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Garlic in Ruminants Feeding

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ABSTRACT

Plant extracts have been used for centuries for various purposes (e.g., traditional medicine, industrial applications, food preservatives) due to their antimicrobial properties and because most of them are categorized as Generally Recognized as Safe (GRAS) for human consumption. The main purpose of ruminant nutritionists is to manipulate the ruminal microbial ecosystems to improve the efficiency of converting feed to animal products. The use of feed additives such as antibiotics has proven to be a useful tool to reduce energy losses (in the form of methane) and nitrogen (in the form of ammonia). Reported that garlic (*Allium sativum*) have many biological activities, such as protective roles in cardiovascular function, as antihypertensive. garlic can have positive effects on the performance of different animals. Garlic has various properties including improve nutrient digestibility, antimicrobial, anti-inflammatory, anti-oxidant and immunostimulant in animal's nutrition. Thus, this review has discussed the effects of garlic in ruminants.

Key words: Garlic, antimicrobial, antioxidative, nutrients digestibility, ruminant

INTRODUCTION

The main purpose of ruminant nutritionists is to manipulate the ruminal microbial ecosystems to improve the efficiency of converting feed to animal products. The use of feed additives such as antibiotics has proven to be a useful tool to reduce energy losses (in the form of methane) and nitrogen (in the form of ammonia). However, the increasing public concern about the use of antibiotics in the animal feed industry made scientists look for alternatives to manipulate gastrointestinal microflora (Syadati *et al*., 2012). Plant extracts have been used for centuries for various purposes (e.g., traditional medicine, industrial applications, food preservatives) due to their antimicrobial properties and because most of them are categorized as Generally Recognized as Safe GRAS for human consumption. It seems that the use of herbal extracts, a natural alternative is the use of antibiotics in animal nutrition (Anonymous, 1994; Schelling, 1984; Davidson and Naidu, 2000; FDA, 2004; Mirzaei-Aghsaghali *et al*., 2012). Garlic (*Allium sativum*) showed positive effects on the performance of different animals. Garlic has various properties including improve nutrient digestibility, antimicrobial, anti-inflammatory, anti-oxidant and immunostimulant in animal's nutrition. Garlic oil is contains several compounds, including sulfur compounds (thiosulfinates, allyl sulfides, glutamylcysteines, allicin), enzymes, free AA (amino acid), sterols, steroids, triterpenoid glycosides, flavonoids, phenols and organoselenium compounds (Lawson, 1996; Reuter *et al.*, 1996). However, its potential effect on modifying rumen microbial fermentation has not been researched until recently. In a continuous culture system, 300 mg L^{-1} of garlic oil reduced the proportions of acetate and branched-chain volatile fatty acids and increased the proportions of propionate and butyrate and the small peptide plus amino acid N(nitrogen) concentration (Busquet *et al.*, 2005a). These changes are consistent with inhibition of methane in the fermentation profile and have the potential to beneficially modify rumen microbial fermentation (Mirzaei-Aghsaghali and Maheri-Sis, 2011). However, garlic oil is a complex mixture of many secondary plant products including allicin ($C_6H_{10}S_2O$), diallyl sulfide ($C_6H_{10}S$), diallyl disulfide ($C_6H_{10}S_2$) and allyl mercaptan (C_3H_6S) (Lawson, 1996), which makes difficult to elucidate the precise mechanism of action on rumen microbial fermentation and its use as a feed additive (Mirzaei-Aghsaghali *et al.*, 2008).

ANTI-MICROBIAL ACTIVITY OF GARLIC

Several studies have pointed out the possibility to use essential oils and/or their components for medical purposes as well as in the food industry for controlling microorganisms responsible for food spoilage (Cantore *et al.*, 2009). Today we know that the essential oils and plant extracts have broad activity against the Gram-positive and Gram-negative Bacteria and also antifungal activity (Kotzekidou *et al.*, 2008). Garlic oil has antimicrobial activity (Feldberg *et al.*, 1988) and a high dose of garlic could have detrimental effects on ruminal fermentation. For instance, Busquet *et al.* (2005b) reported that the molar proportion of acetate was reduced by 11% and NDF digestibility was decreased by 22% when 312 mg of garlic L^{-1} was added to *in vitro* batch culture rumen fermentation at a constant pH (Busquet *et al.*, 2005a). Several studies have suggested that essential oils may be conserve of degradation of amino acid (AA) in rumen by inhibiting microbial deamination (Newbold *et al.*, 2004).

ANTIOXIDANT ACTIVITY OF GARLIC

Have been reported that Garlic and garlic extracts, through their antioxidant activities prevent from free radical damage in the body. Chung (2006) investigated antioxidant properties of garlic compounds (alliin, allyl cysteine, allyl disulfide and allicin) prepared by chemical synthesis or purification. Alliin scavenge superoxide, while allyl cysteine and allyl disulfide did not scavenge superoxide. Allicin suppresses the formation of superoxide by the xanthine/xanthine oxidase system, Likely Through thiol exchange mechanism. garlic compounds Such as Alliin, allyl cysteine and allyl disulfide scavenge hydroxyl radicals. Alliin, allicin and allyl cysteine did not prevent microsomal lipid peroxidation but alliin and allyl cysteine were Scourer of hydroxyl and allyl disulfide was a lipid peroxidation terminator. In summary, allyl disulfide, alliin, allicin and allyl cysteine indicative different patterns of antioxidants as protective compounds against free radical damage (Chung, 2006).

ANTIHYPERTENSIVE ACTIVITY OF GARLIC

Garlic (*Allium sativum*) is reported that have many biological activities, including protective role in cardiovascular function (Mukherjee *et al.*, 2009), as an antihypertensive (Ried *et al.*, 2008). Allicin, a major constituent of garlic, was evaluated for its antihypertensive effects. Chronic oral administration of allicin lowered blood pressure in hypertensive rats (Ali *et al.*, 2000). Allicin also caused pulmonary vasodilatation in lung of rat (Kim-Park and Ku, 2000).

IMMUNOSTIMULANT ACTIVITY OF GARLIC

Although, essential oil and their compounds have been shown to have antimicrobial and antioxidant activity (Hammer *et al.*, 1999), information on their immunomodulatory effects in ruminants is lacking. Yang *et al.* (2007) reported that the Supplementation garlic and juniper berry had no effect on the total number or the differential counts of White Blood Cells (WBC), or on serum amyloid A and haptoglobin concentrations. The concentrations of these 2 acute phase proteins were higher than expected for a mid-lactating cow. Usually, the release of acute phase proteins is attributed to activation of the immune system in conditions such as inflammation, tissue injury and infection (Suffredini *et al.*, 1999). The main function of serum amyloid A is to bind and neutralize endotoxin and carry it to the liver to be excreted in bile. Plasma haptoglobin increases, when bacteria is entered the bloodstream. Haptoglobin binds hemoglobin and prevents utilization of iron contained in hemoglobin by bacteria. The latter need iron for their growth and multiplication. Feeding cattle high proportions of grain has been shown to increase the amount of endotoxin in rumen fluid and its translocation into the bloodstream (Andersen *et al.*, 1994). Yang *et al.* (2007) suggested that the inclusion of a high proportion of grain and the potential translocation of endotoxin into the blood stream might explain the high levels of serum amyloid A and haptoglobin (Table 1).

Burke *et al.* (2009) reported that the garlic or papaya seeds are not recommended as controllers of gastrointestinal nematodes in goats or lambs.

EFFECT OF GARLIC ON RUMEN MICROBIAL FERMENTATION

Ruminants are created a symbiotic relationship with rumen microorganisms which the animal provides nutrients and optimal environmental conditions for the fermentation and microorganisms degraded of fiber and synthesize microbial protein as energy and protein supply for the animal, respectively. However, the symbiotic relationship of energy (methane losses) and protein (losses of ammonia N) is ineffective. These losses not only reduce production performance but also contribute to the release of pollutants to the environment (Mirzaei-Aghsaghali and Maheri-Sis, 2011; Mirzaei-Aghsaghali and Fathi, 2012). Ruminant nutritionists have long been interested to mitigate competition between different microbial populations to improve the efficiency of energy and protein in the rumen. This is achieved by optimizing diet formulation and use of feed additives, that alter the environment and increase or decrease in specific microbial populations in the rumen (Calsamiglia *et al.*, 2006). Some authors examined the main purified active components in garlic

Table 1: Effects of monensin (MON), garlic (GAR) and juniper berry (JUN) essential oils on milk production and composition of lactating dairy cows

	*Treatment					
Item	Control	MON	GAR	JUN	±SE	p-value
White blood cells ($\times10^3$ μL^{-1})	6.30	6.60	6.22	6.21	0.85	<0.95
Lymphocyte (%)	56.40	55.50	52.30	56.90	3.20	<0.79
Neutrophil (%)	32.30	29.80	34.60	30.50	4.20	<0.48
Monocyte (%)	6.40	8.80	6.90	6.10	1.20	<0.33
Eosinophil (%)	4.40	5.40	6.00	5.80	1.40	<0.48
Basophil (%)	0.10	0.30	0.30	0.30	0.20	<0.78
Haptoglobin (μg mL^{-1})	6.81	62.30	54.40	58.30	12.30	<0.78
SAA (μg mL^{-1})	74.80	67.00	79.60	66.40	27.10	<0.96

SAA: Serum amyloid A, *Yang *et al.* (2007)

Table 2: Effects of garlic oil and four purified active components on the molar proportions of acetate, propionate and butyrate

	*Treatment 300 (mg L^{-1})						
Item (M/100 M)	Control	GAR	DAS	DAD	ALM	ALL	±SEM
Acetate	65.3	58.5*	64.3	58.4*	59.4*	65.1	0.39
Propionate	17.3	20.1*	14.1*	20.3*	19.9*	17.5	0.28
Butyrate	13.1	16.9*	12.9	16.6*	16.0*	13.2	0.48

GAR: Garlic oil, DAS: Diallyl sulfate, DAD: Diallyl disulfate, ALM: Allyl mercaptan, ALL: Allicin, *Means differed from the control ($p<0.05$), *Busquet *et al*. (2005b)

oil, allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan presumably play the major role in its antimicrobial activity, *in vitro* to determine their effect on rumen microbial fermentation. the study revealed (Table 2) that Garlic oil, diallyl disulfide and allyl mercaptan reduced acetate and methane proportions and increased propionate and butyrate proportions to the same extent, suggesting that they play the major roll in rumen microbial modulation while allicin and diallyl sulfide had minor effects on rumen microbial fermentation (Busquet *et al*., 2005b).

Ferme *et al*. (2004) reported that garlic is modified the microbial population profile in a continuous culture experiment, reducing of the *Prevotella* spp. (mainly *P. ruminantium* and *P. bryantii*) than the overall microbial population in the rumen. *Prevotella* spp. is mainly responsible for protein degradation and amino acid deamination, suggesting a mechanism of action of garlic oil on protein metabolism where the activity of dehydrogenases which is necessary for deamination is suppressed when methane inhibitors are used (Hino and Russell, 1985). Busquet *et al*. (2005b) investigated the effect of garlic oil and four of its compounds on rumen microbial fermentation and reported that the garlic oil, diallyl disulfide (30 and 300 mg L^{-1} culture fluid) and allyl mercaptan (300 mg L^{-1} culture fluid) resulted in lower molar proportion of acetate and higher proportions of propionate and butyrate.

Antimicrobial activity of garlic has been attributed to its sulfur compounds, particularly allicin (Feldberg *et al*., 1988). However, pure Allicin is a volatile molecule, which is hardly soluble in aqueous solutions and is fairly responsive, quickly, under different conditions is converted to other compounds. The stability of allicin is higher at low temperature and pH, whereas it decreases as temperature and pH increase (Lawson, 1996). For this reason, Amagase *et al*. (2001) suggested that allicin might not contribute to the *in vivo* effects of garlic under physiological conditions. the numerous studies has been suggested that antimicrobial activity of allyl sulfides of garlic oil increases with each addition Sulfur atom the antimicrobial activity of garlic oil and more than the activity of its main compounds individually, suggesting that the effect is the result of synergy among the different compounds (O'Gara *et al*., 2000). In general, additives were not detrimental for rumen microbial fermentation. The lack of detrimental effects in the long-term continuous culture study could be due to the longer adaptation time allowed to the rumen microflora, which may allow replacement of the inhibited microbial population by other resistant bacterial groups. Oh *et al*. (1967) reported that high doses of different plant essential oils, *in vitro* fermentation of mixed rumen microorganisms, is caused inhibition of rumen microbial fermentation. Busquet *et al*. (2005a) suggested that anti methanogenic effect of garlic and its active components was due to direct inhibition of Archaea microorganisms in the rumen (Busquet *et al*., 2005a). A series of short-term studies *in vitro* fermentation is used to illustrate the effects of potentially useful extracts and selected oils and their active components are studied as long-term continuous culture

Table 3: Effect of two doses of monensin, cinnamaldehyde and garlic oil on VFA concentrations in continuous culture

	*Treatment							
Item	Control	MON	MON10	CIN	CIN10	GAR	GAR10	±SEM
Total VFA, mM	87.4[b]	89.7[b]	104.4[a]	85.7[b]	88.0[b]	93.8[ab]	94.3[ab]	3.28
Individual VFA								
Acetate	61.2[a]	60.2[ab]	45.9[d]	55.8[c]	57.0[bc]	58.5[abc]	46.8[d]	0.94
Propionate	20.5[d]	22.4[cd]	45.1[a]	24.2[bc]	21.6[cd]	22.6[cd]	27.4[b]	1.24
Butyrate	10.8[c]	10.3[c]	4.3[d]	13.1[bc]	14.3[b]	11.3[bc]	19.4[a]	1.47
BCVFA	1.0[a]	0.9[bc]	0.6[d]	0.9[a]	1.1[a]	1.0[a]	0.7[cd]	0.03
Acetate:propionate	3.0[a]	2.7[ab]	1.0[d]	2.3[b]	2.6[ab]	2.6[ab]	1.7[c]	0.14

Means within a row with different superscripts differ at $p<0.05$, MO: 1.25 mg L^{-1} of monensin MON10: 12.5 mg L^{-1} of monensin, CIN: 31.2 mg L^{-1} of cinnamaldehyde, CIN10: 312.0 mg L^{-1} of cinnamaldehyde, GAR: 31.2 mg L^{-1} of Garlic oil, GAR10: 312.0 mg L^{-1} of garlic oil. BCVFA: Branched-chain VFA (includes isobutyrate and isovalerate), *Busquet *et al*. (2005a)

fermentation studies (Busquet *et al*., 2005c; Cardozo *et al*., 2004). Garlic oil reduced the proportions of acetate and branched chain fatty acids and increased the proportions of propionate and butyrate (Table 3) (Busquet *et al*., 2005a, 2006).

EFFECT OF GARLIC ON RUMEN pH

Many studies in vitro, using cow rumen fluid showed that diets containing 60% alfalfa hay and 40% concentrate needs to provide of dairy cattle requirements. Cardozo *et al*. (2005) tested the effects of garlic oil in vitro using rumen fluid and 10% straw: 90% corn-barley-based diets formulated for beef cattle at different pH (7.0 vs. 5.5). Whereas at pH 7.0 garlic oil resulted in lower ammonia N and total Volatile fatty acids concentrations, at pH 5.5 the ammonia N concentration was also reduced but the total Volatile fatty acids concentration and propionate proportion increased and the acetate proportion and acetate to propionate ratio decreased compared with Control treatments without garlic, Indicates a change in rumen microbial fermentation. The effect of pH in the response of garlic oil maybe related to the dissociated (hydrophilic) or undissociated (hydrophobic) of the active molecules. Only the undissociated, hydrophobic form of the molecule is able to interact with the lipid bilayer of the cell membrane, diffuse through it and interact with enzymes in the cytoplasm. As pH decreases, acids tend to become undissociated and more hydrophobic, thereby interacting more easily with cell membranes and exerting their antimicrobial effect (Cardozo *et al*., 2005). In addition, it appears that bacteria in the low pH more susceptible to the effects of essential oils (Skandamis and Nychas, 2000). Yang *et al*. (2007) reported that the ruminal pH and total volatile fatty acids concentration were unaffected by the addition of garlic. Benchaar *et al*. (2006) observed that supplementation of cows with a mixture of essential oil compounds had no effect on concentration of total Volatile fatty acids or on molar proportions of individual Volatile fatty acids.

EFFECT OF GARLIC OIL ON METHANE PRODUCTION AND ENERGY METABOLISM

Volatile fatty acids are the end products of rumen microbial fermentation and represent the main supply of metabolizable energy for ruminants (Van Soest, 1982). Therefore, a reduction in their production would be nutritionally unfavorable for the animal. Busquet *et al*. (2005a) suggest that the main effects of garlic oil and its compounds are focused on carbohydrate metabolism in the rumen, whereas the effects observed on N metabolism may be a consequence of the effects observed in the energy metabolism (Busquet *et al*., 2005a). Klevenhusen *et al*. (2011a) investigated of the effects on energy and protein utilisation as well as methane emission from, sheep and reported that the DADS improved fibre digestion and body energy retention and to limited CH4 formation in relation to digestible fibre intake, while lovastatin remained ineffective.

Methane (CH_4) is one of the three main Greenhouse Gases (GHG), together with carbon dioxide (CO_2) and nitrous oxide (N_2O). The production of GHG from livestock and their impact on climate changes are a major concern worldwide. It has been reported that enteric CH_4 is the most important GHG emitted (50-60%), at the farm scale, in ruminant production systems. Methane represents also a significant energy loss to the animal ranging from 2-12% of Gross Energy (GE) intake. So, reducing enteric CH_4 production in ruminants without altering animal products, it is desirable to reduce global greenhouse gas emissions and to improve feed conversion efficiency. Many biologically active molecules present in essential oils have antimicrobial properties that are capable to affect rumen fermentations. Among them, it has recently been shown that garlic oil and some of its components decreased CH_4 production *in vitro* (Mirzaei-Aghsaghali and Maheri-Sis, 2011). This interaction has been demonstrated by the loss of activity of some thiol-containing enzymes (i.e., papain, alcohol dehydrogenases) and by the reaction between different organosulfur compounds and cysteine to form other substances by a thiol-disulfide exchange reaction (Reuter *et al*., 1996). Although, the mechanism of action by which garlic could decrease methane production is not known, it could be related to the capacity of sulfur compounds to inhibition of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) by a thiol-disulfide exchange reaction (Gebhardt and Beck, 1996). Several studies suggested that essential oil and their active components may conserve amino acid from ruminal degradation by inhibiting microbial deamination (Newbold *et al*., 2004). Busquet *et al*. (2005a) investigate the effect of garlic oil and four of its compounds on rumen microbial fermentation and suggest that the main effects of garlic oil and its compounds are focused on the decrease in acetate and the increase in butyrate proportions, whereas the effects on the proportion of propionate are more variable. This fact suggests that, in contrast with the mechanism of action of monensin (which specifically inhibits rumen gram-positive bacteria), the mechanism of action of garlic oil and its compounds is more related to a direct inhibition of methanogenesis. Busquet *et al*. (2005a) investigate the effects of Cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture and reported that the garlic has no stimulatory effects on gram-negative bacteria compared with the control, although monensin resulted in a 2-fold increase, suggesting that both additives had different effects on gram-negative organisms (Busquet *et al*., 2005a). In fact, the effects observed with garlic were similar to those found in methane inhibitors such as amicloral or 2-bromoethanesulfonic acid (Martin and Macy, 1985) which, in contrast with monensin, directly inhibits methanogenic Archaea or the metabolic pathways of methane synthesis. Disposal of excess hydrogen produced by a direct inhibition of methane production results in increased concentrations of other hydrogen sinks such as propionate and butyrate (Demeyer and Nevel, 1975). Conversely, the production of acetate in the rumen results in large quantities of hydrogen and depends on the availability of reducing equivalents such as NAD^+. It has been observed that high partial pressures of H_2 and high $NADH/NAD^+$ ratios in the rumen derived from the inhibition of methanogenesis results in a decrease in acetate production (Miller, 1995). The fermentation pattern observed in garlic is consistent with the hypothesis that its mode of action is as a methane inhibitor. This activity is mediated through the inhibition of the methane-producing microorganisms.

Busquet *et al*. (2005a, 2006) investigated the plant extracts affect in vitro rumen microbial fermentation and reported that the garlic oil (300 and 3,000 mg L^{-1} of culture fluid) and benzyl salicylate (300 and 3,000 mg L^{-1} of culture fluid) reduced acetate and increased propionate and butyrate proportions, suggesting that methane production was inhibited. In fact, in vitro studies demonstrated that garlic reduced the CH_4 (μmol) volatile fatty acids (μmol) ratio from 0.20-0.05. Klevenhusen *et al*. (2011b) investigated the garlic oil and its principal component diallyl disulfide fail to mitigate methane but improve digestibility in sheep and reported that, Dietary supplementation with garlic oil or diallyl disulfide had no influence on the amount of CH_4 produced (27 g day^{-1}). When rates of CH_4 production kg^{-1} organic matter digested were compared, diallyl disulfide but not garlic oil, tended to decrease CH_4 production compared to control. As diallyl disulfide supplementation only tended to decrease CH_4 formation relative to organic matter digested, it too is limited use as a means of CH_4 mitigation.

EFFECT OF GARLIC ON NUTRIENT DIGESTIBILITY AND INTAKE

Yang *et al*. (2007) reported that the Although supplementation with garlic or Juniper Berry increased ruminal digestibilities of Dry Matter (DM) and Organic Matter (OM) as compared with the control diet, the total-tract digestibilities of DM and OM remained unaffected by feeding these 2 essential oil to cows. Similar intakes of N between the control diet and diets supplemented with garlic and juniper berry are consistent with the lack of influence of this essential oil on dry matter intake (Table 4). The decrease in the duodenal flow of dietary N and the increase in ruminal N digestibility with garlic or juniper berry supplementation suggest that the proteolytic activity in the rumen was stimulated. However, ruminal concentration of NH_3-N was not increased by garlic and juniper berry supplementation. In dairy cows, feed intake could be influenced by a number of factors, such as body weight, lactation stage, physical fill, digestion, passage rate, or fermentation metabolites (Allen, 2000). Benchaar *et al*. (2006) reported that the lack of influence of essential oil

Table 4: Effects of monensin (MON), garlic (GAR) and juniper berry (JUN) essential oils on milk production and composition of lactating dairy cows

	*Treatment					
Item	Control	MON	GAR	JUN	SE	p-value
Actual	29.0	28.90	29.90	29.40	2.10	0.44
4% FCM1	25.1	23.30	27.60	26.90	2.50	0.06
SCM2	25.0	23.50	27.00	26.50	2.40	0.06
Milk fat						
%	3.14[a]	2.68[b]	3.46[a]	3.40[a]	0.31	0.01
kg day^{-1}	0.90[a]	0.78[b]	1.04[a]	1.01[a]	0.12	0.03
Milk CP						
%	3.31	3.25	3.23	3.28	0.05	0.48
kg day^{-1}	0.96	0.94	0.96	0.96	0.06	0.56
Milk lactose						
%	4.44	4.47	4.46	4.46	0.07	0.53
kg day^{-1}	1.29	1.30	1.33	1.31	0.11	0.47
Milk efficiency						
Actual:DM	1.40	1.45	1.47	1.43	0.09	0.77
FCM:DM	1.21	1.17	1.35	1.31	0.10	0.19
SCC (105 mL^{-1})	4.69	4.07	4.00	2.46	2.02	0.68

Means within a row with different superscripts differ at p<0.05, MON: 1.25 mg L^{-1} MON, MON10: 12.5 mg L^{-1}, MON, CIN: 31.2 mg L^{-1}, CIN, CIN10: 312.0 mg L^{-1} CIN, GAR: 31.2 mg L^{-1}, GAR and GAR10: 312.0 mg L^{-1}GAR, BCVFA: Branched-chain VFA, includes isobutyrate and isovalerate, *Yang *et al*. (2007)

on total-tract digestibilities of DM and OM is in agreement with previous observations with dairy cows. Busquet *et al.* (2005a) investigate the effect of garlic oil and four of its compounds on rumen microbial fermentation and reported that in the gas production trial (garlic oil 300, diallyl disulfide 300, allyl mercaptan 300 mg L^{-1} of culture fluid) and monensin decreased total Volatile Fatty Acid (VFA) concentration and had lower disappearance of Dry Matter (DM) and lower Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) digestibilities compared with the control.

Yang *et al.* (2007) suggested that the increased of rumen fermented organic matter (RFOM) with garlic supplementation suggests increased energy availability to ruminal microorganisms. Castillejos *et al.* (2007) observed no change in bacterial N flow in a dual-flow continuous culture supplied with garlic (312 mg L^{-1}) or a mixture of essential oil compounds (5, 50 and 500 mg L^{-1}). Benchaar *et al.* (2007) also reported no change in duodenal bacterial flow. The digestibility of dietary protein in the small intestine is usually higher than that of microbial protein (Van Soest, 1994). However, Yang *et al.* (2007) reported that the digestibility of CP in the intestine was not reduced, even though the proportion (% of total N flow at the duodenum) of dietary N was lower and that of microbial N was higher for diets supplemented with garlic (38 and 58%, respectively) and juniper berry (39 and 58%, respectively) compared with the control (48 and 49%, respectively; data not shown). Digestibility of microbial N in the small intestine is relatively constant (80%), whereas that of escaped feed N varies substantially from 50 to 100 % depending on the feed source (NRC, 2001). Klevenhusen *et al.* (2011a) investigate the Garlic oil and their principal component diallyl disulfides fail to mitigate methane and reported that, diallyl disulfide supplementation improved digestibility and energy use efficiency.

EFFECT OF GARLIC ON N METABOLISM AND RUMINAL MICROBIAL PROTEIN SYNTHESIS

Yang *et al.* (2007) reported that the supplementation with garlic, Juniper Berry, or monensin had no effect on N intake. Similar intakes of N between the control diet and diets supplemented with garlic and juniper berry are consistent with the lack of influence of this essential oil on dry matter intake. The decrease in the duodenal flow of dietary N and the increase in ruminal N digestibility with garlic or juniper berry supplementation suggest that the proteolytic activity in the rumen was stimulated. However, ruminal concentration of NH_3-N was not increased by garlic and juniper berry supplementation (Yang *et al.*, 2007). For example, Busquet *et al.* (2005a) reported no changes in protein degradation when garlic was added at 31.2 or 312.0 mg L^{-1} in a continuous culture system maintained at constant pH. Busquet *et al.* (2005a) reported that the diallyl disulfide, an increase on the ammonia N concentration at 300 mg L^{-1} culture fluid was observed in the batch fermentation and gas production trials. In contrast, in the continuous culture system, diallyl disulfide 300 mg L^{-1} culture fluid tended to increase the small peptide plus amino acid N concentration but had no effect on ammonia N concentration or other rumen microbial N metabolism parameters, which makes it difficult to determine which process was stimulated or inhibited during protein degradation. In the continuous culture trial, 300 mg L^{-1} culture fluid of allyl mercaptan tended to increase the ammonia N concentration, similarly to the observed increase in the ammonia N concentration in the batch fermentation trial at the same concentration. However, allyl mercaptan 300 mg L^{-1} culture fluid had no effects on ammonia N concentration in the gas production trial. Benchaar *et al.* (2006) showed no effect on in situ ruminal degradability of N of soybean meal incubated in the rumen of lactating cows supplemented with 2 g day^{-1} of a mixture of essential oil compounds (Benchaar *et al.*, 2006). Molero *et al.* (2004) speculated that the effects of essential oil on N metabolism may result from the inhibition of proteolytic activity or a decrease in the attachment and colonization of feed by proteolytic microbes. Cardozo *et al.* (2004) reported that garlic oil in continuous culture reduced ammonia N and increased peptide and amino acid N concentrations, suggesting that deamination were inhibited.

EFFECT OF GARLIC ON MILK PRODUCTION AND MILK COMPOSITIONS

Yang *et al.* (2007) investigate the effect of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows reported that the milk fat content increase with garlic in diet. The mechanism by which garlic increased milk fat content is not clear, because dry matter intake and fiber digestibility in the rumen were not different between cows fed garlic and those fed the control diet. In contrast, Benchaar *et al.* (2006) observed no change in milk yield and milk composition in cows supplemented with 2 g day^{-1} or 750 mg day^{-1} of a commercial mixture of essential oil compounds, respectively. However, the type and the dose of essential oil supplement were different between the current study. reported that the Milk protein and milk lactose contents as well as their yields were not affected by the treatments. Similarly, milk efficiency, expressed either as kilograms of milk or as Fat-corrected Milk (FCM) per kilogram of dry matter intake, was not different among the treatments.

EFFECT OF GARLIC ON CHOLESTEROL PROFILE

Numerous studies have demonstrated that inhibitory effects of garlic organosulfur compounds on cholesterol biosynthesis in the hepatocytes by inhibition of the HMG-CoA reductase (Gebhardt and Beck, 1996; Cho and Xu, 2000), which could explain an inhibitory effect of garlic oil on methanogenic Archaea. This hypothesis was supported by the findings of Miller and Wolin (2001) that demonstrated that lovastatin and mevastatin (decrease cholesterol production in humans by inhibiting HMG-CoA reductase) have the specifically potential to inhibit rumen methanogenic Archaea without affecting on the rumen bacteria, Because of their different membrane lipid composition (Miller and Wolin, 2001). To try to confirm the hypothesis of the inhibitory effect of garlic oil on methanogenic Archaea, lovastatin was included as positive control in the gas production trial to compare the effects of the antibiotic on rumen microbial fermentation patterns with the ones observed for garlic oil and its compounds under the same in vitro conditions. Reported Although the concentration used for lovastatin (5 mg L^{-1}) in the gas production trial was higher than that recommended by Miller and Wolin (2001) (4 mg L^{-1}), lovastatin had no effects on methane production or other microbial fermentation parameters. The difference between the results observed by Miller and Wolin (2001) and the present trial with lovastatin could be due to the different in vitro systems used (pure cultures of rumen bacteria vs. mixed ruminal bacteria) (Busquet *et al.*, 2005a).

CONCLUSION

Garlic showed positive effects on the performance of different animals. Garlic oil and active components tested at high doses have inhibited rumen microbial fermentation, confirming their antimicrobial activity. Garlic oil is potential option for application as additives for ruminants. Although garlic oil is identified for its health benefits (antiparasitic, insecticidal, anticancer, antioxidant, immunomodulatory, anti-inflammatory and hypoglycemic), its antimicrobial activity against a wide spectrum of Gram-positive and Gram-negative bacteria is its most prominent activity and has been thoroughly studied.

REFERENCES

Ali, M., K.K. Al-Qattan, F. Al-Enezi, R.M.A. Khanafer and T. Mustafa, 2000. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. Prostaglandins, Leukotrienes Essent. Fatty Acids, 62: 253-259.

Allen, M.S., 2000. Effects of diet on short term regulation of feed intake by lactating dairy cattle. J. Dairy Sci., 83: 1598-1624.

Amagase, H., B.L. Petesch, H. Matsuura, S. Kasuga and Y. Itakura, 2001. Intake of garlic and its bioactive components. J. Nutr., 131: 955S-962S.

Andersen, P.H., B. Bergelin and K.A. Christensen, 1994. Effect of feeding regimen on concentration of free endotoxin in ruminal fluid of cattle. J. Anim. Sci., 72: 487-491.

Anonymous, 1994. Garlic. Lawrence Review of Natural Products.

Benchaar, C., H.V. Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette and P.V. Chouinard, 2007. Effects of essential oils on digestion, ruminal fermentation, ruminal microbial populations, milk production and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci., 90: 886-897.

Benchaar, C., H.V. Petit, R. Berthiaume, T.D. Whyte and P.Y. Chouinard, 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production and milk composition in dairy cows. J. Dairy Sci., 89: 4352-4364.

Burke, J.M., A. Wells, P. Casey and J.E. Miller, 2009. Garlic and papaya lack control over gastrointestinal nematodes in goats and lambs. Vet. Parasitol., 159: 171-174.

Busquet, M., S. Calsamiglia, A. Ferret, P.W. Cardozo and C. Kamel, 2005a. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. J. Dairy Sci., 88: 2508-2516.

Busquet, M., S. Calsamiglia, A. Ferret and C. Kamel, 2005b. Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. Anim. Feed Sci. Technol., 123-124: 597-613.

Busquet, M., S. Calsamiglia, A. Ferret, M.D. Carro and C. Kamel, 2005c. Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci., 88: 4393-4404.

Busquet, M., S. Calsamiglia, A. Ferret and C. Kamel, 2006. Plant extracts effect *in vitro* rumen microbial fermentation. J. Dairy Sci., 89: 761-771.

Calsamiglia, S., L. Castillejos and M. Busquet, 2006. Alternatives to Antimicrobial Growth Promoters in Cattle. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C. and J. Wiseman (Eds.). Nottingham University Press, Nottingham, UK., pp: 129-167.

Cantore, P.L., V. Shanmugaiah and N.S. Iacobellis, 2009. Antibacterial activity of essential oil components and their potential use in seed disinfection. J. Agric. Food Chem., 57: 9454-9461.

Cardozo, P.W., S. Calsamiglia, A. Ferret and C. Kamel, 2004. Effects of natural plant extracts on protein degradation and fermentation profiles in continuous culture. J. Anim. Sci., 82: 3230-3236.

Cardozo, P.W., S. Calsamiglia, A. Ferret and C. Kamel, 2005. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high cocncentrate diet for beef cattle. J. Dairy Sci., 83: 2572-2579.

Castillejos, L., S. Calsamiglia, A. Ferrer and R. Losa, 2007. Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 132: 186-201.

Cho, S.B.H. and S. Xu, 2000. Effects of allyl mercaptan and various allium-derived compounds on cholesterol synthesis and secretion in Hep-G_2 cells. Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol., 126: 195-201.

Chung, L.Y., 2006. The antioxidant properties of garlic compounds: Allyl cysteine, alliin, allicin and allyl disulfide. J. Med. Food, 9: 205-213.

Davidson, P.M. and A.S. Naidu, 2000. Phyto-Phenols. In: Natural Food Antimicrobial System, Naidu, A.S. (Ed.). CRC Press, Boca Raton, FL., ISBN: 8493-2047, pp: 265-294.

Demeyer, D.I. and C.I. Van Nevel, 1975. Methanogenesis, an Intergrated Part of Carbohydrate Fermentation and its Control. In: Digestion and Metabolism in the Ruminant, McDonald, L.W. and A.C.I. Warner (Eds.). The University of New England Publishing Unit, Armidale, Australia, pp: 366-382.

FDA, 2004. CFR: Code of federal regulations title 21. US Food and Drug Administration, 21 CFR 184.

Feldberg, K.S., S.C. Chay, A.N. Kotik, M. Nadler, Z. Neuwirth, D.C. Sunderstrom and N.H. Thompson, 1988. *In vitro* mechanism of inhibition of bacterial cell growth by Allicin. Antimicrob. Agents Chemother., 32: 1763-1768.

Ferme, D., M. Banjac, S. Calsamiglia, M. Busquet, C. Kamel and G. Avgustin, 2004. The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. Folia Microbiol., 49: 151-155.

Gebhardt, R. and H. Beck, 1996. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids, 31: 1269-1276.

Hammer, K.A., C.F. Carson and T.V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. J. Applied Microbiol., 86: 985-990.

Hino, T. and J.B. Russell, 1985. Effect of reducing-equivalent disposal and NADH/NAD on deamination of amino acids by intact rumen microorganisms and their cell extracts. Applied Environ. Microbiol., 50: 1368-1374.

Kim-Park, S. and D.D. Ku, 2000. Garlic elicits a nitric oxide-dependent relaxation and inhibits hypoxic pulmonary vasoconstriction in rats. Clin. Exp. Pharm. Physiol., 27: 780-786.

Klevenhusen, F., J.O. Zeitz, S. Duval, M. Kreuzer and C.R. Soliva, 2011a. Garlic oil and its principal component diallyl disulfide fail to mitigate methane but improve digestibility in sheep. Anim. Feed Sci. Tech., 166-167: 356-363.

Klevenhusen, F., S. Duval, J.O. Zeitz, M. Kreuzer and C.R. Soliva, 2011b. Diallyl disulphide and lovastatin: Effects on energy and protein utilisation in, as well as methane emission from, sheep. Arch. Anim. Nutr., 65: 255-266.

Kotzekidou, P., P. Giannakidis and A. Boulamatsis, 2008. Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens *in vitro* on the fate of inoculated pathogens in chocolate. LWT-Food Sci. Technol., 41: 119-127.

Lawson, L.D., 1996. The Composition and Chemistry of Garlic Cloves and Processed Garlic. In: Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species, Koch, H.P. and L.D. Lawson (Eds.). Williams and Wilkins, Boltimore, pp: 37-108.

Martin, S.A. and J.M. Macy, 1985. Effects of monensin, pyromellitic diimide and 2-bromoethanosulfonic acid on rumen fermentation *in vitro*. J. Anim. Sci., 60: 544-550.

Miller, T.L., 1995. Ecology of Methane Production and Hydrogen Sinks in the Rumen. In: Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction, Engelhardt, W., S. Leonhard-Marek, G. Breves and D. Giesecke (Ed.). Ferdinand Enke Verlag, Stuttgart, Germany, pp: 317-331.

Miller, T.L. and M.J. Wolin, 2001. Inhibition of growth of methane-producing bacteria of the ruminant forestomach by hydroxymethylglutary-SCoA reductase inhibitors. J. Dairy Sci., 84: 1445-1448.

Mirzaei-Aghsaghali, A and H. Fathi, 2012. Lactose in ruminants feeding: A review. Ann. Biol. Res., 3: 645-650.

Mirzaei-Aghsaghali, A., N. Maheri-Sis, A. Mirza-Aghazadeh, A.R. Safaei and A. Aghajanzadeh-Golshani, 2008. Nutritional value of alfalfa varieties for ruminants with emphasis on different measuring methods: A review. Res. Biol. Sci., 3: 1227-1241.

Mirzaei-Aghsaghali, A. and N. Maheri-Sis, 2011. Factors affecting mitigation of methane emission from ruminants I: Feeding strategies. Asian J. Anim. Vet. Adv., 6: 888-908.

Mirzaei-Aghsaghali, A., S.A. Syadati and H. Fathi, 2012. Some of thyme (*Thymus vulgaris*) properties in ruminant's nutrition. Ann. Biol. Res., 3: 1191-1195.

Molero, R., M. Ibara, C. Caalsamiglia, A. Ferret and R. Losa, 2004. Effects of a specific blend of essential oil compounds on dry matter and crude protein degradability in heifers fed diets with different forage to concentrate ratios. Anim. Feed Sci. Technol., 114: 91-104.

Mukherjee, S., I. Lekli, S. Goswami and D.K. Das, 2009. Freshly crushed garlic is a superior cardioprotective agent than processed garlic. J. Agric. Food Chem., 57: 7137-7144.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academy of Sciences, Washington, DC., USA.

Newbold, C.J., F.M. McInotch, P. Williams, R. Losa and R.J. Wallace, 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 114: 105-112.

O'Gara, E.A., D.J. Hill and D.J. Maslin, 2000. Activities of garlic oil, garlic powder and their diallyl constituents against *Helicobacter pylori*. Applied Environ. Microbiol., 66: 2269-2273.

Oh, H.K., T. Sakai, M.B. Jones and W.M. Longhurst, 1967. Effect of various essential oils isolated from Douglas fir needles upon sheep and deer rumen microbial activity. Appled Microbiol., 15: 777-784.

Reuter, H.D., H.P. Koch and L.D. Lawson, 1996. Therapeutic Effects and Applications of Garlic and its Preparations. In: Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species, Koch, H.P. and L.D. Lawson (Eds.). Williams and Wilkins, Baltimore, MD., pp: 135-213.

Ried, K., O.R. Frank, N.P. Stocks, P. Fakler and T. Sullivan, 2008. Effect of garlic on blood pressure: A systematic review and meta-analysis. BMC Cardiovasc Disord., Vol. 8. 10.1186/1471-2261-8-13

Schelling, G.T., 1984. Monensin mode of action in the rumen. J. Anim. Sci., 58: 1518-1527.

Skandamis, P.N. and G.J.E. Nychas, 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs and oregano essential oil concentrations. Applied Environ. Microbiol., 66: 1646-1653.

Suffredini, A.F., G. Fantuzzi, R. Badolato, J.J. Oppenheim and N. O'Grady, 1999. New insights into the biology of the acute phase response. J. Clin. Immunol., 19: 203-214.

Syadati, S.A., A. Mirzaei-Aghsaghali, H. Fathi and J. Davuodi, 2012. Importance essential fatty acids (n-6 and n-3) in animal nutrition: I: Ruminant. Ann. Biol. Res., 3: 1161-1176.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O and B Books Inc., Corvalis.

Van Soest, P.J., 1994. Nitrogen Metabolism. In: Nutritional Ecology of the Ruminant, Van Soest, P.J. (Ed.). Cornell University Press, Ithaca, New York, pp: 290-311.

Yang, W.Z., C. Benchaar, B.N. Ametaj, A.V. Chaves, M.L. He and T.A. McAllister, 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. J. Dairy Sci., 90: 5671-5681.