

Effect of herbs and spices (plant extracts) on rumen microbial activities: a review

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ABSTRACT: Ruminant livestock are important sources of animal protein and calories, also are sources of global greenhouse emissions. There is increasing interest in evaluating the potential of natural antimicrobials such as plant extracts to modify rumen microbial fermentation due to reduction on the use of antibiotics in animal feeds and with ban on antibiotics in the Europe. These compounds are known to have an impact on rumen microbial metabolism by inducing changes in the fermentation conditions and also affecting ruminant productivity. The methods to effectively reduce methane production in the reticulo-rumen include processing of feeds, altering the diet, supplementation of unsaturated fatty acids, defaunation, supplementation of organic acids, halogenated compounds, ionophores, microbial feed additives (probiotics), plant extracts and their secondary metabolites. All these strategies to be validated in vivo studies in specific dose(s) to make it economically viable. This review aimed to reveal the impact of these natural plants extracts on rumen microorganisms. Plants normally contain a high to medium content of secondary compounds such as essential oil, saponins, organosulphur and tannins, which exert a specific effect against or favour rumen microorganism and reduce methane, leaving less or no distortion on the remaining rumen biomass. Their actions include direct action on microbial metabolism and deprivation of substrates for microbial growth. These plant extracts require more long term studies *in vivo* to determine their effectiveness in commercial systems. For sustainability, the feed additive would need to be effective over long periods of time, non-toxic for animals, the environment, the consumers and maybe standard use in animal feeds.

Keywords: Fermentation, methanogenesis, microbial population, rumen microorganism

Introduction

The digestive system of ruminant animals posses different microorganisms that have a great influence on the transformation of feed into end-products ([McSweeney & Mackie, 2012](#)). Thus, it is important to study microbial ecology of gastro-intestinal tract of these ruminants, investigation of the organisms present (abundance and diversity), their activity and their relationship and the host ([McSweeney & Mackie, 2012](#)).

Methane expelled by ruminants is on of the most important source of global warming, having a

great impact on the environmental. Greenhouse gas emission, especially methane from ruminants, has been recognized as an important issue worldwide as it is a driver for global warming and climate change ([Kim et al., 2012](#)). It has been reported that livestock production and its by-products are responsible for at least 51 percent of global warming gases ([Goodland & Anhang, 2009](#)). Gas emissions from the livestock sector are estimated at between 4.1 and 7.1 billion tonnes of carbon dioxide equivalents per year, equating to 15-24% of total global anthropogenic green house gas emissions ([Wanapat et al., 2013](#)). Ruminant

nutritionists have examined or evaluated many substances such as essential oil, plant and plant extracts, plant secondary metabolites, etc... to in order to manipulate the rumen microbial ecosystem to reduce methane emission ([Kim et al., 2012](#)). Thus, from the aforementioned parameters there is need to search for more natural alternatives to modify/manipulate the rumen environment the need to identify feed additives to modify ruminal fermentation characteristics and increase the efficiency of feed utilization, and inhibiting the ruminal methanogenesis.

Microbial fermentation and physical breakdown during rumination are a combination of factors responsible for feed degradation in the rumen ([Hart et al., 2008](#)). Fermentation by microorganisms is carried out by a mixed population of bacteria and ciliate protozoa, together with smaller (Archae) and metabolically important population of anaerobic fungi ([Dehority, 2010](#)).

Non-therapeutic levels of antibiotics are usually included in the diet to increase feed efficiency and prevent disease in livestock production ([Benchaar et al., 2008a](#), [Zawadzki et al., 2011](#), [Valero et al., 2014b](#)). The use of antibiotics in livestock diet for disease prevention has been banned in the European Union because of the emergence of multi-drug resistant bacteria that may pose health challenge ([Russell & Houlihan, 2003](#)). Restrictions placed on the usage of this antibiotics and consumers health concern on the use of antibiotics in animal feeds had increased interest in the exploration of alternatives to antibiotics in manipulating rumen fermentation ([Yildiz et al., 2015](#), [Tomkins et al., 2015](#)). As a result, studies are concentrated on the use of substances such as probiotics, prebiotics, organic acids, enzymes and plant extracts ([Geraci et al., 2012](#), [Kim et al., 2012](#), [Patra & Saxena, 2010](#)). Natural plants products are thus considered in this review as alternatives to antibiotics in manipulating rumen fermentation and their effects on rumen microbial population ([Prado et al., 2016](#), [Cruz et al., 2014](#), [Valero et al., 2014a](#)). Therefore, decreasing enteric methane production by ruminants without altering animal performance is desirable as way of reducing global green house gas emissions and as a strategy for improving feed conversion efficiency ([Martin et al., 2010](#)).

[Murray et al. \(1976\)](#) reported that rumen is the origin of the most enteric methane produced by ruminants. Anaerobic digestion of feed components in the rumen by microorganisms results in the production of volatile fatty acids, mainly acetate, propionate and butyrate ([Martin et al., 2010](#)). These volatile fatty acids are used by ruminants as energy source. [Martin et al. \(2010\)](#) reported that protozoa are largely responsible for methane production in the rumen ecosystem and some lipids, saponins, tannins and ionophores are toxic to protozoa. [Newbold et al. \(2004\)](#) has shown that methane production has reduced by almost 50% by removal of protozoa from rumen depending on the feed administered.

A great variety of secondary compounds (saponins, organosulphurs, gingerol, flavonoids, alkaloids and tannins) are produced by plants to provide protection against any kind of attack ([Iason, 2005](#)). These compounds have been shown to exert specific effect against rumen protozoa while leaving the rest of the rumen biomass unaltered ([Wang et al., 2000](#)).

Essential oils

Essential oils are volatile, aromatic compounds extracted from plants by solvent extraction or pressure extraction under liquid carbon dioxide, steam distillation ([Greathead, 2003](#)). They are a variable mixtures terpenoids and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, and sometimes N- and S-containing compounds, coumarins, and homologues of phenylpropanoids ([Dorman & Deans, 2000](#)). They have been shown to exhibit antimicrobial properties against different types of microorganisms including bacteria, protozoa, and fungi ([Helander et al., 1998](#), [Greathead, 2003](#), [Busquet et al., 2006](#), [Macheboeuf et al., 2008](#)).

The main effects of essential oils in the rumen include reduction of protein and starch degradation and inhibition of amino acid degradation, due to selective action on certain rumen microorganisms, especially some bacteria ([Yildiz et al., 2015](#)). It has been reported that they affect rumen bacterial colonization especially in starch rich substrates and inhibition of hyper ammonia producing bacteria involved in amino acid deamination ([Hart et al., 2008](#)). The main antimicrobial mechanisms of

essential oils are on cell membrane ([Calsamiglia et al., 2007](#)) affecting electron transport, ion gradients, protein translocation, phosphorylation steps, and other enzyme-dependent reactions ([Ultee et al., 2000](#), [Dorman & Deans, 2000](#), [Ultee et al., 1999](#)). [Chao et al. \(2000\)](#) have reported that Gram-negative bacteria tended to have a higher resistance to essential oils than Gram-positive bacteria due to

the barrier of the cell wall structure not tolerating the inflow of the secondary metabolites easily. A lot of successes have been achieved by researcher on using essential oils to improve rumen fermentation, inhibition of methanogenesis, improve metabolism of protein and feed utilization efficiency ([Patra, 2011](#), [Baraka & Abdl-Rahman, 2012](#)).

Table 1. Results from different articles using plant extracts and essential oil on rumen microorganisms and observations

Name of Authors	Title of the research	Outcome with respect to rumen microbes and products
Wang et al. (2000)	Effect of steroidal saponin from <i>Yucca schidigera</i> extract on ruminal microbes	<p>Steroidal saponin isolated from <i>Yucca schidigera</i> repeatedly and substantially depressed the activity of cellulolytic ruminal bacteria and fungi whereas its effect on the non-cellulolytic ruminal bacteria examined were variable and species dependent.</p> <p>Among the four non-cellulolytic species examined in their study, bacterial responses included prolonged lag time (<i>Prevotella bryantii</i> and <i>Streptococcus bovis</i>), increased cell lysis subsequent to attainment of stationary phase (<i>Prevotella bryantii</i> and <i>Ruminobacter amylophilus</i>) and, extensive growth prior to stationary phase (<i>Selenomonas ruminantium</i>).</p> <p>All the Gram-positive bacteria examined were inhibited in this trial, whereas the Gram-negative species were unaffected.</p>
McIntosh et al. (2003)	Effects of essential oils on ruminal microorganisms and their protein metabolism	<p>The research demonstrated that commercial blend of essential oil compounds containing thymol inhibited the growth of a specific group of ammonia hyper-producing bacteria especially <i>Clostridium sticklandii</i> and <i>Peptostreptococcus anaerobius</i>. Blend of essential oil had no effect on protozoans <i>in vitro</i> but significantly decreased the growth of the anaerobic rumen fungus <i>Neocallimastix frontalis</i>.</p> <p>The research also found out that some pure strains of rumen bacteria could adapt to growth in the presence of essential oil when introduced in a stepwise way by increasing the essential oil concentrations. Bacteria did significantly differ in their sensitivity to blend of essential oil as the growth of <i>Clostridium sticklandii</i> decreased by 50% at a concentration of 36 mg/l while <i>Streptococcus bovis</i> growth was unaffected until concentrations reached 240 mg/l.</p>
Patra et al. (2006)	Effect of plant extracts on <i>in vitro</i> methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo	<p><i>Terminalia chebula</i>, <i>Acacia concinna</i> and <i>Azadirachta indica</i> extracts resulted in a significant reduction in protozoa count. Other extracts used had no influence on total protozoal counts. Similar trends were observed for small and large entodiniomorphid protozoa counts.</p> <p>Holotrich protozoal were not affected by the extracts.</p>
Benchaa et al. (2007)	Effects of essential oils and their components on <i>in vitro</i> rumen microbial fermentation.	<p>Rumen Gram-negative bacteria were sensitive to carvacrol and eugenol while Gram-positive bacteria were not. Carvacrol and eugenol also inhibited propionate-producing bacteria and favoured butyrate-producing bacteria.</p> <p>Inhibition of the growth of amylolytic bacteria accounts for reduction in the molar proportion of propionate observed in the study.</p> <p>None of the essential oil tested and compounds examined affected ammonia concentration, suggesting that deaminative activity of ruminal bacteria was not affected by essential oil and essential oil compounds.</p> <p>Compounds Phenolics used in the study exhibit broad spectrum antimicrobial activity against rumen microbes at high concentrations.</p>
Fraser et al. (2007)	Assessment of the Effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems	<p>Protozoa numbers were found to decrease with the use of essential oil from cinnamon (<i>cinnamomum verum</i>) leaf than with control in both the rumen simulation technique (Rusitec) and dual-flow (DF) fermenter used.</p>

Table 1. Collections of various results from different articles using plant extracts on rumen microorganisms and observations (Continuation)

Name of Authors	Title of the research	Outcome with respect to rumen microbes and products
Öztürk et al. (2012)	Effects of olive leaf extract on rumen microbial fermentation in <i>in vitro</i> semi-continuous culture system (RUSITEC)	There was significant decrease in the number of rumen ciliate protozoan while the numbers of bacteria and starch degradation increase with reduction in NH ₃ production. Olive leaf (<i>Olea europaea</i>) extract inhibit the growth of both Gram positive and Gram negative bacteria.
Kim et al. (2012)	Effects of plants extracts on microbial population, methane emission and ruminal fermentation characteristics in <i>in vitro</i>	<p>Worm wood (<i>Artemisia absinthium</i>), garlic (<i>Alium sativum</i>), onion (<i>Alium cepa</i>), ginger (<i>Zingiber officinalis</i>), mandarin orange (<i>Citrus reticulata</i>), and honeysuckle (<i>Lonicera sempervirens</i>) were the plant extracts used and they all decreased the population of ciliate-associated methanogen, thereby, reducing methane emissions.</p> <p>Garlic and ginger extracts were found to have decreased the protozoa population resulting in reduction of methane emission in the rumen and thus inhibiting methanogenesis.</p> <p>The addition of wormwood, garlic, mandarin orange and honeysuckle extracts increased the population of <i>Fibrobacter succinogenes</i> in particular to a greater extent than other fibrolytic microorganisms.</p> <p>The <i>Ruminococcus albus</i> diversity in added onion extract increased, while other extracts did not influence the <i>R. albus</i> community.</p> <p><i>Ruminococcus flavefaciens</i> population decreased with the addition of wormwood and garlic extracts, but increased with the addition of other extracts.</p>
Patra & Yu (2012)	Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations	<p>Total gas and methane production by the ruminal cultures decreased linearly with increasing doses of all essential oils. All the essential oils decreased the abundance of archaea, protozoa, and major cellulolytic bacteria (<i>Fibrobacter succinogenes</i>, <i>Ruminococcus flavefaciens</i>, and <i>R. albus</i>) as determined by quantitative real-time PCR.</p> <p>On the basis of denaturing gradient gel electrophoresis analysis, different essential oils changed the composition of both archaeal and bacterial communities to different extents.</p>
Sirohi et al. (2012)	Efficacy of different methanolic plant extracts on anti-methanogenesis, rumen fermentation and gas production kinetics in <i>in vitro</i>	Methanolic extracts of three plants, mehandi (<i>Lawsonia inermis</i>), jaiphal (<i>Myristica fragrans</i>) and green chili (<i>Capsicum annum</i>) were found to have no significant effect on protozoan populations.
Oskoueian et al. (2013)	Effects of flavonoids on rumen fermentation activity, methane production, and microbial population	<p>Addition of naringin and quercetin had no significant effects on the population of bacteria, fungi, <i>Fibrobacter succinogenes</i>, <i>Ruminococcus albus</i>, and <i>Ruminococcus flavefaciens</i> when compared to the control.</p> <p>The flavonoids significantly suppressed the population of total protozoa and total methanogens. The addition of flavone, myricetin, catechin, rutin, and kaempferol significantly reduced the population of almost all of the rumen microorganism.</p>
Kim et al. (2014)	Effects of coconut materials on <i>in vitro</i> ruminal methanogenesis and fermentation characteristics	Researchers found out that coconut (<i>Cocos nucifera</i>) oil and coconut powder decreased in the diversity of methanogens and ciliate-associated methanogens compared to the treatment that has no addition of the oil and the powder.
Yildiz et al. (2015)	Determination of the effects of some plant extracts on rumen fermentation and protozoal counts by Hohenheim <i>in vitro</i> gas production technique	<p>Essence oils of <i>Thymu vulgaris</i>, <i>Oreanum vulgare</i> and <i>Syzygium aromaticum</i> tested demonstrated antibacterial effects on <i>in vitro</i> conditions and lead to reduced protozoal counts.</p> <p><i>Zingiber officinale</i>, compared to the positive and negative controls, had higher protozoal while other essence oils had reduced protozoal counts.</p>

Saponins

Saponins are secondary compounds produced mainly by plants, but also by lower marine animals and some bacteria ([Riguera, 1997](#), [Yoshiki et al., 1998](#)). They are natural detergents defined chemically as high-molecular-weight glycosides in which sugars are linked to a hydrophobic aglycone (sapogenic) which may be triterpenoid or steroidal in nature ([Hart et al., 2008](#)). The complex and diverse structures of saponin arise from the variability of the aglycon structure, the nature of the side chains and the attachment position of these moieties on the aglycone. [Chwalek et al. 2004](#), [Jung et al. 2004](#) have reported that the type of linkage and the composition of sugar of the saponin are related directly to their biological activity. They reduced protein degradation and favour microbial protein and biomass synthesis ([Makkar et al., 1999](#)), which resulted in reduced availability of hydrogen for CH₄ production ([Dijkstra et al., 2007](#)). It has been reported that saponins tend to have dramatic effect on the activity of rumen protozoa ([Teferedegne et al., 1999](#)).

Saponins affect protozoa by forming complexes with sterols in the protozoal membrane surface ([Wina et al., 2005](#)). The membranes become impaired and ultimately disintegrate ([Wallace et al., 2002](#)). Fermentation in the rumen is also modified by suppression of rumen protozoa and selectively inhibiting some bacteria ([Jayanegara et al., 2009](#)). Thus, saponins rich plants have potential for enhancing flow of microbial protein from the rumen, increasing the efficiency of feed utilisation, and decreasing methanogenesis ([Jayanegara et al., 2009](#)).

Organosulphurs

Garlic (*Allium sativum*) has been used medicinally since ancient times in nearly every known civilization ([Rivlin, 2006](#)). *Allium* species contains many sulphur-containing compounds ([Hart et al., 2008](#)) produced from thiosulphates during the steam treatment of the plant ([Pentz & Siegers, 1996](#)). The major sulphur-containing compounds in garlic are γ -glutamyl-S-allyl-L-cysteins and S-allyl-L-cysteine sulfoxides (alliin), which are converted into thiosulphinates (such as allicin) through enzymic reactions when raw garlic is cut or crushed ([Stoll & Seebeck, 1948](#)). During processing of garlic, a broad range of different

organosulphur compounds are produced which includes thiosulphinates, volatile organosulphurs and water-soluble organosulphurs ([Iberl et al., 1990](#), [Freeman & Kodera, 1995](#), [Kodera et al., 2002](#)).

Garlic products are classified into four groups which are garlic essential oil, garlic oil macerate, garlic powder and garlic extract ([Amagase, 2006](#)). Due to the complexity of the chemical structure of garlic, their final concentration in each preparation varies significantly and also depends heavily upon the processing method used ([Hart et al., 2008](#)). The main antimicrobial effect is the ability to interact with the sulphydryl groups (-SH) of other active compounds ([Reuter et al., 1996](#)) resulting in instability of the cell membrane of some specific microbial groups ([Calsamiglia et al., 2007](#)).

The main antimicrobial constituents of garlic have been tested *in vitro* to determine their effect on rumen microbial fermentation. [Busquet et al. \(2005\)](#) tested the effect of allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan as well as garlic oil and found that garlic oil, diallyl disulphide and allyl mercaptan reduced acetate and methane while there was increase in propionate and butyrate concentrations. In contrast, allicin and diallyl sulfide had minor effects on rumen microbial fermentation (i.e the result of the direct inhibition of Archaea microorganisms in the rumen through the inhibition of HMG-CoA reductase ([Calsamiglia et al., 2007](#), [Kamel et al., 2008](#)).

Tannins

Tannins are secondary plant compounds with various molecular weights ([Jayanegara et al., 2012](#)). They are found in cell walls, within vacuoles in stems, bark, leaves, flowers, or seeds in dicotyledonous plants ([Archana et al., 2010](#)). Based on their molecular structure they are classified into hydrolysable (polyesters of gallic acid and various sugars) and condensed tannins (polymers of flavonoids) ([McSweeney et al., 2001](#)). [Frutos et al. \(2004\)](#) described hydrolysable tannins as been made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid). Condensed tannins are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3,4-diol) which usually have a higher molecular weight than hydrolysable tannins. It is believed that tannins decrease the rate

of protein degradation in the rumen, slowed down the rates of both solubilisation and degradation of forage protein to ammonia (Patra & Saxena, 2011).

The effects of tannins on ruminal bacteria are reported to be dependent upon the species of microorganism and type or source of tannin (Jones et al., 1994, Sivakumaran et al., 2004). It has been observed that the growth of proteolytic bacteria such as *Butyrivibrio fibrisolvens*, *Ruminobacter amylophilus* and *Streptococcus bovis* were reduced by condensed tannins of sainfoin (*Onobrychis viciifolia*) and had little effect on *Prevotella ruminicola* (Jones et al., 1994).

Tannins are also thought to suppress internal parasites and methanogenesis in ruminants (Patra & Saxena, 2011, Jayanegara et al., 2012). The inhibitory effects of tannins on rumen methanogenesis have been attributed to their direct effects on methanogenic archaea and protozoa, indirectly leading to a depression of fibre degradation in the rumen (Patra & Saxena, 2011). Wang et al. (2009) had shown that the addition of 0.5 g L⁻¹ phlorotannins to rumen cultures inhibited the growth of *Fibrobacter succinogenes*, but had minimal effect on *Ruminococcus flavefaciens* and *R. albus*, whereas the growth of *Selenomonas ruminantium*, *S. bovis*, *Ruminobacter amylophilus* and *Prevotella bryantii* were stimulated.

Tannic acid had been demonstrated to inhibit cellulolysis and zoospore attachment to cellulose of the fungus *Neocallimastix frontalis* strain RE1 (Muhammed et al., 1995). The mechanisms of inhibition of rumen protozoa are not known (Patra & Saxena, 2011), but tannins have been demonstrated to linearly increase the population of protozoal in rumen fluid of sheep fed with lucern hay (Salem et al., 1997). Benchaar et al. (2008b) did not observe any effect on protozoal numbers in dairy cattle fed quebracho tannins.

Conclusion

Different strategies have been explored to mitigate methane production using feed additives in ruminants. Plant extracts (essential oils, saponins, tannins and organosulphur compounds) have been exploited to manipulate rumen fermentation. Careful selection and combination of these additives may affect manipulation of protein degradation and methanogenesis in the rumen.

Effects of each extract is varied which is as a result of the concentration and nature of the active ingredient in the extract, diversity of forage available, intake, age of plant and other environmental influences. However, a universal extract that works under different conditions may not exist because effects are diet and pH dependent. All substances require more *in vivo* studies over a long period of time to determine whether the effect will be sustained. Successful implementation will also depend on the cost-effectiveness and practicality in commercial farm systems.

Conflict Interest

There was no conflict interest among the authors.

Acknowledgement

This review was written when the first author was under the financial support of TWAS (The world Academy of Science) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazilian Scientific and Technological Development Council).

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Received in January 21 2016

Accepted in February 22, 2016

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