

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

**A. Hodjatpanah-montazeri^{1*}, M. Danesh Mesgaran¹, A. Vakili¹,
B. Ghorbani² and F. Tabatabaie¹**

¹*Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, P O Box 91775-1163, Iran.*

²*Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Iran.*

Authors' contributions

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

Original Research Article

Received 9th July 2013
Accepted 7th October 2013
Published 24th January 2014

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 *in vitro* dry matter disappearance (IVDMD) in comparison with the control. Partitioning factor

*Corresponding author: Email: a.amontazeri@gmail.com;

(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 extract; monensin; methane 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, diallyl 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 CO₂, 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 °C. 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 *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 $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} was the dependent variable; μ was the population mean for the variable; T_i was the effect of treatment i; e_{ij} 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								SEM	P
	Control	MO	TU20	TU40	TU80	GA20	GA40	GA80		
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

¹MO= 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)

²After 96h incubation

³After 24h incubation

⁴In vitro dry matter disappearance after 24h incubation

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

⁶After 24h incubation

⁷Asymptotic gas production

⁸Rate 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. García-González 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.).

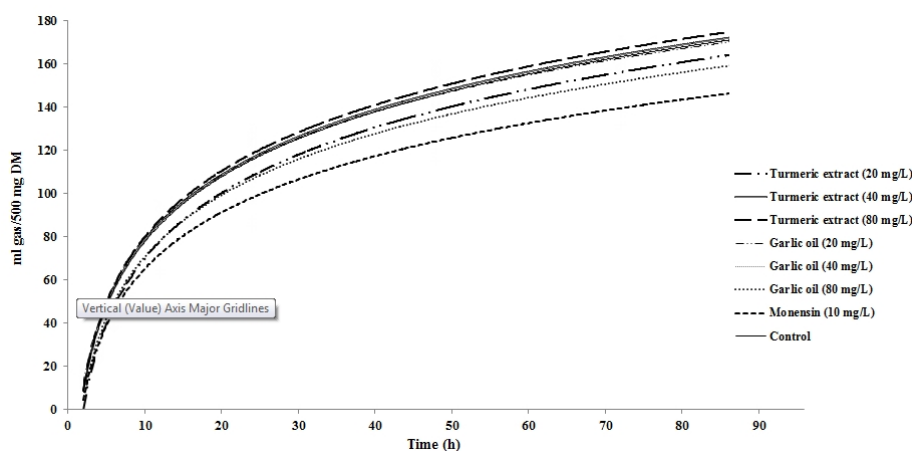


Fig. 1. Gas production profile of different treatments. As the standard errors were the same for all of the treatments, they are not shown in the figure. (SEM=1.46, $R^2=0.98$)

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.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical and financial support of Ferdowsi University of Mashhad.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Grainger C, Beauchemin KA. Can enteric methane emissions from ruminants be lowered without lowering their production? *Anim. Feed Sci. Technol.* 2011;166–167:308–320.
2. Eckard RJ, Grainger C, de Klein CAM. Options for the abatement of methane and nitrous oxide from ruminant production: a review. *Livest. Sci.* 2010;130:47–56.
3. Ipharraguerre IR, Clark JH. Usefulness of ionophores for lactating dairy cows: A review. *Anim. Feed Sci. Technol.* 2003;106:39–57.
4. Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA, Beauchemin KA. A review of plant-derived essential oils in ruminant nutrition and production. *Anim. Feed Sci. Technol.* 2008;145:209–228.
5. Balandrin MF, Klocke JA. Natural plant chemicals: Sources of industrial and medicinal materials. *Science.* 1985;228:1154–1160.
6. Yang WZ, Benchaar C, Ametaj BN, Chaves AV, He ML, McAllister TA. 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.* 2007;90:5671–5681.
7. Macheboeuf D, Morgavi DP, Papon Y, Mousset JL, Arturo-Schaan M. Dose-response effects of essential oils on in vitro fermentation activity of the rumen microbial population. *Anim. Feed Sci. Technol.* 2008;145:335–350.
8. Kongmun P, Wanapat M, Pakdee P, Nawanukrew C. Effect of coconut oil and garlic powder on in vitro fermentation using gas production technique. *Livest. Sci.* 2010;127:38–44.
9. Jahani-Azizabadi H, Danesh Mesgaran M, Vakili A, Rezayazdi K, Hashemi M. Effect of various medicinal plant essential oils obtained from semi-arid climate on rumen fermentation characteristics of a high forage diet using in vitro batch culture. *Afr. J. Microbiol. Res.* 2011;5(27):4812-4819.
10. Lawson L. The composition and chemistry of garlic cloves and processed garlic. In: Koch HP, Lawson LD, editors. *Garlic. The Science and Therapeutic Application of Allium sativum L. and Related Species.* Williams and Wilkins, Baltimore. MD; 2006.

11. Busquet M, Calsamiglia S, Ferret A, Cardozo PW, Kamel C. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. *J. Dairy Sci.* 2005;88:2508–2516.
12. Busquet M, Calsamiglia S, Ferret A, Carro MD, Kamel C. Effect of garlic oil and four of its compounds on rumen microbial fermentation. *J. Dairy Sci.* 2005;88:4393–4404.
13. Busquet M, Calsamiglia S, Ferret A, Kamel C. Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. *Anim. Feed Sci. Technol.* 2005;123:597–613.
14. Chaves AV, Stanford K, Dugan MER, Gibson LL, McAllister TA, Van Herk F, Benchaar C. Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livest. Sci.* 2008;117:215-224.
15. Anassori E, Dalir-Naghadeh B, Pirmohammadi R, Taghizadeh A, Asri-Rezaei S, Maham M, Farahmand-Azar S, Farhoomand P. Garlic: A potential alternative for monensin as a rumen modifier. *Livest. Sci.* 2011;142:276-287.
16. Gilda S, Kanitkar M, Bhonde R, Paradkar A. Activity of water-soluble turmeric extract using hydrophilic excipients. *LWT-Food Sci Technol.* 2010;43:59–66.
17. Negi PS, Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *J. Agri. Food Chem.* 1999;47:4297-4300.
18. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev.* 2009;14(2):141-153.
19. Hodjatpanah AA, Danesh Mesgaran M, Vakili AR. Effects of diets containing monensin, garlic oil or turmeric powder on ruminal and blood metabolite responses of sheep. *J. Anim. Vet. Adv.* 2010;9(24):3104–3108.
20. Vorlaphim T, Phonvisay M, Khotsakdee J, Vasupen K, Bureenok S, Wongsuthavas S, Alhaidary A, Mohamed HE, Beynen AC, Yuangklang C. Influence of dietary curcumin on rumen fermentation, macronutrient digestion and nitrogen balance in beef cattle. *Am. J. Agric. Biol. Sci.* 2011;5:256-260.
21. Benchaar C, Chaves AV, Fraser GR, Wang Y, Beauchemin KA, McAllister TA. Effects of essential oils and their components on in vitro rumen microbial fermentation. *Can. J. Anim. Sci.* 2007;87:413–419.
22. Castillejos L, Calsamiglia S, Ferret A. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. *J. Dairy Sci.* 2006;89:2649–2658.
23. Menke KH, Steingass H. Estimation of energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* 1988;28:7-55.
24. Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agr. Sci.* 1979;92:499-503.
25. SAS. Statistical Analysis Systems user's guide. Version 8.2. SAS Institute, Inc., Cary, NC, USA; 1999.
26. Chaves AV, He ML, Yang WZ, Hristov AN, McAllister TA, Benchaar C. Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. *Can. J. Anim. Sci.* 2008;89:97–104.
27. Benchaar C, Greathead H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Anim. Feed Sci. Technol.* 2011;166-167:338-355.
28. Domesick EJ, Martin SA. Effects of lailomycin propionate and monensin on the in vitro mixed ruminal microorganism fermentation. *J. Anim. Sci.* 1999;77:2305–2312.

29. Garc'ia-Gonz'alez R, L'opez S, Fern'andez M, Bodas R, Gonz'alez JS. Screening the activity of plants and spices for decreasing ruminal methane production in vitro. *Anim. Feed Sci. Technol.* 2008;147:36-52.
30. Beauchemin KA, McGinn SM. Methane emissions from beef cattle: effects of fumaric acid, essential oil and canola oil. *J. Anim. Sci.* 2006;84:1489–1496.
31. Castillejos L, Calsamiglia S, Martin-Tereso J, Ter Wijlen H. In vitro evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. *Anim. Feed Sci. Technol.* 2008;145:259–270.
32. Busquet M, Calsamiglia S, Ferret A, Kamel C. Plant extracts affect in vitro rumen microbial fermentation. *J. Dairy Sci.* 2006;89:761–771.
33. Kilic U, Boga M, Gorgulu M, Şahan Z. The effects of different compounds in some essential oils on in vitro gas production. *J. Anim. Feed Sci. Technol.* 2011;20:626–636.

© 2014 Hodjatpanah-montazeri et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=397&id=32&aid=3429>