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THE EFFECTS OF PLANTS EXTRACTS ON FERMENTATION AND METHANE PRODUCTION UNDER *IN VITRO* CONDITIONS

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ABSTRACT
Three experiments were carried out to investigate the effects of green tea (GRT), turmeric (TUR) and garlic (GAR) extracts at three different dose levels on methane (CH_4) production and fermentation in 24 h rumen batch culture experiments. Treatments were control (CON) or CON plus the supplement at 10, 20 or 30 g/kg of diet dry matter (DM). Neutral detergent fiber (NDF) and DM digestibilities were not affected by dietary treatments. Relative to CON, none of the supplementations had an effect on CH_4 production. Relative to CON, the proportion of propionate decreased ($P<0.05$) with the addition TUR at 10 and 20 g/kg DM GRT but increased ($P<0.05$) with the GAR, particularly when added at 20 g/kg of diet DM. Except for the reduction ($P<0.05$) in acetate proportion with the GAR at 20 g/kg DM, none of the supplements had effects on acetate proportion relative to CON. GRT supplementation at 20 and 30 g/kg DM increased ($P<0.05$) ammonia (NH_3-N) concentration relative to CON. Our results show the plant extracts tested in these studies had no significant effects on rumen CH_4 production or rumen microbial fermentation.

KEYWORDS: Methane, green tea, turmeric, garlic, *in vitro*.

INTRODUCTION

Fermentation of feeds in the rumen is the largest source of methane (CH_4) from enteric fermentation, and CH_4 is one of the important gases contributing to the greenhouse effect. Ruminant livestock constitute worldwide the most important source of anthropogenic emissions of CH_4, accounts for 17-37% of global anthropogenic CH_4 (Knapp *et al*., 2014), since CH_4 production is a natural and inevitable outcome of rumen fermentation. Besides its effect on the environment, CH_4 production in the rumen also represents a significant feed energy loss (2-15%), depending upon types of diets (Johnson and Johnson, 1995). Therefore, reducing ruminal CH_4 not only improves the efficiency of nutrient utilization but also helps protect the environment from warming. A number of CH_4 inhibitors have been reported to decrease enteric CH_4 production (Patra, 2012; Hristov *et al*., 2013). However, each of them often exerts adverse effects on feed digestion and rumen fermentation when added at high enough doses to achieve effective CH_4 redutions (Patra and Yu, 2013). In addition, some of these inhibitors are toxic to animals and/or decrease rumen fermentation (Patra, 2012; Hristov *et al*., 2013). In recent years, some plant extracts rich in flavonoid polyphenol or organosulphurs compounds have arisen as attractive rumen modifiers to improve rumen microbial metabolism as well as reduce CH_4 production in ruminants (Soliva *et al*., 2011; Abarghuei *et al*., 2013; Kim *et al*., 2015; Aemiro *et al*., 2016). Flavonoid polyphenol have been shown to serve as alternative hydrogen (H_2) sink during their microbial degradation (Chesson et al., 1982) and therefore may work as methanogen inhibitors in the rumen (Busquet *et al*., 2005). The aim of this study therefore was to assess the effects of adding flavonoid polyphenol rich plant extracts (Green tea (GRT), Turmeric (TUR), or garlic (GAR)) to ruminant diets at low doses on rumen CH_4 production and fermentation under *in vitro* conditions.

MATERIALS & METHODS

Materials

The three plant extracts used in this research were green tea (*Camellia sinensis*), turmeric (*Curcuma longa*) and garlic (*Allium sativum*) extract. Plant extracts were purchased from Bulk Supplements Co. (Henderson, NV, USA).

Experimental design

Three separate experiments were performed to evaluate green tea (GRT), turmeric (TUR), and garlic (GAR) extracts at three different levels for their effects on rumen CH_4 production and fermentation in a 24-hour batch culture system. Treatments were control without extract (CON) or CON plus the extract at either 10, 20 or 30 g/kg of diet DM.

Ruminal contents were collected 3 h after morning feeding from a ruminally fistulated Holstein cow fed a total mixed ration (TMR) composed of 750 g/kg alfalfa-hay mix, 150 g/kg ground corn, 50 g/kg soy hulls, and 50 g/kg soybean meal (DM basis). The rumen contents were brought to the laboratory in a plastic bag under anaerobic conditions, strained through 2 layers of cheesecloth, and used within 15 min. Cultures were maintained in 250 ml ANKOM gas jars containing 3.0g finely grounded diet, 40 ml of strained

ruminal fluid, 160 ml of media, and 8 ml of reducing solution according to Goering and Van Soest (1970). The diet was composed (on a DM basis) of 500 g/kg timothy hay, 320 g/kg ground corn, 70 g/kg soybean meal, 100 g/kg soy hulls, and 10 g/kg mineral-vitamin mix. Each jar was gassed with CO_2 before sealing, connected to a Tedlar gas collection bag (CEL Scientific Corp., Santa Fe Springs, CA, USA) by the vent valve on the jars and then placed in a water bath at 38º C for 24 hours. ANKOM gas jars were programed to release gasses from jars into connected bags when the psi exceeds 1.0. Every two hours, the jars were shook by hand for 20 seconds. After 24 hours, gas bags were disconnected from jars and analyzed for gas composition within 24 hours. All treatments were incubated in triplicate. To estimate diet digestibility, approximately one gram of the diet was weighed into a mobile nylon bag (5 x 10-cm Dacron bags that had a 50-µm pore size; Ankom Inc., Fairport, NY; USA) and placed in each of the ANKOM jars. After 24 hours incubation, the bags were removed and rinsed in cold water for a total of six rinses. The bags were then dried in an oven at 55ºC for 48 h, placed in a desiccator for 3 h, and weighed. The samplers and diets were then analyzed for DM (AOAC, 2000) and NDF (Van Soest *et al*., 1991).

In vitro gas production

Three separate samples were collected from each bag by a 1mL gas-tight needle syringe (27G 1 1/4; Fisher Scientific, Chicago, IL, USA) and analyzed for gas composition using gas chromatography (SRI 8610C, Torrance, CA, USA) equipped with TCD detector (6' x1/8' S.S. ShinCarbon) and ST 80/800 column (2 m x2 mm internal diameter). The oven temperature was programmed at 38°C for 5 min, then increased at 5°C/min to 270°C and held for 5 min. Argon was used as a carrier gas and peaks (CO_2, H_2, N_2, O_2 and CH_4 were identified by comparing the retention times with those of the corresponding standard (Scotty Analyzed Gases 14, Sigma-Aldrich, St Louis, MO, USA).

The relative proportion of each gas in collected gas bags was calculated using the response factor (RF) equation:

$$RF = (CC_{i}/Area_{i}) \times (Area_{ref}/CC)$$

Where RF is the response factor, CC_i is the proportion of gas in the sample of the gas being tested, $Area_i$ is the area of gas *i* peak, CC_{ref} is the proportion of the reference gas (helium) in the internal standard, and $Area_{ref}$ is the area of the peak of the reference gas.

The relative proportion of each gas in collected gas bags was calculated in milliliters using Avogadro's law, as follows:

$$N = P(V/RT)$$

Where *N* is the amount of gas produced in moles, *P* is the pressure in Kilopascal, *V* is the head-space volume in the gas jars in liters, *T* is the temperature in Kelvin, and *R* is the gas constant.

Measurement of in vitro fermentation parameters

Two 5-mL samples were withdrawn from each culture flask at 24 h, while being stirred with a magnetic bar under a stream of CO_2 for volatile fatty acid (VFA) and ammonia-N determination. Collected samples were placed immediately in an ice bath and then stored at -20°C until analyses. The pH was measured immediately after samples were collected from each jar with a portable pH meter (averaged 6.5).

Samples for VFA's analysis were mixed with 1 mL of freshly prepared 25% meta-phosphoric acid, centrifuged (IEC Centra GP8R, Needham Heights, MA, USA) at 20,000g at 4°C for 20 min and supernatant fluid was then collected and store -20°C until further analysis. Samples for VFA analysis were prepared as described by Jenkins (1987), using 2-ethylbutyric acid as an internal standard. A Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) equipped with a flame-ionization detector and 30-m SP-2560 fused silica capillary column (RestekStabil WAX DA column, Bellefonte, PA, USA) was utilized for VFA analysis. The helium carrier gas was maintained at a linear velocity of 23 cm/s. The oven temperature was programmed to 65°C for 3 min, increased at 12°C/min to a final temperature of 225°C, which was held for 9 min. The column temperature was maintained at 65°C and flameionization detector temperature at 225°C. For ammonia-N, the 5 mL collected sample was centrifuged at 20,000g (IEC CentraGP8R, Needham Heights, MA, USA) at 4°C for 10 min. The supernatant was then acidified with 0.5 mL of 0.1 N HCL and stored at -20°C until analysis. Acidified samples were thawed and analyzed for ammonia-N, as outlined by Cotta and Russell (1982).

Statistical analysis

Data were analyzed using the MIXED procedures of SAS (Version 9.1, Statistical Analysis System 2003) using treatment as the fixed effect and replicate as the random effect. Differences among treatment means were tested using PDIFF. All results were expressed as least-square means and significance was declared at $P<0.05$.

RESULTS & DISCUSSION

The effects of GRT dose levels on diet digestibility, gas production, NH_3-N and VFA proportions are presented in Table 1. Addition of GRT to cultures did not affect ($P>0.05$) diet digestibility and total gas production. The *iso*butyrate, *iso*valerate, and butyrate proportions were also not affected ($P>0.05$) by GRT addition; howeover, the valerate proportion increased ($P<0.05$) with the 20 and 30 g/kg DM GRT addition. These results suggested that GRT had no inhibitory effects on microbial fermentation. The effect of GRT on rumen fermentation is mainly related to its flavonoid polyphenolic compounds. Green tea extracts contain flavonoid polyphenolic compounds that account for 30% of the dry weight of leaves (Mukhtar and Ahmad, 2000). The flavonoid polyphenols in green tea leaves are known as catechins and account for nearly 80–90% of the total polyphenol content (Riemersma *et al*., 2001). Green tea flavonoid polyphenols as antioxidant may have an important role in the rumen ecosystem. Aemiro *et al*. (2016) reported that VFA concentration was reduced by 8.6–15.9% with increasing concentrations of green tea extract (20-50 g/kg DM). Kondo *et al*. (2004) also indicated that the addition of green tea grounds reduced VFA production. The discrepancy among the studies may be related to several factors such as diet composition, the

period of adaptation to the product, dose levels, and/or the level of active components in the green tea extracts (Bodas *et al*., 2012).

TABLE 1. The effects of green tea (GRT) addition at different levels on gas production and fermentation parameters in rumen cultures

	GRT doses (g/kg)				
Item	CON	10	20	30	MSE
Diet digestibility, %					
Dry matter	51.4	52.2	48.6	52.7	1.74
Neutral detergent fiber	33.3	34.1	31.2	33.1	1.27
Ammonia-N, mg/dL	5.4^{c}	5.7^{cb}	7.0^{ba}	7.5^{a}	0.47
Gas production, mL					
Total	267.9	269.1	267.9	273	4.27
H_2	7.2	11.2	7.6	8.1	2.31
CH_4	97.5	79.9	82.2	84.4	9.49
CO_2	139.5	149.7	145.5	153.6	7.44
VFA, mole/100 mole					
Acetate	47.2	47.0	44.7	46.0	0.89
Propionate	25.5^{a}	25.7^{a}	24.3^{b}	24.6^{b}	0.20
*Iso*butyrate	1.2	0.8	1.3	0.8	0.30
Butyrate	21.8	21.4	22.2	21.3	0.60
*Iso*valerate	1.2	1.0	1.2	1.1	0.06
Valerate	3.2^{b}	4.1^{b}	6.4^{a}	6.1^{a}	0.48

VFA = volatile fatty acids, CH4 = methane gas, CO2 = carbon dioxide gas, H2 = hydrogen gas, MSE = mean standard error, CON = control

$^{a\,b\,c}$ rows with different superscripts are statistically different at $P < 0.05$

In present study, addition of GRT at 20 and 30 g/kg DM to cultures increased ($P<0.05$) NH_3-N concentration. The increase in NH_3-N concentration might be related to greater crude protein intake with the 20 and 30 g/kg GRT diets. Green tea extracts contain 18-35% crude protein of the dry weight of leaves. Phenolic compounds can bind and precipitate macromolecules, such as dietary proteins, thereby reducing protein digestibility (Bravo, 1998). The lack of effect of GRT supplementation on reducing of NH_3-N concentration could be related to phenolic structure of GRT. The catechins are low molecular weight phenols (monomers). The protein-binding capacity of phenolic compounds is due to the high degree of hydroxylation of phenolic polymers, whereas low molecular weight phenols are unable to precipitate proteins (Bravo, 1998). Sarni-Manchado *et al*. (1999) reported that protein binding of polymeric condensed tannins was stronger than that of low molecular weight oligomers and monomers. On the other hand, Aemiro *et al*. (2016) reported that *in vitro* NH_3-N concentrations decreased with increasing concentrations of green tea extract. In present study, addition of GRT at 20 and 30 g/kg DM to cultures decreased ($P<0.05$) propionate concentration. These results could be related to utilization of H_2 by flavonoid polyphenols, such as tea catechins, during their microbial fermentation. Propionate formation is known to be a H_2 sink when H_2 is not utilized in other production. In present study, GRT addition to cultures tended ($P<0.16$) to decrease CH_4 production compared to CON suggesting that GRT could have effects on CH_4 emission in a dose-dependent manner. A decrease in CH_4 production by phytochemicals may be confounded with several factors such as suppression of protozoa, archaea and H_2-producing microbial populations and/or decreased fiber digestion in the rumen. However, the green tea extract in this study apparently did not result in a net H_2 formation, and hence had no adverse effects on overall digestion. It was reported that the antibacterial activity of (+)-catechin was very weak (Sakanaka *et al*., 1989; Kajiya et al., 2004). Bravo *et al.* (1994) noted that tea catechins disappeared in large intestine, suggesting that certain microorganisms can degrade tea catechins under anaerobic condition. The decrease in ruminal CH_4 production with flavonoid polyphenols such as catechin could be linked to its role as an alternative H_2 sink during the degradation of its metabolites. Flavonoid polyphenols such as catechin act as an antireductant under the anaerobic conditions, in contrast to their well-known antioxidant role during oxidative stress. According to Becker *et al*. (2014), during the microbial fermentation of catechin this accounted for the acceptance of six H_2 atoms per catechin molecule. Becker *et al*. (2014) reported that 1.0 mol catechin prevented the emission of 1.2 mol CH_4. Kim *et al*. (2015) reported that *Camelia japonica* extract decreased CH_4 concentration in 24-h *in vitro* batch culture incubations. Addionally, Aemiro *et al*. (2016) also reported 7.4–13.5% decreases in the emission of CH_4 when green tea extract was added to Corriedale wethers rations at 10, 25 and 40g/Kg DM intake.

The effects of TUR dose levels on diet digestibility, gas production, NH_3-N and VFA are presented in Table 2. Although the addition of TUR to cultures at 10 and 20 g/kg DM did not affect ($P>0.05$) diet digestibility and NH_3-N concentration, TUR addition to cultures increased ($P<0.05$) total gas production and valerate proportion and tended ($P<0.16$) to increase CO_2 production. The *iso*butyrate, *iso*valerate, and butyrate proportions were also not affected ($P>0.05$) by TUR addition. These results suggested that TUR improved the microbial fermentation

possibly due to the ability of rumen micromicroorganisms to degrade components in TUR and utilize them as an energy source (Wachenheim *et al*. 1992; Hart *et al*. 2008). TUR contains 3-5% curcuminoids (50-60% curcumin) and up to 5% essential oils and resins. Degradation of curcumin, a flavonoid polyphenol, by gut microbes has been reported (Vitaglione *et al*., 2012). The metabolic and energetic role of polyphenols for microbe growth is unclear. Previous studies (Jung and Fahey, 1983; Grabber and Jung 1991) suggested that part of energy derived from degradation of polyphenols can be channelled towards VFA synthesis, but conversely, Theodorou *et al.* (1987) indicated that rumen degradation of polyphenols and other aromatic compounds is quite small. Previous studies have reported that plant extracts with flavonoid polyphenol can potentiality improve rumen fermentation at relatively low and moderate concentrations (Jiménez-Peralta *et al*., 2011; Abarghuei *et al*., 2013).

TABLE 2. The effects of turmeric extract (TUR) addition at different levels on gas production and fermentation parameters in rumen cultures.

	TUR doses (g/kg)				
Item	CON	10	20	30	MSE
Diet digestibility, %					
Dry matter	54.3	54.8	54.3	54.4	0.81
Neutral detergent fiber	24.2	24.0	23.4	24.2	1.31
Ammonia-N, mg/dL	8.6	9.2	8.9	7.3	0.70
Gas production, mL					
Total	234.7[a]	248.0[b]	248.8[b]	234.1[a]	3.30
H_2	2.6	3.6	2.3	4.0	0.89
CH_4	84.0	85.8	86.6	82.2	2.50
CO_2	128.5[ab]	136.8[a]	136.8[a]	124.6[b]	4.40
VFA, mole/100 mole					
Acetate	44.4	43.6	45.5	44.7	1.18
Propionate	31.1[a]	29.9[b]	29.1[b]	29.4[b]	0.36
*Iso*butyrate	0.6	0.5	0.5	0.5	0.04
Butyrate	18.8	19.2	18.6	18.2	0.96
*Iso*valerate	0.7	0.7	0.6	0.6	0.05
Valerate	4.5[a]	6.1[b]	5.7[b]	6.5[b]	0.33

VFA = volatile fatty acids, CH4 = methane gas, CO2 = carbon dioxide gas, H2 = hydrogen gas, MSE = mean standard error, CON = control

[a b] rows with different superscripts are statistically different at $P < 0.05$

In the present study, addition of TUR to cultures decreased ($P<0.05$) propionate proportion. This result may also be due to H_2 utilization during the microbial degradation of flavonoid polyphenols found in curcumin. Methane production was not affected ($P>0.05$) by TUR addition. However, at the 10 and 20 g/kg DM TUR addition, total gas production increased ($P<0.05$) and CO_2 tended to increase ($P<0.11$) while propionate proportion decreased without changes in H_2 concentration suggesting that TUR may have some positive effects on CH_4 emission. During the fermentation, H_2 could be consumed by the degradation of flavonoid polyphenols in curcumin. Previous reports suggested that some flavonoid polyphenols are partially hydrogenated and thus possibly used as H_2 sinks by rumen bacteria (Chesson *et al*., 1982; Walle *et al*., 2004; Williamson and Clifford, 2010). A lack of significantly effects on CH_4 emission in the present experiment however may be related to low inclusion rates. Pawar *et al.* (2014) showed that 667 and 833 mg/L of turmeric oil decreased CH_4 emmission and 167 and 333 mg/L of turmeric oil had no effects on CH_4 emmission.

The effects of GAR dose levels on diet digestibility, gas production, NH_3-N and VFA are presented in Table 3. GAR had no effects ($P>0.05$) on DM digestibility and total gas production suggesting that GAR did not have detrimental effects on diet fermentability. Similarly, Yang *et al.* (2007) reported the supplementation of garlic oil had no effects on DM, OM, and NDF digestibilities. Patra *et al.* (2007) also reported the feeding of garlic bulb at the rate of 1% of DM intake to buffalo did not affect VFA. Klevenhusen *et al.* (2011b) reported no effects of garlic oil on VFA profile in the ruminal fluid from sheep fed a 50:50 forage: concentrate diet and supplemented daily with 5 g of garlic oil for 19 days. However, Wanapat *et al.* (2008) found that the supplementation of diet with garlic powder resulted in reductions in VFA concentrations in Holstein Friesian crossbred steers. The numerical reductions in NH_3-N concentration with GAR addition, relative to CON, could be related to decline in deamination and/or bacteria use of peptides and amino acids as nitrogen source. Generally, when proteins are protected from ruminal deamination, NH_3-N declines and ruminants would have more amino acids available for absorption in the lower gut. Cardozo et al. (2005) reported that garlic oil (at 0, 3, 3, 30, and 300 mg/L) decreased NH_3-N concentration by inhibiting both peptidolysis and deamination processes in 24-h *in vitro* batch culture incubations. Ferme *et al.* (2004) reported that garlic oil reduced the population of *Prevotella* spp. (mainly *P. ruminicola* and *P. bryantii*), the most abundant proteolytic and deaminating bacterium in

the rumen (Wallace *et al*., 2002). It has been also reported that CH_4 inhibitors such as garlic oil can reduce dehydrogenase activity (Hino and Russell, 1985) which may explain how garlic oil reduces the deamination rate leading to a decrease in ruminal NH_3-N production. Castillejos *et al.* (2006) and Wanapat *et al.* (2008) reported that garlic powder had no effect on ruminal fluid concentration of NH_3-N. Methane production was not affected (P>0.05) by GAR addition. However, the observed changes in acetate and propionate proportions at the 20g/kg DM and the numerical changes in these parameters at the 30g/kg DM GAR addition to cultures and the unchanged H_2 concentrations suggest GAR could have positive effects on CH_4 emission. Patra *et al.* (2010) and Staerfl *et al*. (2010) reported that extracts of garlic bulbs or garlic bulbs mixed with the diet reduced CH_4 production. Other studies also observed significant CH_4 suppressing effects of garlic oil or its compounds (diallyl disulfide and allicin) supplemented in batch cultures (Busquet *et al*., 2005; Macheboeuf *et al*., 2006), Rusitec fermenters (Soliva *et al*., 2011), or *in vivo* (Kongmun *et al*., 2011). In contrast, no effects on CH_4 production were observed by others (Kamel *et al*., 2008; Kongmun *et al*., 2010; and Patra *et al*., 2011).

TABLE 3. The effects of garlic extract (GAR) addition at different levels on gas production and fermentation parameters in rumen cultures.

	GAR doses (g/kg)				
Item	CON	10	20	30	MSE
Diet digestibility, %					
Dry matter	53.6	54.5	52.7	53.0	2.29
Neutral detergent fiber	28.3	27.2	25.1	25.6	2.84
Ammonia-N, mg/dL	6.9	6.0	5.8	5.8	0.57
Gas production, mL					
Total	379.2	381.1	387.1	376.5	7.07
H_2	4.3	4.8	2.6	2.7	1.24
CH_4	123.8	116.1	120.8	118.9	3.51
CO_2	229.6	230.4	234.9	218.3	7.08
VFA, mole/100 mole					
Acetate	42.8^{a}	45.9^{a}	32.5^{b}	38.2^{ab}	3.17
Propionate	30.5^{a}	31.2^{a}	38.6^{b}	35.0^{ab}	2.19
*Iso*butyrate	1.4	0.8	1.2	1.4	0.32
Butyrate	18.6^{ab}	16.8^{a}	21.2^{b}	17.6^{a}	1.15
*Iso*valerate	2.5^{a}	1.2^{b}	1.3^{b}	1.0^{b}	0.24
Valerate	4.2^{a}	4.2^{a}	5.3^{a}	6.9^{b}	0.42

VFA = volatile fatty acids, CH4 = methane gas, CO2 = carbon dioxide gas, H2 = hydrogen gas, MSE = mean standard error, CON = control

$^{a\,b}$ rows with different superscripts are statistically different at $P < 0.05$

The garlic and its main components may affect methanogenesis by inhibiting growth, development and/or activity of rumen methanogenic microbes both directly by affecting methanogens and indirectly by reducing the number of protozoa associated with methanogens. It was hypothesised that garlic derived organosulphur compounds might inhibit the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase which catalyses synthesis of isoprenoid units in the membranes of methanogenic archaea (Busquet *et al*., 2005; Soliva *et al*., 2011). A lack of significantly effect on CH_4 emission in the present experiment might be related to the dosages of GAR used. Via analysis by real-time polymerase chain reaction, McAllister and Newbold (2008) observed that allicin at 20 mg/ L decreased methanogen numbers in Rusitec, but not at 2 mg/ L. Klevenhusen *et al.* (2011a) reported a decrease in CH_4 production when sheep fed a 50:50 forage:concentrate diet and supplemented with diallyl disulfide (4 g per day), but no effects were reported with lower doses of diallyl disulfide (2 g per day; Klevenhusen *et al*., 2011b). Addition of GAR at the 20 g/kg DM dose to cultures increased (P<0.05) the proportions of propionate and butyrate and reduced the proportion of acetate compared with the CON. On the other hand, addition of GAR at the 30 g/kg DM dose to cultures tended (P<0.13) to increase the proportion of propionate and tended (P<0.16) to reduce the proportion of acetate compared with the CON. These results could be more probably explained by the reduced populations of the protozoans or methanogenic *Archea* population. The inhibition of protozoa by GAR and GAR component's may favour propionate producer bacterial species, thereby increasing accumulation of propionate in the rumen (Wallace *et al*., 2002). Previous *in vitro* and *in vivo* studies showed that supplementing a basal diet with raw garlic or garlic oil reduced number of total protozoans (Wanapat *et al*., 2008; Kongmun *et al*., 2010). Inhibition of CH_4 production via reducing methanogenic Archaea population usually increases the concentration of H_2, which is rechannelled to other H_2 sinks such as propionate, resulting in increased concentration of propionate. Similar to the present study, Busquet *et al.* (2005) reported a decreased proportion of acetate and increased proportion of propionate and butyrate by inclusion of 300 mg/l garlic oil in continuous culture system. Wanapat *et al.* (2008) found that the supplementation of diet with garlic powder resulted in a reduction of the proportion of acetate, but the proportion of propionate and butyrate were increased. GAR and GAR component's effects on rumen fermentation may depend on rumen pH and diet

composition. For example, Cardozo *et al.* (2005) found that garlic oil had a more pronounced impact on rumen VFA profile at low compared with high rumen pH (5.5 versus 7.0), and proposed that the status of the molecules (*i.e.* dissociated or undissociated) maybe dependent on rumen pH. However, batch cultures are usually highly buffered to enable ruminal microorganisms to grow for several hours without removal of fermentation products. Also, in present study, final pH in the cultures moved in a narrow range (6.54-6.56).

CONCLUSION

Results from this study showed that plant extracts used in these studies had no significant effects on rumen CH_4 production or rumen microbial fermentation. Although the addition of these supplements had no effects on CH_4 production, they tended to reduce rumen methanogenesis without adversely affecting rumen fermentation. The lack of effects on CH_4 production by supplements may be related to their low inclusion rates in treatment diets. Future studies need to be aimed at finding a suitable effective dose of these supplements for inhibiting ruminal methanogenesis.

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