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Review

Garlic and its Bioactive Compounds: Implications for Methane Emissions and Ruminant Nutrition

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Simple Summary: Methane (CH_4) produced by ruminants contributes as a source of anthropogenic greenhouse gases (GHG). Plant-derived bioactive compounds have been investigated for their potential to reduce CH_4 emissions from ruminant livestock. Garlic contains bioactive organosulphur compounds, which have been reported to be effective in reducing CH_4 emissions, but they have demonstrated inconsistent effects in reducing CH_4 production in the rumen. This might be because different types of garlic-based supplements vary in their concentrations of bioactive compounds. Therefore, further investigation is needed, such as the mode of action and persistence of the bioactive compound, to determine whether these compounds can be used successfully to inhibit rumen methanogenesis. The present review discusses garlic and its potential contribution to reducing CH_4 production by ruminant animals and discusses how differences in the diet and the bioactive compound concentration in garlic might contribute to these differences.

Abstract: Methane (CH_4) emission from enteric fermentation of ruminant livestock is a source of greenhouse gases (GHG) and has become a significant concern for global warming. Methane emission is also associated with poor feed efficiency. Therefore, research has focused on identifying dietary mitigation strategies to decrease CH_4 emissions from ruminants. In recent years, plant-derived bioactive compounds have been investigated for their potential to reduce CH_4 emissions from ruminant livestock. The organosulphur content of garlic has been observed to decrease CH_4 emission and increase propionate concentration in anaerobic fermentations (*in vitro*) and in the rumen (*in vivo*). However, the mode of action of CH_4 reduction is not completely clear and the response *in vivo* is inconsistent. It might be affected by variation in the concentration and effect of individual substances in garlic. The composition of the diet that is being fed to the animal may also contribute to these differences. This review provides a summary of the effect of garlic and its bioactive compounds on CH_4 emissions by ruminants. Additionally, this review aims to provide an insight into garlic and its bioactive compounds in terms of efficacy, safety, consistency and possible mode of action, deriving data from both *in vivo* and *in vitro* studies.

Keywords: garlic, greenhouse gas, ruminant, organosulphur, plant-derived bioactive compounds

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1. Greenhouse Gas Emissions from Ruminants

1.1. Greenhouse Gas Emissions from Ruminants and the Contribution of Methane

Ruminants play essential roles in sustainable agriculture, among which is the conversion of renewable resources (grassland, natural pasture, crop residues or other co-products) into edible food for humans [1]. Worldwide demand for meat and milk is projected to grow by 73% and 58%, respectively, in 2050 compared to 2010, due to continued world population expansion, the emergence of the middle class, increasing incomes and urbanisation with more emphasis on the developing countries [1-3]. Ruminant production needs to provide high-quality food to meet the increasing demands of a growing global population, which can adapt to climate changes and, at the same time, decrease the negative impact on the environment, such as methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) emissions and avoid changes in land use such as forest conversion to pasture.

The livestock sector plays a vital role in climate change, with greenhouse gas (GHG) emissions along livestock supply chains producing 7 gigatonnes CO_2 equivalents per annum, equalling 14,5% of all human-induced emissions [1,4]. Ruminant production systems are a source of greenhouse gases from various activities in the supply chain (**Figure 1**). Microbial fermentation of feed in the gastrointestinal tract, known as enteric fermentation, is the primary source of CH_4 emissions from ruminants. Enteric fermentation is the main agricultural source of CH_4, comprising 39% from dairy, 38% from beef and 23% from sheep, with emissions from slurry stores and livestock manure handling and spreading accounting for most of the remaining 15%. It is the third largest contributor of GHG after energy and industry [1]. In addition, enteric fermentation in ruminants is the largest source of anthropogenic CH_4 emissions contributing between 20 and 25% [5]. Methane emissions from ruminants, in particular, have been a global discussion topic as the global warming potential of CH_4 is 28 times greater than CO_2 [6-8]. Ruminants also produce large amounts of CO_2, with 4:1 CH_4 to CO_2 ratio, contributing to ruminants' total contribution of 8% to anthropogenic GHG emissions [9].

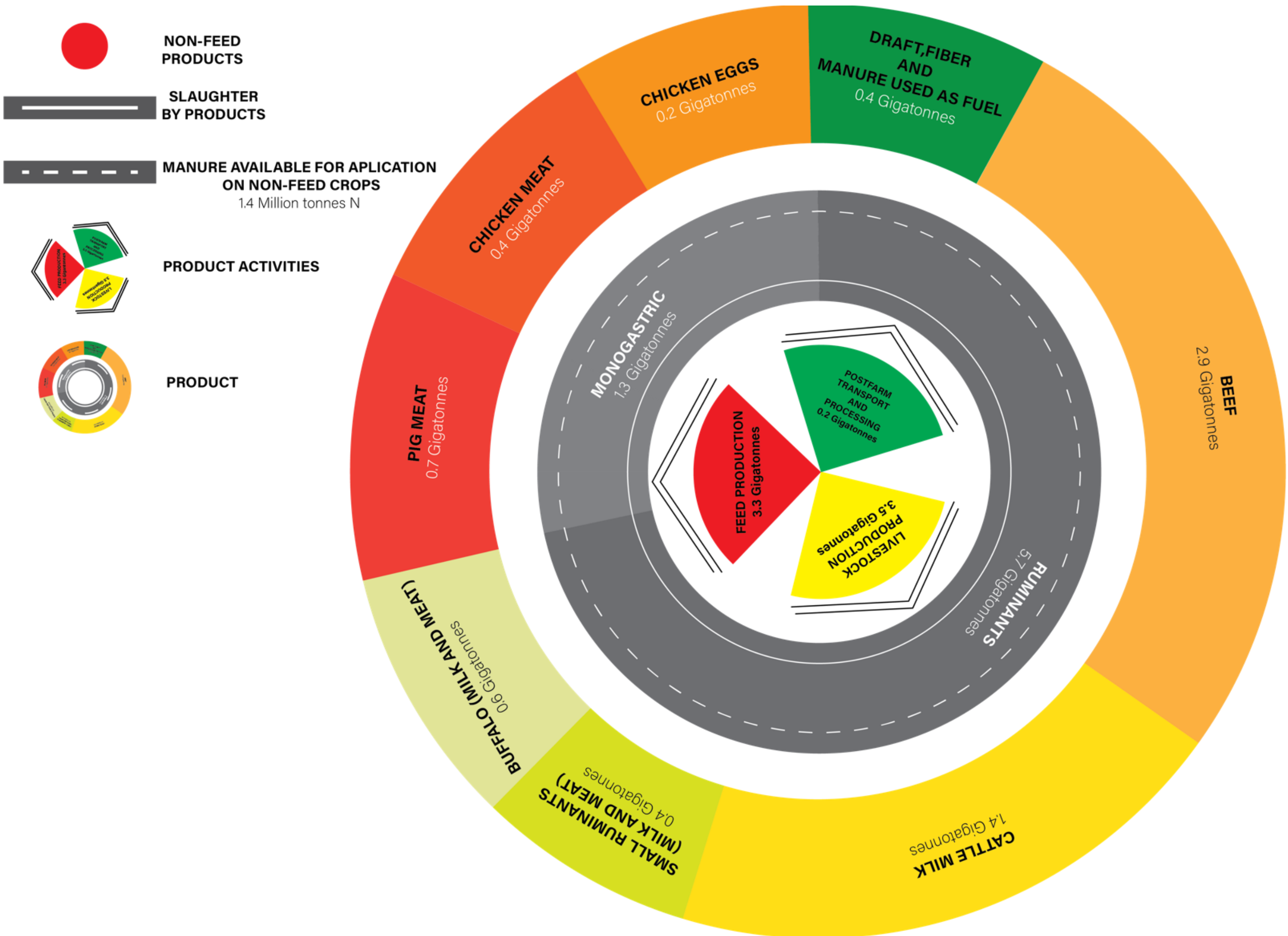

Figure 1. Global livestock emissions from supply chains, production activities and products (adapted from **[1]**). This figure is excluded from the CC BY license under which this article is published.

1.2. Global Targets for the Mitigation of CH_4 Emissions

Greenhouse gas emissions must be decreased by 80-90% compared with the emissions in 1990 in developed countries by 2050, according to the European Council Directorate-General for Climate Action European Council Directorate-General for Climate Action [10]. However, agricultural CH_4 emissions are projected to increase by about 30% by 2050 compared to 2010 under FAOSTAT policies, with a range of 20 to 50% in the integrated assessment model (IAMs) [11,12]. At the same time, the planet will need 70% more food by 2050, and it is predicted that this dramatic increase in production will also cause a 30-40% rise in agricultural emissions due to growth of the human population and rise in income driving an increased demand for animal protein [13-15]. Therefore, food production systems are under pressure to meet these food demands and climate-smart, sustainable, and environmentally friendly production practices are essential. The various sectors are also challenged with developing more resilient food supply chains under changing climatic conditions while providing safe, affordable, and nutritious foods. Therefore, innovative solutions in climate action and the implementation of appropriate enteric CH_4 mitigation strategies are required for sustainable food production from ruminants [16].

Global agricultural CH_4 emissions need to decrease by 24–47% (interquartile range), and CO_2 emissions need to reach net-zero by mid-century if warming is to be limited to 1.5°C [13]. More than 100 countries have recently set targets within the agriculture sector as part of national climate mitigation strategies and commitments. However, only a few (including industrialised countries) have specific targets or are currently designing policies to promote absolute reductions in the agricultural CH_4 emissions in all sectors [17]. Consequently, policy efforts will need to intensify for the agriculture sector to contribute effectively to limiting the global temperature increase to 1.5°C, the ambitious end of the Paris Agreement temperature goals, [18].

A further challenge in mitigating GHG from the agriculture sector is the rising demand for milk and meat [2,19,20]. While a number of the technical solutions are available (such as feed quality, animal health, animal production and herd management), adoption of these interventions might be hindered by the high-cost of investing to infrastructure and strategies of precision nutrition [1,15,16]. This latter point is critical because there are limited incentives for adopting GHG mitigation technologies under the current emission trading schemes in developed countries; therefore, supportive policies from multi-stakeholders such as adequate institutional and pro-active governance are needed to fulfil the sector's mitigation potential [1,16,19]. This means decreases in GHG emissions need to be viewed holistically, and emissions trade-offs across every stage of different supply chains should be considered for policy-making around GHG mitigation [1]. In the long-term, any remaining anthropogenic CH_4 emissions, e.g., linked to food production, must be offset through negative emission options such as using dietary supplements to reduce GHG emissions from ruminants, improved pastures and management systems [21].

1.3. The Role of Ruminants' Diet in Mitigation of CH_4 Emissions

Dietary manipulation is an attractive and effective way to mitigate CH_4 emissions due to the direct effect of diet on rumen fermentation patterns that could lead to decreased CH_4 production [22-24]. *In vitro* and *in vivo* studies [26-28] have demonstrated that rumen fermentation measures, such as volatile fatty acids (VFA) concentration, gas/CH_4 production, dry matter digestibility (DMD) relates to the rumen microbial population, which in turn depends on the ruminant diet.

A large number of studies have focused on dietary strategies to mitigate CH_4 emissions from ruminants [15,25,26]. Dietary supplements are used in livestock production to enhance feed-use efficiency, ruminant product quality and performance and health of the animal [27]. Recent advances in understanding methanogenesis have promoted and explored feed additives that can decrease CH_4 emissions to varying degrees, including using dietary lipids, medium-chain fatty acids, polyunsaturated fatty acids, probiotics, plant-derived bioactive compounds, and essential oils [28-32]. Ionophores such as monensin have also been reported to inhibit rumen methanogenesis [45,46]. However, since the European Union (EU) banned antibiotics as feed additives in 2006 due to concerns of antimicrobial resistance in food supply chains [33], interest in using plant-based feed additives (essential oils, plant extracts, and plant-derived bioactive compounds) has increased [34].

Feed manipulation is an attractive and effective way to mitigate ruminant-derived CH_4 emissions, due to the direct influence of feed on rumen fermentation patterns which can lead to decreased CH_4 production. Garlic contains a number of active metabolites that could impact on rumen fermentation, decreasing CH_4 emissions by rumen microbes and increasing propionate production within the rumen [35-37]. A detailed review of the literature around the potential use of garlic to decrease CH_4 emissions is presented in Section 3 of this review.

2. An Introduction to Rumen CH_4 Synthesis

2.1. The Rumen Microbiome and Metabolic Pathways of CH_4 Synthesis in the Rumen

Ruminants have a unique digestive system, comprised of four chambers: the reticulum, rumen, omasum, and abomasum [38,39]. The most significant among four chambers (approx. 80% of the total volume) is the rumen, which contains a diverse and dynamic population of microorganisms that allow ruminants to break down plant material containing cellulose and hemicellulose via anaerobic fermentation [38,40]. Bacteria and protozoa account for the most significant fraction of microbial biomass (50%-70%), followed by fungi (8-20%) [41,42]. These microorganisms harbouring in the rumen make up a complex microbial ecosystem, living in a symbiotic relationship with the ruminant hosts, which assists with the efficient conversion of plant biomass (rich in structural polysaccharides) into VFA which serve as an essential energy resource for the host [41,43]. For large herbivores such as dairy cow and beef cattle, this energy resource makes up 70% of the dietary energy [41].

According to Sirohi*, et al.* [44], rumen bacteria are the most diverse group accounting for 10^{10}-10^{11} cells/ml of rumen contents: archaea, mainly methanogens, account for 10^{7}-10^{9} cells/ml, fungi account for 10^{3}-10^{6} cells/ml, and protozoa account for 10^{4}-10^{6} cells/ml. Most of the bacteria in the rumen are strict anaerobes; they are actively involved in the breakdown of lignocellulosic feed ingredients through different enzymatic activities; which are also classiffied as fibrolytic, amylolytic, proteolytic, lipolytic, ureolytic and tanniolytic bacteria [45-48].

To date, very few methanogenic species have been isolated from the rumen; Holotrich ciliate protozoa are highly active in the rumen and produce H_2 that methanogens use to produce CH_4. The interactions between bacteria and protozoa are essential and could play a critical role in the CH_4 production pathways [42,49]. The removal of protozoa from the rumen is associated with decreased CH_4 emission [42,50].

In the symbiotic relationship between the ruminant and the rumen microbial ecosystem, ruminants maintain the rumen in an anaerobic state with a stable temperature of around 39°C, and a pH ideal for microbial growth [51-53]. Production of CH_4 in ruminants starts with different ruminal microorganisms, bacteria, protozoa, and fungi when they hydrolyse and ferment complex feed components such as proteins and polysaccharides into simple products, including amino acids, sugars and alcohols [54].

The products are further fermented to VFA, H_2 and CO_2 by both the primary fermenters and other microbes that cannot hydrolyse complex polymers by themselves [55]. It enables the high conversion efficiency of cellulose and hemicellulose, and CH_4 represents a by-product of this process produced by certain microbes (methanogens) [56]. It is estimated that a cow produces 250-500 g/d CH_4 [57]. The gaseous waste products of enteric fermentation, CO_2 and CH_4, are mainly removed from the rumen by eructation [52]. Methane synthesis in the reticulorumen is an evolutionary adaptation that enables the rumen ecosystem to dispose of excess H_2, which may otherwise accumulate and inhibit carbohydrate fermentation and fibre degradation [58]. Disposal of excess H_2 produced by direct inhibition of CH_4 production results in increased concentrations of other H_2 sinks such as propionate and butyrate [59]. Methanogens are at the bottom of this trophic chain and use the end products of fermentation as substrates (**Figure 2**).

Methanogens are anaerobic microorganisms that have three coenzymes that have not been observed in any other microorganisms, which allow them to produce CH_4 from methyl coenzyme M [60]. It has been estimated that there are between 360-1000 species, however until this point, only 6 genera have been identified and 8 species have been cultured [53,61]. The predominant genus in the rumen is *Methanobrevibacter* and from this genus the most predominant species are *ruminantium*, *smithii* and *mobile [60]*. Most methanogens grow at pH between 6 and 8, although some species can survive in a wider range from 3-9.2 [49,62].

Three types of methanogenic pathways are involved in CH_4 synthesis, namely hydrogenotrophic (reduction of CO_2 coupled to the oxidation of H_2), methylotrophic (conversion of methyl-group containing compounds) and acetoclastic [63]. The hydrogenotrophic pathway is generally recognized as the main pathway to remove H_2, through which methanogens can utilize H_2 as electron donor to reduce CO_2 to CH_4. Newly recognized methanogens use a range of methyl donor compounds and CO_2 for CH_4 production, suggesting that other pathways maybe identified [64]. The draft genome of *Candidatus Methanomethylophilus Mx1201*, a methanogen isolated from the human gut belonging to the rumen cluster C, more recently categorized into the order *Methanomassiliicoccales* [65], contains genes for methylotrophic methanogenesis from methanol and tri-, di- and monomethylamine [66]. In artificial systems, such as biogas production facilities, acetate is recognized as an important substrate for methanogens, which is referred to as acetoclastic methanogenesis [67]. A comprehensive understanding of the functionality of methanogens and their CH_4 producing pathways may provide insights into effective CH_4 abatement strategies.

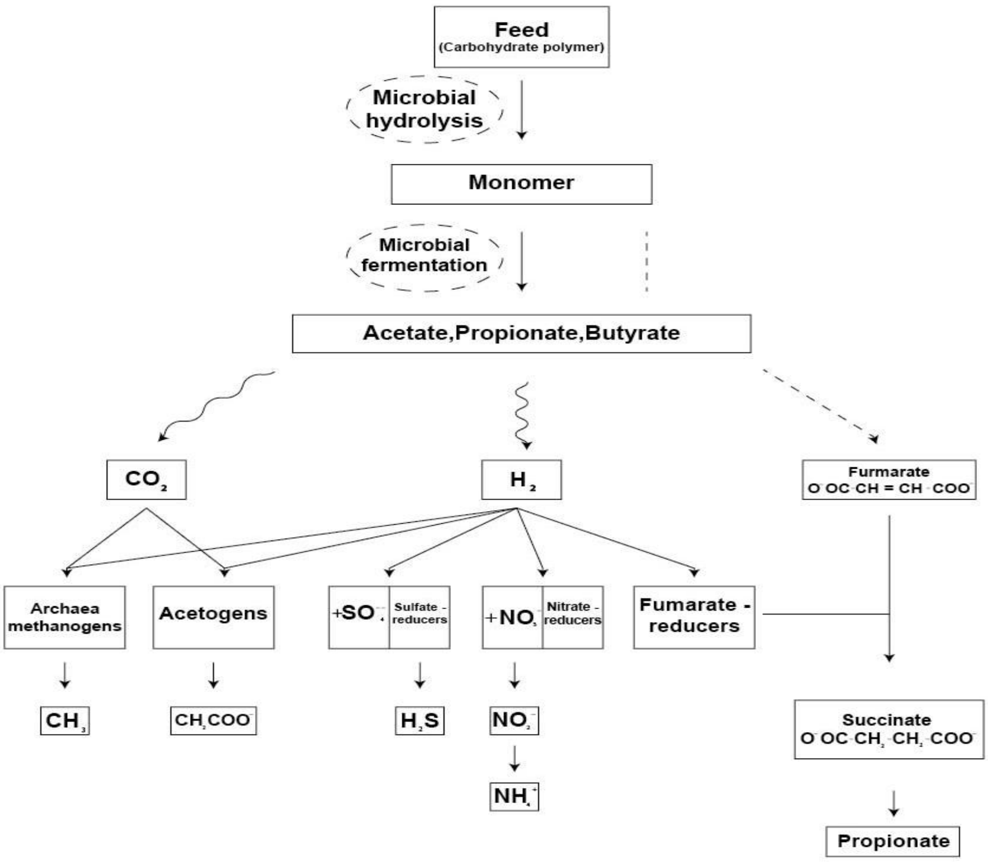

Figure 2. Biochemical pathways for CH_4 synthesis (adapted from [24]).

2.2. Targeted Manipulation of Ruminant Metabolic Pathways to Reduce CH_4 Synthesis

Methane production in the rumen can represent a loss of up to 12% digestible energy (Johnson and Johnson, 1995). Decreasing enteric CH_4 emissions by ruminant animals without compromising animal production is desirable as a strategy both to decrease global warming effects and to improve feed conversion efficiency [16,68]. The type of feed and the presence of electron acceptors other than CO_2 in the rumen will significantly influence the presence and activity of H_2 producers and users [54,57]. This is because pathways other than methanogenesis can also consume H_2 and thus potentially compete with and decrease methanogenesis in the rumen [54].

Dietary manipulation may rechannel the H_2 produced during normal ruminal fermentation from CH_4 production to propionate synthesis in the rumen [69,70]. However, the rumen ecosystem is very complex, and the ability of this system to efficiently convert complex carbohydrates to VFA is partly due to the effective removal of H_2 by reducing CO_2 to produce CH_4. Thus, inhibition of methanogenesis is often short-lived, as the system's ecology is such that it often returns to the initial level of CH_4 production through various adaptive mechanisms [58]. Issues surrounding chemical residues, toxicity, and high cost, can also limit the utilization of this strategy in animal production [71].

Another potential pathway is a targeted effect on certain microbial populations [31,72]. Plant-derived bioactive compounds are volatile components and aromatic lipophilic compounds which contain chemical constituents and functional groups such as terpenoids, phenolics and phenols, which have potent antimicrobial activities. [32,73-76]. Methanogenesis decreases with the application of plant-derived bioactive compounds, primarily by reducing protozoa. Methanogenesis decreases by disrupting cell membranes due to the lipophilic nature of plant-derived bioactive compounds, decreasing protozoa and methanogens [72,77]. Therefore, inclusion of plant-derived bioactive compounds in ruminant diets are a potential strategy to mitigate rumen CH_4 synthesis [78].

A targeted approach to reducing CH_4 emissions by dietary manipulation will therefore need to: i) have a long-term effect that overcomes adaptation to dietary changes, and ii) not have a detrimental effect on the digestion of other dietary nutrients, which may occur if the rumen microbiome is altered in any way.

3. Garlic and Ruminant CH_4 Emissions

3.1. The Need to Exploit Plant-derived bioactive compounds

In livestock production, the use of antibiotics as growth promotors in animal feed is highly objectionable due to their residual effects and the risk of antimicrobial resistance development [79][110]. Garlic (*Allium sativum*) has been applied pharmaceutically since ancient times in nearly every known civilization and has been widely used as a foodstuff in the world and is "generally recognized as safe" (GRAS) as a food flavouring agent by the U.S. FDA, making them ideal candidates to use as feed additives in livestock production [80]. However, plant-derived bioactive compounds also exhibit antimicrobial activity and therefore, can affect the rumen microbial ecosystem directly [34,81-83].

Antimicrobial properties of organosulphur compounds from garlic have shown a bactericidal effect [84-87] and hence garlic extract and some of their compounds have been extensively investigated as a potential way to modify the rumen microbiome. Garlic is a prevalent plant for bacteria agent to alter microbe ecosystem in cattle digestive tract. [88]. **Table 1** shows previously reported antimicrobial activities from garlic and its compounds (antifungal, antiprotozoal, antibacterial). The complex composition of garlic also involves a paradoxical outcome in the GIT microbiome [89], as the same time garlic is rich in indigestible polysaccharides, such as fructans, which act as a prebiotic for specific GIT microbiota [90].

In recent years, plant-derived bioactive compounds (e.g. organosulphur, saponins, and tannins) with diverse biological activities have been investigated for their potential as alternatives to growth-promoting antibiotics in ruminant production [73,91,92], and their potential mechanism of action as rumen modulators and CH_4 inhibitors [92,93]. To date, garlic supplementation in ruminant diets has shown a variable CH_4 reduction both *in vitro* and *in vivo* studies [88,94,95], these are summarised in **Table 2.**

3.2. Effect of Garlic on CH_4 Emissions: *In Vitro* Assessments

Based on batch culture and dual flow continuous culture studies, the supplementation of garlic oil (300 mg/L) and allicin, (a sulphur-containing bioactive compound in garlic; 300 mg/L) decreased CH_4 yield (mL/g dry matter (DM)) by 73.6% and 19.5%, respectively, compared with control basal diets consisting of 50:50 forage:concentrate ratio, over 24 h [35]. The inclusion of garlic extracts at 1% of total volume of rumen fluid containing 0.3 g of timothy grass decreased CH_4 yield (mL/g DM) by 20% compared to control for 24 h incubation [96]. Garlic powder supplementation at 16 mg/200 mg of substrate resulted in reducing CH_4 yield (mL/g DM) by 21% with basal diets comprising 60:40 forage:concentrate ratio over 72 h using swamp buffalo rumen fluid in batch cultures [29]. The supplementation of a combination of garlic oil at 0.25 g/L, nitrate at 5 mM, and saponin at 0,6 g/L reduced CH_4 yield (mL/g DM) by 65% at day 2 and by 40% at day 18 compared with control basal diet consisting of 50:50 forage:concentrate ratio in batch cultures [48].

The effects of a combination of garlic powder and bitter orange (*Citrus aurantium*) extract (Mootral) using a semi-continuous in vitro fermentation (RUSITEC) demonstrated that the treatment effectively decreased CH_4 yield by 96% (mL/g DM) by altering the archaeal community without exhibiting any negative effects on fermentation [97]. The study showed that a mixture of garlic and citrus extracts effectively decreased CH_4 production in all feeding regimens without adversely affecting nutrient digestibility. Furthermore, a mixture of garlic and citrus extracts supplementation improved rumen fermentation by increasing the production of total VFA.

The supplementation of bulb of garlic decreased CH_4 yield (mL/g DM) by 55% at 0.5 ml/30 ml in batch culture using rumen liquor of buffalo as inoculum without affecting the protozoa population [98]. The inclusion of garlic at the rate of 135 mg/g of substrate resulted in more than 20% inhibition in CH_4 yield (mL/g DM), with no effect on gas production and a slight increase (2%) in *in vitro* DM degradability [99]; although such inclusion rate it is rather unrealistic to be applied at commercial level. The effect of the inclusion of garlic oil on CH_4 and VFA production based on *in vitro* is also influenced by diet and dose-dependent factors [100].

Some studies on ruminants have shown that garlic extracts improved nutrient use efficiency by decreasing energy loss as CH_4 or ammonia nitrogen in continuous rumen culture [37,101,102]. Almost complete inhibition of methanogenesis have demonstrated using garlic oil distillate without affecting feed organic matter degradation in experiments using rumen simulation techniques (RUSITEC) [103]. These studies have consistently shown the reduction potential of CH_4 by garlic supplementation [48,104], while the effect on short-chain fatty acids (SCFA) is more variable. Previous studies also observed an increase in total SCFA concentrations with moderate garlic oil concentrations [35]. Besides, most studies reported an increase in the molar proportion of butyrate, often accompanied by a decrease in acetate proportion, whereas the effects on other SCFA and digestibility can vary [35,48,105].

Variations in the concentration and effect of individual substances in garlic extract and the type of diet can contribute to these differences [35,106]. Since different garlic varieties can vary substantially in different concentrations in compounds that affect CH_4 emissions, the potential effect of the efficacy of garlic feeding on reducing CH_4 emissions may also depend on the variety [29,107]. However, the role of garlic still remains unclear due to limited data on the mode of action; and further research could shed light into their properties as bioactives.

3.3. Effect of Garlic on CH_4 Emissions: *In Vivo* Assessments

Based on an *in vivo* study, the supplementation of a feed additive based on citrus and garlic extracts (Mootral), at 15 g/d in steers diets, decreased 23% in CH_4 yield after 12 weeks [108]. Steers (n=20) receiving the Mootral treatment had lower CH_4 production than the steers receiving the control treatment over time with no effect on DMI, average daily gain, and feed conversion efficiency. Dietary supplementation of allicin at 2 g/d for 42 d decreased CH_4 yield (mL/g DM) by 6% compared to a control diet in sheep [109]. The inclusion of garlic extract directly affects rumen archaea, which are the microorganisms primarily responsible for CH_4 synthesis in the rumen [35]. This hypothesis is supported by further *in vivo* research that reported the effect of garlic oil on the diversity of methanogenic archaea in the rumen of sheep [110]. The supplementation of garlic oil at different doses (20 g -35 g/kg DM/day) resulted in CH_4 reduction (mmol/L of VFA) at 21.96 [111]. A decrease in CH_4 production scaled to digested NDF intake when diallyl disulphide (DAD) was supplemented at 4 g/d in sheep [112]. The supplementation of 7% coconut oil and 100 g/d of garlic powder in buffalo improved the rumen ecology (by increasing amylolytic and proteolytic bacteria while protozoal population decreased by 68-75% and decreased the CH_4 yield (g/kg DMI) by 9% without changing nutrient digestibility [113]. Other studies demonstrated no long-lasting effects on CH_4 production when anti-methanogenic treatments (essential garlic oil and linseed oil at 3 μL/kg BW and 1.6 mL/kg BW, respectively) were given to neonatal lambs [114]. However, early-life intervention induced modifications in the composition of the rumen bacterial community of lambs that persisted after the intervention ceased with little or no effect on archaeal and protozoal communities [114].

Feeding garlic bulbs at the rate of 1% of DMI resulted in 11% inhibition in CH_4 yield (g/kg DMI) in sheep (fed a diet with 50:50 concentrate to roughage ratio) along with an increase in nutrient digestibility. Methane was decreased up to 31% when supplemented with garlic powder at the rate of 2% of DMI without affecting the digestibility of nutrients and milk composition compared to the control group in lactating murrah buffaloes [115]. The supplementation of freeze-dried garlic leaves (FDGL) at 2.5 g/kg DM/day of sheep diet resulted in a reduction of CH_4 yield (g/kg DMI) by 9.7% [116].

The use of antibiotics in livestock production as growth promoters in animal feed are highly objectionable because of their residual effects and the risk of developing antimicrobial resistance. However, garlic (*Allium sativum*) has been used medicinally since ancient times and has been widely used as a food ingredient in the world and known as "generally recognized as safe" (GRAS) as a food flavouring agent by FDA, the United States, making it an ideal candidate for use as a feed additive in livestock production. In addition, bioactive compounds derived from plants also have antimicrobial activity and, therefore, can affect the rumen microbial ecosystem. Although it might be argued that there is a risk of microbes developing resistance to garlic bioactive compounds after long exposure periods, something has not been investigated yet. The antimicrobial properties of organosulfur compounds from garlic have shown a bactericidal effect. Garlic extract and some of its compounds tested at high dose have been studied extensively as potential means to modify the rumen microbiome. Reports on the effect of garlic on CH_4 emissions both *in vitro* and *in vivo* are inconsistent between studies and applications in terms of efficient livestock production and limited ability to maintain its effects over longer periods of time. This may be due to the effect of garlic supplementation on rumen fermentation depending on the type and dosage of garlic components which vary in bioactive components, substrate composition and composition of microbial population in the inoculum.

Table 1. Antifungal, antiprotozoal, antibacterial, antiviral of garlic and its compounds

Form	Garlic bioactive compound (mode of action)	Antibacterial	Antiprotozoal	Antifungal	Reference
DAS[1]					
DAS[1] (purity, 97%)	Diallyl sulphide (binding to thiol-containing proteins/enzymes in bacterial cells)	*Cronobacter sakazakii*	ND[2]	ND[2]	[117]
Garlic extracts					
Garlic extracts	ND[2]	ND[2]	*Taenia taeniaeformis*, *Hymenolepis microstoma*, *H. diminuta*, *Echinostoma caproni*, and *Fasciola hepatica*	ND[2]	[118]
Garlic extracts	Thiosulfinates and Allicin (thiol enzyme inhibition and preventing the parasite's RNA, DNA and protein synthesis)	ND[2]	*Blastocystis* spp	ND[2]	[119]
Garlic extracts	DATS[3] (affecting the fungal cell wall and causing irreversible ultrastructural changes in the fungal cells, leading to loss of structural integrity)	ND[2]	ND[2]	*Trichophyton verrucosum*, *T.* mentagrophytes, *T. rubrum*, *Botrytis cinerea*, *Candida* species, *Epidermophyton floccosum*, *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifera*, *Microsporum gypseum*, *M. audouinii*, *Alternaria alternate*, *Neofabraea alba,* and *Penicillium expansum*	[120]
Garlic extracts	Allicin (oxidative interaction with important thiol-containing enzymes)	*Bacillus*, *Escherichia*, *Mycobacterium*, *Pseudomonas*, *Staphylococcus and Streptococcus*	ND[2]	*Aspergillus niger*, *Penicillium cyclopium and Fusarium oxysporum*	[121]

Garlic extracts	Allicin (reacts with cysteine-containing Burkholderia enzymes involved in key biosynthetic pathways)	*B. cenocepacia* C6433	ND[2]	ND[2]	[122]
Garlic extracts	Allicin (interferes with RNA production and lipid synthesis)	*Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*	ND[2]	*Candida albicans*	[123]
Garlic extracts	Allicin (interferes with RNA production and lipid synthesis)	*S. aureus*	ND[2]	ND[2]	[124]
Garlic extracts	Spasmolytic effect was most likely mediated through Ca^{2+}-channel inhibition	*Salmonella enteritidis, Escherichia coli, Proteus mirabilis* and *Enterococcus faecalis*	ND[2]	ND[2]	[125]
Garlic extracts	Allicin (reduced serum total oxidative status, malondialdehyde and nitric oxide production, and increased total thiols)	ND[2]	ND[2]	*Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa*	[126]
Garlic extracts	ND[2]	*Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria, Pseudomonas, Salmonella,* and *Staphy lococcus*	ND[2]	*Candida albicans*	[127]
Garlic oil					
Garlic oil	DAS[1] (the presence of the allyl group is fundamental for the antimicrobial activity of these sulphide derivatives when they are present in *Allium*)	*Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*	ND[2]	ND[2]	[128]
Garlic oil	Ajoene (inhibiting the human glutathione reductase and *T. cruzi* trypanothione reductase)	ND[2]	*Cochlospermum planchonii, Plasmodium, Giardia, Leishmania,* and *Trypanosoma.*	ND[2]	[129]
Garlic oil	DAS[1] (the richness in sulphur atoms may have contributed to the effectiveness of the EO activity)	*Staphylococus aureus,Salmonella Typhimurium, Listeria monocytogenes,*	ND[2]	ND[2]	[130]

Garlic oil	Allicin (inactivation of allicin by cysteine groups of mucin or other gastrointestinal bacteria)	*Escherichia coli, Campylobacter jejuni* *Campylobacter jejuni*	ND[2]	ND[2]	[131]

DAS[1] : Diallyl sulphide ; ND[2]: Not Determined; DATS[3]: Diallyl Trisulphide

Table 2. Effect of garlic on CH_4 emissions based on *in vitro* and *in vivo*

Type of Study	Garlic form supplementation	Level of supply	Basal diet	CH_4 yield	Reference
In Vitro					
Batch culture					
Batch culture (sheep rumen fluid)	Garlic and citrus extracts	0%, 10% and 20% of DMI	Concentrate and grass at 50 : 50 ratio	↓11% (from 11.12 mL/g DM to 9.89 mL/g DM)	[132]
Batch culture (sheep rumen fluid)	Bulb of garlic	70 mg	450 mg DM[5] substrate (a mixture of lucerne hay (500 g/kg), grass hay (200 g/kg) and barley (300 g/kg))	↓9.8% (from 1.32 mmol/g DM to 1. mmol/g DM)	[99]
Batch culture (sheep rumen fluid)	ALL[7] and ; DAD[12]	0.5, 5 and 10 mg/l	1:1 alfalfa hay:concentrate either (HF[10] inoculum; 700:300 alfalfa hay:concentrate; 4 sheep) or HC[11] inoculum, 300:700 alfalfa hay:concentrate; 4 sheep)	ND[6]	[37]
Batch culture (sheep rumen fluid)	Garlic oil	0, 20, 60, 180 or 540 mg/L	300 mg MC[13] (500:500 alfalfa hay:concentrate) and the other 4 were fed HC[11] (150:850 barley straw:concentrate)	↓12.1% (from 0.262 mmol/L of VFA 0.257 mmol/L of VFA)	[100]
Batch culture (cow rumen fluid)	Garlic extracts	1% of total volume	0.3 g of timothy	↓ 20% (from 40.2 mL/g DM to 32.5 mL/g DM)	[96]
Batch culture (buffalo rumen fluid)	Coconut oil and garlic powder	, 16:0, 8:4, 4:8 and 0: mg	200 mg DM[5] (60:40 roughage (R) and concentrate (C) ratio were used as substrates)	ND[6]	[29]

Batch culture (sheep rumen fluid)	Garlic oil and cinnamaldehyde	0, 20, 60, 180 and 540 mg/L	Forages and concentrates 50: 50 alfalfa hay: concentrate diet (MC[13]) and 15: 85 barley straw: concentrate diet (HC[11]).	ND[6]	[106]
Batch culture and dual flow continuous culture (cow rumen fluid)	Garlic oil	3, 30, 300, and 3000 mg/L	50:50 forage:concentrate diet	↓73.6% (from 0.20 mmol/L of VFA to 0.07 mmol/L of VFA)	[35]
Batch culture (cow rumen fluid)	Combination of garlic oil, nitrate, and saponin	Garlic oil (0.25g/L), nitrate (5mM), and quillaja saponin (0.6g/L)	400mg of ground feed substrate. The feed substrate is a mixture of alfalfa hay and a dairy concentrate feed at a 50:50 ratio	↓65% at day 2 (from 29.1 mL/g DM 10.3 mL/g DM) and by 40% at day 1 (from 21.4 mL/g DM to 13 mL/g DM)	[48]
Batch culture (cow rumen fluid) CCF[14]	Garlic powder	2 – 6 % of DMI[2]	Concentrate and wheat straw at a 50: 50 ratios	ND[6]	[115]
CCF[14] (goat rumen fluid) Rusitec[15]	PTS[16]	200 µL/L/day	Alfalfa hay and concentrate in a 50:50 ratio.	↓48% (from 249 mmol/L of VFA to 129 mmol/L of VFA)	[133]
Rusitec[15] (cow rumen fluid)	Mootral (garlic and citrus extract)	1 – 2 g	7 g hay and 3 g concentrate	↓ 96% (from 10.70 mL/g DM to 0.40 mL/g DM)	[97]
Rusitec[15] (cow rumen fluid)	Garlic oil	300 mg/l	A basal diet (15 g DM[5]/d) consisting of ryegrass hay, barley and soyabean meal (1:0·7:0·3)	↓ 91% (from 7.96 mL/g DM to 0.73 mL/g DM)	[103]
In Vivo Buffalo					
Buffalo	Coconut oil and garlic powder	7% coconut oil plus 100 g/d of garlic powder	Rice straw ad libitum, concentrate 0.5 % BW[1]	↓ 9% (from 27.5 mmol/L of VFA to 25 mmol/L of VFA)	[113]

Buffalo	Garlic powder	2% of DMI[2]	Concentrate and roughage diet which comprised of concentrate mixture, berseem, and wheat straw. Wheat straw and concentrate mixture at a ratio of 60: 40	↓33% (from 40.70 g/kg DMI to 27 g/kg DMI)	[115]
Buffalo	A mixture of (garlic and soapnut in 2 : 1 ratio	2 % of DMI[2]	50% wheat straw and 50% concentrate	↓12.6% (from 36.30 g/kg DMI to 31.72 g/kg DMI)	[134]
Buffalo	Mixture of garlic bulb and peppermint oil	2.5% of DMI[2]		↓7.4% (from 29.17 g/kg DMI to 27.01 g/kg DMI)	[135]
Cattle					
Cattle	Mootral (garlic and citrus extract)	15 g/d	TMR[3] at a ratio of 47% forage and 53% concentrate	↓23.2% (from 19.4 g/kg DMI to 14.9 g/kg DMI)	[108]
Cattle	Garlic powder	40 g/d	Concentrate at 5 g/kg BW[1] with UTRS[4] fed ad libitum	↓5% (from 29.3 mmol/L of VFA to 27.9 mmol/L of VFA)	[88]
		200 g/d			[136]
Cattle	A mixture of mangosteen peel, garlic, and urea pellet	200 g/d	Rice straw ad libitum and concentrate was fed at 0.5% of BW[1]		[136]
Cattle			Concentrate at 0.5% of BW[1] while rice straw was fed ad libitum.	↓6.5% (from 27.6 mmol/L of VFA to 25.8 mmol/L of VFA)	[137]
Goat					
Goat	Garlic oil	20 – 35 g	600 g/kg DM[5] of concentrate and 400 g/kg DM[5] of cowpea/maize silage in a ratio of 1:3	ND[6]	[111]
Sheep					

Sheep	ALL[7]	2 g/head day	TMR[3]	↓7.7% (from 66.1 g/kg DMI to 61 g/kg DMI)	[109]
Sheep	FDGL[8]	2.5 g/ (kg BW[1]0.75·d)	Mixed hay plus concentrate at 60:40 ratio	↓ 10% (from 28.05 g/kg DMI to 25.34 g/kg DMI)	[116]
Sheep	Garlic powder	0.5% concentrate (DM[5])	Concentrate to rice straw at ratio of 30:70	↓ 6.6% (from 42.3 g/kg DMI to 39.5 g/kg DMI)	[138]
Sheep	Combined garlic essential oil and linseed oil	Linseed oil (1.6 mL/kg BW[1]) and garlic essential oil (3 μL/kg BW[1]	Free access to a natural grassland hay 921.1 g DM[5]/kg and concentrate 889.0 g DM[5]/kg	↓19.6% (from 19.68 g/kg DMI to 15.81 g/kg DMI)	[114]

BW[1]: Body weight; DMI[2]: Dry matter intake; TMR[3]: total mix ratio; UTRS[4]: Urea Treated Rice Straw; DM[5]: Dry Matter; ND[6]: Not determined; ALL[7]: Allicin; FDGL[8]: Freeze Dried Garlic Leaves; DMD[9]: Dry Matter Digestibility; HF[10]: High Forage; HC[11]: High Concentrate; DAD[12]: Diallyl Disulphide; MC[13]: Medium Concentrate; CCF[14]: Continuous-Culture Fermenters; Rusitec[15]: Rumen simulation technique; PTS[16]: Propyl Propane Thiosulfinate

4. Bioactive Compounds in Garlic that Decrease CH_4 Emissions and the Potential Effect on Biochemical Pathways

Garlic contains the organosulphur compounds allicin ($C_6H_{10}S_2O$), alliin ($C_6H_{11}NO_3S$), diallyl sulphide ($C_6H_{10}S$), diallyl disulphide ($C_6H_{10}S_2$), and allyl mercaptan (C_3H_6S) [139-142] (**Figure 3**). These compounds are widely known for their unique therapeutic properties and health benefits as they act as antioxidants to scavenge free radicals [143]. Garlic-derived organosulphur compounds demonstrate different biochemical pathways that may provoke multiple inhibitions [144]. One potential pathway for the direct inhibition of the methanogenesis by garlic is via the inhibition of CH_4 producing microorganisms such as archaea [144]. Archaea possess unique glycerol-containing membrane lipids linked to long-chain isoprenoid alcohols, which are essential for cell membrane stability. The synthesis of isoprenoid units in methanogenic archaea is catalyzed by the enzyme hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase. Garlic oil is a potent inhibitor of HMG-CoA reductase Gebhardt and Beck [144], as a result, the synthesis of isoprenoid units is inhibited, the membrane becomes unstable, and cells die. The effect of garlic bioactive compounds in ruminants have been reported in **Table 3**.

Diallyl sulphide (DAS) has shown small effects on rumen microbial fermentation [35]. It has been suggested in various studies that the antimicrobial potency of allyl sulphides in garlic oil increases with each additional S atom [145,146]. This could explain why supplementation of DAD (which contains 2 S atoms) resulted in more potent effects compared with diallyl sulphide (DAS) (containing 1 S atom). Supplementation of DAD at 80 μL/L/day and propyl propane thiosulphinate (PTS) at 200 μL/L/day strongly inhibited CH_4 yield (g/kg DMI) by 62% and 96%, respectively) in batch cultures after 24 h incubation of the ruminal fluid of goats [133].

Supplementation of allicin at 2 g/head/day effectively enhanced OM, N, NDF, and ADF digestibility and decreased daily CH_4 yield (g/kg DMI) in ewes, probably by decreasing the population of ruminal protozoa and methanogens [109]. Supplementary allicin can also decrease the ruminal concentration of ammonia by 14% but can increase the total VFA produced by up to 14.3% [101,109,112]. Significant increases in the populations of *F. succinogenes, R. flavefaciens, and B. fibrisolven*s in ewes supplemented with allicin have also been observed [136]. It is well established that CH_4 production has been positively correlated with more acetate production and negatively correlated with increased propionate production [147] because propionate synthesis is a main pathway for H_2 consumption, representing a competitive and alternative pathway to methanogenesis [71,148]. Allicin has been found to alter rumen VFA production so that less acetate and more propionate and butyrate is produced, and this may be due to an abundance of the *Prevotellaceae* and *Veillonellaceae* families [113]. *Prevotellaceae* is one of the predominant families in rumen fluid, and it is well known to produce propionate by utilizing H_2 produced during carbohydrate fermentation [149].

Dietary garlic constituents are transformed into various metabolites in a biological system.Busquet, Calsamiglia, Ferret, Carro and Kamel [35] observed that allyl mercaptan is a common metabolite of allium-derived compounds as obtained after incubation of allicin and other allyl sulphides in fresh blood at 37°C or gastric fluids [139]. Diallyl disulphide and allyl mercaptan resulted in a less potent effect than garlic oil in increasing *in vitro* rumen fermentation and decreasing CH_4 production, suggesting a possible synergistic effect between the different compounds present in the garlic oil [35]. In the specific case of garlic oil, the CH_4 mitigating effect may be directly attributed to the toxicity of organosulphur compounds, such as diallyl sulphide and allicin, to the methanogens [150].

Garlic extracts have demonstrated effectively decreased CH_4 production and improved rumen fermentation by increasing the production of total VFA at 200 g/kg of the feed [132]. Supplementation with garlic extracts has been associated with a lower abundance of the family *Methanobacteriaceae*, the major CH_4 producer in the rumen [97] [83]. This was connected to the toxicity of organosulphur compounds of garlic, such as diallyl sulphide and allicin, in inhibiting certain sulphydryl-containing enzymes essential for the metabolic activities of methanogenic archaea [48]. This interaction has been demonstrated by the loss of activity of some thiol-containing enzymes (eg papain and alcohol dehydrogenases) and by the reaction between different organosulphur compounds and cysteine to form other substances by a thiol-disulphide exchange reaction [145].

The constituents of dietary garlic are converted into various metabolites in biological systems, which can cause synergistic effects between different compounds in garlic. It can therefore cause different forms of garlic to have different bioactive components. This compound can potentially impact CH_4 reduction, which is directly related to the toxicity of organosulfur compounds to methanogens.

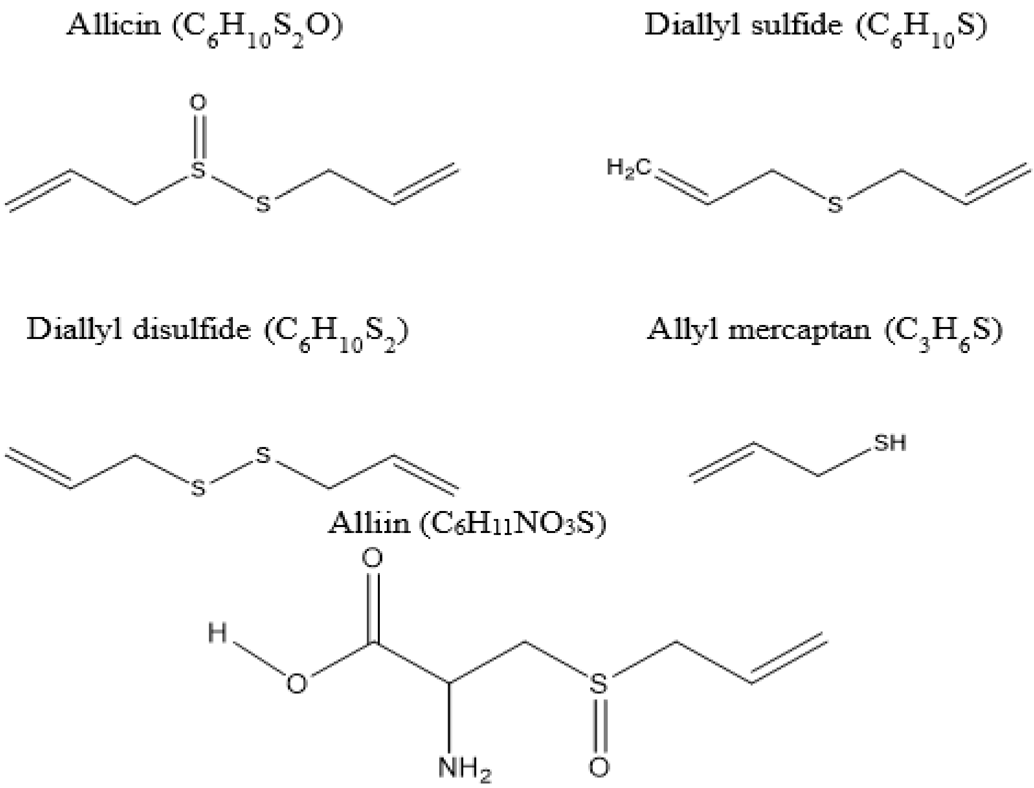

Figure 3. Chemical structures of allicin ($C_6H_{10}S_2O$), diallyl sulphide ($C_6H_{10}S$), diallyl disulphide ($C_6H_{10}S_2$), allyl mercaptan (C_3H_6S), and alliin ($C_6H_{11}NO_3S$)

Table 3. The effect of bioactive compounds in ruminants

Animal	Basal diet	Garlic form supplementation	Bioactive Compound	Level of supply	Effects	Reference
Buffalo						
Buffalo	Concentrate was offered at 0.5% of BW[9] while rice straw was given on ad libitum basis	Coconut oil and garlic powder	ND[1]	7% coconut oil plus 100 g/d of garlic powder	↑ BUN[22]; C_3[16]; Total bacteria population; Amylolytic and proteolytic bacteria; rumen ecology ↓ CH_4; Total VFA[4]; C_2[19]; C_2[19]/ C_3[16] ratio; protozoal population	[113]
Buffalo	Concentrate and roughage diet which comprised of concentrate mixture, berseem, and wheat straw.	Garlic powder	ND[1]	2% of DMI[8]	↑ Milk production; Digestibility ↓ CH_4	[115]
Buffalo	Wheat straw and concentrate mixture at a ratio of 60: 40	A mixture of (garlic and soapnut in 2 : 1 ratio	ND[1]	2% of DMI[8]	↑ urinary nitrogen; feed conversion efficiency	[134]
Buffalo	50% wheat straw and 50% concentrate mixture	A mixture of garlic bulb and peppermint oil	ND[1]	2.5% of DMI[8]	↓ CH_4; faecal nitrogen ↓ CH_4	[135]
Cattle						
Cattle	TMR[7] according to the National Academies of Sciences, Engineering, and Medicine	Mootral (garlic and citrus extract)	ALL and flavonoid	15 g/d	↓ CH_4	[108]

					• CO_2 and O_2 did not differ between treatments	
Cattle	Concentrate at 5 g/kg BW[9] UTRS[13] fed ad libitum	Garlic powder	ALL[14], ajoene, S-allylcysteine, DAD[15], S-methylcysteine sulfoxide and S-allylcysteine	40 g/d	DMI[8], average daily gain, and feed efficiency remained similar in control and supplemented steers. ↑ pH; C_3[16]; rumen fermentation efficiency ↓ CP[17] digestibility; NH_3-N; C_2[19]; CH_4; Population sizes of bacteria and protozoa; proteolytic bacteria; amylolytic and cellulolytic bacteria	[88]
Cattle	Rice straw ad libitum and concentrate was fed at 0.5% of BW[9]		A mixture of mangosteen peel, garlic, and urea pellet	200 g/d	↑ NH_3-N; C_3[16] ; bacterial population; rumen fermentation, microbial protein synthesis ↓ CH_4; protozoa population	[136]
Cow						
Cow	TMR[7]	Garlic essential oil	ALL[14]	5 g/kg DM[2]	↑ Feed digestibility ↓ The flow of bypass protein to the small intestine	[151]

Cow	TMR[7]	DAD	DAD[15]	DAD[15] was fed at levels of 56 mg/kg DM[2] and 200 mg/kg DM[2] in Exp. 1 and Exp. 2, respectively. This is equivalent to 1.0 or 3.3 g/cow per day		[152]
Cow	Fed with *ad libitum* with UTRS[13] and concentrate at 0.5 g kg^{-1} body weight (BW), twice daily	Garlic powder	ND[1]	80 g d^{-1}	↑ C_3[16]; N retention and absorption ↓ C_2[19]/ C_3[16]; Protozoa	[153]
Goat						
Goat	600 g/kg DM[2] of concentrate and 400 g/kg DM[2] of cowpea/maize silage in a ratio of 1:3 respectively	Garlic oil	ND[1]	20 – 35 g	↑ ADF[5]& lignin digestibility, total VFA[4], FCR[6], NH_3-N, digestibility ↓ CH_4; Protozoa	[111]
Goat	Grass hay (Leymus chinensis, 0.38 kg/d dry matter (DM[2])) and concentrate (0.22 kg/d DM[2])	Garlic oil	ND[1]	0.8 g/d		[154]
Sheep						

Ewe	TMR[7]	ALL[14]	ALL[14]	2 g/d	OM[11]; N; NDF[12]; ADF[5] digestibility ↓ CH_4; protozoa and methanogens.	[109]
Ewe	TMR[7] based on barley-based diet	Garlic oil	ALM[23] (26%), allyl trisulphide (18%), ALL[14] (1.5%)	0.02 g/kg DM[2]	↑ *Methanosphaera stadtmanae, Methanobrevibacter smithii* Alter the diversity of rumen methanogens without affecting the methanogenic capacity of the rumen	[110]
Lamb	A barley-based concentrate diet ad libitum.	Garlic essential oil	ND[1]	200 mg/kg DM[2]	• No effects on intake and ruminal fermentation characteristics compared to lambs fed unsupplemented diet • The addition of garlic did not affect carcass characteristics, meat quality, and had small effects on FA[22] composition of back fat and liver It seems unlikely that these minor changes will have any impact on the health properties of lamb meat	[104]
Lamb	Free access to a natural grassland hay [921.1 g dry matter (DM[2])/kg and concentrate (889.0 g DM[2]/kg]	Combined garlic essential oil and linseed oil	ND[1]	Linseed oil (1.6 mL/kg BW[9]) and garlic essential oil (3 μL/kg BW[9]	↓ CH_4; VFA[4] • A long-term early-life intervention induced modifications in the composition of the rumen bacterial community	[114]

					• There was no persistency of the early-life intervention on methanogenesis	
Lamb	According to Ministry of Agriculture of P. R. China, 2004	Garlic skin	ND[1]	80 g/kg DM[2]	↑ ADG[3]; VFA[4]; *Prevotella, Bulleidia, Howardella, Methanosphaera* ↓ Fretibacterium • Favorably regulated pyrimidine metabolism, purine metabolism, vitamin B6 and B1 metabolism. • High correlations between uctuant rumen microbiota and metabolites	[92]
Sheep	Control diet (basal total mixed ration with no additive=CTR)	raw garlic or garlic oil	ND[1]	Dose of raw garlic (75 versus 100 g/kg DM) and garlic oil (500 versus 750 mg/kg DM)	C_3[16]; C_2[19]/ C_3[16] ratio ↓ NDF[12]; ADF[5] by garlic oil supplementation; Protozoa in a dose-independent manner; NH_3	[107]
Sheep	Mixed hay (Hay-diet, as control) and hay plus garlic stem and leaf silage diet (GS-diet, at ratio of 9:1)	Garlic stem and leaf silage	ND[1]	66 g/kg BW[9]0.75 /d DM[2]	↑ Nitrogen digestibility; C_3[16]; C_5[18]; Glucose; plasma LeuTR and WBPS NEFA[20]	[102]
Sheep	Meadow hay (3rd cut, vented) and concentrate (barley grain and soybean meal; 700:300) offered in a 1:1 ratio	Garlic oil	DAD[15]	5g garlic oil or 2g DAD[15]/kg DM[2]	↑ digestibility and energy use efficiency ↓ concentrate intake; Low palatability	[112]

Sheep	Mixed hay plus concentrate at 60:40 ratio	FDGL[10]	ALL[14]	2.5 g/ (kg $BW^{9}0.75$.d)	↑ ↓	NH_3-N; Glucose CH_4; DM[2] ingested	[116]
Sheep	Forage to concentrate ratio of 1:1.	Bulb of garlic	ND[1]	1% of DM[2]	↑	Nutrient digestibility (DM[2], OM[11], NDF[12], ADF[5] and cellulose)	[94]
Ram	Concentrate to rice straw was 30:70 (as-fed basis).	Garlic powder	ND[1]	0.5% concentrate (DM[2])	↓	CH_4; Serum glutamic oxaloacetic transaminase	[138]

ND[1]: Not determined; DM[2]: Dry Matter; ADG[3]: Average Daily Gain ; VFA[4]: Volatile Fatty Acid ; ADF[5] : Acid Detergent Fibre ; FCR[6] : Feed Conversion Ratio ; TMR[7] : Total Mix Ratio ; 442
DMI[8] : Dry Matter Intake ; BW[9] : Body Weight ; FDGL[10] : Freeze Dried Garlic Leaves ; OM[11] : Organic Matter ; NDF[12] : Neutral Detergent Fibre ; UTRS[13] : Urea Treated Rice Straw ; 443
ALL[14] : Allicin ; DAD[15] : Diallyl Disulphide ; C_3[16]: Propionate; CP[17] : Crude Protein ; C_5[18] : Butyrate ; C_2[19]: Acetate; NEFA[20] : Plasma non-esterified fatty acids ; BUN[21] : Blood urea nitro- 444
gen ; FA[22] : Fatty Acid; ALM[23] : Allyl Mercaptan 445

5. Nutritive Value of Garlic in Ruminants

5.1. Chemical Composition of Garlic

Garlic contains volatile oils and protein, comprising 1-3.6 g/kg and 160-170 g/kg respectively [139]. In addition, it is a rich source of sulphur, potassium, phosphorus, magnesium, sodium, and calcium [121]. The sulphur content in garlic varies from 5 to 37 g/kg of DM [121]. Garlic products can be classified into garlic essential oils, garlic oil macerate, garlic powder, and garlic extract [155].

5.2. Effects Garlic on Rumen Fermentation

Garlic powder and garlic oil exhibit activities on modifying rumen fermentation parameters, improving nutrient digestibility, decreasing rumen protozoa numbers, and decreasing CH_4 emissions and the effect of garlic extracts on the rumen microbiome have been comprehensively investigated [151,153]. The latest findings on the effect of garlic on ruminant animal productivity is summarised for both *in vitro* (**Table 4**) and *in vivo* determinations (**Table 5**).

Supplementation of garlic oil at 0.8 g/d did not greatly affect ruminal fermentation parameters (total VFA concentration and individual VFA molar proportions) but increased ammonia and microbial crude protein [154]. In addition, garlic oil altered rumen fatty acid profile by increasing t11-18:1 (TVA) and c9, t11-CLA. This appeared to be achieved as a consequence of inhibition of the final step of biohydrogenation which can lead to the accumulation of TVA in the rumen, [154]. Garlic powder supplementation at 80 g/d in steers could enhance ruminal propionate production and successfully reduce acetate/propionate (C_2: C_3) ratio by 10%, decreasing protozoa population as well as increasing N retention and absorption in ruminants [92]. Similarly, Ahmed, Yano, Fujimori, Kand, Hanada, Nishida and Fukuma [132] showed the same finding in *in vitro* studies, that the supplementation of garlic and citrus extract at 20% of the substrate could improve the production of total VFA and propionate and reduce C_2: C_3 ratio by 27%.

The effect of garlic oil and other organosulphur compounds (diallyl disulphide and allyl mercaptan) on rumen microbial fermentation in batch culture have been reported as resulting in lower molar proportions of acetate and higher proportions of propionate and butyrate upon supplementation of diallyl disulphide (DAD) (30 and 300 mg L-1 culture fluid) and allyl mercaptan (300 mg L-1 culture fluid) [35]. Moreover, there was a decrease in CH_4 yield (mL/g DM) of 73.6, 68.5 and 19.5% upon administration of garlic oil, DAD, and allyl mercaptan at 300 mg/L respectively, which may help to improve the efficiency of energy use in rumen fermentation [35]. The effects of cinnamaldehyde and garlic oil have been investigated on rumen fermentation in a dual-flow continuous culture [156]. They reported that the inclusion of garlic oil at 312 mg/L increased the small peptide plus amino acid N concentration and the proportion of propionate and butyrate and decreased the proportion of acetate and branch-chained VFA, which indicate that garlic oil affected the fermentation profile and can be used as modulators of rumen microbial fermentation [156]. However, in the experiment of Kamel, Greathead, Tejido, Ranilla and Carro [37], three levels of DAD (0,5, 5, and 10 mg/L) were investigated, but none of the treatments had a suppressing effect on CH_4 production. Furthermore, DAD supplementation at 56 mg/kg DM and 200 mg/kg levels failed to decrease CH_4 production *in vivo [152]*. Other studies reported that DAD supplementation in sheep only tended to decrease CH_4 yield relative to OM digested and that its potential to reduce CH_4 production in sheep was low; despite that, it improved digestibility and energy use efficiency by promoting growth of anaerobic rumen fungi which might increase fiber digestion [112].

Reports of garlic's effect on rumen fermentation are inconsistent between studies. This might be effect by various factors such as the dose administered, the composition of the substrate, and the composition of the microbial population in the inoculum [100]. Garlic oil and garlic powder tested at high doses showed the highest impact in reducing CH_4 emission. However, the dose level needs to be considered on how much it can be fed at the farm level.

5.3. Effects of Garlic on Rumen Microbiota

Garlic has been found to modify the microbial population profile in continuous culture experiments, reducing specifically the *Provotella spp* (mainly *P.ruminantium* and *P. briyantii*) while other microbial populations remain unaffected [93,157]. *Provotella spp* is mainly responsible for protein degradation and amino acid deamination, suggesting that garlic oil may also affect protein metabolism in which dehydrogenase activity is required to suppress deamination when using CH_4 inhibitors [158].

Endo and ectosymbiotic methanogens of protozoa can contribute around 25% of CH_4 emission from sheep rumen fluid, but the effect of garlic by-products on protozoa numbers differed in different studies [49,145]. The effect of garlic powder supplementation at 4 mg/200 mg DM *in vitro* fermentation systems have shown a decreased protozoa population by 60% [29]. Supplementing a basal diet with raw garlic or garlic oil at 500 mg/kg DM efficiently decreased the total protozoa in sheep by 35% [107]. Most studies of the effect of garlic components on the population of methanogens were carried out *in vitro*. Inclusion of garlic oil at 100 mg/L and 250 mg/L decreased methanogenic bacterial activity by 68.5% and 69% respectively Chaves, He, Yang, Hristov, McAllister and Benchaar [105]. Supplementation of garlic oil at 1 g/L effectively reduced the *in vitro* abundance of *F. succinogenes, R. flavefaciens,* and *R. albus* without affecting total bacteria and could reduce the abundance of archaea and protozoa population by 16.5 % and 8% respectively Patra and Yu [32]. In addition, the increase in the population of those three cellulolytic bacteria (*F. succinogenes, R. flavefaciens,* and *R. albus)* could be more probably explained by the reduced populations of the protozoa that engulf bacteria [32].

Observations of the reduction of methanogens coincide with those of *in vitro* results. In addition, the decreased population of protozoa could also be responsible for the reduction in methanogens, as the total methanogen population declined in absolute number as well as in proportion to the total bacterial population in the absence of protozoa [159]. Garlic powder supplementation at 80 g/d did not affect the amylolytic or cellulolytic bacteria population, but decreased protozoa population by 41% Wanapat, Khejornsart, Pakdee and Wanapat [153]. Supplementation of plant extracts (mixture garlic and citrus extract) at 10% and 20% of the substrate reduced *Methanobacteriaceae*, which is the major CH_4 producer in the rumen by 94.07 and 92.70 respectively Ahmed, Yano, Fujimori, Kand, Hanada, Nishida and Fukuma [132]. Furthermore, 20% PE effectively increased the abundance of H_2-consuming groups such as *Prevotellaceae* and *Veillonellaceae* and reduced some H_2-producing bacteria.

Garlic showed positive effects on rumen fermentation, improving nutrient digestibility, alter the rumen microbiome by decreasing protozoa and decreasing CH_4 emissions. Besides the effect are inconsistent between studies. Therefore, future research should also clarify the mode of action of CH_4 from bioactive compounds.

Table **4.** *In vitro* trials that studied the effect of garlic in ruminant productivity

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In Vitro Studies	Basal diet (Forage and concentrate ratio)	Garlic Form	Level of supply	Effects	Reference
Batch culture					
Batch culture	1000 g grass/kg ration + 0 g concentrate/kg ration (100:0), 80:20, 60:40, 40:60, and 20:80	Mixture of garlic and citrus extracts	200 g/kg of the feed	↑ Gas and CO_2; NH_3-N; Total VFA[17]: C_3[2] and C_5[3] pH; C_2[1] • Did not interfere with OM[18] and fibre digestibility • Altering rumen fermentation	[132]
Batch culture	0.5 g DM[11] of a 10:90 forage: concentrate	Garlic extract	0, 0.3, 3, 30, and 300 mg/L	↓ C_2[1]/ C_3[2] ratio; pH; C_3[2] ↓ Total VFA[17]; NH_3-N; C_2[1]	[101]
Batch culture	Grass and concentrate mixture (50:50)	*Sapindus rarak* extract with or without garlic extract	1.8 g/kg *Sapindus rarak* extract + 0.25 ppm garlic extract	↑ C_3; ruminal fermentation based on feed digestibility, fermentation products, and rumen bacterial population ↓ Crude digestibility; C_2[1]; Protozoa	[95]
Batch culture	450 mg DM[11] substrate (a mixture of lucerne hay (500 g/kg), grass hay (200 g/kg) and barley (300 g/kg))	Bulb of garlic	70 mg	DM[11] digestibility ↓ CH_4; C_2[1]/ C_3[2]	[99]

Batch culture	1:1 alfalfa hay:concentrate either (HF[13] inoculum; 700:300 alfalfa hay: concentrate; 4 sheep) or (HC[10] inoculum, 300:700 alfalfa hay:concentrate; 4 sheep)	ALL[14] and DAD[15]	0.5, 5 and 10 mg/l	↑ ↓	C_2/C_3 ratio at HC[10] pH; CH_4/ VFA[17]	[37]
Batch culture	300 mg (MC[9]; 500:500 alfalfa hay:concentrate) and the other 4 were fed (HC[10]; 150:850 barley straw:concentrate)	Garlic oil	0, 20, 60, 180 or 540 mg/L	 ↓	C_2[1]/ C_3[2] ratio; C_5[3] by garlic oil at 60, 180 and 540 mg/L with diet MC[9] Total VFA[17] by garlic oil 540 for MC[9] diet; C_2[1] by increasing doses of garlic oil; CH_4	[100]
Batch culture	0.3 g of timothy	Garlic extracts	1% of total volume	↑	Total VFA[17]; fibrolytic bacteria; *F. succinogens* C_2[1]/ C_3[2] ratio; ciliate-associated methanogen; *R. flavefaciens*	[96]
Batch culture	200 mg DM[11] (60:40 roughage (R) and concentrate (C) ratio were used as substrates)	Coconut oil and Garlic powder	0:0, 16:0, 8:4, 4:8 and 0:16 mg	↑ ↓	C_3[2]; *Ruminococcus albus* at 8:4 mg ; at 8:4 and 0:16 mg could improve ruminal fluid fermentation in terms of VFA[17] profile Gas production; NH_3-N; Total VFA[17]; C_2[1]: C_3[2] ratio; CH_4; Protozoa	[29]
Batch culture	Forages and concentrates 50: 50 alfalfa hay: concentrate diet (MC[9]) and the other four received a 15: 85 barley	garlic oil and cinnamaldehyde	0, 20, 60, 180 and 540 mg/L	↑ ↓	VFA[17] CH_4/ VFA[17] ratio the effectiveness of garlic oil and cinnamaldehyde to manipulate ruminal fermentation may depend on the characteristics of the diet fed to	[106]

	straw: concentrate diet (HC[10]).			the animals, which highlights the importance of testing these additives with different diet types	
Batch culture and dual flow continuous culture	50:50 forage:concentrate diet	Garlic oil	3, 30, 300, and 3000 mg/L	Batch culture ↑ C_3[2]; C_5[3] with supplementation of Garlic oil (30 and 300 mg/L), DAD[15] (30 and 300 mg/L), and ALM[16] (300 mg/L) C_2[1] with supplementation of Garlic oil (30 and 300 mg/L), DAD[15] (30 and 300 mg/L), and ALM[16] (300 mg/L) Dual flow Continuous Culture: ↑ Efficiency of energy use in the rumen ↓ CH_4	[35]
Batch culture	200 mg substrate	Bulb of garlic	30 mg	↑ Gas production ↓ CH_4 Inhibited methanogenesis without adversely affecting other rumen characteristics	[98]
Batch culture	400mg of ground feed substrate. The feed substrate is a mixture of alfalfa hay and a dairy concentrate feed at a 50:50 ratio	Combination of garlic oil, nitrate, and saponin	garlic oil (0.25g/L), nitrate (5mM), and quillaja saponin (0.6g/L)	↑ NH_3-N by nitrate at days 10 and 18 ↓ CH_4; Feed digestion by the combinations (binary and ternary) of garlic oil with the other inhibitors at days 10 and 18; NH_3-N by saponin, alone or in combinations, and garlic oil alone at	[48]

				day 2; Total VFA[17] by garlic oil alone or garlic oil-saponin combination; Methanogens	
Batch culture	Concentrate and wheat straw at a 50: 50 ratio	Garlic powder	2 – 6 % of DMI[8]	↓ CH_4; C_3[2]; C_5[3]	[115]
CCF[6]					
CCF[6]	Alfalfa hay and concentrate in a 50:50 ratio.	PTS[7]	200 μL/L/day	↑ *Prevotella; Methanobrevibacter* and *Methanosphaera* ↓ CH_4; methanogenic archaea; Methanomicrobiales	[133]
CCF[6]	50:50 alfalfa hay:concentrate	Garlic oil	312 mg/L	C_3[2]; C_5[3]; Small peptide; NH_3-N ↓ C_2; VFA[17]	[156]
Rusitec[4]					
Rusitec[4]	7 g hay and 3 g concentrate	Mootral (garlic and citrus extract)	1 – 2 g	SCFA[5]; C_5[3] ↓ CH_4; *Methanobacteriacea*	[97]
Rusitec[4]	A basal diet (15 g DM[11]/d) consisting of ryegrass hay, barley and soyabean meal (1:0·7:0·3)	Garlic oil	300 mg/l	↑ Bacterial population ↓ CH_4; Protozoa; NDF[12]	[103]

C_2[1]: Acetate; C_3[2] : Propionate ; C_5[3] : Butyrate ; Rusitec[4] : Rumen Simulation Technique ; SCFA[5] : Short Chain Fatty Acid ; CCF[6] : Continuous-Culture Fermenters ; PTS[7] : Propyl Propane Thiosulfinate ; DMI[8] : Dry Matter Intake ; MC[9] : Medium Concentrate ; HC[10] : High-Concentrate ; DM[11]: Dry Matter; NDF[12] : Neutral Detergent Fibre ; HF[13] : High Forage ; ALL[14] : Allicin ; DAD[15] : Diallyl Disulphide ; ALM[16] : Allyl Mercaptan ; VFA[17]: Volatile Fatty Acid ; OM[18] : Organic Matter

Table 5. *In vivo* trials that studied the effect of garlic in ruminant productivity 551

In Vivo Studies	Basal diet (Forage and concentrate ratio)	Garlic form supplementation	Level of supply	Effects in Ruminant Productivity	References
Buffalo					
Buffalo	Concentrate was offered at 0.5% of BW[7] while rice straw was given on ad libitum basis	Coconut oil and garlic powder	7% coconut oil plus 100 g/d of garlic powder	↑ BUN[18]; C_3[15]; Total bacteria population; Amylolytic and proteolytic bacteria; rumen ecology ↓ CH_4; Total VFA[3]; C_2[14]; C_2[14]/ C_3[15] ratio; protozoal population	[113]
Buffalo	Concentrate and roughage diet which comprised of concentrate mixture, berseem, and wheat straw.	Garlic powder	2% of DMI[12]	↑ Milk production; Digestibility ↓ CH_4	[115]
Cattle					
Cattle	TMR[6] according to the National Academies of Sciences, Engineering, and Medicine	Mootral (garlic and citrus extract)	15 g/d	↓ CH_4 • CO_2 and O_2 did not differ between treatments DMI[12], average daily gain, and feed efficiency remained similar in control and supplemented steers	[108]
Cattle	Concentrate at 5 g/kg BW[1] with UTRS[13] fed ad libitum	Garlic powder	40 g/d	↑ pH; C_3[15]; rumen fermentation efficiency	[88]

				$\downarrow$ CP[20] digestibility; NH_3-N; C_2[14]; CH_4 ; Population sizes of bacteria and protozoa ; proteolytic bacteria ; amylolytic and cellulolytic bacteria	
Cow					
Cow	TMR[6]	DAD[16]	DAD[16] was fed at levels of 56 mg/kg DM[1] and 200 mg/kg DM[1] in Exp. 1 and Exp. 2, respectively. This is equivalent to 1.0 or 3.3 g/cow per day		[152]
Cow	Fed with *ad libitum* with urea-treated rice straw and concentrate at 0.5 g kg^{-1} body weight (BW[7]), twice daily	Garlic powder	80 g d^{-1}	C_3[15]; N retention and absorption C_2[14]/ C_3[15]; Protozoa	[153]
Cow	TMR[6]	Garlic essential oil	5 g/kg DM[1]	$\uparrow$ Feed digestibility $\downarrow$ The flow of bypass protein to the small intestine	[151]
Goat					

Goat	600 g/kg DM[1] of concentrate and 400 g/kg DM[1] of cow-pea/maize silage in a ratio of 1:3 respectively	Garlic oil	20 – 35 g	↑ ADF[4] & lignin digestibility, total VFA[3], FCR[5], NH_3-N, digestibility ↓ CH_4, protozoa	[111]
Goat	grass hay (*Leymus chinensis*, 0.38 kg/d DM) and concentrate (0.22 kg/d DM[1])	Garlic oil	0.8 g/d		[154]
Sheep					
Ewe	TMR[6] based on barley-based diet	Garlic oil	0.02 g/kg DM[1]	↑ *Methanosphaera stadtmanae, Methanobrevibacter smithii* Alter the diversity of rumen methanogens without affecting the methanogenic capacity of the rumen	[110]
Ewe	TMR[6]	ALL[9]	2 g/head day	OM[10]; N; NDF[11]; ADF[4] digestibility ↓ CH_4; protozoa and methanogens	[109]
Lamb	A barley-based concentrate diet ad libitum.	Garlic essential oil	200 mg/kg DM[1]	• No effects on intake and ruminal fermentation characteristics compared to lambs fed unsupplemented diet • The addition of garlic did not affect carcass characteristics, meat quality, and had small effects on FA[19] composition of back fat and liver It seems unlikely that these minor changes will have any impact on the health properties of lamb meat	• [104]

Lamb	Free access to a natural grassland hay [921.1 g dry matter (DM[1])/kg and concentrate (889.0 g DM[1]/kg	Combined garlic essential oil and linseed oil	Linseed oil (1.6 mL/kg BW[7]) and garlic essential oil (3 μL/kg BW[7]	↓ CH_4; VFA[3] • A long-term early-life intervention induced modifications in the composition of the rumen bacterial community • There was no persistency of the early-life intervention on methanogenesis	[114]
Lamb	According to Ministry of Agriculture of P. R. China, 2004	Garlic skin	80 g/kg DM[1]	ADG[2]; VFA[3]; *Prevotella, Bulleidia, Howardella, Methanosphaera* ↓ *Fretibacterium* • Favourably regulated pyrimidine metabolism, purine metabolism, vitamin B6 and B1 metabolism • High correlations between uctuant rumen microbiota and metabolites	[92]
Sheep					
Sheep	Control diet (basal total mixed ration with no additive=CTR)	Raw garlic or garlic oil	Dose of raw garlic (75 versus 100 g/kg DM[1]) and garlic oil (500 versus 750 mg/kg DM[1])	C_3[15]; C_2[14]/ C_3[15] ratio • NDF[11]; ADF[4] by garlic oil supplementation; Protozoa in a dose-independent manner; NH_3	[107]
Sheep	Mixed hay (Hay-diet, as control) and hay plus garlic stem and leaf silage diet (GS-diet, at ratio of 9:1)	Garlic stem and leaf silage	66 g/kg $BW^{0.75}$ /d DM[1]	↑ Nitrogen digestibility; C_3[15]; C_5[17]; Glucose; plasma LeuTR and WBPS ↓ Plasma non-esterified fatty acids (NEFA[21])	[102]

Sheep	Meadow hay (3rd cut, vented) and concentrate (barley grain and soybean meal; 700:300) offered in a 1:1 ratio	Garlic oil	5g garlic oil or 2g DAD[16]/kg dietary DM[1]	↑ ↓	digestibility and energy use efficiency concentrate intake; Low palatability	[112]
Sheep	Mixed hay plus concentrate at 60:40 ratio	FDGL[8]	2.5 g/ (kg BW[7]$^{0.75}$·d)	↑ ↓	NH_3-N; Glucose CH_4; DM[1] ingested	[116]
Sheep	Forage to concentrate ratio of 1:1.	Bulb of garlic	1% of DM[1]	↑	Nutrient digestibility (DM[1], OM[10], NDF[11], ADF[4] and cellulose)	[94]

DM[1]: Dry Matter; ADG[2]: Average Daily Gain; VFA[3]: Volatile Fatty Acid; ADF[4]: Acid Detergent Fibre; FCR[5] : Feed Conversion Ratio ; TMR[6] : Total Mix Ratio ; BW[7] : Body Weight ; FDGL[8] : Freeze dried garlic leaves ; ALL[9] : Allicin ; OM[10] : Organic Matter ; NDF[11] : Neutral Detergent Fibre ; DMI[12] : Dry Matter Intake ; UTRS[13] : Urea Treated Rice Straw ; C_2[14]: Acetate; C_3[15] : Propionate ; DAD[16] : Diallyl Disulphide ; C_5[17] : Butyrate ; BUN[18] : Blood urea nitrogen ; FA[19] : Fatty Aci; CP[20] : Crude Protein; NEFA[21] : Plasma non-esterified fatty acids

6. Conclusion and Future Perspectives

Significant amounts of research have been conducted into decreasing CH_4 emissions from ruminants, as this is a contributor to global warming. Understanding rumen function and dynamics have been found to be important in determining dietary strategies to mitigate rumen CH_4 production. Interactions between bacteria and protozoa are crucial play a critical role in CH_4 production pathways. The main target of dietary manipulation is either via direct inhibition of methanogens, or by altering metabolic pathways leading to the reduction of substrates for methanogenesis. Garlic and its bioactive compounds such as allicin ($C_6H_{10}S_2O$), diallyl sulphide ($C_6H_{10}S$), diallyl disulphide ($C_6H_{10}S_2$), and allyl mercaptan (C_3H_6S) have demonstrated inconsistent effects in decreasing CH_4 production during rumen fermentation. This may be due to various reasons; firstly, different types of garlic contain different amounts of bioactive compounds. Secondly, the composition of the basal diet can affect the action of garlic bioactives by affecting on rumen metabolism. However, generally increasing the dietary dose of garlic and/or its bioactive compounds results in a decrease CH_4 production. Further research is needed to understand how organosulfur compounds within garlic products affect methanogens and their pathways, providing insight into effective CH_4 reduction strategies. Generally, there will not be a single "silver bullet" for agricultural GHG emissions. Rather, this approach will have a shorter-term impact, but could be combined with other dietary strategies to prevent adverse effects on rumen digestibility and fermentation. There are real opportunities for future innovative industries based on developing garlic for use in agriculture. Given the far-reaching consequences of rumen fermentation on ruminant nutrition, food production and the environment, it is not surprising that many studies have been undertaken to understand microbial populations in the rumen and ultimately manipulate them to maximize productivity while reducing the environmental burden of ruminants.

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