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Ginger (*Zingiber officinale* Roscoe), a natural additive with antimethanogenic properties in ruminants

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ABSTRACT

Objective: To describe ginger (*Zingiber officinale* Roscoe) properties and its potential to reduce ruminal methane production.

Design/methodology/approach: A literature search and review of scientific articles published over the past 20 years was conducted, focusing on the use of ginger as an antimicrobial agent in animals of zootechnical interest, as well as its application as an antimethanogenic additive in ruminants.

Results: The metabolite profile of ginger is described, along with the effects of ethanolic, methanolic, and/or aqueous extracts, essential oils, and ginger meal in *in vitro* assays evaluating antimicrobial and antimethanogenic activity in cattle, sheep, and goats. Additionally, the scope and potential areas of opportunity for its field application are discussed.

Limitations on study/implications: No *in vivo* studies have been conducted on the use of ginger as an antimethanogenic additive in ruminants.

Findings/conclusions: Ginger rhizome meal has been shown to reduce methane production *in vitro*, suggesting its potential as an antimethanogenic additive. However, *in vivo* studies are needed to confirm this effect, identify the active compounds responsible, and clarify their mechanism of action in the rumen.

Keywords: Ruminal methanogenesis, ruminal microbiota, greenhouse gases, gingerols and shogaols.

INTRODUCTION

The livestock sector contributes to greenhouse gas (GHG) emissions, with cattle production being responsible for 62% of global livestock-related emissions, equivalent to 5 gigatonnes of CO_2-e. Additionally, enteric fermentation accounts for 44% of methane (CH_4) emissions (FAO, 2022). Methane production not only contributes to global warming

but also compromises livestock productivity, as between 4% and 15% of the total energy consumed by the animal is lost during CH_4 synthesis (Bodas *et al.*, 2008).

Many of the strategies aimed at reducing CH_4 production in ruminants focus on modifying ruminal characteristics through dietary interventions (Patra, 2012), as the ruminal microbiota is directly responsible for the biosynthesis of this gas (Patra *et al.*, 2017). However, none of these strategies have proven entirely effective, as their impact on CH_4 reduction is temporary, often costly, and may even be toxic to the ruminant (Moss *et al.*, 2000; Patra, 2012). Therefore, it is essential to search for new dietary alternatives with the potential to reduce ruminal methanogenesis, without generating harmful residues that negatively affect the animal or pose risks to consumers of milk and meat. One natural alternative could be the inclusion of ginger (*Zingiber officinale* Roscoe) in the diet of domestic ruminants, as it exhibits antimicrobial properties (Mao *et al.*, 2019) and acts as a modulator of microbial populations (Teng *et al.*, 2018). The antimicrobial effect of this plant has been tested in various animal species of zootechnical interest, including some ruminants (Kim *et al.*, 2012; Soroor & Moeini, 2015), with reports of reduced ruminal CH_4 production resulting from the use of both ginger rhizome meal (Patra *et al.*, 2010; Soroor & Moeini, 2015; Khejornsart *et al.*, 2021; Altınçekiç *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023) and aerial part extracts (Kim *et al.*, 2012).

Although the antimicrobial and antimethanogenic effects of ginger in ruminants have been documented, its mechanism of action remains unknown, and thus, the effectiveness of its application under field conditions is still uncertain. Based on this context, the objective of this review is to contribute to the understanding of ruminal methanogenesis and its environmental impact, as well as to examine the effect of dietary ginger on CH_4 production in the rumen, its inclusion levels, the most suitable form of presentation in complete diets, its potential limitations, and its utilization as a feed additive.

MATERIALS AND METHODS

A systematic review was conducted on scientific articles addressing the chemical properties of ginger, its use as an antimicrobial agent in animals of zootechnical interest, and its use as an antimethanogenic additive in ruminants, published over the past 20 years. Articles were obtained from academic repositories and search engines such as Google Scholar, NCBI, and Scopus, using the following keywords individually or in combination, in English: "*Zingiber officinale*," "ginger," "ginger meal," "ginger extract," "ginger properties," "gingerols," "shogaols," "ruminant," "ruminal methanogenesis," "rumen microbiota," "greenhouse gases," "methane."

RESULTS AND DISCUSSION

Ruminal Methane

According to the UN (2023), the global population is expected to reach 10.4 billion by the year 2100, which will result in a greater demand for food inputs, including animal-based protein. Globally, cattle are among the main providers of milk and meat, both of which are important sources of high biological value protein (FAO, 2022). However, the cattle sector contributes more than 5 gigatonnes of CO_2-e emissions within the livestock industry —6.5 and 8.5 times more than buffaloes and small ruminants, respectively (Figure 1). Of the total GHG emissions from livestock, 44% corresponds to enteric CH_4 produced by cattle (FAO, 2022).

Ruminal Methanogenesis

In the rumen, CH_4 is synthesized by methanogenic archaea, which reduce remnants of carbon dioxide (CO_2), hydrogen (H_2), formic acid, and methylamines through various metabolic pathways. These compounds are produced as a result of the fermentative activity of other microorganisms (Patra *et al.*, 2017). Methanogenic archaea are found in the rumen both free-living interacting as part of a microbial consortium composed of ruminal bacteria and fungi within biofilms that cover feed particles and as endosymbiotic and ectosymbiotic associates, mainly with ciliated protozoa (Figure 2; Valle *et al.*, 2015). It is estimated that between 9 and 37% of ruminal CH_4 is produced by these microorganisms (Newbold *et al.*, 2015; Eckard *et al.*, 2010; Huws *et al.*, 2018). Therefore, studying dietary strategies aimed at reducing methanogenesis necessarily involves considering the various groups within this complex microbial consortium. Reducing CH_4 production would not only decrease greenhouse gas emissions from the sector but also improve feed efficiency and productivity of production systems (Johnson and Johnson, 1995).

Some strategies to reduce ruminal CH_4 production have involved the use of ionophores, the addition of chemical compounds such as bromochloromethane and nitrocompounds, or energy and protein supplementation in low-quality diets. More natural alternatives include the use of forage legumes with high condensed tannin content and/or the addition of secondary metabolites such as saponins, tannins, and essential oils (Patra, 2012).

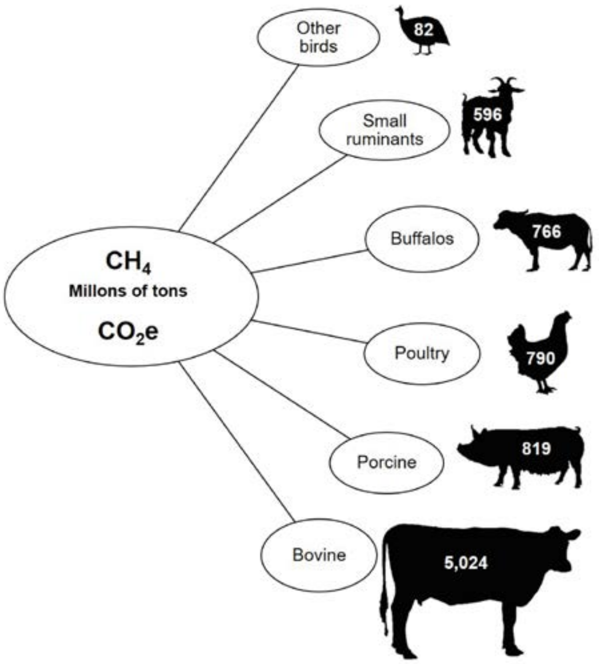

Figure 1. Methane production from the main species of zootechnical interest, presented in million tonnes of CO_2-equivalents. Prepared based on data from the FAO (2022).

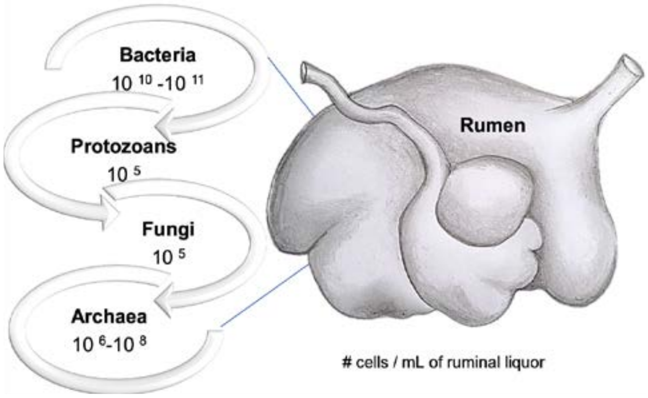

Figure 2. Estimated proportion of bacteria, protozoa, fungi, and archaea comprising the ruminal microbiota. Illustration by the authors.

However, some of these strategies do not guarantee the welfare and/or productivity of the ruminant or have only a partial or short-lived effect (Moss *et al.*, 2000), most likely due to the resilience of the microbial consortium. With the aim of utilizing plant resources with intrinsic natural properties that can modify the ruminal microbiota and consequently reduce CH_4 production, a review of the scientific literature on ginger was conducted, which could have potential as an additive or supplement.

Antimicrobial and Antimethanogenic Activity of Ginger in Ruminants

Ginger (*Zingiber officinale*) is a tropical and subtropical plant native to China and India, attributed with various biological activities, including antimicrobial properties (Figure 3A; Mao *et al.*, 2019). Its biological activity is due to the content of different secondary metabolites, such as terpenes, sesquiterpenes, monoterpenoids, and phenolic compounds, mainly 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol (Figure 3B). These latter compounds are the most abundant and provide ginger with its characteristic aroma and pungent flavor (Sanwal *et al.*, 2010; Mao *et al.*, 2019).

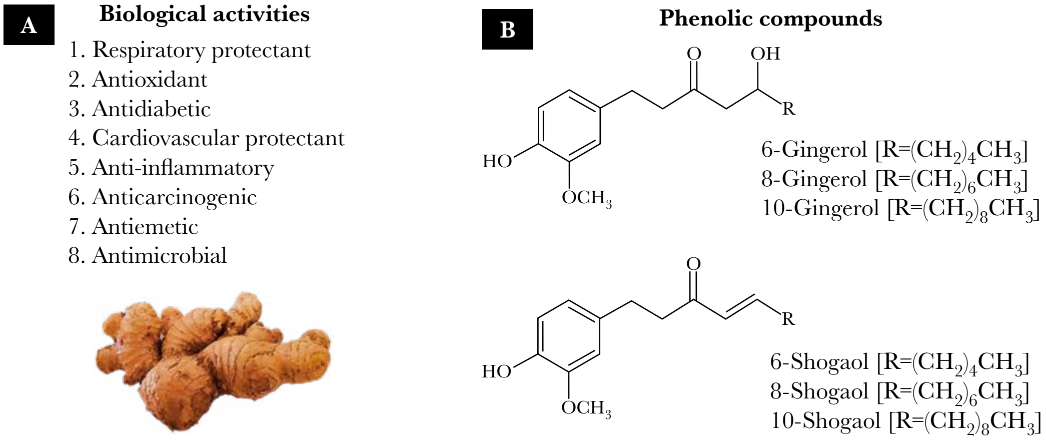

Figure 3. Biological activities (A) and main phenolic compounds of ginger (B). Illustration by the authors based on information from Mao *et al.* (2019).

Gingerols, the main metabolites of fresh ginger, are chemically known as phenolic β-hydroxyketones and are precursors of other bioactive compounds in ginger (Gopi *et al.*, 2016). Their importance lies in their antimicrobial potential and inhibition of biofilm formation (Kumar *et al.*, 2014; Kim *et al.*, 2015; Borges *et al.*, 2016). Shogaols are products of the dehydration of the β-hydroxyketone group of gingerols (Shukla *et al.*, 2019); therefore, they are more abundant in dried or dehydrated ginger. Shogaols are considered the metabolite with the greatest bioactive potential even though they are found in lower concentrations (Samwal *et al.*, 2010). Shogaols are thermodynamically more stable than gingerols at high temperatures (Hu *et al.*, 2011), an important characteristic when emphasizing on the extraction of a metabolite of interest. The amount and ratio of gingerol/shogaol will depend on the physical state of the rhizome, whether fresh (Jacotet-Navarro *et al.*, 2016; An *et al.*, 2020) or dried (Ok *et al.*, 2012; Guo *et al.*, 2017), post-harvest storage time (Sanwal *et al.*, 2010; Yeh *et al.*, 2014), among other factors.

The preparation of ginger extracts is affected by drying, maceration, and root extraction methods (Delasasso *et al.*, 2022), type and concentration of solvent used (Jacotet-Navarro *et al.*, 2016; Ko *et al.*, 2019; Shukla *et al.*, 2019; An *et al.*, 2020; Tanweer *et al.*, 2020), type of industrial processing, drying and extraction time and temperature, pH (Ok *et al.*, 2012), and the origin of the ginger crop (Yudthavorasit *et al.*, 2014; Ozola *et al.*, 2019).

The antimicrobial effect of ginger has been tested in various animal species. In mice, a modulatory effect of microRNAs contained in exosome-like nanomembranes from ginger on the intestinal microbial populations of the rodent was described (Teng *et al.*, 2018). In livestock species, antimicrobial effects have been evidenced in laying hens and broiler chickens, as well as a modulatory effect on intestinal microorganisms in quail (Dieumou *et al.*, 2009; Shanoon *et al.*, 2012; Salmanzadeh, 2015). In ruminants, *in vitro* studies demonstrated an antimicrobial effect on ciliated protozoa associated with methanogens (Kim *et al.*, 2012), as well as a reduction in total ruminal bacteria (Soroor and Moeini, 2015); however, studies using aqueous, ethanolic, and/or methanolic ginger extracts reported an increase in ruminal protozoa numbers in an *in vitro* assay with ruminal fluid from buffaloes (Patra *et al.*, 2010).

Regarding the effect of ginger on the reduction of ruminal CH_4 production, *in vitro* studies have shown that essential oils (Altınçekic *et al.*, 2021) and ginger powder (Soroor and Moeini, 2015; Kurniawati *et al.*, 2018; Khejornsart *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023) can reduce the production of this gas by up to 27% and 46%, respectively, without significantly affecting other fermentation variables. These results were dose-dependent; however, no changes in ruminal CH_4 production were observed when ginger extracts were used (Patra *et al.*, 2010).

Optimal Dose of Ginger in the Diet of Ruminants

An optimal dose of ginger in the diet of ruminants for reducing ruminal methanogenesis has not been established, due to the variability of results among studies. This variability originates from the form in which ginger is incorporated and the processing it undergoes, which alters its chemical profile (see previous section). In this regard, ginger rhizome powder is the form that has shown the most promising results in reducing CH_4 in the rumen

(Soroor and Moeini, 2015; Khejornsart *et al.*, 2021; Altınçekiç *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023), compared to other presentations such as extracts from the aerial part of the plant (Kim *et al.*, 2012), or ethanolic, methanolic, and/or aqueous extracts (Patra *et al.*, 2010), and essential oils (Kurniawati *et al.*, 2018) from the root. Gutiérrez-Fidencio *et al.* (2023) demonstrated through *in vitro* studies using ruminal fluid from cattle in southeastern Mexico that the inclusion of 5% to 40% ginger rhizome powder reduced CH_4 production by 45.9% and 52.1%, respectively, as lower concentrations had no effect. In fact, this study reported the highest CH_4 reduction compared to others conducted with ginger powder at inclusion rates of 7.5% (Khejornsart *et al.*, 2021) and between 15% and 30% (Soroor and Moeini, 2015). Although the effect on ruminal methanogenesis is promising and Gutiérrez-Fidencio *et al.* (2023) confirmed this effect using different ginger powders of various origins, further *in vivo* studies are needed before ginger powder can be recommended as a natural anti-methanogenic additive for ruminant livestock.

Efficient Utilization of the Qualities of Ginger Rhizome

To date, there are no studies identifying which metabolites or other components of ginger are responsible for its anti-methanogenic activity in ruminants. It is crucial to determine which molecules cause this effect in order to obtain consistent results with ginger —although this also applies to any foliage or plant-based additive. In the case of ginger, this will require chemical analysis of its phenolic compound profile using high-precision techniques such as HPLC. Additionally, it is important to consider that the composition of ginger's main metabolites changes depending on whether it is used fresh or dried. For example, in ginger juice, the most abundant metabolite is gingerol (Gopi *et al.*, 2016; Wohlmuth *et al.*, 2005), whereas dehydration transforms gingerol into shogaol (Ozola *et al.*, 2019; Teng *et al.*, 2019).

The gingerol and shogaol content in ginger (Ghasemzadeh *et al.*, 2018; Mao *et al.*, 2019; Dalsasso *et al.*, 2022) can also be affected by several factors, such as the genotype or variety (Wohlmuth *et al.*, 2005; Sanwal *et al.*, 2010; Pawar *et al.*, 2011; Salmon *et al.*, 2012), the cultivation origin (Yudthavorasit *et al.*, 2014; Ozola *et al.*, 2019), and agroclimatic conditions during cultivation, such as altitude, temperature, soil pH, and phosphorus availability (Sahoo *et al.*, 2023). Other factors include the plant's stage of maturity (Bailey-Shaw *et al.*, 2008), post-harvest storage time and conditions (Sanwal *et al.*, 2010; Yeh *et al.*, 2014), and finally the dehydration process, whether by heat or lyophilization (Ok and Jeong, 2012; An *et al.*, 2016). Identifying the agent responsible for the antimethanogenic effect would allow for the manipulation of certain cultivation conditions and/or extraction processes to optimize ginger's chemical profile.

Preference for Diets Containing Ginger

The presence of gingerols and shogaols gives ginger a sweet aroma that may be attractive to cattle (Ginane *et al.*, 2011), which is an advantage for its use as a feed additive in ruminants. However, these same metabolites also produce an astringent sensation that could affect intake (Wohlmuth *et al.*, 2005), since ruminants rely on their senses of taste and smell, in addition to sight and touch, to select their food (Harper *et al.*, 2016). Proposing ginger rhizome powder as an additive for livestock feeding implies that it must be palatable to the animal; therefore, preference and feeding behavior tests will be necessary to assess the acceptance of ginger in the diet and thus determine its viability as an antimethanogenic additive for ruminants.

Perspectives on Understanding the Effect of Ginger on the Rumen Microbiota

The use of ginger in ruminant diets could have an effect on the rumen microbiota by impacting microbial populations directly involved in CH_4 synthesis or by altering the metabolism of other microorganisms, thereby reducing CH_4 precursor substrates. This could be studied using highly sensitive molecular techniques, such as digital droplet PCR (ddPCR), to analyze the proportions of microbial groups and/or subgroups (Ángeles-Mayorga *et al.*, 2022). On the other hand, for a more in-depth analysis, it is necessary to employ Next Generation Sequencing (NGS) tools, either through the generation of metaprofiles, which typically provide an overview of microorganisms from phylum to family (and sometimes genus) levels, with over 80% identification certainty using databases such as Greengenes (DeSantis *et al.*, 2006) or the Ribosomal Database Project (Cole *et al.*, 2014); or through untargeted sequencing approaches aimed at obtaining full metagenomes, which include the total DNA of the complex rumen fluid sample.

Therefore, analyzing the changes in microbial populations due to the effect of ginger and/or any of its components or metabolites would allow us to elucidate the cause of the anti-methanogenic effect. Ideally, the application of molecular and/or genomic tools would enable the evaluation of the effect of additives or dietary components in ruminants, not only ginger, which has been the focus of this review.

CONCLUSIONS

Ginger reduces ruminal methane production in cattle and sheep under *in vitro* conditions. It is more effective to provide ginger in the form of rhizome powder, with an appropriate inclusion rate of 5% in total mixed rations. However, the mechanism and the metabolite responsible for the anti-methanogenic effect of ginger are still unknown. This could be analyzed through HPLC chemical profiling, as well as molecular and genomic analyses of the rumen microbiota; the latter would help to understand the underlying basis of ginger's effect on ruminal methanogenesis. *In vivo* diet trials using ginger and preference tests will be necessary to propose the use of rhizome powder as an anti-methanogenic feed additive.

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