Effects and Mechanisms of Brassica Forage Crops on Methane Emission Reduction in Ruminants (Postprint)

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Abstract

Methane is a greenhouse gas produced during the degradation of feed by microorganisms in the digestive tract of ruminants. Methane emissions from ruminants not only cause energy loss from feed but also exacerbate the Earth' s greenhouse effect. Brassica (Brassica spp.) forage crops are primarily used for catch cropping and have high nutritional value. Under different seasonal conditions, housed feeding or grazing, and mixed or sole feeding situations, Brassica forage crops can significantly reduce methane emissions from ruminants, demonstrating good prospects for promotion and application. Regarding the reasons for this emission reduction, previous studies have conducted investigations on feed chemical composition, rumen fermentation metabolic parameters, and rumen microorganisms, but the underlying mechanism remains unclear. This paper reviews the research progress on methane emission reduction by Brassica forage crops in ruminants and discusses the potential mitigation mechanisms.

Full Text

Mitigation of Methane Emissions with Forage Brassicas in Ruminants and Possible Mechanisms

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Abstract: Methane is a greenhouse gas produced during microbial degradation of feed in the ruminant digestive tract. Methane emissions from ruminants not only cause substantial feed energy losses but also exacerbate global greenhouse effects. Forage brassicas (Brassica spp.) are important catch crops with high nutritional value. When fed to ruminants either alone or mixed with other feeds, under grazing or housed conditions, and across different seasons, these crops can significantly reduce methane emissions, demonstrating excellent potential for practical application. Previous research has explored potential causes for this reduction, examining feed chemical composition, rumen fermentation parameters, and rumen microorganisms, yet the underlying mechanisms remain unclear. This review summarizes research progress on methane mitigation by forage brassicas in ruminants and discusses possible mechanisms.

Keywords: forage brassicas; methane emissions; mitigation; ruminants; effect; mechanism

Global climate change has garnered widespread attention worldwide. Greenhouse gases emitted from human activities, including industrial and agricultural production, are the primary drivers of global warming. Methane is a significant contributor to the greenhouse effect, with 21 times the global warming potential of carbon dioxide [1]. Global atmospheric methane emissions total 500–600 million tons annually, with a half-life of 8.4 years [2]. Agriculture accounts for 62% of methane emissions from human activities, with ruminants contributing 58% of agricultural emissions [3]. In China, agricultural greenhouse gas emissions represent 11% of the national total, with enteric fermentation and manure from livestock contributing 54% of agricultural emissions [4]. From 1949 to 2003, methane emissions from livestock increased at an annual rate of 2.4% [5], highlighting livestock as a major methane source. While monogastric animals emit minimal methane, ruminants are the primary contributors [6]. Beyond environmental impacts, methane emissions represent a loss of 3.9%–10.7% of ingested metabolizable energy [7], making methane mitigation a key research focus in animal science. Current efforts aim to elucidate mitigation mechanisms and develop simple, effective, low-cost, and safe strategies. Forage brassicas are important catch forages that have shown remarkable methane-reducing potential [8]. This review synthesizes research on methane mitigation by forage brassicas and explores possible mechanisms.

1 Methane Emissions from Ruminants and Mitigation Measures

Methane from ruminants is primarily produced in the rumen [9]. Feed degradation by rumen microorganisms generates short-chain fatty acids, ammonia, carbon dioxide, methane, and hydrogen. Methanogens utilize hydrogen and carbon dioxide to produce methane. Fermentation type influences methane production, with propionate generating less methane than acetate and butyrate [10]. Methanogens thrive at pH 6.0–7.5, with a lower limit of 5.5–6.5. Lower rumen pH also increases propionate production. Rumen outflow rate affects methane formation by reducing feed degradation time, increasing dissolved hydrogen concentration, and favoring propionate formation [10].

Methane mitigation can be achieved by reducing absolute methane production or decreasing emission intensity (methane per unit of animal product). Reducing production can involve adding electron acceptors that bind with fermentation-generated hydrogen instead of carbon dioxide, or directly inhibiting methanogens. Common electron acceptors include nitrates and sulfates. Direct inhibition utilizes ionophore antibiotics, lipids, plant extracts, chemical inhibitors [11], and defaunation methods. Reducing emission intensity involves altering diet composition, improving digestibility, optimizing management, enhancing animal health and welfare, increasing reproductive efficiency, and breeding high-yielding or low-methane livestock [12-13]. These approaches are practical and publicly acceptable. Using alternative forages is particularly simple and cost-effective. Compared to perennial ryegrass (*Lolium perenne*), forage brassicas can substantially reduce methane emissions by approximately 30% [14], demonstrating excellent application prospects.

2.1 Forage Brassicas

Forage brassicas belong to the Brassicaceae family and include many species cultivated worldwide, primarily kale (*Brassica oleracea*), turnip (*B. campestris*), forage rape (*B. napus*), and swede (*B. napus* ssp. *rapifera*) [15]. Major species in China include swede (*B. napobrassica* Mill.), cabbage (*B. oleracea* L.), Chinese cabbage [*B. campestris* L. ssp. *chinensis* Makino (var. *communis* Tsen et Lee)] [16], forage rape (*B. campestris*), and turnip (*B. rapa*) [17]. Currently, forage rape is being actively promoted in China [18].

Forage brassicas exhibit strong cold tolerance and are mesophytic plants with modest soil requirements and short growth cycles, making them ideal for catch cropping [15] and providing green forage in late autumn and early spring [17]. The Ministry of Agriculture' s *National Planting Structure Adjustment Plan (2016-2020)* [19] advocated developing forage rape in winter-fallow fields in southern China and as a relay crop after spring wheat in northeast China. In northwest China, where growing seasons are "one season with surplus, two seasons insufficient," brassicas can be planted after wheat and soybean harvests, utilizing idle land, water, heat, and light resources to increase feed supply [20-21].

Forage brassicas yield high biomass, with forage rape producing up to 47 t/ha of fresh forage, indicating broad promotion potential in China [18]. As dicotyledonous plants, brassicas are highly nutritious before lignification. Forage rape contains 3%-14% crude protein, 1.6%-2.3% ether extract, 6.4%-6.6% ash, 54%-57% neutral detergent fiber, and 32%-35% acid detergent fiber [22]. Brassicas demonstrate high feeding value for ruminants, with digestibility and metabolizable energy exceeding most forages and supporting good animal performance. Under grazing conditions, sheep fed turnips and forage rape had lower daily gains than those on white clover (*Trifolium repens*) but higher than those on ryegrass [15].

2.2 Methane Mitigation Effects of Forage Brassicas

Research on methane mitigation by forage brassicas remains limited. In vitro studies have been conducted primarily in Australia, the United States, and New Zealand, while animal trials have focused mainly on New Zealand with some Australian work.

Durmic et al. [23] conducted in vitro batch rumen fermentation with 10 forages including brassicas, finding that arrowleaf clover (*T. vesiculosum*) produced the most methane. Turnip and a turnip-forage rape hybrid (*B. napus* cv. Winfred) reduced methane by 30% compared to arrowleaf clover, though variation between samples of the same forage was substantial. Broccoli (*B. oleracea*) and a forage rape-cabbage hybrid (*B. campestris* × *B. napus*) showed no significant difference from arrowleaf clover, and methane production did not differ between high- and low-glucosinolate (GSL) broccoli varieties. Dillard et al. [24] used a continuous culture system to study nutrient digestibility, short-chain fatty acids, and methane production from brassica diets where half the substrate was orchardgrass (*Dactylis glomerata*) and half was either forage rape, oilseed rape (*B. napus*), turnip, or annual ryegrass (*L. multiflorum*). Total short-chain fatty acid concentration, pH, and acetate molar proportion did not differ between brassicas and annual ryegrass, but forage rape and oilseed rape had lower propionate proportions, while forage rape had higher butyrate proportions. Brassicas produced less methane than annual ryegrass, even when expressed per gram of organic matter, neutral detergent fiber, digestible organic matter, or digestible neutral detergent fiber. Sun et al. [25] freeze-dried and ground kale, turnip, forage rape, swede (*B. napus* ssp. *rapifera*), and perennial ryegrass as substrates for in vitro batch culture, finding brassicas had lower acetate-to-propionate ratios but no significant difference in methane production compared to ryegrass, without substantial hydrogen accumulation that would indicate methanogen inhibition. Inconsistent results across in vitro studies, even between samples of the same forage within a single experiment, preclude definitive conclusions.

Animal trials have been more extensive. New Zealand researchers conducted a series of eight experiments [14]. Experiment 1 (winter) fed sheep four common brassicas—kale, turnip, forage rape, and swede—as sole diets for 7 weeks. Compared to perennial ryegrass, methane yield (g/kg DMI) decreased by 10%, 6%, 25%, and 23%, respectively. Apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber was higher for brassicas. When expressed per unit digestible dry matter, the four brassicas reduced emissions by 27%, 23%, 39%, and 43%. Subsequent experiments consistently demonstrated mitigation effects [8]. Experiment 2 (winter) fed forage rape as the sole diet for 7 weeks, reducing methane yield by 30%; extended feeding to 15 weeks still reduced yield by 22% [26]. This trial also measured nutrient digestibility, energy balance, nitrogen balance, rumen degradation kinetics, fermentation parameters, and digesta flow rate, revealing higher nutritional value for forage rape than ryegrass and potentially better animal performance alongside reduced emissions.

Experiment 3 (winter) compared housed and grazing sheep fed forage rape as the sole diet versus ryegrass. In housed sheep, methane yield decreased by 37% at week 7 and 31% at week 12; in grazing sheep, reductions were 32% and 34% at weeks 7 and 12, respectively [27]. Grazing sheep on forage rape had daily gains of 315–365 g, exceeding those on ryegrass (210–307 g), with higher dressing percentage (46.2% vs. 42.9%). Methane per kg liveweight gain was 41.9–48.2 g for forage rape versus 62.5–123.8 g for ryegrass [27].

Experiment 4 (summer) fed bulb turnip, leaf turnip, forage radish (*Raphanus sativus*), and forage rape as sole diets to housed sheep for 4 weeks. Compared to a ryegrass/white clover mixture (92% ryegrass, 6% white clover, 2% weeds by fresh weight), methane yield decreased by 20%, 33%, 22%, and 10%, respectively [28]. Experiment 5 (winter) fed forage rape as the sole diet to housed sheep, measuring methane from day 33 to 66 in seven periods, yielding only 11.5–14.2 g/kg DMI [29]. Experiment 6 (summer) fed fresh leaf turnip, first-cut forage rape, second-cut forage rape, and bulb turnip to housed sheep for 32 days. Compared to a ryegrass/white clover mixture (67%:33%), leaf turnip, first-cut rape, and second-cut rape reduced methane yield by 15%, 16%, and 13%, while bulb turnip increased emissions by 13% [25].

Experiment 7 (winter) fed housed sheep five mixed diets containing fresh forage rape and perennial ryegrass at ratios of 100%, 75%, 50%, and 25% rape DM. After 24 days, methane yield decreased linearly by 64%, 43%, 27%, and 9% compared to the pure ryegrass diet [30]. This trial also measured nutrient digestibility, energy and nitrogen balance, and rumen fermentation parameters, finding maximum nitrogen retention at 50% rape DM. Experiment 8 (winter) fed fresh forage rape as the sole diet to heifers for over 32 days, reducing methane yield by 43% compared to a mixed pasture (96% ryegrass, 3% white clover, 1% weeds) [31].

Williams et al. [32] conducted a summer trial feeding forage brassicas (*B. napus* cv. Winfred) to dairy cows. Lactating cows 5 months post-calving were fed either a control diet of fresh chicory, alfalfa haylage, and cracked corn (41% chicory, 34% alfalfa, 25% corn on DM basis) or a test diet where chicory was replaced isometrically with fresh forage brassica. During days 6–10 of a 10-day feeding period, brassica-fed cows produced 21% less methane per unit DMI and 27% less per unit milk yield than chicory-fed cows.

Thus, except in isolated cases, forage brassicas consistently reduce methane emissions across different seasons, feeding systems, and inclusion levels, with mitigation effects persisting through 15 weeks of continuous feeding.

3.1 Feed Chemical Composition

Correlation analyses across multiple trials [8,33–37] examined relationships between methane yield and chemical components including ash, crude protein, ether extract, soluble carbohydrates, pectin, starch, acid detergent fiber, neutral detergent fiber, lignin, ratios of fermentable to structural carbohydrates, nitrates, and sulfates. Only crude protein (n=287, r=-0.584, P<0.001) and nitrates (n=210, r=-0.530, P<0.001) showed moderate negative correlations with methane yield. Forage brassicas contain 7.1-750 mmol nitrate/kg DM (or 0.1-10.5 g/kg DM) [15], a wide range. Theoretically, 1 mol nitrate can reduce 1 mol methane [38]; in Sun et al.' s trial [14], nitrates could maximally reduce methane by only 0.1-0.6 g/kg DMI compared to perennial ryegrass. In some cases, brassica nitrate content was far lower than ryegrass or below detection limits [14]. Therefore, while nitrates may contribute partially to reduced emissions, they are not the fundamental cause. Crude protein and nitrates are highly correlated (n=50, r=0.584, P<0.001), which may explain the negative correlation with methane yield. Multiple regression analysis of methane yield against chemical components yielded an adjusted R^2 of 0.450 (DF=134, P<0.001), with acid detergent fiber as a positive predictor and water-soluble carbohydrates as negative predictors. Thus, chemical composition only partially explains variation in methane yield from brassicas, with structural and water-soluble carbohydrates playing some role.

3.2 Rumen Fermentation Parameters

Correlation analysis of 218 sheep fed forage rape across eight experiments [8,33-37] revealed moderate correlations between methane yield and pre-feeding acetate-to-propionate ratio (r=0.393), (acetate+butyrate)-to-propionate ratio (r=0.411), acetate (r=0.420), propionate (r=0.350), and valerate (r=0.473) concentrations, as well as post-feeding butyrate (r=0.295) and propionate (r=0.322) concentrations. Multiple regression showed that in pre-feeding samples, only volatile fatty acid concentrations, acetate/propionate, and (acetate+butyrate)/propionate were significant, with an adjusted R^2 of 0.417 (DF=202, P<0.001). In post-feeding samples, the adjusted R^2 was 0.418 (DF=133, P<0.001), with only acetate concentration significant (P=0.042). These results indicate that rumen fermentation type only partially explains methane emissions from brassicas. Ammonia was unrelated to methane emissions, confirming it does not participate in methane metabolism.

When sheep were fed forage rape, rumen pH was lower than with perennial ryegrass at all sampling times over 24 hours, averaging 6.02 versus 6.71 for ryegrass [26]. While rumen pH is thought to affect methane emissions, dietary buffer supplementation trials failed to confirm that low pH explains brassicas' methane reduction [29].

3.3 Effects of Forage Brassicas on Rumen Microorganisms

Sheep fed forage rape showed markedly different relative abundances of bacteria, methanogens, and protozoa compared to those fed perennial ryegrass [26]. Hydrogen-producing bacteria such as *Ruminococcus*, unclassified *Ruminococcaceae*, and *Clostridiales* were less abundant, indicating reduced hydrogen production. Less hydrogen, combined with higher propionate, may reduce methane formation [10].

Methylotrophic methanogens were more abundant in rape-fed sheep [26]. These methanogens utilize hydrogen and methanol to produce methane. Forage rape contains substantial pectin [14,26]; methanol generated from pectin methyl groups can serve as a substrate for methylotrophic methanogens [33], which should increase rather than decrease methane emissions. Thus, pectin and methylotrophic methanogens cannot explain reduced emissions. Protozoal populations changed little, with only minor changes in *Eudiplodinium* [26], suggesting protozoa are not responsible.

3.4 Methanogen Inhibitory Substances

Although in vitro methane production results were inconsistent [23-25], the absence of substantial hydrogen release [25] indicates methanogens were not inhibited, suggesting brassicas lack direct methanogen inhibitors or contain them at negligible levels. No difference in methane production between high- and low-GSL varieties [23] further indicates GSL does not act as a methanogen inhibitor.

3.5.1 GSL and SMCO

GSL and SMCO are two classes of plant secondary metabolites widespread in brassicas that act as antinutritional factors [15]. Forage brassicas contain both GSL and SMCO, whereas perennial ryegrass contains virtually none.

GSL are sulfur-containing, anionic, hydrophilic plant secondary metabolites with a core structure comprising -D-glucose linked to a sulfonated aldoxime group and an amino acid-derived side chain [34]. Sun et al. [14] identified 18 GSLs across four brassica species—kale, turnip, forage rape, and swede—each containing 9-16 GSLs, with 3-4 GSLs accounting for over 80% of total content. Each species had distinct predominant GSLs, though 4-pentenyl glucosinolate was generally abundant, exceeding 40% of total content in forage rape, swede, and turnip, while sinigrin exceeded 30% in kale. GSLs are physiologically inactive until hydrolyzed by -thioglucosidase (myrosinase) into bioactive compounds. GSLs and myrosinase exist in different cells or cellular compartments; mechanical damage brings them into contact, generating isothiocyanates (ITC), thiocyanates, nitriles, epithionitriles, and oxazolidine-2-thiones, with ITC being the primary product [35].

SMCO is a non-protein amino acid present at 1%-2% DM in brassicas [39]. When plant tissues are damaged, vacuolar cysteine sulfoxide lyase is released, degrading SMCO into ammonia, pyruvate, and methanesulfinic acid. In the rumen, SMCO is completely converted to dimethyl sulfoxide [15], which binds to protein thiol groups, inactivating proteins, reducing hemoglobin content, and potentially causing anemia while affecting other protein synthesis. SMCO can increase plasma growth hormone and thyroxine levels, stimulating protein synthesis to replace inactivated proteins. Its effect on methane emissions has not been reported.

3.5.3 Possible Roles of GSL and SMCO in Methane Mitigation

The in vitro studies discussed above showed no methane reduction from brassicas, indicating that normal dietary concentrations of GSL and SMCO do not directly inhibit rumen methanogenesis.

Free triiodothyronine (FT3) is the active form of thyroid hormone T3. Feeding forage brassicas increases serum FT3 concentration [40]. Injecting 300 µg FT3 every 2 days into castrated male sheep increased blood FT3 levels and reduced mean retention time (MRT) of digesta [41]. Pinares-Patino et al. [42] found that sheep with shorter rumen retention times (particularly solid-phase) produced less methane when fed alfalfa. Goopy et al. [43] selected sheep for high and low methane yields, finding low-methane sheep had smaller rumen volumes and shorter liquid- and solid-phase retention times. Artificially elevating FT3 via injection shortened retention time and reduced methane yield by 8% [41]. Similarly, increasing ambient temperature raised blood FT3 and reduced retention time and methane yield [44].

Therefore, we hypothesize that under normal feeding conditions, GSL and/or SMCO and their metabolites do not directly inhibit methanogens at their natural concentrations. Instead, they increase FT3 concentration, which reduces digesta retention time and consequently lowers methane emissions.

4 Summary

Forage brassicas substantially reduce methane emissions from ruminants. Except in isolated cases, mitigation effects persist across seasons, feeding systems, and inclusion levels, with effects maintained during continuous feeding for up to 15 weeks. Current research has excluded several potential mechanisms, but the precise mode of action remains unclear. Routine chemical components cannot fully explain reduced emissions, and direct methanogen inhibitors are absent. Nitrates may partially contribute, but are not the primary cause. Altered rumen microbial communities and fermentation patterns may only partially explain the effect. Rumen pH decreases but not enough to inhibit methanogens. The role of secondary metabolites is unclear, but literature suggests GSL and SMCO metabolites may be involved. These compounds may stimulate FT3 secretion, reducing digesta retention time and methane emissions. This hypothesis requires experimental validation.

Feeding forage brassicas is a simple, practical, low-cost, and pollution-free mitigation strategy. China has numerous brassica forages, including forage rape, swede, cabbage, and Chinese cabbage, with forage rape currently being actively promoted. Utilizing forage brassicas represents an important pathway for methane mitigation in Chinese ruminant production. If the role of GSL and SMCO metabolites is confirmed, brassica by-products such as rapeseed meal could also be developed as methane mitigation agents.

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