

## The Effect of Medicinal Plants as a Feed Additive in Ruminant Nutrition

### Review Article

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### ABSTRACT

Since the use of antibiotics in animal feeds has been banned, researches on alternative natural products that modulate ruminal fermentation have been intensified. Natural compounds such as plant extracts have been considered to be replaced antibiotics as safe and sustainable alternatives. Plant extracts especially essential oils and saponins have strong antimicrobial, antiparasitic, antiprotozoal and anti-inflammatory properties. These natural compounds have been modulated the ruminal fermentation to improve nutrient utilization in ruminants. Therefore, the purpose of this review is to provide an overview of the literature surrounding use of herbal plants as feed additives in ruminant nutrition.

**KEY WORDS** aromatic plant, essential oil, fermentation, ruminant, saponin.

### INTRODUCTION

Antibiotic feed additives, such as ionophore antibiotic monensin, have been used widely in ruminant production systems for many years, to improve daily gain and feed conversion rates. However, the use of antibiotics in animal nutrition has been prohibited in the European Union since January 2006 because of the potential for the selection of antibiotic-resistant bacterial strains by the risk of antibiotic residues in milk and meat products exists. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Plant extracts offer a unique opportunity in this regard (Wallace, 2004), as many plants produce secondary metabolites, such as saponins and tannins, which have antimicrobial properties. These compounds have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Wang *et al.* 1998; Hristov *et al.* 1999). The secondary metabolites differing from the ubiquitous primary metabolites (e.g. carbohydrates, protein, fats and nucleic acids) in that their distri-

bution is limited. Plant secondary metabolites are a natural resource that is largely unexploited in conventional animal production system. Essential oils (EO) are complex mixture of secondary metabolites consisting of low-boiling point, phenylpropens and terpenes. They are particularly associated with plants defined as herbs and spices (Greathead, 2003). It has been suggested that one of the reason for the beneficial effects of plant extracts is related to the influence on microbial fermentation. The beneficial effects have also been attributed to the binding of ammino to saponins. Finally, herbs can contribute to nutrient requirements, stimulate the endocrine system and affect intermediate nutrient metabolism (Wang *et al.* 1998; Wenk, 2000).

### Definition and chemistry

#### Aromatic plants

Aromatic plants, also known as herbs and spices, have been used in the Middle East since approximately 5000 BC for their preservation and medicinal properties, in addition to enhancing the aroma and flavor of foods (Li, 2006). Their

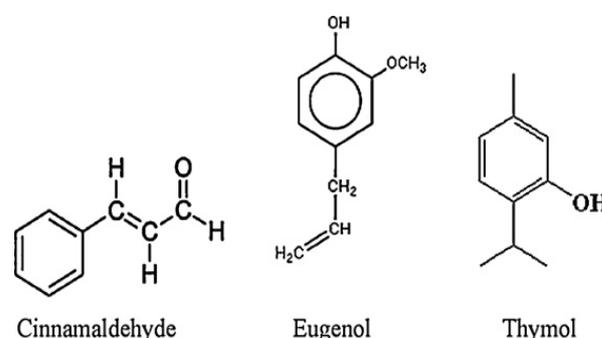
use continues undiminished today and according to the world health organization (WHO) nearly 80% of the plant's population, especially in developing countries still depends on plant produced medicines for their health care (Gurib-Fakim, 2006). Additionally, feed additives derived from plants, also called phytonics or phytobiotics, botanicals can be included in animal diets to improve their productivity and the properties of the resulting feed and animal products. Among these natural additives, aromatic plants, their extracts and their essential oils have been examined due to their advantages over the antibiotics as growth promoters. They are residue free and generally recognized as safe (Brenes and Roura, 2010).

Many herbs and spices can be found worldwide, with many originating from the Mediterranean area, either in the wild or cultivated, such as rosemary, oregano, sage, thymus (Negi, 2012). Some bioactive compounds show therapeutic value, such as antioxidant and antiseptic activities (Li, 2006). Thus, they may reduce the risk of cancer or cardiovascular diseases and may find application as treatments in curing or managing a wide range of ailments such as respiratory diseases and stomach or inflammatory disorders (Kadri *et al.* 2011). Generally, the bioactive components in the aromatic plants possess the ability to protect the body from damage caused by free radicals induced oxidative stress by quenching singlet oxygen and inducing cytochrome or other enzymes (Couladis *et al.* 2003). Moreover, herbs and spices can inhibit oxidative rancidity and delay the development of off-flavor in some products. They also contain antimicrobial compounds which contribute to the retardation of microbial growth on foods, especially snack foods and meat products (Elgayyar *et al.* 2001; Li, 2006).

### Essential oil

Essential oils, also known as volatile or ethereal oils, occur in edible, medicinal, and herbal plants. As these aromatic compounds are largely volatile, they are commonly extracted by steam distillation or solvent extraction (Greathead, 2003). There are more than 3000 plants used for their essential oils of which about 300 are used commercially as flavors and fragrances. Essential oils can be extracted from many parts of a plant, including the leaves, flowers, stem, seeds, roots and bark. However, the composition of the essential oil can be varying among different parts of the same plant (Dorman and Deans, 2000). For instance, essential oils obtained from the seeds of coriander (*Coriandrum sativum*) have a different composition from the essential oil of cilantro, which is obtained from the immature leaves of the same plant (Delaquis *et al.* 2002). Chemical differences among the essential oils extracted from individual plants, or different varieties of plants, also exist and are attributed to genetically determined properties,

age of the plant, and the environment in which the plant grows (Cosentino *et al.* 1999). For instance, Martinez *et al.* (2006) observed that the concentration of carvacrol, thymol, p-cymene, and  $\gamma$ -terpinene in thyme essential oils varied widely depending on the species of the thyme plant. Chemically, essential oils are variable mixtures of principally terpenoids, mainly monoterpenes (C<sub>10</sub>), sesquiterpenes (C<sub>15</sub>), although diterpenes (C<sub>20</sub>) may also be presented, and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally N- and S-containing compounds, coumarins and homologues of phenylpropanoids (Dorman and Deans, 2000). They have shown biological activities such as anti-oxidative, fungicidal and antimicrobial activity. Essential oils are the most concentrated form of phytobiotics, which are increasingly used in the animal feed industry.



**Figure 1** Examples of chemical structures of some essential oil compounds

### Saponins

Saponins are one of the most important active substances found in plant extracts. The detergent action of saponins kills rumen protozoa. The susceptibility of ruminal ciliate protozoans to saponins is probably expected due to the presence of cholesterol in eukaryotic (including protozoa) cells membranes, but not in prokaryotic bacterial cells, as saponins exhibit an affinity towards cholesterol (Klita *et al.* 1996). Suppression or elimination of protozoa may enhance the flow of microbial proteins from the rumen, increase the efficiency of feed utilization and increase the nutrition of the animal, provided that the loss of protozoa does not impair the fiber breakdown (Newbold *et al.* 1997). The ultimate effect of feeding saponin containing plant materials increased animal production as it was observed in the positive response to the inclusion feeding of antibiotics or other synthetic chemicals (Sultana *et al.* 2012). Numerous studies have been conducted to determine the effects of feeding ruminants with saponin-rich plants (Makkar *et al.* 1998; Wang *et al.* 2000; Sultana *et al.* 2012). These results have indicated that the saponins have strong antiprotozoal activity and might serve as an alternative to, infeed antibiotics or

growth hormone used for ruminants through its defaunating properties.

Saponins are a group of naturally occurring compounds which have properties resembling soaps and detergents. They are complex and chemically diverse group of compounds, mainly of plant origin, but also accruing in a number of marine animals. Their physiological effects are as diverse as their chemical structures and properties. Saponins have a number of common and characteristics properties which included bitter taste, formation of stable foams in aqueous solution, hemolysis of red blood cells, toxicity of cold-blooded animals such as fish, snails, insects, an ability to interact with bile acids, cholesterol or other 3- $\beta$ -hydroxy steroids in aqueous or alcoholic solutions to form mixed micelles or coprecipitates (Cheeke, 1995).

## Herbal plant properties

### Antimicrobial activity

Limited data are available on the effect of plant extracts on rumen microbial fermentation. Oh *et al.* (1967) showed that essential oil extracted from Douglas fir needles (*Pseudotsuga menziesii*) exerted a general inhibitory effect on ruminal bacteria activity *in vitro*. Nagy and Tengerdy (1968) were the first to investigate effects of essential oils on ruminal microbial fermentation, as gas production, *in vitro*.

Nagy and Tengerdy (1968) observed that essential oils extracted from sagebrush (*Artemisia tridentate*) markedly inhibited activity of ruminal bacteria *in vitro*. The antimicrobial properties of EO have been demonstrated against a wide range of microorganisms, including bacteria, protozoa and fungi (Chao *et al.* 2000). Essential oils have also been exploited for their activity against a wide variety of food-borne pathogens.

For example, *Escherichia coli* O157:H7 was inhibited by oregano oil and its two main components carvacrol and thymol (Elgayyar *et al.* 2001). Lambert *et al.* (2001) observed that the combination of thymol and carvacrol exhibited higher antibacterial activity than either compound alone and that the inhibitory effect of oregano essential oil is mainly due to the additive antibacterial action of these two compounds. Delaquis *et al.* (2002) examined the antibacterial activity of crude oils and the distilled fractions of dill (*Anethum graveolens*), coriander (seeds of *Coriandrum sativum*), cilantro (leaves of immature *C. sativum*), and eucalyptus (*Eucalyptus dives*) against some common Gram- positive and Gram- negative food spoilage bacteria (i.e. *Salmonella typhimurium*, *Listeria monocytogenes*, *S. aureus*, *P. fragi*, *Serratia grimesii*, *Enterobacter agglomerans*, *Bacillus cereus*). Results showed that the magnitude and the spectrum of antibacterial activity of these individual fractions frequently exceeded those of the crude oils.

It is generally accepted that phenolic compound have the greatest antimicrobial activity among the secondary metabolites found in essential oils (Dorman and Deans, 2000). Such examples are the monoterpenes carvacrol and thymol and the phenyl propene eugenol (Benchaar *et al.* 2009). In addition, non-phenolic secondary metabolites found in essential oil have a variable antimicrobial capacity. Some researchers (Dorman and Deans, 2000) reported that cinnamaldehyde, a non-phenolic phenylpropene exhibits strong antimicrobial activity. Some aromatic plants and their extracts have been reported to stimulate the growth of certain bacteria.

Previous *in vitro* continuous culture studies conducted in our laboratory (Cardozo *et al.* 2004; Busquet *et al.* 2005) with doses from 0.22 to 2.2 mg/L of culture fluid of different plant extracts and secondary metabolites demonstrated the potential of some extracts to modify rumen microbial fermentation. As already mentioned the antimicrobial properties of the aromatic plants are partially attributed to their essential oils. It is suggested that the hydrophobicity of the essential oils and their components is an important characteristic that enables the essential oils to accumulate in the lipid bilayer of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Solorzano-Santos and Miranda-Novales, 2012). Ruminant nutritionists were interested in essential oil mainly because of their role in reducing the palatability of some plant species.

### Effects on utilization of nutrients

Feeding ruminants in intensive production systems, especially for dairy production, requires supplies of high levels of energy and proteins. Plant extracts form dietary proteins complexes that also protect them from microbial fermentation. Once they bypass the rumen, the complexes dissociate under the acidic conditions in the abomasum and proteins become available to dissociate under the acidic conditions in the abomasums and proteins become available to the host animal. Evidence for *in vivo* antimicrobial activity of aromatic plants essential oils have not yet been confirmed in ruminants, although oils have been used to manipulate ruminal metabolism in order to improve feed efficiency and animal productivity (Benchaar *et al.* 2009). Nevertheless, results from *in vitro* batch culture studies provide evidence that essential oils or their components have the potential to improve nitrogen and / or energy utilization in ruminants by altering microbial populations. These responses are only observed with high doses of essential oils, which also can inhibit the process of ruminal fermentation causing a decline in total volatile acid production, although rumen microbial populations adapt after long term exposure to these substances (Lorenzi *et al.* 2009). Fernandez *et al.* (1997)

showed that a commercial product of blended essential oil compounds inhibited the degradation of protein in the rumen, thus potentially increasing the protein supply to the post-ruminal tract.

Plant extracts (especially saponins) destroy rumen fungi along with the protozoa and have foam-stabilizing properties that may enhance bloat, especially under high protein feeding regimes (Hofmann *et al.* 2003). Makkar *et al.* (1998) investigated effects of *Yucca schidigera* (YS), *Quilajajasaponaria* and *Acacia auriculiformis* saponin containing on rumen fermentation. Ammonia levels and protozoal counts at 24 h in an *in vitro* rumen fermentation system were also reduced by saponins; decreases were as high as 30 and 63%, respectively.

#### Effects on fermentation and methane emission

Ruminal methane production represents a loss of 2 to 15% dietary energy in ruminant animals. Reducing ruminal methane production could improve animal performance. Essential oil extracted has a direct effect on bacterial cell and membranes due to their hydrophobic nature and lipophobic character, so they had a high affinity for lipids of bacterial cell membranes (Benchaar *et al.* 2008). It was reported that essential oils (Dong *et al.* 2010) decreased methane production. Antibacterial activities of phytochemical products were also reported from a variety of nonphenolic substances (Dong *et al.* 2010). Garcia-Gonzalez *et al.* (2008) reported that all of the phytochemical products caused significant reduction of methane production in the mixed grass diet, whereas only one or two of the phytochemical products exhibited inhibitory effects on methane production in other types of diets. Therefore, the inhibitory effects of the phytochemical products on methane production appear to be diet type dependent, which may have been due to different dietary nutrient composition and balance. It seems that the inhibitory effects of the dietary nutrient composition are balance. It seems that the inhibitory effects of the phytochemical products on methane production are lessened in more nutritionally-balanced diets. Similarly, Busquet *et al.* (2005) reported that garlic oil (312 mg/L) reduce acetate and increased propionate proportions in a manner consistent with decreased methane production *in vitro*. Patra *et al.* (2005) reported that ethanol and methanol extracts of cloves and the methanol extract of fennel also inhibited methane production *in vitro*. Studies showed saponins decreased methane production; Saponins also have anti-protozoal effects (Hue *et al.* 2005). One possible mechanism to explain the effect of saponins on protozoa is that saponins can change cell membrane permeability. Since protozoa produce a large amount of hydrogen, methanogens are attached to the surface protozoa (Lee *et al.* 2000) to utilize hydrogen. The reduction of protozoa numbers, therefore, favors a decrease in methane

production. Some studies reported that the extracts of *Acacia concinna*, *Azadirachta indica* and *Terminaliachebula* reduced total protozoa counts significantly. Yañez-Ruiz *et al.* (2004) and Ozturk *et al.* (2012) reported that protozoa concentrations in the rumen of animals, fed by oliveleaf were lower than in animals fed by standard diets.

#### Effects of volatile fatty acid production

Volatile fatty acids (VFA) are the end products of rumen microbial fermentation and important energy substrate for ruminants and approximately 70% of the metabolizable energy of ruminants has been supplied by VFA (Christaki *et al.* 2012). It is interesting to point out that when supplied at high levels (3000 mg/L) most essential oils and their secondary constituents reduced the total VFA concentration compared with control, which was consistent with their antimicrobial activity (Davidson and Naidu, 2000). Total VFA productions have been reported as lower, higher, and not different when plant extracts or plant essential oil were tested (Christaki *et al.* 2012).

These differing results may be partially explained by the experimental conditions of these studies, including type of diets, plant species and / or their active substances used and pH values of rumen fluid. Supplementation, while essential oil has increased ruminant total VFA concentration, may indicate improved feed digestion, in a limited number of studies. Mohammad *et al.* (2004) reported that increasing levels (i.e., from 0.17 to 1.7 g/L) of cyclodextrin encapsulated horseradish linearly increased total VFA concentration in batch culture. When the same product was fed to cattle, there was a very small increase in total VFA, but no change in feed digestibility. In one such study, addition of 1.5 mg/L mixture of essential oil increased total VFA concentration in continuous cultures maintained at constant pH, although there was no concomitant increase in organic matter digestibility (Castillejos *et al.* 2005). Evans and Martin (2000) observed that thymol, a primary component of some essential oils, modified the concentration of VFA *in vitro* incubations of ruminal fluid. When thymol was added to ruminal fluid at the level of 400 mg/mL, final pH and acetate to propionate ratio increased while propionate and lactate decreased. Two *in vivo* studies with mixture of essential oil reported no effects in sheep feeding (110 mg/day) or cattle (1 g/day) on total VFA concentration or proportions (Newbold *et al.* 2004; Beauchemin and McGinn, 2006). Busquet *et al.* (2006) studied the effect of various plant extracts (i.e. Anise oil, Cade oil, Capsicum oil, Cinnamum oil, Galric oil, Oregano oil, Tea tree oil and Yucca) and secondary metabolites (i.e. *Anethol*, *Benzyl salicylate*, *Carvacrol*, *Carvone*, *Cinnamaldehyde* and *Eugenol*) on ruminal fermentation in a 24 h batch culture. Each treatment was supplied at varying doses up to 3 g/L of culture fluid. None

of essential oils or secondary metabolites increased total VFA concentration but, at the highest concentration, most treatments decreased total VFA concentration, a possible reflection of decreased feed digestion.

## CONCLUSION

The main reasons for the wide use of antibiotic feed additives are beneficial effects on health status, performance, nutrient and energy utilization. The trend toward more natural animal production systems has led to an increasingly critical attitude on the part of consumers about in-feed antimicrobial agents. It is a common misconception that all plant extracts made from plants are safe because they are natural or organic. There are evidences that some essential oils reduce the rate of deamination of amino acids, rate of ammonia production and the number of ammonia producing bacteria. Therefore, natural plant extracts can be used to manipulate ruminal fermentation by selective modulation of certain microbial species. The possible use of natural plant products as a productivity enhancer provides cheaper, safer, sustainable and more consumer acceptable alternatives to synthetic compounds. Utilization of phytomedicines will not only improve the health of animals, but also support the farmer's income through productions.

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