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In vitro Effect of Garlic Oil and Turmeric Extract on Methane Production from Gas Test Medium

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Authors' contributions

This work was carried out in collaboration between all authors and all authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the effect of garlic oil and turmeric extract on rumen fermentation and methane emission using an in vitro gas production technique.
Study Design: Completely randomized design.
Place and Duration of Study: Department of animal science, Faculty of agriculture, Ferdowsi University of Mashhad, between November 2012 and June 2013.
Methodology: Treatments were: control (no additives); 10 mg/L of cultural fluid of monensin (MO) as a positive control; 20, 40 and 80 mg/L of cultural fluid of garlic oil (GA20, GA40 and GA80 respectively); 20, 40 and 80 mg/L of cultural fluid of turmeric extract (TU20, TU40 and TU80 respectively). All treatments were incubated for 96 h and gas production was recorded by a pressure transducer at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. At 24 h post incubation, pH of cultural medium and methane production were determined.
Results: Supplementation of MO and GA80 resulted in a significant reduction in total gas production compared with the control. Furthermore, addition of garlic oil at 80 mg/L strongly decreased methane production during 24 h post incubation (43% lower than the control). Mid and high concentrations of turmeric extract (TU40 and TU80) caused to increase in vitro dry matter disappearance (IVDMD) in comparison with the control. Partitioning factor

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(PF = mg DM disappeared/ ml gas produced after 24 h incubation) and pH of the cultural medium were higher using MO, TU20 and GA80 compared with that of the control. Lower extent of gas production (b) and gas production rate (c) were observed in MO and GA80 compared with those of the control.
Conclusion: Present data indicated that using natural additives might alter rumen fermentation pattern as observed in methane emission from a mixed diet.

Keywords: Garlic oil; turmeric extrac;, monensin; me thane production; fermentation; gas production.

1. INTRODUCTION

Methane is one of the greenhouse gasses, which is normally produced during the anaerobic enteric fermentation of feeds in many animals including ruminants [1]. In order to elaborate efficiency of converting feed to animal production consumable by human and decreasing methane emission, utilization of feed additives in ruminant ration were proposed by ruminant nutritionists [2]. Supplementation of diets with ionophores such as monensin lessens losses of energy and nitrogen in the form of methane and ammonia respectively [3]. However, in recent years scientists are seeking alternatives for these ionophores because of the increasing concern about appearance of their residue in milk and meat [4]. Plant secondary metabolites are a group of organic compounds that seems to have no direct function in plant growth and development [5]. In accordance to antimicrobial properties of plant extracts, many studies have focused on their potential for modification of ruminal fermentation during the last decade [6,7,8,9]. Garlic oil consists of different molecules that are found in the plant or as the consequence of changes occurring during oil extraction including allicin, diallyl sulfide, dialyl disulfide and allyl mercaptan among others [10]. It was demonstrated that these compounds might impact rumen fermentation by reducing the proportion of acetate to propionate and also inhibit in vitro methanogenesis [11,12,13]. Furthermore, some in vivo studies also assessed the effect of garlic oil on ruminal fermentation [6,14,15]. Anassori et al. [15] reported the potential of garlic oil to improve rumen fermentation efficiency through increasing the production of propionate and reducing protozoa population. Turmeric is an Indian spice derived from the rhizomes of the plant *Curcuma longa Linn* [16]. Despite of vast studies about antimicrobial and anti-inflammatory characteristics of turmeric [17,18]; only few studies evaluated its effects on rumen fermentation [19,20]. This study was conducted to investigate the effects of garlic oil and turmeric extract on methane production, in vitro dry matter disappearance (IVDMD), partitioning factor (PF), medium pH and gas production parameters of a mixed diet.

2. MATERIALS AND METHODS

2.1 Extracts Preparation

For the preparation of turmeric extract, fresh rhizomes of turmeric (*Curcuma longa Linn.*) were obtained from local market. A hundred g of sample was grinded and extracted with 400 ml ethanol using Soxhlet extraction apparatus (140 °C, 60 min). Garlic oil was acquired from Atrineh Saziba Co. (Iran). Monensin was purchased from Behroodatrak Company (Iran), which produces this product under a license by Elanco Division, Eli Lilly Canada Inc.

2.2 Experimental Treatments

Garlic oil and turmeric extract were dissolved in ethanol (96%, wt/vol) as 50, 100 and or 200 mg per 40 ml. Monensin solution was prepared by dissolving 25 mg monensin in 40 ml ethanol (96%, wt/vol). Benchaar, et al. [21] verified that less than 2% ethanol in cultural medium was not effective on in vitro fermentation. Based on the noted study, in order to omit the effect of ethanol on fermentation, the amount of each stock solution in each fermentation bottle was considered as 0.8 ml (1.6%). Also, equal volumes of ethanol were added to the control bottles. Treatments were as follows: control (no additives), 10 mg/L of cultural fluid of monensin (MO) as a positive control [22]; 20, 40 and 80 mg/L of cultural fluid of garlic oil (GA20, GA40 and GA80, respectively) or 20, 40 and 80 mg/L of cultural fluid of turmeric extract (TU20, TU40 and TU80, respectively).

2.3 Gas Production Technique

Rumen inoculum was obtained before the morning feeding from two ruminally fistulated sheep. Animals were fed 0.5 kg of alfalfa hay and 0.5 kg of concentrate (24% corn grain, 20.4% barley grains, 27% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.5% mineral and vitamin premix and 0.2% salt). All experimental procedures were approved by Advisory committee of Ferdowsi University of Mashhad. Pooled rumen fluid was squeezed through 4 layers of cheesecloth into an insulated thermos. Under continuous flushing of CO_2, 50 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2:1, buffer were prepared as proposed by Menke and Steingass [23]) was dispensed with pipetor pump into a 125 ml serum bottle containing 0.5 g DM of a mixed diet (Table 1). Eight bottles were considered for each treatment in two separate runs. Garlic oil, turmeric extract or monensin solution was added to each bottle to achieve the final concentrations needed. Bottles were sealed with rubber stopper and aluminum cap and placed in a shaking water bath for 96 h at 38.6 ºC. For each run of test, two bottles containing incubation medium without any substrate were incubated as the blanks to correct the gas production resulting from the activity of the rumen fluid.

Table 1. Ingredients and chemical composition of the mixed diet used in *vitro* gas production trial

Item	Percentage
Ingredients (% of DM)	
Alfalfa	45.0
Corn grain	15.0
Barley grain	19.0
Cottonseed meal	6.0
Soybean meal	4.0
Sugar beet pulp	3.0
Wheat bran	5.0
Calcium carbonate	1.0
Salt	1.0
Vitamin-mineral mix[1]	1.0
Chemical composition (g/kg DM)	
CP	155.0
NDF	289.0
ME (Kcal/kg DM)	2.8

Composition of vitamin-mineral mix: Ca, 196.0 g kg^{-1}; P, 96.0 g kg^{-1}; Mg, 19.0 g kg^{-1}; Fe, 3.0 g kg^{-1}; Na, 71.0 g kg^{-1}; Cu, 0.3 g kg^{-1}; Mn, 2.0 g kg^{-1}; Zn, 3.0 g kg^{-1}; Co, g kg^{-1}; I, 0.1 g kg^{-1}; Se, 0.01 g kg^{-1}; and Vit A, 500000 IU kg^{-1}; Vit D, 100000 IU kg^{-1}; Vit E, 100 IU kg^{-1}

Gas pressure was measured by a pressure transducer at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after the incubation. At 24 h post incubation, methane production was determined in four bottles of each treatment in each run using a multiple gas detector (SR2-BIO System, SEWERIN, Germany), then they were opened to measure medium pH using a pH meter (Metrhom pH meter, Model 691). Finally, the content of each bottle were filtered and the residual was oven dried (60 °C for 48 h) and used to calculate in vitro dry matter disappearance.

2.4 Calculation and Statistical Analysis

Gas pressure was converted into volume using an experimentally calibrated curve and cumulative gas production data were fitted to a model of $Y= b (1-e^{-ct})$ [24]; where: Y was potential of gas production at time (t); b was gas production from fermentable fraction (ml/0.5 g DM); c was gas production rate (ml/h); and t was incubation time (h). In vitro dry matter disappearance was considered as g DM disappeared after 24 h incubation. Moreover, partitioning factor was calculated as mg DM disappeared per ml gas produced after 24 h incubation.

Statistical analysis was performed using GLM procedure of SAS [25]. The model used for the analysis was $Yij = \mu + Ti + eij$, where Yij was the dependent variable; μ was the population mean for the variable; Ti was the effect of treatment i; eij was the random error associated with the observation ij. Treatments were compared with control using the Duncan test at $P= 0.05$.

3. RESULTS AND DISCUSSIONS

Gas production after 96 h incubation, methane production, IVDMD, PF, pH after 24 h of incubation and gas production kinetic are presented in (Table 2). Addition of monensin and garlic oil at 80 mg/L of cultural fluid resulted in a significant reduction in total gas production after 96 h incubation compared with that of the control. However, all the levels of turmeric extract raised up the volume of produced gas after 96 h incubation. Relative to the control, the addition of garlic oil at 80 mg/L strongly decreased methane production during 24 h of the incubation. Busquet et al. [12] evaluated the effects of garlic oil and some of its compounds on in vitro rumen fermentation. They reported that 300 mg/L of garlic oil applied directly into the fermentation flasks significantly suppressed total gas and methane production by 20 and 74 percent, respectively. Our findings confirmed these as GA80 decreased the total gas volume and methane production by 15 and 43 percent, respectively. Furthermore, the inhibitory effect of garlic oil on methane production was larger than that of the monensin (0.93 vs. 1.55 mmol/g DM). These results confirmed the previous findings in which garlic oil showed higher antimethanogenic activity than monensin [12,26]. This phenomenon could be due to the different mechanism of action of these additives. Monensin reduces methanogenesis through inhibition of rumen Gram positive bacteria whereas garlic oil may inhibit rumen methanogens directly [27].

Table 2. Effects of monensin and different doses of turmeric extract or garlic oil on *in vitro* ruminal fermentations and gas production kinetic of a mixed diet

	Treatments[1]									
	Control	**MO**	**TU20**	**TU40**	**TU80**	**GA20**	**GA40**	**GA80**	**SEM**	**P**
Total gas[2] (mmol/g DM)	9.14^{c}	8.38^{b}	9.75^{d}	10.15^{e}	10.52^{f}	9.38^{c}	9.48^{cd}	7.77^{a}	0.18	<0.001
Methane[3] (mmol/g DM)	1.62^{bc}	1.55^{b}	1.75^{de}	1.85^{ef}	1.93^{f}	1.68^{cd}	1.75^{de}	0.93^{a}	0.05	<0.001
Methane (mmol/mmol gas)	0.177^{bc}	0.185^{c}	0.180^{bc}	0.182^{bc}	0.175^{b}	0.182^{bc}	0.185^{c}	0.117^{a}	0.004	<0.001
IVDMD[4]	0.61^{b}	0.57^{a}	0.62^{bc}	0.64^{cd}	0.65^{d}	0.64^{cd}	0.62^{bc}	0.62^{bc}	0.01	0.01
PF[5]	2.46^{a}	3.08^{d}	2.72^{b}	2.57^{a}	2.58^{a}	2.50^{a}	2.47^{a}	2.87^{c}	0.06	<0.001
pH[6]	6.35^{a}	6.40^{c}	6.39^{b}	6.36^{a}	6.35^{a}	6.37^{ab}	6.35^{a}	6.40^{c}	0.01	0.04
b[7] (ml/0.5 g DM)	165.49^{cd}	148.61^{a}	163.47^{c}	166.97^{cd}	169.28^{d}	163.61^{c}	167.77^{cd}	158.52^{b}	2.25	0.002
c[8] (ml/h)	0.058^{de}	0.046^{a}	0.048^{b}	0.058^{de}	0.059^{e}	0.062^{f}	0.057^{d}	0.050^{c}	0.0007	<0.001

[1]MO= monensin (10 mg/L), TU20=turmeric extract (20 mg/L), TU40=turmeric extract (40 mg/L), TU80=turmeric extract (80 mg/L), GA20=garlic oil (20mg/L), GA40=garlic oil (40mg/L), GA80=garlic oil (80mg/L)

[2]After 96h incubation

[3]After 24h incubation

[4]In vitro dry matter disappearance after 24h incubation

[5] Partitioning factor (mg DM disappeared/ ml gas produced after 24h incubation).

[6]After 24h incubation

[7]Asymptotic gas production

[8]Rate of gas production

[a,...,f] Means within the same row having different letters are significantly different (P < 0.05).

Application of TU40 and TU80 improved IVDMD compared with that of the control which was consistent with their stimulatory effect on gas production. Moreover, monensin diminished IVDMD as well as total gas and methane production. Some methane inhibitors may have adverse effect on dry matter digestibility [28]. Among the additives evaluated in the present study, only the highest dose of garlic oil (80 mg/L) reduced methane emission without any negative effect on IVDMD. These data did not confirm the findings of Busquet et al. [12] who observed a reduction in methane production and dry matter digestibility by adding 300 mg/L garlic oil in 17 h batch culture incubation. This inconsistency could be due to higher dose of garlic oil applied in that study compared with current experiment. Partitioning factor of MO, TU20 and GA80 was significantly higher than the data obtained from the control. However, other levels of applied extracts (TU40, TU80, GA20 and GA40) tended to improve the PF. Garc´ıa-Gonz´alez et al. [29] also noticed an improvement in PF by monensin under in vitro conditions. Beauchemin and McGinn [30] observed that a reduction in methane production had an adverse relationship with substrate disappearance. It could be interpreted that supplementation of GA80 might enhance efficiency of feed fermentation through decreasing losses of energy in the form of methane.

Under the present study conditions, inclusion of MO, 20 mg/L of turmeric extract and 80 mg/L garlic oil significantly increased the pH of the cultural medium. Similarly, Castillejos et al. [31] evaluated the different doses of monensin and ten essential oils using an in vitro rumen microbial fermentation and observed an increase in pH of the medium by application of monensin in most cases. However, in the latter study the effect of essential oils on pH were variable. Furthermore, in the study of Busquet et al. [32] only high doses (300 and 3000 mg/L) of different plant extracts including garlic oil increased the pH significantly compared with the data obtained from the control.

Supplementation of MO and GA80 significantly decreased the asymptotic gas production (b) compared with that of the control. In accordance with a decline in the disappearance of DM by addition of MO, a reduction was expected in the cumulative gas production. Furthermore, the rate of gas production decreased by using MO in the cultural medium. Our results are in agreement with a recent in vitro gas production experiment in which, garlic oil at dose of 150 ppm reduced gas production rate (c) in different feed samples [33]. Moreover, a slow gas production trend was observed for MO among treatments (Fig. 1.).

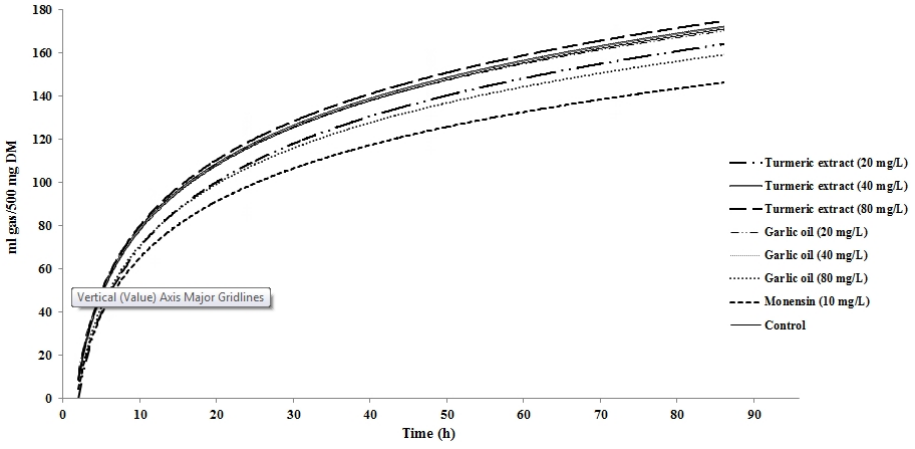

Fig. 1. Gas production profile of different treatments. As the standard errors were the same for all of the treatments, they are not shown in the figure. (SEM=1.46, R^2=0.98)

4. CONCLUSION

The decrease in total gas and methane production observed with the use of garlic oil in the current study indicate its potential to inhibit methanogenesis of a mixed diet. Despite of depletion in total gas production and IVDMD by application of monensin, PF raised. It is concluded that garlic oil when applied in the highest dosage (80 mg/L) is more effective in modifying ruminal fermentation than other applied treatments. Moreover, results indicated that turmeric extract has a moderate effect on fermentation potential even used with different levels.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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