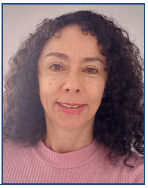
REVIEW ARTICLE / PREGLEDNI ČLANAK

Essential oils as modifiers of rumen metabolism and reducers of methane gas production



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Abstract

The demand for food in response to the increasing human population has challenged livestock farmers to increase food production. In this process, environmental health has been affected, not only by anthropogenic activities, but also by increasing populations of production animals. Various human activities and natural processes, such as rumen fermentation, produce methane and carbon dioxide, two gases that produce the greenhouse effect. The interest in mitigating the production of these gases has led to research that can assess the most effective measures to do so without affecting animal production. Among them, the use of certain essential oils has shown good results, not only for reducing the population of methanogenic bacteria, but also for modifying rumen metabolism to make it more efficient while reducing methane production. The literature has stated that there is still much to be researched, mainly in vivo studies, and concerning the duration of the effects achieved, to ensure that efforts to reduce methane production do not affect feed conversion..

Key words: *global warming; environmental pollution; ruminants; plants*

Introduction

The production of greenhouse gases (GHG) comes from both anthropogenic activities and natural processes, including the gases emitted in eructation, product of ruminal fermentation, and through faecal matter. The most important gases are CO_2 and methane (CH_4), (Castro-Montoya et al., 2015). Although CO_2 is produced in greater quantities, CH_4 generates a 25–34 times greater impact on global warming than the former (McGratha et al., 2018; Benchaar and Greathead, 2011). Additionally, the contribution of methane caused by the digestive fermentation of ruminants is calculated between 16% and 25% of the total production of GHG (Wu et al., 2018; Cobellis et al., 2016). Although this figure does not exceed other GHG sources, it has raised serious questions about bovine production. At the same time, the demand for food for a constantly growing human population has led to an increase in livestock production of meat and milk, posing a challenge for animal production to produce more food while promoting sustainable and safe production that minimises negative impacts on the ecosystem (Silva et al.,

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2018; Belanche et al., 2020; Ángeles-Mayorga et al., 2022).

Several years ago, research began on phytometabolites that could generate beneficial effects on ruminal function. Essential oils (EO) were found to reduce methane gas (MG) production, as by modifying the microbial population or ruminal metabolism (Kholif et al., 2018). In this regard, two general characteristics have been highlighted in the use of EOs in ruminant nutrition. The first is that the beneficial effects are frequently additive or synergistic, so that the use of EO associations gives better results than using them separately or as isolated metabolites. The second is that the effects can vary from beneficial at certain concentrations to adverse in higher amounts (Kholif et al., 2012; Morsy et al., 2016), so their use requires prior knowledge. Starting from the variability of the results reported in different studies, and identifying the factors that can influence the results, the objective of this review was to collect information that would clarify the benefits of using of EO in animal nutrition, with the aim of reducing MG production without affecting bovine production.

Rumen physiology and methane gas production

Rumen microorganisms are able to break down cellulose efficiently, producing volatile fatty acids (VFAs) such as acetate, propionate and butyrate, as well as formic acid and carbon dioxide. Within ruminal microorganisms, the methanogenic archaea capture H_2 to reduce CO_2 and thereby produce ATP and methane (CH_4). This is not used by the ruminant and is a waste product, constituting a calculated loss of energy between 2%-12% of the gross energy ingested in the feed (Cobellis et al., 2016; Danielsson et al., 2017; McGratha et al., 2018). Of the total MG produced by ruminants, 87% comes from the rumen and is emitted in eructation, while 13% is produced in the large intestine and is eliminated in the faeces (Cobellis et al., 2016). Three coenzymes (F420, M, and B) participate in methanogenesis and H_2 is consumed in this process, so fermentation with high H_2 production leads to greater MG production (Cobellis et al., 2016). This is how high fibre and low-quality forage leads to higher MG production while feed rich in fermentable carbohydrates reduce pH and MG production because they create a less favourable environment for methanogens that are free in the rumen or adhered to the feed, the protozoa or the ruminal mucosa (Bodas et al., 2012; Patra, 2012; Aguiar-Zalzano and Rojas-Bourrillon, 2014). It is possible that the highest GHG emission reported in Latin America and the Caribbean are due to the low quality of forage offered to bovines in those regions (Ángeles-Mayorga et al., 2022).

The amount of MG produced by a ruminant depends on various factors, including the animal traits (animal size, size of the rumen, volume of feed consumed, stage of production), others are related to diet and the characteristics of the rumen biomass (Pinares-Patiño et al., 2013; Aguiar-Zalzano and Rojas-Bourrillon, 2014). The relationship with the genetic characteristics of the animals has been related to the size of the rumen, indicating that the smaller its size, the lower the production of MG, and their fermentative characteristics (Pinares-Patiño et al., 2013).

On the other hand, there is a discrepancy as to whether the quantity of archaea in the rumen, which is normally 108 cells/mL, influences the amount of MG produced in ruminants. Wallace et al. (2015) indicated that since MG production is a source of ATP for bacteria, the amount in which they are found determines the amount of MG produced; on the contrary, Ángeles-Mayorga et al. (2022) and Shi et al. (2014) claimed that it is the metabolic activity and not the quantity, that influences the greater or lesser production of MG. It seems that both factors impact the levels of MG emitted by ruminants (Cobellis et al., 2016).

Patra (2012) reported that *Methanobrevibacter* and *Methanosaricina* are the two most dominant genera within the methanogens, while Danielsson et al. (2012, 2017) related a greater population of *Methanobrevibacter gottschalkii* with greater MG production, and the abundance of *M. ruminantium* with reduced MG production. Genomics studies indicate that there are differences between these two bacteria in the gene that encodes the expression of enzymes mediating MG production (Kittelmann et al., 2014). *Proteobacteria* of the *Succinivibrionaceae* family produce succinate and very little methane in the process of propionate formation, which explains the lower amount of MT in the presence of a higher proportion of these bacteria (Danielsson et al., 2017). Actinobacteria of the *Bifidobacteriaceae* and *Coriobacteriaceae* families produce lactic and acetic acid with high hydrogen release, which leads to increased methane formation (Moss et al., 2000). Finally, *Prevotella*, a genus of anaerobic bacteria, constitutes a large part of the ruminal bacteria, with wide versatility in terms of the substrates it uses, making it difficult to understand its metabolism and relate it to high or low MG production (Danielsson et al., 2017). This information is useful when seeking to modify the rumen biomass, since it would allow for determination of which genera or families of bacteria should be the target of EO action.

As mentioned, the other determining factor in the production of MG relates to the metabolic characteristics of ruminants. A greater production of acetate and butyrate leads to a greater release of H_2 and with it a greater amount of substrate for the production of methane. On the other hand, fermentation leads to a greater proportion of propionate and generates less H_2 and therefore less formation of MG (Moss et al., 2000; Bodas et al., 2012; Kittelmann et al., 2014). Although MG production is related to higher fibre digestibility and thus better feed conversion, studies are not conclusive in indicating whether lower MG production with certain diets has lower digestibility of feed, since elements that are not digested in the rumen can undergo postruminal degradation (Goopy et al., 2014). Feed with low crude protein and high neutral detergent fibre increases the retention time of content in the rumen, with lower VFA production and higher methane production per unit of milk or meat (Ángeles-Mayorga et al., 2022).

Effect of some essential oils and their secondary metabolites at the rumen level

Essential oils (EO) are aromatic compounds obtained from plants as liquid extracts, generally through steam stripping techniques or with the use of solvents. These oils contain lipophilic and volatile secondary metabolites that act on plants, giving them their characteristic aroma and flavour among many other functions. They vary in composition and quantity, among species within the same genus of plants, depending on soil type, time of year, and the biological age of plants, and therefore their production is neither constant nor equivalent in all cases (Martínez, 2003; Benchaar and Greathead, 2011; Silva et al., 2018).

Several of the EOs tested in experimental studies have proven to have an antibacterial effect, and therefore have been used as rumen fermentation modifiers (Benchaar and Greathead, 2011; Castro-Montoya et al., 2015) (Table 1). Consequently, future research should continue to determine if the use of EO in ruminants constitutes a favourable effect in environmental terms and regarding profitability for producers. However, not all studies have found a parallel relationship between methanogenic bacteria reduction and MG production, or between MG production and ruminal fermentation (Castro-Montoya et al., 2015).

Among the mechanisms of action of the metabolites present in EOs on microorganisms, the following stand out:

- Increased permeability of the cell membrane leading to loss of its content, imbalance of H+ and K+ gradients, and cell lysis or, alternatively, depletion of bacterial ATP, by mechanisms similar to those of ionophore compounds (Lambert et al., 2001; Di Pasqua et al., 2006; Paparella et al., 2008);
- The presence of oxygen and sulphur in the EO metabolites, and their hydrophobic nature, allows them to accumulate in the bacterial membrane, which could alter the structure or ionic transport (Munchberg et al., 2007; Benchaar and Greathead, 2011);
- Alteration of the proportion of saturated and unsaturated fatty acids in the cell membrane and alteration of its selectivity (Di Pasqua et al., 2006);
- Inhibition of glycolysis enzymes, preventing microorganisms from efficiently using glucose for ATP production (Gill and Holley, 2004);
- Blocking the entry of glucose into the bacterial cell (Gill and Holley, 2004);
- Increased production of propionate in greater quantities than acetate, achiev-

Table 1. Effect of some essential oils on the population of ruminal microorganism

ESSENTIAL OIL or METABOLITE	TYPE OF MICROORGANISM					REFERENCE
	Archaea	*Fibrobacter succinogenes*	*Ruminococcus albus*	*Ruminococcus flavefaciens*	*Protozoa*	
Syzygium aromaticum	D	D	D	D	NR	Cobellis et al., 2016
Eugenia spp.	D	D	D	D	D	Patra, 2012
Eucalyptus globulus	D	D	D	NE	I	Cobellis et al., 2016
Allium sativum L.	D	D	D	D	NR	
Thymus capitatus L.	D	D	D	D	D	
Mentha piperita L.	D	D	D	D	D	
Rosemary officinalis	NE	I	NE	NE	NE	
Cinamaldehído	NR	NR	NR	NR	NE	Benchaar et al., 2008
Eugenol	NR	NR	NR	NR	NE	

D= Decreased the population; NE= No effect; I= Increased the population; NR= No report ing more effective use of feed by ruminants. Such effects have been enhanced when EOs are supplied with enzymatic products with amylase activity (Andreazzi et al., 2018; Silva et al., 2018);

- Reduction of protein and starch degradation, as well as fatty acids as a consequence of the controlling effect of some bacterial colonies, such as ammonia-producing bacteria whose main action is to deaminate amino acids, as well as protozoa (Hart et al., 2008);
- Altered energy metabolism in *Streptococcus bovis, Selenomonas ruminantium and Methanobrevibacter smithii* (*Archeae*) (Evans and Martin, 2004).

Accordingly, the effect of EOs is caused by the presence of secondary metabolites, each having a specific chemical composition and functions, which can enhance one another. However, metabolites found in higher concentrations may exert a dominant effect on others. Alcohols, aldehydes, hydrocarbons, ketones, esters and ethers are found, most of which are fat-soluble, and are grouped as terpenes, terpenoids and phenylpropanoids, among the most important (Cobellis et al., 2016; Kholif et al. al., 2018; Wu et al., 2018; Hart et al., 2019).

Some of the reported effects of EO are:

- **Thymol** is a phenolic secondary metabolite with antibacterial action, present in thyme. At concentrations of 400 mg/L, MG production and glucose entry into the bacterial cell decreased, rumen fermentation was inhibited, and rumen pH increased with decreased volatile fatty acid (VFA) production. At concentrations of 45 μg/mL, it reduced lactate production in *Streptococcus bovis* but not in *S. ruminantium*, though this was achieved at twice the concentration (Evans and Martin, 2000). Others report that at 300 mg/L, the production of MG, acetate and propionate decreases, and the result using thyme EO was better than when using isolated thymol in the same concentration (Macheboeuf et al., 2008), which is corroborated in many studies on the synergistic effect of the metabolites present in an EO or extract exceeding that found with the isolated metabolites.
- **p-Cymene** is a monoterpene that, at concentrations of 20 mg/L, shows a reducing effect on MG production without affecting VFA production, suggesting a different mechanism of action from that proposed for thymol, possibly exerting an inhibitory effect on the methanogenic bacteria (Chaves et al., 2008) since the metabolite is located in the bacterial membrane. This alters its ultrastructure at the level of the lipid fraction, culminating in an alteration of ion exchange across the membrane. The aforementioned effect was determined for p-cymene, carvacrol, thymol, and gamma terpinene, and it was found that they can enter bacteria through the membrane, interacting with cellular structures (Cristani et al., 2007).
- **Carvacrol** is a phenolic compound found in oregano (*Origanum vulgari*) and thyme (*Thymus vulgaris*), with an antibacterial effect due to the presence of a phenol OH-group (Benchaar and Greathead, 2011). Others have reported that it decreases the production of MG at different concentrations between 225–750 mg/L associated with thymol. Specifically, at a concentration of 268 mg/L, it alters the production of propionate without reducing that of MG, while above 300 mg/L, it reduces the production of VFA and MG simultaneously, reaching an inhibition of fermentation at higher concentrations (Macheboeuf et al., 2008).
- **Eugenol** is a phenolic monoterpene found mainly in the EO of cloves

(*Syzygium aromaticum)* and cinnamon (*Cinnamomum cassia*), with antibacterial action (Benchaar and Greathead, 2011). The alcoholic extracts obtained from clove reduced the production of MG, but also decreased the digestibility of food, probably due to the presence of tannins. A reduction in carboxymethylcellulase, xylanase and acetylesterase was found when clove extract was used. Additionally, there was an increase in the acetate:propionate ratio and it generated an antiprotozoal effect due to the reduction of entodyniomorphs and holothrichos (Patra et al., 2010). Chaves et al. (2008) evaluated the essential oils of cinnamon leaf, garlic (*Allium sativum*) and juniper berry (*Juniperus communis*), finding that the first two reduced the production of propionate and MG, without affecting the nitrogen utilisation capacity, because they do not affect the processes of bacterial deamination in the rumen. Likewise, it is indicated that the MG-reducing effect of cinnamon EO is greater (70%) than that generated by monensin (57%). Other studies reported a reduction in VFA formation and ruminal fermentation, with high concentrations of eugenol (600–900 ppm). On the other hand, evaluating concentrations of 400 mg/L and 500 mg/L of eugenol, there was a 30% and 35% reduction in MG production respectively, finding no changes in VFA production (Benchaar and Greathead, 2011).

- **Cinnamaldehyde**, is the main component of the EO of cinnamon (*Cinnamomum cassia*) of the non-phenolic phenylpropane type, with an antibacterial effect similar to that of thymol and carvacrol but lacking the hydroxyl group. Its effect is not due to alteration of the bacterial membrane but to the inactivation of microbial enzymes by the action of their carbonyl group (Benchaar and Greathead, 2011). At a concentration of 132 ppm, it does not influence the production of MG, acetate or propionate; at twice that concentration it reduces methanogenesis but not VFA production, indicating that its mechanism of action is not related to fermentation. At three times the initial dose, the production of MG and VFA decreased, and at 662 ppm, the reduction of both components is notorious on rumen metabolism (Macheboeuf et al., 2008). When comparing the antimethanogenic effect of cinnamon EO with the effect of isolated cinnamaldehyde, using the same concentration, a greater action of EO is found, probably due to a synergistic effect with eugenol, another metabolite present in cinnamon (Burt, 2004).
- A meta-analysis by Patra (2010), related the reduction of CH_4 with the reduction of the rumen protozoan population, confirming the report of Belanche et al. (2020).
- A commercial mixture of EO from coriander seeds (*Coriandrum sativum)* + geranyl acetate + eugenol and geraniol, was evaluated in 149 bovine females, who showed acceptance of the product and in no case did it lead to a reduction in consumption. The cows showed higher feed consumption and increased milk production of expected compositional quality. There was a 6% reduction in CH_4 production per cow per day and 20% less per kg of milk produced. This last parameter is more useful to consider since reducing MG production based on production is the aim, and also because it is expected that having less CH_4 produced ensures more usable carbon for the animal and therefore less energy loss (Hart et al.,

2019). Due to the presence of monoterpenes such as geraniol, this mixture had antibacterial, antifungal and antiprotozoal activity; however, there are indications that there is no modification of ruminal fermentation, or of VFA production when bovines are supplemented with it. This suggests that the productive benefits are not due to an effect on rumen metabolism.

Effects of the use of EO on MG production in ruminants

As mentioned, in light of the strong demand for food currently in place and expected to continue in future decades, the production of meat and milk must continue to increase. However, due to limitations of the use of antibacterials as growth promoters, alternatives that improve the digestibility of feed, primarily in ruminants, are being sought. This also includes the potential use of EO within the ration of animals or as isolated metabolites (Silva et al., 2018; Hart et al., 2019). There are many effects generated by the secondary metabolites present in some EOs, but in relation to the topic that is dealt with here, the efficacy in reducing the production of MG has focused on three aspects:

- antimicrobial effect evident in a reduction of archaea or rumen protozoa;
- ability to partially modify the metabolism of the rumen microbiome;
- ability to modify the products of bacterial metabolism, thus reducing MG precursors (Castro-Montoya et al., 2015; Cobellis et al., 2016).

In a complementary way and considering that it has been shown that CH_4 production is higher in bovine production where feed conversion and productivity are low, the process of searching for alternatives to reduce MG production also aims to simultaneously improve ruminal metabolism and feed conversion (Benetel et al., 2022).

Regarding the reduction of rumen MG production with the use of EO, the following has been found:

- **Garlic oil** (*Allium sativum*). The main components of this are sulfur derivatives with cysteine, among which alliin stands out. Due to enzymatic action, when garlic is macerated it releases allicin, a thiosulfate that constitutes the active metabolite. Busquet et al. (2005), tested the effect of garlic oil and four of its main components (diallyl sulfide and disulfide, allicin and allyl mercaptan) on MG production and ruminal fermentation. They found that garlic oil and diallyl disulfate decreased the ratio of acetate to propionate and butyrate, while diallyl sulfide only increased butyrate. Garlic oil, diallyl disulfide and allyl mercaptan, in concentrations of 300 ppm, decreased MG production by 73.6%, 68.5% and 19.5%, respectively, with a decrease in the production of acetate and an increase in the production of propionate and butyrate. This was also confirmed by Chaves et al. (2008).
- **Peppermint oil** (*Mentha piperita*). Similar as with other EOs, some studies show no effects on MG production while others report that there are effects. Testing concentrations between 0.3–2 mL of peppermint EO/L of cell culture, Agarwal et al. (2009), found a reduction in the protozoan population and in MG production in a quantity directly proportional to the concentration of EO used, but with a decrease in the digestibility of dry matter. In the highest concentrations, VFA production was also decreased.
- **EO of oregano** (*Origanum vulgare*) and **cinnamon** (*Cinnamomum verum*) was reported by Macheboeuf et al. (2008) to

reduce MG production by up to 98%. A 12% reduction was achieved with **dill** (*Anethum graveolens*). As for the **oregano** and **peppermint** in doses of 1 g/L, Patra and Yu (2012) found that it reduced MG production, but also reduced feed digestion and fermentation.

- A commercial EO prepared with a mixture of 10% **coriander seed** (*Coriandrum sativum*) oil, 7% **eugenol**, 7% **geranyl acetate**, 6% **geraniol** and fumaric acid as a preservative has been studied in an *in vivo* study in dairy and beef cows (Belanche et al., 2020). It showed a reducing effect on MG production two weeks after starting the supply, and this was sustained for six weeks in total. It is noteworthy that the reported results were achieved at low doses compared to those used in similar studies (Castro-Montoya, 2015). In the meta-analysis carried out by Belanche et al. (2020), it was found that the use of this EO reduced MG production between 8.8% and 12.9%, without affecting digestibility or milk production.
- **EO of Oregano** (*Origanum vulgare*) and **white thyme** (*Thymus mastichina*) reduced total gas production in the rumen by 75% without affecting the digestibility of the diet components. The effect may be due to the reduction of methanogenic bacteria but not of those responsible for ruminal fermentation, since digestion was favourable and H_2 production was lower (Benetel et al., 2022). The same author found that at low ruminal pH, the effect of Eos was better at reducing MG production. This is explained because at a lower pH (approximately 5.5), bacterial metabolism of carbohydrates results in a lower C_2:C_3 ratio, which gives lower methane production, since the C_3 production pathway consumes H+, compared to the C_2 and C_4 pathways. Therefore, having more C_3, the cellulolytic bacteria compete with the Archea for substrate and there is less production of CH_4.
- **Citrus essential oil**. Citrus EOs contain 28 components, among which D-limonene, b-phellandrene, 3-carene, nilalol and g-treponeme stand out. However, each varies depending on the factors mentioned above. Limonene has a higher antimicrobial activity against Gram - bacteria. This oil decreases the production of ammonium and acetate and despite the fact that it induces a decrease in VFA, after two weeks of stopping the addition of EO, the levels returned to normal. Likewise, the pH increased and MG production decreased. This does not last over time, possibly because the microorganisms adapt and the effect is lost, therefore intermittent administration is suggested (Wu et al., 2018). In a study carried out with the inclusion of orange EO in beef steers fed with *Cynodon dactylon*, a reduction in MG production of up to 12% was found, but there was also a reduction in digestibility (Jiménez-Ocampo et al., 2022).

Finally, there are several studies that demonstrate the effect of different inclusion times with EO in ruminants (MG production, ruminal fermentation, feed conversion, milk quality, among others), which would that a minimum time of four weeks is required to observe the favourable effects of its inclusion, though they can be achieved in less time in some cases (Belance et al., 2020). Achieving the reducing effect of MG production, after several days of supply, the rumen biome seems to adapt and some reduction of the effect is shown (Benchaar and Greathead, 2011; Blanch et al., 2016). Therefore, this requires specifying the research in both aspects since it would determine the short and long-term efficacy with the supplementation with EO.

Although the objective of this review is focused on EOs, other scientific efforts aimed at finding alternatives to reduce MG production are also important. Among them, the development of compounds that attack the enzymes participating in the reduction of CO_2 to CH_4 is important. It has been shown that 3-nitrooxypropanol (3NOP) is capable of reducing MG production between 25% and 84%, according to several studies by McGratha et al. (2018) on beef and dairy cattle, and sheep. 3NOP targets the active site of methylcoenzyme M reductase that catalyses the last step of methanogenesis, inhibiting it. This active site corresponds to a nickel molecule, preventing it from carrying out the required electron transfer (Martínez-Fernández et al., 2014). Likewise, other proposals focus on the use of forage (grasses and legumes) that can decrease MG production due to the presence of secondary metabolites, as reported by Ángeles-Mayorga et al. (2022) whose *in vitro* studies found that *Enterolobium cyclocarpum, Guazuma ulmifolia, Lysiloma latisiliquum, Leucaena leucocephala* and *Piscidia piscipula* contain condensed tannins that reduce CH_4 production. Finally, studies carried out with selenium nanoparticles as a fertiliser for *Festuca arundinacea* Schreb improved the digestibility of forage and thereby reduced GHG production (González-Lemus et al., 2022).

Conclusions

Several studies have shown that favourable results with the administration of EO to reduce MG production in *in vitro* methods. Although they are valuable as a starting point for the development of other studies, they are not decisive unless their benefits are reproduced through *in vivo* tests. Likewise, in some cases, the lower methane production was temporary, suggesting that rumen microorganisms adapt to the inclusions, causing the MG production to increase again, and making it necessary to carry out studies for longer periods of time.

A common denominator is found in the reported research: the correlation between the concentration of EO and the observed effects. In general terms, with lower concentrations no effect is found, in medium concentrations there is a reduction of MG or VFA, depending on the mechanism of action, and in high concentrations there is a reduction of both, and a compromise of ruminal fermentation and feed digestibility. Likewise, there is agreement in the fact that the reducing effect of MG production by EOs, is greater than that of each separate component, which can indicate an additive effect between different metabolites. There are various factors involved MG production, so a better standardisation of tests would make it possible to establish with greater certainty the real efficacy of the EOs individually, in combination with others, or of the isolated metabolites.

Finding a single explanation of how EOs can help reduce MG production seems difficult, due to the multifactorial effect related to the reduction of the methanogenic rumen microbiota, the specific reduction of rumen protozoa, modification of rumen metabolism, or the rumen microenvironment, which are among the identified factors. Finally, efforts to mitigate MG production cannot be limited to a single strategy, but instead to a combination of all those that show results *in vivo*, since there are many factors that can be modified to benefit the well-being of the ecosystem.

References

1. AGARWAL, N., C. SHEKHAR, R. KUMAR, L. C. CHAUDHARY and D. N. KAMRA (2009): Effect of peppermint (Mentha piperita) oil on in vitro methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Feed Sci. Technol. 148, 321-327. 10.1016/j.anifeedsci.2008.04.004
2. AGUIAR-ZALZANO, E. and A. ROJAS-BOURRILLON (2014): Métodos utilizados para reducir la producción de metano endógeno en rumiantes. Nutrición Animal Tropical 8, 72-90.
3. ANDREAZZI, A. S. R., M. N. PEREIRA, R. B. REIS, R. A. N. PEREIRA, N. N., MORAIS JUNIOR, T. S. ACEDO, R. G. HERMES, and C. S. CORTINHAS (2018): Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. J. Dairy Sci. 101, 7199-7207. 10.3168/jds.2017-14331
4. ÁNGELES-MAYORGA, Y., E. R. CEN-CEN, M. M. CROSBY-GALVÁN, B. J. E. RAMÍREZ-BRIBIESCA, B. CANADELARIA-MARTÍNEZ, A. SÁNCHEZ-VILLARREAL and M. RAMÍREZ MELLA (2022): Foliage of tropical trees and shrubs and their secondary metabolitesmodify in vitro ruminal fermentation,methane and gas production without a tight correlation with themicrobiota. Animals. 12, 2628. https://www.mdpi.com/journal/animals. Consulted 24-03-2023. doi.org/10.3390/ani12192628
5. BELANCHE, A., C. J. NEWBOLD, B. ZWEIFEL and D. R. YAÑEZ-RUIZ (2020): A Meta-analysis Describing the Effects of the Essential oils Blend Agolin Ruminant on Performance, Rumen Fermentation and Methane Emissions in Dairy Cows Animals 10, 620; Consulted 11-04-2023. 10.3390/ani10040620.
6. BENCHAAR, C. and H. GREATHEAD (2011): Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166, 338-355. 10.1016/j.anifeedsci.2011.04.024
7. BENCHAAR, C., T. A. McALLISTER and P. Y. CHOUINARD (2008): Digestion, ruminal fermentation, ciliate protozoal populations, andmilk production fromdairy cows fed cinnamaldehyde, quebracho condensed tannin, or Yucca schidigera saponin extracts. J. Dairy Sci. 91, 4765-4777. 10.3168/jds.2008-1338
8. BENETEL, G., S. T. DOS SANTOS, G. M. FUGUNDES, K. C. WELTER, F.A. MELO, I. A. A. G. LOBO, J. P. MUIR and I. C. S. BUENO (2022): Essential Oils as In Vitro Ruminal Fermentation Manipulators to Mitigate Methane Emission by Beef Cattle Grazing Tropical Grasses Molecules 27, 7. https://www.mdpi.com/journal/molecules. Consulted 11-04-2023. 10.3390/molecules27072227
9. BLANCH, M., M. J. M. RANILLA, A. VISO, M. VÁZQUEZ-ANON and A. BACH (2016): Influence of a mixture of cinnamaldehyde and garlic oil on rumen fermentation, feeding behavior and performance of lactating dairy cow. Anim. Feed Sci. Technol. 219, 313-332. 10.1016/j.anifeedsci.2016.07.002
10. BODAS, R., N. PRIETO, R. GARCÍA-GONZÁLEZ, S. ANDRÉS, F. J. GIRÁLDEZ and S. LÓPEZ (2012): Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Technol. 176, 78-93. 10.1016/j.anifeedsci.2012.07.010
11. BURT, S. (2004): Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microb. 94, 223-253. 10.1016/j.ijfoodmicro.2004.03.022
12. BUSQUET, M., S. CALSAMIGLIA, A. FERRET, M. D. CARRO and C. KAMEL (2005): Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci. 88, 4393-4404. 10.3168/jds.S0022-0302(05)73126-X
13. CASTRO-MONTOYA, J., N. A. PEIREN, J. W. CONE, B. ZWEIFEL, V. B. FIEVEZ and S. DE CAMPENEERE (2015): In vivo and in vitro effects of a blend of essential oils on rumen methane mitigation. Liv. Sci.180, 134-142. 10.1016/j.livsci.2015.08.010
14. CHAVES, A. V., M. L. HE, W. Z. YANG, A. N. HRISTOV, T.A. MCALLISTER and C. BENCHAAR (2008): Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. Can. J. Anim. Sci. 88, 117-122. 10.4141/CJAS07061
15. COBELLIS, G., M. TRABALZA-MARINUCCI, Z. YU, M. CORZO-MARTÍNEZ, M., N. CORZO and M. VILLAMIEL (2016): Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Biological properties of onions and garlic. Trends Food Sci. Technol. 18, 609-625. 10.1016/j.scitotenv.2015.12.103
16. CRISTANI, M., M. D'ARRIGO, G. MANDALARI, F. CASTELLI, M. G. SARPIETRO, D. MICIELI, D., V. VENUTI, G. BISIGNANO, A. SAIJA and D. TROMBETTA (2007): Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J. Agric. Food Chem. 55, 6300–6308. 10.1021/jf070094x
17. DANIELSSON, R., J. DICKSVED, L. SUN, H. GONDA, B. MÜLLER, A. SCHNÜRER and J. BERTILSON (2017): Methane Production in Dairy Cows Correlates with Rumen Methanogenic and Bacterial Community Structure. Front. Microbiol. 8, 226, 1-15. 10.3389/fmicb.2017.00226
18. DANIELSSON, R., A. SCHNÜRER, V. ARTHURSON and J. BERTILSSON (2012): Methanogenic population and CH4 production in swedish dairy cows fed different levels of forage. Appl. Environ. Microbiol. 78, 6172-6179. 10.1128/AEM.00675-12
19. DI PASQUA, R., N. HOSKINS, G. BETTS and G. MAURIELLO (2006): Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J. Agric. Food Chem. 54, 2745-2749. 10.1021/jf0527221

20. EVANS, J. D. and S. A. MARTIN (2000): Effects of thymol on ruminal microorganisms. Curr. Microbiol. 41, 336-340. 10.1007/s002840010145
21. GILL, A. O. and R. A. HOLLEY (2004): Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against L-monocytogenes and Lactobacillus sakei. Appl. Environ. Microbiol. 70, 5750-5755. 10.1128/AEM.70.10.5750-5755.2004
22. GONZÁLEZ-LEMUS, U., G. MEDINA-PÉREZ, A. PELÁEZ-ACERO and R. G. CAMPOS-MONTIEL, R. G. (2022): Decrease of Greenhouse Gases during an In Vitro Ruminal Digestibility Test of Forage (Festuca arundinacea) Conditioned with Selenium Nanoparticles. Nanomaterials 12, 3823. 10.3390/nano12213823. Consulted 7-01-2023. 10.3390/nano12213823
23. GOOPY, J. P., A. DONALDSON, R. HEGARTY, P. E. VERCOE, F. HAYNES, M. BARNETT and V. H. ODDY (2014): Low-methane yield sheep have smaller rumens and shorter rumen retention time. Br. J. Nutr. 111, 578-585. 10.1017/S0007114513002936
24. HART, K. J., D. R. YAÑEZ-RUIZ, S. M. DUVAL, N. R. MCEWAN and C. J. NEWBOLD (2008). Plant extracts to manipulate rumen fermentation. Anim. Feed Sci. Technol. 147, 8-35. 10.1016/j.anifeedsci.2007.09.007
25. JIMÉNEZ-OCAMPO, R., M. D. MONTOYA-FLORES, G. PAMANES-CARRASCO, E. HERRERA-TORRES, J. ARANGO, M. ESTARRÓN-ESPINOSA, C. F. AGUILAR-PÉREZ, E. E. ARAIZA-ROSALES, M. GUERRERO-CERVANTES and C. KU-VERA (2022): Impact of orange essential oil on enteric methane emissions of heifers fed bermudagrass hay. Front. Vet. Sci. 9. https://www.frontiersin.org/articles/10.3389/fvets.2022.863910/full. Consulted 15-01-2023. 10.3389/fvets.2022.863910
26. HART, K. J., H. G. JONES, K. E. WADDAMS, H. J. WORGAN, B. ZWEIFEL and J. NEWBOLD (2019): An Essential Oil Blend Decreases Methane Emissions and Increases Milk Yield in Dairy Cows Open J. Anim. Sci.. 9, 259-267. 10.4236/ojas.2019.93022
27. KHOLIF, S. M., T. A. MORSY, M. M. ABDO, O. H. MATLOUP and A. A. ABU EL-ELLA (2012): Effect of supplementing lactating goats rations with garlic, cinnamon or ginger oils on milk yield, milk composition and milk fatty acids profile. J. Life Sci. 27-34. 10.1080/09751270.2012.11885191
28. KITTELMANN, S., C. S. PINARES-PATIÑO, H. SEEDORF, M. R. KIRK, S. GANESH, J. C. MCEWAN and P. JANSSEN (2014): Two different bacterial community types are linked with the low methane emission trait in sheep. PLoS One 9(7):e103171. Consulted 7-01-2023. 10.1371/journal.pone.0103171
29. LAMBERT, R. J. W., P. N. SKANDAMIS, P. COOTE and G. J. E. NYCHAS (2001): A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91, 453-462. 10.1046/j.1365-2672.2001.01428.x
30. MACHEBOEUF, D., D. P. MORGAVI, Y. PAPON, J. L. MOUSSET and M. ARTURO-SCHAAN, (2008): Dose–response effects of essential oils on in vitro fermentation activity of the rumen microbial population. Anim. Feed Sci. Technol. 145, 335-350. 10.1016/j.anifeedsci.2007.05.044
31. MARTÍNEZ, M. A. (2003): Aceites Esenciales. http://www.med-informatica.com/OBSERVAMED/Descripciones/AceitesEsencialesUdeA_esencias2001b.pdf. Consultated 18-01-2023
32. MARTÍNEZ-FERNÁNDEZ, G., L. ABECIA, A. ARCO, A., G. CANTALAPIEDRA-HIJAR, A.I. MARTÍN-GARCÍA, E. MOLINA-ALCAIDE, M. KINDERMANN, S. DUVAL and D. R. YÁÑEZ-RUIZ (2014): Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. J. Dairy Sci. 97, 6:3790–3799. 10.3168/jds.2013-7398
33. McGRATHA, J., S. M. DUVALA, L. F. M. TAMASSIAA, M. KINDERMANNA, R. T. STEMMLERA, N. GOUVEAA, T. S. ACEDOA, I. IMMIGA N. WILLIAMSA and P. CELI (2018): Nutritional strategies in ruminants: A lifetime approach. Research in Veterinary Science 116 (2018) 28–39. 10.1016/j.rvsc.2017.09.011
34. MORSY, T. A., A. E. KHOLIF, S. M. KHOLIF, A. M. KHOLIF, X. SUN and A. Z. M. SALEM, (2016): Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Ann. Anim. Sci. 16, 209–222. https://doi.org/10.1515/aoas-2015-0039
35. MOSS, A. R., J. P. JOUANY and D. J. NEWBOLD (2000): Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231-253. 10.1051/animres:2000119
36. MUNCHBERG, U., A. ANWAR, S. MECKLENBURG and C. JACOB (2007): Polysulfides as biologically active ingredients of garlic. Org. Biomol. Chem. 5, 1505-1518. 10.1039/b703832a
37. PAPARELLA, A., L. TACCOGNA, I. AGUZZI, C. CHAVES-LOPEZ, A. SERIO, F. MARSILIO and G. SUZZI (2008): Flow cytometric assessment of the antimicrobial activity of essential oils against *Listeria monocytogenes*. Food Control 19, 1174–1182. 10.1016/j.foodcont.2008.01.002
38. PATRA, A. K. and Z. YU (2012): Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl. Environ. Microb. 78, 4271-4280. 10.1128/AEM.00309-12
39. PATRA, A. K., D. N. KAMRA and N. AGARWAL (2010): Effects of extracts of spices on rumen methanogenesis, enzyme activities and fermentation of feeds in vitro. J. Sci. Food Agric. 90, 511520. 10.1002/jsfa.3849.
40. PATRA, A. K. (2010): Meta-analyses of e_ects of phytochemicals on digestibility and rumen fermentation characteristics associated with methanogenesis. J. Sci. Food Agri. 90, 2700-2708. 10.1002/jsfa.4143

41. PINARES-PATIÑO, C. S., M. J. ULYATT, K .R. LASSEY, T. N. BARRY and C. W. HOLMES, (2003): Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. J. Agri. Sci.140, 205-214. 10.1017/S0021859603003046
42. SHI, W., C. D. MOON, S. C. LEAHY et al. (2014): Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. Genome Res. 24, 1517-1525. 10.1101/gr.168245.113
43. SILVA, G. G., C. S. TAKIYA, T. A. DEL VALLE, E. F. DE JESUS, N. T. GRIGOLETTO, B. NAKADONARI, C. S. CORTHINHAS, T. S. ACEDO and F. O. RENNÓ (2018): Nutrient digestibility, ruminal fermentation, and milk yield in dairy cows fed a blend of essential oils and amylase. J. Dairy Sci. 101, 9815-9826. 10.3168/jds.2018-14789
44. WALLACE, R. J., J. A. ROOKE, N. MCKAIN, C. A. DUTHIE, J. J. HYSLOP, D. W. ROSS, A. WATERHOUSE, M. WATSON and R. ROEHE (2015): The rumen microbial metagenome associated with high methane production in cattle. BMC Genomics 16:839. 10.1186/s12864-015-2032-0
45. WU, P., Z. B. LIU, W. F. HE, S. B. YU, G. GAO and J. K. WANG (2018): Intermittent feeding of citrus essential oils as a potential strategy to decrease methane production by reducing microbial adaptation. J. Cleaner Prod. 194, 704e713. 10.1016/j.jclepro.2018.05.167.

Esencijalna ulja kao modifikatori metabolizma buraga i reduktori proizvodnje plina metana

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Potražnja za hranom kao odgovor na povećanje svjetske populacije zahtjeva povećanje proizvodnje hrane na polju uzgoja stoke. To je utjecalo na zdravlje okoliša, ne samo zbog antropogenih aktivnosti, već i zbog povećanja populacije proizvodnih životinja. Različite aktivnosti koje obavljaju ljudi i prirodni procesi, poput fermentacije buraga proizvode metan i ugljični dioksid, dva plina koja uzrokuju učinak staklenika. Interes za smanjenje proizvodnje ovih plinova doveo je do istraživanja koja mogu procijeniti koje su najučinkovitije mjere za to, bez utjecaja na uzgoj životinja. Među njima, uporaba nekih esencijalnih ulja pokazala je dobre rezultate, ne samo smanjujući populaciju metanogenih bakterija, već i modificirajući metabolizam buraga čineći ga učinkovitijim, istovremeno smanjujući proizvodnju metana. Uspoređujući neka prethodna istraživanja, zaključuje se da ima još puno prostora za istraživanje, ponajprije u *in vivo* studijama, kao i svemu povezanom s trajanjem postignutih učinaka, pazeći da nastojanja da se smanji proizvodnja metana ne utječu na konverziju hrane za životinje.

Ključne riječi: *globalno zatopljenje, zagađenje okoliša, preživači, biljke*