A Review on Essential Oils as a Natural Alternative to Ionophores in Ruminant Diets

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Abstract

The potential threat to human health, associated with the use of antibiotics and chemical feed additives in animal feed has prompted legislation in the European Union to ban antimicrobial growth promoters such as monensin. This has accelerated investigations into the effectiveness of natural alternatives to ionophores such as plant extracts, more specifically essential oils. Ionophores, which are a potent antibiotic against gram positive bacteria, have been used for many years to improve the efficiency of energy and protein utilization by ruminants. Ionophores exert these effects by modifying ruminal fermentation through increased propionate production, decreased methane production and a decrease in the activity of proteolytic and obligate amino acid fermenting bacteria. Through its mode of action ionophores also contribute to a reduction in the incidence of metabolic disorders such as ruminal acidosis, bloat, laminitis and ketosis. A number of studies, mostly *in vitro*, have shown similarities in the antimicrobial and ruminal fermentation effects of ionophores and essential oils. This makes essential oils attractive alternatives to antibiotics to manipulate ruminal microbial activity. Many questions however still remain, since the effects of different combinations of essential oils seem to be dependent on the diet and feed ingredients, ruminal pH and dosage level. Furthermore whether or not the effect observed *in vitro* carry over to a much larger and diverse ecosystem such as a rumen needs to be investigated further. Essential oils hold promise as a natural alternative to antibiotics in ruminant nutrition to improve overall feed efficiency and productivity.

Introduction

The utilization of ionophores, a potent antibiotic against gram positive bacteria has been proven to be effective in improving the efficiency of energy and protein utilization by ruminants (Van Nevel and Demeyer, 1988). However, the use of ionophore antibiotics in animal feeds is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria due to excessive use of these products. For these reasons there has been a search for alternative products including yeasts, organic acids, plant extracts, probiotics and antibodies to replace antibiotics (Calsamiglia *et al*., 2006). The potential use of essential oils in ruminant diets was first reported during the 1960's by researchers (Borchers, 1965; Oh *et al*., 1967; 1968; Nagy and Tendergy, 1968). As a result of the approval of antibiotics and ionophores in animal feed as additives in the 1970's, few studies were published thereafter on the use of essential oils as feed additives (Broderick and Balthrop, 1979). Only after the ban on antibiotics being used as feed additives was announced by the EU, has there been a renewed interest in the use of essential oils as feed additives in the animal feed industry.

The purpose of this paper is to review available research reports involving ionophores and essential oils as feed additives for ruminants. Emphasis will be placed on the mode of action, effects on carbohydrate and protein metabolism, origin and classification, and activity of essential oils and effects on ruminant performance. The paper concludes with recommendations on future research needs.

Ionophores

The term ionophore was first used in 1968 to refer to all carboxylic polyethers that fit the classical definition of antibiotics (Pressman, 1968). These are naturally occurring compounds which are fermentation products of microbes, actinomycetes (Bagg, 1997). These fermentation products, ionophores, were originally developed as coccidiostats for the poultry industry (Richardson *et al*., 1976). Since about the middle of the 70's ionophores have been used extensively in ruminant feeds. Ionophores were used for the manipulation of ruminal fermentation and/or as a product of weight gain of growing ruminants. It also helped with the improvement of efficiency of feed utilization and this came as a result of the manipulation of rumen fermentation. There are several ionophores that are used commercially or that have been investigated in the past for use in ruminants. They are monensin, lasalocid, tetronarsin, salinomycin, lysocellin, narasin, nigericin, laidlomycin and valynomycin. Of all of these the best known and used in South Africa is monensin and lasalocid.

Ionophores are lipophilic compounds that are toxic to many bacteria, protozoa, fungi and higher organisms such as ruminants (Russell and Strobel, 1989). Ionophores are able to penetrate biological membranes because they are lipophilic. Their toxicity arises from their capacity to penetrate membranes and subsequently alter the flux of ions from and into the cell. At the level of microbial membranes, ionophores interfere with the flux of ions into and out of microbial cells. This is achieved in two ways, either by the formation of ion-ionophere complexes that function selective ion carriers or by the creation of selective pore that promotes a less specific influx and efflux of ions. In the first instance monensin and lasalocid are ionophores that function as selective ion carriers and gramicidin an ionophore that creates a selective pore for less specific ions (Bergen and Bates, 1984; Russell and Strobel, 1989). The only ionophores that are used are the ones that act as mobile carriers. Within the mobile carrier group, two different types of ionophores are found. The first are ionophores that serve as antiporters, they exchange H^+ cations for monovalent cations whereas the uniporters transport cations into the cell without an exchange for H^+. Examples of antiporters are monensin and nigericin and a uniporters is valynomycin (Fellner *et al*., 1997).

Monensin preferentially binds with Na^+ but also has the capacity to bind with K^+ and transport it across the cell membrane (Russell and Strobel, 1989). On the other side, tetronasin is highly selective to bind to divalent cations and its selectivity favours Ca^{++} and Mg^{++} in that sequence (Fellner *et al*., 1997). As there is an increase in the ion flux from the activity of the ionophores, there is also an increase in the activity of the Na/K pump and H^+ ATPase system of the bacterial cells. These two systems activity increases significantly in order to maintain the ion balance and the intracellular pH of the cell by pumping out Na/K and H^+. As a result there is an increase for energy for maintenance function of the bacterial cells. The use of energy for maintenance purposes depletes the cell of ATP. This depletion of energy compromises the bacterial cell's capacity to grow and to reproduce (Bergen and Bates, 1984).

The bacterial cell's sensitivity to the ionophore determines the severity of the effect of the ionophore. This in turn has been shown to depend on the permeability of the cell's membrane to the ionophore being used (Russell, 1996). Gram-positive bacteria have a less complex cell membrane, lacking a highly impermeable outer membrane; where as gram-negative bacteria have this outer membrane. This difference in membrane structure is what makes gram-positive bacteria more susceptible to the antibiotic effects of ionophores (Russell, 1996).

Ionophores and carbohydrate metabolism

The rumen is an anaerobic ecosystem where in ruminal microorganisms (bacteria, protozoa and fungi) ferment carbohydrates and proteins taken in by the host animal to obtain energy and nutrients required for their growth. Some of the microbes' end products of fermentation are sources of nutrients to the host. These include the volatile fatty acids (VFA) and microbial protein (energy and N sources). There are also other fermentation end products that in some cases are useless to the host like heat, methane and ammonia. These end products represent a loss of energy and protein to the environment and thus won't be used for maintenance or production (Owens and Goetsch, 1988). Gram-positive bacteria found in the rumen are involved in fermentation processes that produce end products like acetate, butyrate, formate, lactate, hydrogen and ammonia (Russell and Strobel, 1989; Russell, 1996). Some of these fermentation processes are coupled with the production of methane (CH_4). The last few years there has been a growing concern of the part that CH_4 plays in the greenhouse effect and how the production could be reduced from farm animals (Moss, 1993). Methane acts as a receiver for H^+ ions that is released during fermentation, and with the flow of H^+ to C to form CH_4, energy is lost. Gram-negative bacteria in the rumen are involved in fermentation pathways that are associated with the production of propionate and succinate. When gram-negative bacteria predominates the rumen environment, less CH_4 is produced due mainly to the decreased availability of H^+ ions and formate (Bergen and Bates, 1984). From this one can conclude that there are major benefits in feeding ionophores to ruminants. One of the benefits is the shift in VFA production. This shift is from acetate to propionate and the associated decrease in CH_4 production. This is because of a shift from acetate-producers (gram-positive bacteria) to propionate-producers (gram-negative bacteria).

The shift from acetate to propionate could be seen in a study done by Sauer *et al*. (1997). They fed 240 mg /d of monensin to lactating dairy cows in a TMR that contained 650 g /kg of roughage and 350 g /kg of concentrate, all on a dry matter (DM) basis. In this study monensin decreased the acetate to propionate ratio and the CH_4 output by 19 and 21% respectively, whereas the molar proportion of propionate was increased by 17%. The same could be seen in feedlot animals being fed diets that are typically fed to dairy cows. Feedlot steers were fed a diet containing approximately 500 g of forage and 500 g of grain concentrate per kg of DM. The studies showed that ionophores significantly increased propionate production. In one study a maximum increase of 76% over the control treatment was found (Van Maanen *et al*., 1978; Rogers and Davis, 1982).

As mentioned earlier a considerable amount of propionate is produced in the rumen when carbohydrates are fed to the animals. When the propionate passes across the rumen wall, a small percentage is converted to lactate. The remainder is transported via the portal vein to the liver where it is converted to glucose. The conversion of propionate to glucose yields a net gain of 17 moles of adenosine triphosphate (ATP). For acetate there is only a 12 mole increase in net gain of ATP. Thus for increases in production (e.g. growth and milk yield) it is more economical to have increased propionate production. However, when it comes to milk solids other VFA's become more important. When farmers get paid for milk yield and not milk solid content, the increase in propionate is economically better. Acetate is responsible for the production of milk fat. It gets taken up by the mammary gland from the blood and is then converted to milk fat. Because of a drop in acetate, a decrease in milk fat will follow, meaning a decrease in farm revenue. Thus the conditions under which ionophores are fed to diary animals should be taken into consideration because of the negative impact it can have on the milk price received per litre of milk produced.

In a feedlot environment the shift from acetate to propionate is preferred. This is due to the fact that more ATP is produced during propionate metabolism than during acetate metabolism. Because of an increase in ATP, more energy is available for maintenance and growth and energy is the main driver of all processes in the animal body.

Increases in concentrates lead to an increase in lactate production. Thus excessive production of lactate through fermentation in the rumen is associated with an increased risk of ruminal acisdosis because of a decrease in ruminal pH (Nocek, 1997). Ionophores also have an influence on lactate producing bacteria. *Streptococcus bovis* are lactate producing bacteria and are thus influenced by ionophore inclusion in the diet. On the other hand, bacteria that utilize lactate as energy source, e.g. *Megasphaera elsdenii* and *Selenomonas ruminantium,* are not influenced by ionophores (Bergen and Bates, 1984). Thus the effect that ionophores have on lactate production and utilization would be beneficial when dairy cows are fed high concentrate diets, the same goes for feedlot cattle, since both experience metabolic disorders associated with high concentrate diets.

Ionophores and protein metabolism

Ionophores not only influence carbohydrate metabolism but also protein metabolism. On farms that follow intensive farming practices, there is a concentration of nutrients because more nutrients are imported as feed and fertilizer than are exported as products like meat and milk. This negative balance of nutrients could have adverse effects on the environment, including animals and humans. This excess of nitrogen could leach into the ground and over time into underground water systems and reservoirs. Most of the N not leaching into the ground is volatilised either as N_2, N_2O, NH_3 or NO_x compounds of which NO_x can contribute to acid rain. Some work done, indicate that ammonia (NH_3) losses can represent up to 70% of the N excreted by beef cattle in open feedlots (Council for Agricultural Science and Technology, 2002). Implementation of comprehensive nutrient management plans on farms that are highly intensive farming practices, may improve efficiency of nutrient utilization, decreasing imported nutrients and decreasing nutrient losses to the environment while at the same time improving farm profitability (Klausner *et al*., 1998; Wang *et al*., 2000a, b).

There are two different ways in which a reduction in manure N can be achieved. This includes, increasing animal performance from the same diet and/or a reduction in the concentration of protein in the diet to achieve the same performance. The first option would result in fewer animals needed or required to produce the same amount of meat and milk. The second option requires an improvement in feed utilization by the animal, thus an improvement in the efficiency of protein utilization. This improvement in efficiency can be reached by adding of feed additives like ionophores to ruminant diets.

Another advantage of ionophores is a decrease in the activity of proteolytic and obligate amino acid fermenting bacteria (Russell, 1996). A consequence of this decreased bacterial activity is a decrease NH_3 concentration. This is because of a reduced breakdown of protein and amino acids (AA) from the feed. This was shown by a number of studies done on lactating dairy cows. The dairy cattle were fed either high grain diets (Haimoud *et al*., 1995) or high forage diets (Haimoud *et al*., 1996; Ruiz *et al*., 2001). These studies showed that ionophores effectively decrease the concentration of NH_3 in the rumen fluid. This effect of ionophores was also proven during the 1970's when *in vivo* studies indicated that the addition of monensin to diets reduced the ruminal concentration of NH_3 (Dinius *et al*., 1976; Poos *et al*., 1979). The same effect was seen with *in vitro* studies. These studies demonstrated a decrease in protein degradation, NH_3 accumulation and microbial protein in pure cultures (Chen and Russell, 1989; Russell *et al*., 1988) and mixed cultures (Russell and Martin, 1984; Van Nevel and Demeyer, 1977; Whetstone *et al*., 1981). Further examination by Chen and Russell (1991) suggested that monensin had greater inhibition on deamination of proteins rather than on proteolysis. This was because of an accumulation of α-amino-N and peptides. Findings like these suggest that a higher proportion of protein in the feed may escape the rumen, having a sparing effect on the dietary protein when monensin is included in the feed of ruminants (Faulkner *et al*., 1985; Muntifering *et al*., 1981). Other studies done however have shown mixed results in terms of the protein sparing effect when feeding ionophores, but total tract N digestibility was increased consistently (Rogers *et al*., 1997).

Ionophores decreases NH_3 which can lead to a decrease in microbial protein synthesis. The largest proportion of crude protein that reaches the lower digestive system of dairy cows fed conventional diets are from microbial origin (Clark *et al*., 1992; Dewhurst *et al*., 2000). When a ionophore induced decrease in microbial protein is observed (in dairy animals this could be seen in reduced performance and a decrease in milk protein percentage) an increase in high quality feed with rumen undegradable protein that will reach the lower digestive tract is required to avoid detrimental impacts on animal performance.

Effect of ionophores on ruminant performance

Dairy cattle

i. Dry matter intake

Results on the effect of ionophores on DMI are variable. Most of the work done on ionophores and DMI interaction was done on dairy cows. Ipharraguerre and Clark (2003) reported in an extensive review that the inclusion of ionophores either decreased DMI or had no effect on DMI of lactating dairy cattle. In 8 of 12 studies no significant differences for DMI were detected between control and ionophore-treated cows. In the remaining four studies, the administration of ionophores to lactating cows decreased DMI from 0.4 (2%) to 1.7 kg (10%) per day and averaged 1.2 kg per day (7%) It was speculated that the different results had to do with the stage of lactation the dairy cattle was in (Ipharraguerre and Clark, 2003). When cows are in a positive energy balance they are generally in late lactation or in the dry period. When the diet is then supplemented with ionophores it may cause an increase in the energy available per unit of feed. The cows are consuming feed according to their energy requirements, causing a decrease in DMI because of the increase in energy per unit of feed (Benchaar *et al*., 2006b). When cows are in early lactation, thus in negative energy balance, the additional energy available, due to the inclusion of ionophores in the diet are used for the improvement of performance or the reduction of body reserve losses or for both. Thus when the animal is in a negative energy balance no effect on DMI will be observed (Benchaar *et al*., 2006b).

ii. *Milk composition and yield*

Administering ionophores to dairy cows either did not affect (18 experiments) or increased (14 experiments) milk production in the studies reviewed by Ipharraguerre and Clark (2003). Within the group of studies that reported a positive response, the increase in milk production ranged from 2.8 (11.2%) to 0.4 kg per day (2.6%) and averaged 1.5 kg per day (7%) when monensin was administered at doses that ranged from 80 to 350 mg per day per cow. They also found that milk fat content was significantly decreased (10 experiments) or numerically (20 experiments). Trails from Australia and New Zealand showed a 7% to 8% increase in milk yield. Increases in milk protein yield but not milk fat was found (Cameron *et al*., 1993; Lynch *et al*., 1990; Lowe *et al*., 1991). A review by McGuffey *et al.* (2001) found the following on monensin: milk yield increased by 1.3 kg /d (5%), milk fat % decreased by 2%, fat yield (kg /d) decreased but no significance was found, milk protein % and protein yield decreased by 5% and 0.026 kg /d (3%).

iii. *Body weight and Body condition score*

The study conducted by Duffield (1997) found that monensin had a significant effect on body condition score (BCS). Cows were classed into three groups according to their BCS. Thin cows had a BCS ≤ 3.0, cows in proper condition had a BCS of 3.25 to 3.75 and cows classified as fat had a BCS ≥ 4.0. For the thin cows no significant production response was found in the first 90 days of lactation. For the cows in proper condition a significant increase in peak milk production of 0.85 kg was found, for the fat cows a significant increase of 1.2 kg /d in milk production was observed for the first 90 days of lactation (Duffield., 1997). Studies summarized by Ipharraguerre and Clark (2003) did not report initial BCS nor changes in BCS.

iv. *Metabolic disorders*

The use of ionophores has been found to reduce the incidence of metabolic disorders like pasture bloat, sub-clinical ketosis, abomasal displacement and ruminal acidosis. Several studies done in Australia and New Zealand found that monensin prevented pasture bloat (Cameron *et al*., 1993; Lynch *et al*., 1990; Lowe *et al*., 1991). During a 30-d control feeding period, the incidence of bloat was 86.3% in bloat susceptible steers. Feeding of monensin (40 mg /kg) reduced the incidence of bloat to 4.2% over 36 d. Removal of monensin from the diet caused incidence of bloat to increase to 24.3% for the next 36 d (McGuffey *et al.* 2001). Work conducted by Duffield (1997) which included 1010 cows from 25 Canadian commercial dairy herds showed that 335 mg of monensin CRC reduced the incidence of sub-clinical ketosis by 50%. The duration of sub-clinical ketosis was also reduced significantly. CRCs containing monensin also reduced the risk of abomasal displacement precalving (Duffield, 1997). Furthermore it was found that ionophores have an impact on reducing ruminal acidosis for feedlot cattle with monensin reducing the time which the rumen pH was below 5.6.

Beef cattle:

i. *Dry matter intake*

Goodrich *et al*. (1984) summarized 228 experiments conducted before 1984 with beef cattle fed monensin and Raun (1990) summarized 37 experiments with beef cattle fed high concentrate diets conducted from 1981 to 1990 in the United States of America. They found that monensin decreased DMI by between 4 to 6.4%. Thirty five European studies reviewed by Nagaraja (1995) found an average decrease of 4% in DMI. A study done by Guan *et al*. (2006) with 36 Angus yearling steers on monensin and rotation of monensin and lasalocid with high and low concentrate diets found that DMI was not significantly influenced on a low concentrate diet ($P > 0.05$). However, with the high concentrate diet, both monensin and rotation between monensin and lasalocid significantly decreased DMI /d ($P < 0.05$).

ii. *Feed efficiency*
Improved feed efficiencies were found to be 5.6% (Raun 1990) to 7.5% (Goodrich *et al*. 1984). In European studies the average improvement in feed efficiency was found to be 8.7% (Nagaraja (1995). In the study by Guan *et al*. monensin and rotation between monensin and lasalocid did not significantly ($P > 0.05$) influence feed efficiency of the low concentrate diet and this is supported by other studies (Guan *et al*., 2009). With the high concentrate diet workers found an improved feed efficiency ($P < 0.05$). Improved feed efficiency observed with ionophores on high concentrate diets is related to the reduction in DMI and due to a direct effect on growth rate.

iii. *Average daily gain*
Monensin increased ADG by 1.6% (Goodrich et al. 1984) and 1.8% (Raun 1990). Nagaraja (1995) found an average increase of 5.2%. Guan *et al*. (2006) reported that on both the high and low concentrate diets no influence was seen on ADG with monensin and the rotation between monensin and lasalocid. This was consistent with other studies (Stock *et al*., 1990; Zinn *et al*., 1994).

Essential oils

The uses of essential oils are becoming more and more popular as replacements for antibiotics in the animal feed industry. This is due to the fact that antibiotics are facing reduced social acceptance from the consumer of animal products. For this reason alternatives for the manipulation of microbes and microbial populations are being investigated. Essential oils are one of the many compounds being investigated due to its antimicrobial activities, resulting in reduced peptidolysis, deamination and methanogenesis. The use of essential oils is objective or target dependent, making selection of different essential oils important in different situations.

Plants produce secondary metabolites through their metabolism. These include an extensive variety of organic compounds that can be classified into three main groups. They are saponins, tannins and essential oils. Of these three groups, essential oils have been shown to have antimicrobial activities. These essential oils are currently considered as being safe for human and animal consumption. They are categorized as GRAS by the USA FDA meaning generally recognized as safe (FDA, 2005). The potential use of essential oils in ruminant diets as feed additives has been reviewed by Calsamiglia *et al*. (2007) and Benchaar *et al*. (2007).

The origin, classification and activity of essential oils

Essential oils are a blend of secondary metabolites obtained from the plant volatile fraction by means of steam distillation (Gershenzon and Croteau, 1991). In general they are classified into two chemical groups that have different origins. Thus different precursors of the primary metabolism are synthesised through separate metabolic pathways. These two groups are terpenoids and phenylpropanoids. Terpenoids are the more numerous and diverse group of the two of secondary metabolites and are derived from an isoprenoid structure (C_5H_8). Within the terpenoid group, the most important components of essential oils belong to the monoterpenoids and the sesquiterpenoid families (Gershenzon and Croteau, 1991). The phenylpropanoids are not the most common compounds of essential oils and are derived from a structure with a chain of three corbons bound to an aromatic ring of 6 carbons.

Just like ionophores, terpenoids and phenylpropanoids develop their action against bacteria through the interaction with the bacteria's cell membrane (Griffen *et al*., 1999; Davidson and Naidu, 2000; Dorman and Deans, 2000). The mode of action is different in ionophores and the essential oil compounds. Where ionophores interact with cations, terpenoids and phenylpropanoids causes conformational changes in the cell's membrane. The result is a leakage of ions across the cell membrane and the loss of the transmembrane ionic gradient (Griffen *et al*., 1999). In most of the cases large amounts of energy is diverted to the function of restoring the transmembrane ionic gradient, reducing the amount of energy for growth and reproduction. In some of these cases the energy demand for keeping the ionic gradient is so large that microbial death occurs (Griffen *et al*., 1999; Ultee *et al*., 1999; Cox *et al*., 2001). The mechanism of action makes essential oils more effective than ionophores. They can interact directly with the cell membrane because of their hydrophobic nature (Smith-Palmer *et al*., 1998; Chao and Young, 2000; Cimanga *et al*., 2002). Because of their small molecular weight in comparison to monensin and other ionophores, essential oils are also effective against gram-negative bacteria. What makes them effective against gram-negative bacteria is their ability to cross the external hydrophilic part of the cell membrane due to their small molecular weight (Calsamiglia *et al*., 2007; Griffen *et al*., 1999; Dorman and Deans., 2000). This property of essential oils of being active against both gram-positive and gram-negative bacteria reduces the selectivity of these compounds against heterogeneous populations of microbes found in the rumen environment. This makes it more difficult to modulate rumen microbial fermentation to study the effects of essential oils on different populations. Other mechanisms of action have also been described, which include the coagulation of some cell constituents, denaturation of proteins and the interaction with DNA (Gustafson *et al*., 1997; Juven *et al*., 1994).

The effects of the main essential oils available to be used as feed additives will be presented briefly. This will be done by separately discussing the active compounds.

Carvacrol and thymol

These two compounds form part of the monoterpenoids. They have strong antimicrobial activity against a wide range of both gram-positive and gram-negative bacteria. They are found in oregano and thyme.

It was reported that thymol inhibits deamination (Brochers, 1965). A similar conclusion was reached by Broderick and Balthrop (1979) after they incubated rumen fluid *in vitro* in thymol. Evans and Martin (2000) reported that thymol affected the energy metabolism of two relevant rumen bacteria grown in pure cultures. The two affected bacteria were *Streptococcus bovis* and *Selenomonas ruminantium.* Thymol reduced methane and lactate concentrations, although at higher doses also reduced overall nutrient digestion and total VFA production. From this there were clear indications that microbial metabolism was inhibited. It was reported that when low doses of thymol (50 mg /ℓ) was used, no effect on *in vitro* rumen microbial fermentation were observed. At higher doses (500 mg /ℓ) total VFA and the ammonia-N concentrations decreased, and a shift in the acetate to propionate ratio, increasing it (Castillejos *et al*., 2006). Results from several *in vitro* studies, suggest that the effects of thymol are dependent on the type of diet being fed and the pH (Castillejos *et al*., 2006; Cardozo *et al*., 2005). Different pH's will lead to shifts in opposite directions of acetate and propionate ratio. Castillejos *et al*. (2006) reported an increase in the acetate to propionate ratio at high pH's (more than 6.4) with a 60:40 lucerne hay to concentrate diet. Cardozo *et al*. (2005) observed changes in the opposite direction from those found by Castillejos. They observed decreases in the acetate to propionate ration when thymol was incubated in rumen fluid from cattle fed a 10:90 straw to concentrate diet. The pH was 5.5 for this diet.

Compounds which contain phenolic structures like thymol are more effective as antimicrobials in comparison with other non-phenolic secondary plant metabolites. What makes secondary plant metabolites containing a phenolic structure more effective is the presence of a hydroxyl group on one of the six carbons of the phenolic structure. Non-phenolic secondary metabolites do not contain a hydroxyl group, making them less effective (Helander *et al*., 1998; Ultee *et al*., 2002).

As a result of the strong and wide-spectrum activity thymol has against both gram-positive and gram-negative bacteria, there exists a narrow margin of security between an optimal dose and a toxic dose. The effects reported were not always in the desired direction (Castillejos *et al*., 2006). This leads to a suggestion that the antimicrobial activity of thymol may in some cases be too strong and non-specific to modulate the fermentation in a complex microbial environment such as the rumen.

Cinnamaldehyde, eugenol and anethol

These three compounds are from the phenylpropanoid group. They have a wide spectrum of antimicrobial activity against both gram-positive and gram-negative bacteria. The compound cinnamaldehyde is the main component of cinnamon oil (*Cinnamomum cassia*) accounting for as much as 75% of the oil composition. Research by Cardozo *et al*. (2004) using a continuous culture experiment suggested that cinnamon oil modified N metabolism of rumen microbes by the inhibition of peptidolysis. The effect it had on VFA concentrations was negligible (Cardozo *et al*., 2004). At higher doses both cinnamon oil and cinnamaldehyde decreased VFA and ammonia-N concentrations. Through this it was observed that cinnamaldehyde had stronger effects than cinnamon oil (Busquet *et al*., 2006). The effect of higher doses on the proportions of individual VFA were however different. Cinnamon oil increased acetate without affecting the molar proportions of propionate and butyrate. Cinnamaldehyde increased the propionate proportion without having an effect on acetate and butyrate proportions. From this can be concluded that other substances contained within the oil may be responsible for the difference in VFA profile.

Inconsistent effects were found on N metabolism when cinnamaldehyde were studied. Some studies reported a change in N metabolism (Cardozo *et al*., 2004; Busquet *et al*., 2005a) while other studies found no effects (Busquet *et al*., 2005b).

Eugenol is one of the main active compounds found in clove buds (*Eugenia caryophyllus* or *Syzygium aromaticum*) and also in cinnamon oils. Eugenol accounts for up to 85 and 8% of these oils (Davidson and Naidu, 2000). A continuous culture study showed that at low doses of clove bud oil, there were lower proportions of acetate and branch-chained VFA. But the study also showed higher proportions of propionate (Busquet *et al*., 2005a).

Clove bud oil also proved to have an effect on N metabolism. It was responsible for increasing peptide N and at the same time decreasing amino acid N concentration. This suggests a decreased peptidolytic activity in the rumen. In general, effects observed with eugenol were similar to effects reported for clove bud oil.

The main active component in aniseed is anethol. Anethol is responsible for the antimicrobial activity of anise oil. The ether group on its aromatic ring gives the anise oil its antimicrobial activity. *In vitro* studies done on anise oil and anethol showed that both decreased total VFA production and they also decreased the proportions of acetate and propionate in the VFA mixture. With this decrease of acetate and propionate, the proportion of butyrate was increased and the *in vitro* studies showed that anethol had a stronger effect when it was compared to anise oil. No affect was observed on the ammonia-N concentrations with both anise oil and anethol (Busquet *et al*., 2005a). The stronger effect of anethol to anise oil can be due to a lower concentration of anethol in the anise oil.

Studies done by Cardozo *et al*. (2006) suggest that anise oil inhibit deamination of amino acids and also reduced the acetate and propionate concentrations in the rumen. They also found with a decrease in the VFA concentrations, that there was a decrease in acetate to propionate ratio. This led to the conclusion that the use of anise oil or anethol may be beneficial to beef production systems.

Capsaicin

Capsicum oil is found in hot peppers, the *Capsicum annum* ssp., and the main component being capsaicin a tetraterpenoid making up 10 – 15% of the capsicum oil (Cichewicz and Thorpe, 1996). When capsicum oil was supplied to rumen fluid from dairy cows used in an *in vitro* study, negligible effects were observed in both short and long term studies (Cardozo *et al*., 2004; Busquet *et al*., 2005a). Work done by Cardozo *et al*. (2005) showed that the effects were different when rumen fluid from beef cattle was used in an *in vitro* system. The beef cattle were fed a diet containing 10:90, straw to concentrate ratio. They reported that at a pH of 7.0 total VFA and ammonia-N concentrations were reduced. They also observed that there was a reduction in the acetate to propionate ratio. In contrast to the effects observed at pH 7.0, they found that at a pH of 5.5, capsicum oil reduced the ammonia-N concentration, increased total VFA production and also the propionate proportions, and reduced the acetate proportion and thus a reduction in the acetate to propionate ratio. From the study done by Cardozo *et al*. (2006) one can conclude that when feeding a high concentrate diets at low pH, nutrient utilization in the rumen may be improved.

There is evidence that capsaicin found in capsicum oil increases DM and water intake by humans and rats (Zafra *et al*., 2003; Calixto *et al*., 2000). From this it can be concluded that it will have the same effect on DM and water intake on ruminants. From all of the above there seems to be potential for the use of capsaicin in beef cattle diets based on its effects of increasing DM intake and its potential effect on rumen microbes, changing their fermentation patterns and products.

Garlic oil

A mixture of a large number of different molecules is found in garlic oil. The large number of molecules can be found in the plant or can be a result of changes that occurs during the extraction and processing of the oil (Lawson. 1996). Garlic oil is known for its many therapeutic properties which includes the following anti-parasitic, insecticidal, and anti-cancer, antioxidant, immunomodulatory and anti-inflammatory properties. The most prominent activity that has been thoroughly studied is probably its antimicrobial properties. Garlic oil has a wide spectrum of antimicrobial properties against both gram-positive and gram-negative bacteria (Reuter *et al*., 1996). Studies done by Busquet *et al*. (2005abc and 2006) have consistently shown that garlic oil reduces the proportions of acetate and branch chain volatile fatty acids (BCVFA) and that it increases the proportions of propionate and butyrate. Thus there is a shift in the acetate to propionate ration, making it useful in beef cattle production.

In vitro studies done demonstrated that garlic oil reduced CH_4. This reduced the CH_4 (µmol): VFA (µmol) ratio from 0.20 to 0.05 (Busquet *et al*., 2005). These results clearly show that garlic oil has a huge effect on methane producing microorganisms. In the metabolic pathway of rumen fermentation, methane is the main hydrogen sink. Thus inhibition of CH_4 synthesis generates reducing equivalents that need to be disposed of, propionate and butyrate being the main alternatives (Van Nevel and Demeyer, 1988).

To identify the main active component of garlic oil that was responsible for the effects observed, four active components were extracted. These active components were allicin, diallyl sulphide, and diallyl disulphide and allyl mercaptan. Garlic oil and these four active compounds thought to play a role in the antimicrobial activity of the oil were tested in, *in vitro* studies to determine their effects on rumen microbial fermentation (Busquet *et al*., 2005c). It was found that garlic oil and the two active compounds diallyl disulphide and allyl mercaptan were responsible for the reduction in acetate and methane and the increase in propionate and butyrate. Kamel *et al*. (2007) reported similar results.

Variable results were observed on N metabolism by garlic oil and the main active compounds. Cardozo *et al*. (2004) suggested from his study that garlic oil inhibited deamination, other workers reported only small and variable effects (Busquet *et al*., 2005bc). Cardozo *et al*. (2005) tested the effect of garlic oil on rumen fluid and a high concentrate diet typically found in feedlot diets at different pH levels, 7.0 vs. 5.5. They found that at a pH level of 7.0, garlic oil resulted in lower ammonia-N and total VFA concentrations. At a pH of 5.5 they observed a reduction in ammonia-N concentration, but total VFA concentration as well as the propionate concentration increased and a decrease in the acetate concentration and the acetate to propionate ratio occured. The control treatment contained no garlic oil in the diet, with comparison between the treatments; a shift in microbial fermentation can therefore be suggested.

Combination of essential oils

The additive, synergistic and or antagonistic effects of a combination of essential oils have been reported by Burt (2004). Many of the products commercially available these days have a combination of one or more different essential oils. But very little information is available on the potential synergies amongst them. Because of all the different combinations that can be used to produce commercial products, only one will be discussed.

A product developed by Pancosma, a company in Switzerland, called XTract 7065 was tested in farm trails in order to characterize its effect on field conditions. XTract 7065 contains a blend of essential oils, consisting of 9.5% eugenol, 5.5% cinnamaldehyde and 3.5% capsicum. XTract 7065 was fed to beef cattle at a rate of 800mg/head/day. The trial showed that XTract 7065 decreased DM intake. The overall result is that more of the energy is available from the same amount of feed resulting in improved feed efficiency and or improved overall gains. This trial showed that XTract 7065, when supplemented in high concentrate diets, improved growth and better performance were seen with steers than with heifers. The manufacturers recommend a dose of 1000mg /head /day to optimize the performance of beef cattle fed a high concentrate diet.

Effect of essential oils on ruminant performance

Dairy cattle

Information on the effects of essential oils on dairy cattle performance is limited. A few trials of short duration (28 d) and not using more than 4 cannulated cows have been reported. This makes it difficult to evaluate the effect of essential oils on lactation performance of dairy cattle (Benchaar *et al.,* 2006a).

i. *Dry matter intake*
   A TMR was fed to 4 Holstein cows which contained a mixture of essential oils. Benchaar *et al.* (2006a) reported that there was no interaction between the additions of essential oils (MEO) and DMI.

ii. *Milk composition and yield*
   Milk and 4% fat corrected milk (FCM) yields were not affected by the addition of MEO to a TMR fed to lactating cows (Yang *et al*., 2007). Results of Benchaar *et al*. (2006b) also found no changes in the milk production and milk composition of cows fed a 2 g /d MEO ruminant supplement.

iii. *Body weight and Body conditioning score*
   No research results could be found on the effect essential oils have on both body weight changes and BCS. Further research is needed on this subject.

iv. *Metabolic disorders*
   As with body weight changes and BCS, no research results could be found on the effect essential oils have on metabolic disorders like pasture bloat, subclinical ketosis, abomasal displacement and ruminal acidosis. Further research is needed on this subject.

Beef cattle:

As is the case with dairy cattle limited information is available on the use of essential oils in feedlots, especially with regard to individual essential oils. There is however information on combinations of essential oil and some performance data for feedlot animals. Research was conducted by Pancosma using XTract 7065 and ADM. The product XTract 7065 contained 17% eugenol, 11% cinnamaldehyde and 7% capsaicin. Thirteen studies incorporating 18 trails were conducted supplementing XTract 7065 and using 884 growing animals. Three trails were conducted on sheep and 15 on beef cattle (Bravo *et al*., 2009). The following results were reported:

i. *Dry matter intake*
   XTract 7065 did not alter the DMI for lambs (P = 0.24) or beef cattle (P = 0.81) (Bravo *et al*., 2009).

ii. *Feed efficiency*
   Because XTract 7065 did not alter DMI, there was a trend for improved efficiency in both the lambs and beef cattle trails. Increases in feed efficiency for lambs were 11.9% and for beef cattle the increases were 2.6% (Bravo *et al*., 2009).

iii. *Average daily gain*
   Bravo *et al*. (2009) found that XTract 7065 tended to improve ADG for both lambs and beef cattle. An increase in ADG of 2.9% was found for beef cattle and ADG increase of 16.8% for lambs was reported.

The effect that essential oils have on DMI is variable. Not all essential oils have an effect on DMI and thus wouldn't have an effect on ADG and feed efficiency (FCR). Some increase DMI, where as others may decrease or have no effect on DMI. Essential oils may have an effect on the palatability of feed and this may be a cause of reduced feed intake. It is suggested that when this might happen, it is better to encapsulate the essential oil. Work done by Busquet *et al*. (2005a) showed that the addition of cinnamaldehyde fed to dairy cattle resulted in a reduction of feed intake, but increased the milk production, although not significantly. It was also found that capsicum oil increased both DM and water intake in animals.

Differences were observed between dairy and beef cattle fed the same concentrations of essential oils. Differences observed were due to the diet composition fed to the cattle. With dairy cattle, the diets usually consist of a 60:40 or 40:60 concentrate to forage ratio. With beef cattle in feedlots the diet ratio is usually 90:10, concentrate to forage ratio. The high concentrate percentage in feedlot diets causes a larger decrease in pH. Because the effects of essential oils on rumen microbes are pH dependant, a decrease in pH increases the antimicrobial effect of essential oils. This is due to conformational changes in the molecular structure of the active compound.

In summary, essential oils vary considerably in chemical structure, source and activity. Consequently their effects on ruminal fermentation and animal performance are inconsistent. More research is needed to identify essential oils that only have desirable effects on rumen function and animal performance.

Future research needed

Over recent years some knowledge has been gained on the use of essential oils as modifiers of microbial fermentation in the rumen. However, there are still several issues that need to be addressed before specific recommendations can be establisheded for commercial use in both dairy and feedlot enterprises. Some of the many limitations of current knowledge that need to be resolved include the following:

1.) Researchers should report the concentrations of the main active compounds in essential oils they use or they should instead use the pure active compound. The recommended doses should be established in units like mg/kg DM or mg /head /day of the active compound rather than units of the oil or extract. This should be done because the active compound in essential oils or the extract can vary widely depending on the plant cultivar being used, the growing conditions under which it is produced or the extraction methods being used (Sivropoulou *et al*., 1996; Marino *et al*., 2001; Burt, 2004).

2.) *In vitro* studies have been very useful in screening the effects of a wide variety of essential oils. The use of *in vitro* studies will still be useful for screening of other useful extracts and to study their specific mechanisms of action. However these methods are not without limitations. One of the limitations is that doses used in *in vitro* systems are usually reported as milligrams per litre. These values are much higher than those reported from *in vivo* studies. This is because the bacterial concentrations found *in vitro* is much lower than the concentrations found *in vivo*.

3.) The microbes in the rumen may adapt to new environmental conditions when essential oils or extracts are used to modify the environment. There are reports that suggest an adaptation to the antimicrobial activity of the active compounds found in essential oils is possible over time (Cardozo *et al*., 2004; Molero *et al*., 2004; Castillejos *et al*., 2007). In *in vitro* studies the adaptation time is generally short thus not allowing the microbes enough time to adapt to the product or extract. Thus enough time should be allowed to evaluate the product.

4.) There are also opportunities to explore other effects of the active compounds and extracts. These opportunities include potential effects on bio-hydrogenation of fatty acids and their activity against pathogenic micro-organisms.

5.) As with other feed additives, the presence of residues in products like milk and meat should be evaluated. No such evaluations have been conducted on the residues of essential oil or of the active compounds they consist of.

6.) The use of feed additives and thus also essential oils can only be justified if there is a beneficial effect larger than the cost of the product. The cost:benefit ratio will depend on the cost of the essential oil, the dose that is required and the most important, the resulting improvement in animal performance.

7.) The effect of essential oils has proven to be diet and feed ingredient dependant. There is an urgent need for studies where typical South African feed ingredients are used such as hominy chop in feedlot diets.

Conclusion

As with ionophores, most of the essential oils and active compounds tested at high doses have inhibited rumen microbial fermentation, thus confirming their antimicrobial activity. In most cases when the concentrations of the active compound were increased detrimental effects were seen. These detrimental effects were observed with concentrations higher than 500 mg /ℓ. At doses in the range of 50 to 500 mg /ℓ, depending on the active compound, some of the essential oils and active compound were able to modify fermentation in the rumen. At these more moderate concentrations changes in VFA production and/or protein metabolism were observed. These observations depend on whether the active compound is used alone or as a mix in combination with other essential oils or active compounds.

Because of the many conditions that exist in the dairy and feedlot enterprises, a universal extract may not exist. This is because many of the active compounds are diet and pH dependant, making the use of one universal compound difficult because not all farmers and feedlot managers feed the same diet to their animals. Thus it actually comes down to the microbial population within the rumen.

As is the case with ionophores, there are advantages in using essential oils as modifiers of rumen fermentation. This can be seen on the effect these compounds have on fermentation, causing a shift from acetate to propionate production and thus decreasing the acetate to propionate ratio. In terms of protein metabolism, some of the active compound's mechanism of action may be related to the inhibition of deamination. Inhibition of peptidolysis has also been suggested by some researchers. Some consumers may pay a premium price for products (meat and milk) that are produced by the use of essential oils in animal feeds. The cost:benefit ratio of the essential oils or active compounds may make it more attractive for producers to use, but only if the benefits are more than those obtained from the use of ionophores.

However, the use of essential oils also may have negative effects. The two most important effects that need further research are the effects of residues in meat and milk and the adaptation of rumen microbes to essential oils. Because of these effects, *in vivo* studies are needed to determine and confirm the mechanisms of action observed in *in vitro* studies. Thus *in vivo* studies are needed to determine the optimal dose in units like mg /kg DM or mg /head /day of the active compound, the potential adaptation of rumen microbes to the action of the active compounds and the time it takes to adapt. Furthermore, the fate of the active compounds and the presence of residues in meat and milk, needs to be studied and also the effect of the compounds on animal performance.

Research results on lactation studies are limited and growth performance data on feedlot animals is practically non-existing, especially with regards to feed ingredients such as hominy chop, which is being used to a large extent in South African feedlots. Essential oils hold promise as a natural alternative, but more research is needed before nutritionists would be able to make practical recommendations. The bottom line is, when ionophores are banned we need to have natural alternatives in place!.

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