Regulatory Effects and Mechanisms of Plant Extracts on Rumen Fermentation, Production Performance, and Methane Production in Ruminants: Postprint

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Abstract

Plant extracts not only possess functions such as regulating rumen fermentation patterns in ruminants, improving nitrogen retention rate, and reducing methane emissions, but also, due to their low toxicity and side effects as well as their characteristics of naturalness, nutritional properties, and biological activity, have become one of the ideal alternatives to antibiotics. This article reviews the latest research progress on the regulatory effects and mechanisms of plant extracts on rumen fermentation in ruminants, aiming to provide a reference basis for future research and product development in this field.

Full Text

Regulation of Plant Extracts on Rumen Fermentation, Performance, and Methane Production in Ruminants and Its Mechanisms

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Abstract: Plant extracts not only regulate rumen fermentation patterns, improve nitrogen retention, and reduce methane emissions in ruminants, but also represent ideal antibiotic alternatives due to their low toxicity, natural properties, nutritional value, and biological activity. This article reviews recent research progress on the regulatory effects of plant extracts on rumen fermentation and their underlying mechanisms, providing a reference basis for future research and product development in this field.

Keywords: plant extract; ruminant; rumen fermentation; regulation; mechanism

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The rumen of ruminants is a complex anaerobic fermentation system that establishes a symbiotic relationship with rumen microorganisms. The rumen provides nutrients and suitable environmental conditions for microbial fermentation, while microorganisms degrade fiber and synthesize microbial protein (MCP), supplying energy and protein to the host animal. However, this fermentation process is also accompanied by energy losses and the production of harmful gases such as carbon dioxide (CO) and methane (CH). Therefore, rational regulation of rumen fermentation has become a research area of high priority for animal nutritionists. Current primary methods for regulating rumen fermentation include appropriate feeding strategies, improving dietary composition and processing, and using feed additives. Feed additive regulation has traditionally relied on antibiotics, but the resulting drug residue problems have become increasingly severe [1]. These residues not only pollute the environment but also endanger human health, making the search for suitable antibiotic alternatives urgent [2].

Plant extracts are mixtures containing one or more active ingredients obtained from plant materials through physical and chemical processing. Due to their low toxicity and natural, nutritional, and bioactive properties, they have attracted considerable attention from researchers. Recent studies have shown that plant extracts can regulate rumen fermentation, with certain extracts altering fermentation patterns to reduce rumen protein degradation and decrease CH gas emissions [3]. Current research on plant extracts for rumen fermentation regulation primarily focuses on reducing CH emissions, improving rumen fermentation patterns, and decreasing rumen protein degradation rates [4-5]. This review summarizes research progress on the regulatory effects of plant extracts on rumen fermentation and their mechanisms to provide references for their application in ruminant nutrition.

1. Effects of Plant Extracts on Rumen Volatile Fatty Acids

Volatile fatty acids (VFAs) are the main products of dietary carbohydrate degradation in the rumen and serve as the primary energy source for ruminants as well as substrates for milk fat and body fat synthesis. VFAs provide more than 60% of the fermentable energy in the rumen, highlighting their critical role in ruminant energy metabolism. In recent years, only a few studies have demonstrated that plant extract supplementation increases rumen VFA concentrations. Wu et al. [6] used an in vitro gas production method to study the effects of adding 5% and 10% flaxseed oil to a diet with a concentrate-to-forage ratio of 30:70, finding that VFA concentrations increased significantly, acetate proportion decreased significantly, propionate proportion increased significantly, and the acetate-to-propionate ratio decreased significantly. Mao [7] added tea saponins (0 or 3 g/d) and soybean oil (0 or 3%) to the diets of weaned goats, reporting that all treatment groups showed significantly increased VFA production. Pilajun et al. [8] supplemented the diet of fistulated buffaloes with mangosteen peel extract at 30 g/kg, resulting in significantly higher VFA concentrations. Mateos et al. [9] investigated the addition of cinnamaldehyde at 180 mg/L to a diet with a concentrate-to-forage ratio of 50:50, observing significantly higher VFA concentrations and acetate proportions compared to the control group.

However, most studies have shown that plant extract supplementation either decreases VFA concentrations or has no effect. Shi et al. [10] used artificial rumen technology to study the addition of 1%, 2%, 3%, and 4% perilla oil to diets, finding no significant effect on VFA concentrations. Liang et al. [11] added sunflower oil, tea oil, and their combinations at 4% of concentrate intake to lactating buffaloes, which decreased rumen fluid VFA concentrations, though the difference was not significant. Thao et al. [12] administered eucalyptus oil at 2 mL/d to freely grazing buffaloes, reporting unchanged VFA concentrations but reduced acetate proportion and increased propionate molar ratio. Khorrami et al. [13] added thymol and cinnamaldehyde (active components of plant extracts) to the basal diet of Holstein cows, finding no effects on VFA concentrations or acetate and butyrate proportions after 21 days. Benhissi et al. [14] used batch culture techniques to study the addition of 2% tannin-rich plant extracts, observing no significant effect on VFA concentrations after 24 h of fermentation.

Although numerous studies indicate that plant extracts reduce rumen VFA concentrations or have no effect, they can alter the molar proportions of individual VFAs. McMurphy et al. [15] reported that saponin supplementation in the diet of 16 steers increased acetate and propionate concentrations. Jin et al. [16] added linseed oil at 475 g/d to the basal diet of Yanbian yellow cattle, which decreased acetate proportion while increasing propionate proportion. Reducing the acetate-to-propionate ratio represents a positive effect of plant extracts on VFA composition, though not all plant extracts improve rumen VFA composition positively. Castillejos et al. [17] reported that eugenol supplementation (500 mg/L) decreased propionate proportion without affecting total VFA concentration. Busquet et al. [18] found that garlic oil (300 and 3,000 mg/L) and benzyl salicylate (300 and 3,000 mg/L) decreased acetate proportion while increasing propionate and butyrate proportions. Supplementation with some plant extracts or their active components can increase butyrate concentration, suggesting these compounds act differently than monensin.

Nevertheless, in vitro results often differ from in vivo findings [19]. The doses of plant extracts applied in vitro far exceed physiologically acceptable levels for live animals. According to meta-analyses, in vitro dose ranges from 0.03 to 500 g/kg [5], whereas in vivo doses range from 0.02–0.75 g/kg for small ruminants, 0.04–0.25 g/kg for beef cattle, and 0.01–0.43 g/kg for dairy cows [4]. Khiaosaard et al. [4] found that plant extracts more effectively reduced acetate, ammonia concentrations, and methane production in small ruminants and beef cattle compared to dairy cows. For example, garlic oil (500 mg/kg) significantly reduced ammonia concentration and increased propionate concentration in sheep [20] but had no effect in dairy cows (250 mg/kg) [21]. Capsaicin (13–60 mg/kg) decreased acetate concentration in beef cattle [22] but did not affect rumen fermentation in dairy cows (9–38 mg/kg) [23].

Rumen microorganisms exhibit adaptive responses to added plant extracts, which challenges the development of long-term effects for plant extracts as feed additives, particularly at low doses. Busquet et al. [24] used 24-h batch cultures to investigate the effects of garlic oil on in vitro rumen microbial fermentation, finding that VFA concentrations decreased at 300 mg/L. However, in subsequent continuous culture experiments, increasing the garlic oil dose to 312 mg/L had no effect on VFA concentrations. Cardozo et al. [25] added cinnamaldehyde, garlic, and anise oils (7.5 mg/kg) in continuous culture, observing altered VFA concentrations during the first 6 days but no effect thereafter. These studies provide strong evidence that microbial flora can adapt to plant extracts, representing a current challenge for commercial development of plant extracts as feed additives.

2. Effects of Plant Extracts on Rumen Nitrogen Metabolism

The symbiotic relationship between ruminants and rumen microflora enables ruminants to utilize non-protein nitrogen as a nitrogen source for MCP synthesis, which provides an excellent amino acid resource for the host to synthesize milk and meat proteins. However, for high-producing ruminants, rumen MCP synthesis cannot meet their requirements, necessitating increased dietary protein supplementation, which raises feed costs. Moreover, inefficient nitrogen utilization by ruminants leads to excessive nitrogen flow into the environment, causing pollution. Therefore, improving nitrogen utilization benefits both ruminants and the environment.

Numerous studies have investigated the effects of plant extracts and their active components on rumen nitrogen metabolism. Zhang [26] added sunflower oil, tea oil, and mixed oils to the diets of 32 lactating dairy cows, reporting that all oil-supplemented groups showed significantly reduced ammonia nitrogen (NH -N) concentrations compared to the control. Wanapat et al. [27] added a mixture of 6% coconut oil and sunflower oil to swamp buffalo diets, significantly reducing NH -N concentration by inhibiting rumen protein degradation. However, some studies have shown that plant extracts do not alter rumen nitrogen metabolism or even increase NH -N concentrations. Kongmun et al. [28] reported that adding 7% coconut oil to buffalo diets had no effect on rumen fluid

NH -N concentration. Cui et al. [29] used in vitro gas production to add 0.01%, 0.10%, and 1.00% phytoecdysteroids to rumen fluid, finding that NH -N concentrations increased at all doses, though not significantly. NH -N concentration is generally considered an important indicator for evaluating nitrogen retention in the rumen; reduced NH -N concentration indicates decreased rumen protein degradation or improved microbial NH -N utilization. The regulatory effects of plant extracts on NH -N concentration depend on their chemical structure and dosage. Jahani-Azizabadi et al. [30] used in vitro gas production to add 140 and 280 L/L of cinnamaldehyde and thymol to a diet with a concentrate-to-forage ratio of 1:1, finding that only thymol reduced NH -N concentration while cinnamaldehyde had no effect. Busquet et al. [18] observed that some plant extracts and their main components could reduce NH -N concentration at high concentrations, with 300 mg/L as the critical effective level, while low doses (3 mg/L) had no effect.

Early research by Borchers [31] added thymol (1 g/L) to rumen fluid containing casein, observing amino acid accumulation and decreased NH -N concentration, indicating inhibited deamination. McIntosh et al. [32] further used in vitro batch culture to ferment casein with rumen fluid from dairy cows fed 1 g/d of mixed plant oils, finding that amino acid deamination was reduced by 9%. These studies suggest that reduced NH -N concentration may result from plant extracts inhibiting amino acid deamination. Wallace [33] reported that supplementing sheep diets with 100 mg/d of mixed plant oils reduced hyper-ammonia-producing bacteria by 77%. Flythe et al. [34] investigated the effects of soluble phenolic-rich alfalfa on rumen protein metabolism, finding that hyper-ammonia-producing bacterial activity was inhibited, which may explain the suppressed deamination.

Another mechanism for decreased NH -N concentration may involve the defaunation effect of plant extracts. Rumen protozoa secrete deaminases that degrade feed proteins to produce NH -N but cannot utilize NH -N for their own protein synthesis. Additionally, protozoa can ingest and digest large amounts of bacterial protein, reducing the quantity of MCP reaching the duodenum, and affect protein hydrolysis and deamination. Therefore, removing protozoa can prevent nitrogen cycling between microorganisms and protozoa, thereby increasing MCP flow to the duodenum [35-37]. Fan et al. [38] used 12 healthy goats to investigate the effects of yucca saponins on rumen nitrogen metabolism, finding that yucca saponin supplementation significantly reduced protozoal numbers and rumen NH -N concentration, decreased urinary nitrogen excretion, and increased the proportion of retained nitrogen to total nitrogen intake, indicating that yucca saponins can defaunate protozoa and improve nitrogen utilization efficiency. Noorian et al. [39] used in vitro gas production to study Echium amoneum extract, reporting decreased NH -N concentration and a trend toward reduced protozoal numbers. Tannins are another plant extract that can improve rumen nitrogen metabolism by forming stable complexes with feed proteins, protecting them from microbial degradation while inhibiting the activity of some proteolytic and fiber-degrading bacteria under certain conditions [40-42].

Long-term in vitro and in vivo fermentation show less significant effects on nitrogen metabolism compared to short-term in vitro fermentation. Newbold et al. [43] and Benchaar et al. [44] reported that adding 110 mg or 2 g of mixed plant oils to rumen fluid from sheep or dairy cows using soybean meal as a fermentation substrate had no effect on rumen nitrogen metabolism. Busquet et al. [45] and Cardozo et al. [46] used dual-flow continuous culture experiments to find that the effects of plant oils and their active components on rumen nitrogen metabolism disappeared after 6–7 days of fermentation, indicating that rumen microbial flora developed adaptation. Therefore, results from 24- or 48-h in vitro fermentation cultures should be interpreted cautiously, as they do not account for potential changes in rumen microbial flora.

3. Antimicrobial Effects of Plant Extracts

Plant extracts are known to have broad-spectrum antimicrobial effects against bacteria, protozoa, archaea, and fungi [47]. Their antibacterial properties are primarily attributed to hydrophobic active compounds such as terpenoids and tannins [48]. Tannins have been reported to delay rumen protein breakdown, improve amino acid utilization in the small intestine, reduce ammonia production and urea nitrogen excretion in the rumen, and slow rumen protein degradation, thereby improving animal nutritional status [49].

Research has shown that plant extracts can improve the rumen environment, enhance microbial activity, promote bacterial protein synthesis, and increase crude fiber digestibility, thereby enhancing performance [50]. The relatively lower rumen pH in beef cattle compared to dairy cows may enhance plant extract activity by increasing hydrophobicity, thereby disrupting bacterial cell membranes [51]. In both in vitro rumen fluid and in vivo experiments with dairy cows, yucca extract supplementation improved dietary nutrient digestibility and milk quality, increased serum immunoglobulin (Ig) G, IgM, and IgA levels, significantly reduced methane production, decreased energy losses, and lowered environmental pollution [52]. Therefore, yucca plant extracts possess strong antiprotozoal activity, reducing protozoal numbers, improving rumen nitrogen utilization, and increasing MCP flow to the small intestine.

Dietary tea saponin supplementation has been reported to significantly reduce rumen pH and NH -N concentration while increasing MCP production and propionate and butyrate concentrations, significantly affecting rumen microflora in dairy cows. When methanogen activity is inhibited, excess hydrogen in the rumen is used for propionate synthesis, increasing propionate concentration and altering rumen fermentation patterns [53-55]. Rumen microorganisms play a crucial role in digestion, and regulating them to improve feed digestibility is important for maximizing rumen digestive efficiency and enhancing performance. Rumen fermentation regulators have achieved good results in production practice, and future research should focus on: (1) investigating specific mechanisms rather than just apparent effects, and (2) understanding rumen microbial activity and metabolic patterns to develop new regulators.

4. Effects of Plant Extracts on Ruminant Performance

Due to differences in chemical properties, dosage, and animal species, plant extracts produce varying effects that can improve animal performance. Yang et al. [56] found that adding 400, 800, or 1,600 mg/d of cinnamaldehyde to the diets of 70-day-old beef cattle resulted in linear increases in daily dry matter intake (DMI) and average daily gain over the experimental period. In dairy cows, cinnamaldehyde supplementation (1 g/d or 50 mg/kg) had no effect on DMI, milk yield, or milk composition [57]. In a series of crossover and randomized design trials, a mixture of cinnamaldehyde and eugenol (525 mg/d per cow) had no effect on dairy cow performance [58]. Wall et al. [59] reported that the same plant extract mixture reduced daily milk yield by 400–600 mg in multiparous cows but increased milk yield in primiparous cows at rates of 200–600 mg/d per cow. Plant extracts also increased DMI only in primiparous cows, indicating that production effects depend on the cow’ s lactation stage. In Tager et al. [60], dietary capsicum supplementation (250 mg/d per cow) had no effect on milk yield. However, recent studies have reported positive effects of capsicum on dairy cow performance. Oh et al. [23] found that solid-form capsicum (250 and 500 mg/d per cow) increased milk and energy-corrected milk (ECM) yields. Stelwagen et al. [61] and Oh et al. [62] reported that rumen-protected capsicum (100 or 200 mg/d per cow) improved milk yield and feed conversion efficiency. Low-dose plant extract supplementation (4–38 mg/kg) in dairy cow diets has been shown to increase milk yield or feed conversion efficiency without significantly affecting rumen fermentation in vitro [63]. Oh et al. [23] found that low-dose plant extracts had no effect on rumen fermentation in dairy cows.

Adding lemongrass to dairy cow forage improved milk flavor and enhanced immunity and health while providing a pleasant lemon aroma that improved the cattle’ s environment, making it more comfortable for production [64]. Researchers also found that spraying anise oil in dairy barns improved cow social behavior and increased milk yield [65].

5. Plant Extracts for Reducing CH Emissions

Rumen fermentation in ruminants accounts for approximately 25% of global CH emissions, raising concerns among animal nutritionists and environmental scientists. CH production also represents a major pathway of energy loss for ruminants [66]. Due to their antimicrobial properties, plant extracts have been widely used in many fields (e.g., traditional medicine, food preservatives) [18]. These antimicrobial characteristics result from abundant secondary metabolites, including saponins, terpenoids, essential oils, tannins, and phenolic compounds. Recent studies have shown that plant extracts can reduce CH emissions from rumen fermentation [18].

As a common plant extract, essential oil is a complex mixture obtained through water or ethanol-water distillation, primarily containing phenols, terpenoids, esters, and aldehyde derivatives with numerous antimicrobial molecules that regulate rumen fermentation while protecting ruminants from pathogens [49]. Many studies have found that dietary essential oil supplementation at certain levels reduces rumen CH production. Sallam et al. [67] used in vitro gas production with a concentrate-to-forage ratio of 1:1 to investigate different eucalyptus oil levels, reporting linear reductions in CH production of 26.8%, 46.8%, 77.3%, and 85.3% at addition levels of 25, 50, 100, and 150 L, respectively. Araujo et al. [68] further studied essential oil from Cordia verbenacea D.C. for its ability to inhibit rumen CH production, finding that essential oil reduced CH production by 30% when added to hay-based fermentation, with CH-reducing capacity equivalent to monensin at levels above 1 mL/mL. Chandrasekharaiah et al. [69] examined 11 addition levels of citronella oil, wintergreen oil, and clove oil on rumen fermentation CH production, reporting linear reductions in CH production for all three oils. Compared to the control, CH production decreased by 39% and 4% when citronella oil and wintergreen oil were added at 2.67 and 0.66 L/mL, respectively, without negative effects on rumen fermentation. Numerous recent studies have confirmed that dietary essential oil supplementation at appropriate levels reduces rumen CH production, making essential oils promising regulators for rumen fermentation and CH reduction.

Saponins are also plant secondary metabolites—high-molecular-weight glycosides composed of triterpenoid or steroidal aglycones (sapogenins) linked to sugar chains, containing one or more C-C bonds [70]. Recent studies have shown that saponins also affect rumen CH production. Rodríguez et al. [71] added Enterolobium cyclocarpum saponins to Pennisetum purpureum grass as a substrate for in vitro fermentation, reporting significantly reduced CH production after 24 h. Goel et al. [72] used in vitro gas production to investigate the effects of 3%, 6%, and 9% saponins on rumen CH production, finding that CH production decreased by 49.66% at the 6% addition level. Wang et al. [73] evaluated the effects of adding 0, 5, 10, 20, 40, and 60 mg/mL of ginsenosides on rumen CH production, reporting reductions of 30.20%, 43.49%, 44.67%, and 75.8% after 8 h; 6.97%, 9.63%, 18.90%, and 61.82% after 12 h; and 2.34%, 9.39%, 6.90%, and 20.73% after 24 h, with CH reduction showing a linear relationship with dosage. These results demonstrate that appropriate ginsenoside levels positively inhibit CH production.

The mechanism by which plant extracts reduce rumen CH production may relate to their antimicrobial activity and selective modification of rumen microflora [37]. The effects of plant extracts on CH production vary with type, dosage, diet composition, and differ between in vitro and in vivo conditions.

6. Summary

Plant extracts can regulate rumen fermentation, improve nutrient utilization, and reduce harmful substance production in ruminants, but their widespread application faces many challenges. Due to the diversity of plant extract types, their biological activity mechanisms vary, including regulation of antioxidant enzymes at transcriptional and translational levels, binding and penetration of pathogen surface receptors, mediation of immune cell signaling pathways, defaunation effects, and reduction of methanogen proliferation. Most current research has short-term effects that may not accurately reflect practical application in animal production. Additionally, determining the optimal concentration for plant extract supplementation is a major limiting factor for practical application. Despite these challenges, plant extracts show promising prospects as novel rumen fermentation regulators. The EU Commission has funded an international project called "Rumen-up," involving multiple European organizations and research institutes, aimed at screening plant extract additives that can reduce CH emissions during rumen production and improve nutrient utilization in ruminants. Selecting plant extracts with positive effects on rumen fermentation from diverse varieties with complex compositions and varying active components will undoubtedly benefit research progress in rumen fermentation regulation.

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Note: Figure translations are in progress. See original paper for figures.