*Garcinia mangostana* L.) peel rich in condensed tannins, flavonoids, and cinnamic acid improves growth performance and plasma antioxidant activity under tropical conditions. Journal of Applied Animal Research. 2022 Dec 31;50(1):307-15 Available at: <https://doi.org/10.1080/09712119.2022.2068557>
- Yaxing Z, Erdene K, Zhibi B, Changjin A, Chen B. Effects of *Allium mongolicum* regel essential oil supplementation on growth performance, nutrient digestibility, rumen fermentation, and bacterial communities in sheep. Frontiers in veterinary science. 2022 Oct 31; 9:92672. Available at: <https://doi.org/10.3389/fvets.2022.926721>
- Xiao L, Yi S, Azad MA, Huang M, Guo X, Kong X. Nutritional Values and In Vitro Fermentation Characteristics of 29 Different Chinese Herbs. Agriculture. 2022 Aug 23;12(9):1285. Available at: <https://doi.org/10.3390/agriculture12091285>
- Soltan YA, Hashem NM, Morsy AS, El-Azrak KM, El-Din AN, Sallam SM. Comparative effects of *Moringa oleifera* root bark and monensin supplementations on ruminal fermentation, nutrient digestibility and growth performance of growing lambs. Animal Feed Science and Technology. 2018 Jan 1; 235:189-201. Available at: <https://doi.org/10.1016/j.anifeedsci.2017.11.021>
- Bhat AR, Ganai AM, Ishfaq A, Beigh YA, Sheikh GG, Masood D. In-vitro effect of *Artemisia absinthium* (Titween) on digestibility and rumen parameters of small ruminants. Indian Journal of Animal Research. 2018;52(4):579-82. Available at: <http://dx.doi.org/10.18805/ijar.v0iOF.6996>
- El Aziz MM, Ashour AS, Melad AS. A review on saponins from medicinal plants: chemistry, isolation, and determination. J. Nanomed. Res. 2019;8(1):282-8. Available at: <http://dx.doi.org/10.18805/ijar.v0iOF.6996>
- Kaur R, Mishra V, Gupta S, Sharma S, Vaishnav A, Singh SV. Industrial and Environmental Applications of Plant-Derived Saponins: An Overview and Future Prospective. Journal of Plant Growth Regulation. 2024 Jan 5:1-5. Available at: <https://doi.org/10.1007/s00344-023-11201-x>
- Jafari S, Ebrahimi M, Goh YM, Rajion MA, Jahromi MF, Al-Jumaili WS. Manipulation of rumen fermentation and methane gas production by plant secondary metabolites (saponin, tannin and essential oil)—a review of ten-year studies. Annals of Animal Science. 2019 Jan 1;19(1):3-29. Available at: <https://doi.org/10.2478/aoas-2018-0037>
- Kim H, Lee HG, Baek YC, Lee S, Seo J. The effects of dietary supplementation with 3-nitrooxypropanol on enteric methane emissions, rumen fermentation, and production performance in ruminants: a meta-analysis. Journal of Animal Science and Technology. 2020 Jan;62(1):31. Available at: <https://doi.org/10.5187%2Fjast.2020.62.1.31>

15. Videv E, Angelova T, Krustanov J. Opportunities to reduce methane emissions (CH₄) in the digestive processes of ruminant animals. Review. Bulgarian Journal of Animal Husbandry/Životnov Dni Nauki. 2024 Jan 1;61(1). Available at: <http://doi.org/10.61308/HDZX9447>
16. Wang B, Ma MP, Diao QY, Tu Y. Saponin-induced shifts in the rumen microbiome and metabolome of young cattle. *Frontiers in microbiology*. 2019 Feb 28; 10:410738. Available at: <http://doi:10.3389/fmicb.2019.00356>
17. Kholif AE. A review of effect of saponins on ruminal fermentation, health and performance of ruminants. *Veterinary Sciences*. 2023 Jul 10;10(7): 450. Available at: <http://doi:10.3390/vetsci10070450>
18. Li J, Monje-Galvan V. In Vitro and In Silico Studies of Antimicrobial Saponins: A Review. *Processes*. 2023 Sep 28;11(10): 2856. Available at: <https://doi.org/10.3390/pr11102856>
19. Soltan YA, Patra AK. Ruminal microbiome manipulation to improve fermentation efficiency in ruminants. *Animal feed science and nutrition-production, health and environment*. 2021 Dec 31. Available at: <http://doi.org/10.5772/intechopen.101582>
20. Ugboogu EA, Elghandour MM, Ikpeazu VO, Buendía GR, Molina OM, Arunsi UO, Emmanuel O, Salem AZ. The potential impacts of dietary plant natural products on the sustainable mitigation of methane emission from livestock farming. *Journal of Cleaner Production*. 2019 Mar 10; 213:915-25. Available at: <http://doi.org/10.1016/j.jclepro.2018.12.233>
21. Beauchemin KA, Ungerfeld EM, Abdalla AL, Alvarez C, Arndt C, Becquet P, Benchaar C, Berndt A, Mauricio RM, McAllister TA, Oyhantçabal W. Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*. 2022 Dec 1;105(12):9297-326. Available at: <https://doi.org/10.3168/jds.2022-22091>
22. Muñoz-Páez KM, Buitrón G. Bioconversion of H₂ and CO₂ from dark fermentation to methane: effect of operating conditions on methane concentration. *Chemosphere*. 2022 Dec 1; 308:136305. Available at: <https://doi.org/10.1016/j.chemosphere.2022.136305>
23. Arndt C, Hristov AN, Price WJ, McClelland SC, Pelaez AM, Cueva SF, Oh J, Dijkstra J, Bannink A, Bayat AR, Crompton LA. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences*. 2022 May 17;119(20):e2111294119. Available at: <https://doi.org/10.1073/pnas.2111294119>
24. Rai S, Acharya-Siwakoti E, Kafle A, Devkota HP, Bhattarai A. Plant-derived saponins: a review of their surfactant properties and applications. *Sci*. 2021 Nov 16;3(4):44. Available at: <https://doi.org/10.3390/sci3040044>
25. Nguyen LT, Fărcaș AC, Socaci SA, Tofană M, Diaconeasa ZM, Pop OL, Salanță LC. An overview of saponins-a bioactive group. 2020. Available at: <http://doi.org/10.15835/buasvmcn-fst:2019.0036>
26. Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*. 2020 Dec;2:1-4. Available at: <https://doi.org/10.1186/s43014-020-0020-5>
27. Ramdani D, Yuniarti E, Jayanegara A, Chaudhry AS. Roles of essential oils, polyphenols, and saponins of medicinal plants as natural additives and anthelmintics in ruminant diets: A systematic review. *Animals*. 2023 Feb 20;13(4):767. Available at: <https://doi.org/10.3390/ani13040767>
28. El Hazzam K, Hafsa J, Sobeh M, Mhada M, Taourirte M, El Kacimi K, Yasri A. An insight into saponins from Quinoa (*Chenopodium quinoa* Willd): A review. *Molecules*. 2020 Feb 27;25(5): 1059. Available at: <https://doi.org/10.3390/molecules25051059>
29. Mieres-Castro D, Mora-Poblete F. Saponins: research progress and their potential role in the post-COVID-19 pandemic Era. *Pharmaceutics*. 2023 Jan 20;15(2):348. Available at: <https://doi.org/10.3390/pharmaceutics15020348>
30. Sari NF, Ray P, Rymer C, Kliem KE, Stergiadis S. Garlic and its bioactive compounds: Implications for methane emissions and ruminant nutrition. *Animals*. 2022 Oct 31;12(21): 2998. Available at: <https://doi.org/10.3390/ani12212998>
31. Hasanah N, Haryuni N. Supplementation of Tannin and Saponin Extracts to Optimize Fermentation in the Rumen and Reduce Methane Gas Emissions. *Bestindo Journal of Animal Nutrition and Forage*. 2024 Mar 27; 1(1): 34-40. <https://bestindolestari.id/journal/index.php/bjanf/article/view/20>
32. Zhang F, Li B, Ban Z, Liang H, Li L, Zhao W, Yan X. Evaluation of origanum oil, hydrolysable tannins and tea saponin in mitigating ruminant methane: In vitro and in vivo methods. *Journal of animal physiology and animal nutrition*. 2021 Jul;105(4):630-8. Available at: <https://doi.org/10.1111/jpn.13501>
33. Ku-Vera JC, Jiménez-Ocampo R, Valencia-Salazar SS, Montoya-Flores MD, Molina-Botero IC, Arango J, Gómez-Bravo CA, Aguilar-Pérez CF, Solorio-Sánchez FJ. Role of secondary plant

- metabolites on enteric methane mitigation in ruminants. *Frontiers in Veterinary Science*. 2020 Aug 27; 7:584. Available at: <https://doi.org/10.3389/fvets.2020.00584>
34. Jadhav RV, Kannan A, Bhar R, Sharma OP, Gulati A, Rajkumar K, Mal G, Singh B, Verma MR. Effect of tea (*Camellia sinensis*) seed saponins on in vitro rumen fermentation, methane production and true digestibility at different forage to concentrate ratios. *Journal of Applied Animal Research*. 2018 Jan 1;46(1):118-24. Available at: <https://doi.org/10.1080/09712119.2016.1270823>
 35. Syamsiyah D, Suharti S, Jayanegara A. Fermentation Characteristics, Digestibility, and Estimation of Ruminant Methane from Saponin: A Quantitative Study. *Jurnal Sain Peternakan Indonesia*. 2023 Jun 30;18(2):76-82. Available at: <https://doi.org/10.31186/jspi.id.18.2.76-82>
 36. Supapong C, Cherdthong A, Seankamsorn A, Khonkhaeng B, Wanapat M, Uriyapongson S, Gunun N, Gunun P, Chanjula P, Polyorach S. In vitro fermentation, digestibility and methane production as influenced by *Delonix regia* seed meal containing tannins and saponins. *Journal of Animal and Feed Sciences*. 2017 Jun 6;26(2):123-30. Available at: <https://doi.org/10.22358/jafs/73890/2017>
 37. Cherdthong A, Prachumchai R, Wanapat M, Foiklang S, Chanjula P. Effects of supplementation with royal poinciana seed meal (*Delonix regia*) on ruminal fermentation pattern, microbial protein synthesis, blood metabolites and mitigation of methane emissions in native Thai beef cattle. *Animals*. 2019 Aug 29;9(9):625. Available at: <https://doi.org/10.3390/ani9090625>
 38. Liu Y, Ma T, Chen D, Zhang N, Si B, Deng K, Tu Y, Diao Q. Effects of tea saponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in Dorper crossbred ewe. *Animals*. 2019 Jan 21;9(1):29. Available at: <https://doi.org/10.3390/ani9010029>
 39. Kim H, Kim BW, Yoo D, Moon J, Kwon I, Lee Y, Seo J. In vitro evaluation of *Aloe saponaria* as a potential feed additive to modulate ruminal fermentation and microbial diversity. *Journal of Applied Animal Research*. 2023 Dec 31;51(1):115-22. Available at: <https://doi.org/10.1080/09712119.2023.2165086>
 40. Levy B, Jami E. Exploring the prokaryotic community associated with the rumen ciliate protozoa population. *Frontiers in microbiology*. 2018 Oct 29; 9:381023. Available at: <https://doi.org/10.3389/fmicb.2018.02526>
 41. Abdullah RH, Al-Galbi HA. Impact of Feeding Tea Leaves Saponins on Some Productive and Serum Biochemical Parameters of Awassi Lambs. *Basrah Journal of Agricultural Sciences*. 2020 Jun 23;33(1):17-25. Available at: <https://doi.org/10.37077/25200860.2020.32.1.02>
 42. Baheg R, El-Bahrawy KA, El-Azrak KM, Samak MA, Sallam SM. Effect of condensed tannins and saponin supplementation on reproductive performance in Barki ewes. *Egyptian Journal of Nutrition and Feeds*. 2017 Aug 1; 20(2): 197-210. Available at: <https://dx.doi.org/10.21608/ejnf.2017.75166>
 43. Yanza YR, Irawan A, Jayanegara A, Ramadhani F, Respati AN, Fitri A, Hidayat C, Niderkorn V, Cieslak A, Szumacher-Strabel M, Hidayat R. Saponin Extracts Utilization as Dietary Additive in Ruminant Nutrition: A Meta-Analysis of In Vivo Studies. *Animals*. 2024 Apr 19;14(8): 1231. Available at: <https://doi.org/10.3390/ani14081231>
 44. Hartinger T, Gresner N, Südekum KH. Does intraruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. *Journal of animal science and biotechnology*. 2018 Dec; 9:1-21. Available at: <https://doi.org/10.1186/s40104-018-0249-x>
 45. Honan M, Feng X, Tricarico JM, Kebreab E. Feed additives as a strategic approach to reduce enteric methane production in cattle: modes of action, effectiveness and safety. *Animal Production Science*. 2021 Feb 2. Available at: <https://doi.org/10.1071/AN20295>
 46. Jayanegara A, Yogiarto Y, Wina E, Sudarman A, Kondo M, Obitsu T, Kreuzer M. Combination effects of plant extract rich in tannins and saponins as feed additives for mitigating in vitro ruminal methane and ammonia formation. *Animals*. 2020 Aug 30;10(9): 1531. Available at: <https://doi.org/10.3390/ani10091531>
 47. Chen J, Cao D, Jiang S, Liu X, Pan W, Cui H, Yang W, Liu Z, Jin J, Zhao Z. Triterpenoid saponins from *Ilex pubescens* promote blood circulation in blood stasis syndrome by regulating sphingolipid metabolism and the PI3K/AKT/eNOS signaling pathway. *Phytomedicine*. 2022 Sep 1; 104:154242. Available at: <https://doi.org/10.1016/j.phymed.2022.154242>
 48. Sharma P, Tyagi A, Bhansali P, Pareek S, Singh V, Ilyas A, Mishra R, Poddar NK. Saponins: Extraction, bio-medicinal properties and way forward to anti-viral representatives. *Food and Chemical Toxicology*. 2021 Apr 1; 150:112075. Available at: <https://doi.org/10.1016/j.fct.2021.112075>
 49. Kunatsa Y, Katerere DR. Checklist of african soapy saponin—Rich plants for possible use in communities' response to global pandemics.

- Plants. 2021 Apr 22;10(5):842. Available at: <https://doi.org/10.3390/plants10050842>
50. Albores-Moreno S, Alayón-Gamboa JA, Ayala-Burgos AJ, Solorio-Sánchez FJ, Aguilar-Pérez CF, Olivera-Castillo L, Ku-Vera JC. Effects of feeding ground pods of *Enterolobium cyclocarpum* Jacq. Griseb on dry matter intake, rumen fermentation, and enteric methane production by Pelibuey sheep fed tropical grass. *Tropical animal health and production*. 2017 Apr; 49:857-66. Available at: <https://doi.org/10.1007/s11250-017-1275-y>
51. Salazar SS, Vázquez AT, Botero IC, Balbuena FJ, Narváez JJ, Campos MR, Avilés LR, Sánchez FJ, Vera JC. Potential of *Samanea saman* pod meal for enteric methane mitigation in crossbred heifers fed low-quality tropical grass. *Agricultural and Forest Meteorology*. 2018 Aug 15;258:108-16. Available at: <https://doi.org/10.1016/j.agrformet.2017.12.262>
52. Lileikis T, Nainienė R, Bliznikas S, Uchockis V. Dietary Ruminant Enteric Methane Mitigation Strategies: Current Findings, Potential Risks and Applicability. *Animals*. 2023 Aug 10;13(16):2586. Available at: <https://doi.org/10.3390/ani13162586>