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Effects of the Thyme Extract on the Ruminal Methane Production

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Abstract: An experiment was conducted to study the effects of Thyme Methanol Extract (TME), on rumen methanogenesis *in vitro*. Different levels (0, 0.25 and 0.5 mL) of TME were incubated for 2, 4, 12, 16, 24, 48, 72 and 96 h in diluted ruminal fluid with a 100% forage diet (alfalfa) to evaluate their effects on rumen methane production. This experiment was carried out based on Complete Random Design (CRD) with three treatments and three replicates. Treatment A is 0 mL of TME and diet (control), Treatment B is 0.25 mL of TME and diet and Treatment C is 0.5 mL TME and diet. Result indicated that the methanol extract of thyme (0.25 and 0.50 mL) reduced methane production in all of the incubation times without 2 h ($p<0.05$).

Key words: Ether extract, methanol, thyme, methane

INTRODUCTION

With the development of large-scale agriculture in the mid-20th century, farming became a big business for some companies and ruminant one of the important animals for meet of human nutritional demands. Atmospheric methane concentrations have leveled off while the world population of ruminants has increased at an accelerated rate. World ruminant populations were increasing and this rate has increased to 16.96 million head/year in the 2005. There was a strong relationship between change in atmospheric methane concentrations and the world ruminant populations. This change in relationship between the atmosphere and ruminant numbers suggests that the role of ruminants in greenhouse gases may be less significant than originally thought, with other sources and sinks playing a larger role in global methane accounting. Ruminants establish a symbiotic relationship with rumen microorganisms by which the animal provides nutrients and optimal environmental conditions for the fermentation of feeds and microorganisms degrade fiber and synthesize microbial protein as an energy and protein supply for the animal, respectively. However, this symbiotic relationship has energy (losses of methane) and protein (losses of ammonia N) inefficiencies. Van Nevel and Demeyer (1988). These losses not only reduce production performance, but also contribute to the release of pollutants to the environment (Tamminga, 1996). Antibiotic ionophores have been very successful in reducing these energy and protein losses in the rumen (Van Nevel and Demeyer, 1988). However, the use of antibiotics in animal feeds is facing reduced social acceptance because of the appearance of residues and resistant strains of bacteria and their use has been banned in the European Union since January 2006 by Directive 1831/2003/CEE, European Commission (2003). For this reason, scientists have become interested in evaluating other alternatives to modulate rumen fermentation, including the use of yeasts, organic acids, plant extracts, probiotics and antibodies by Calsamiglia *et al.* (2006). Thyme contains active compounds such as thymol and carvacrol by Calsamiglia *et al.* (2007). Thymol is a monoterpene [5-methyl-2-(1-methyleth-yl)phenol; C10H14O] with strong antimicrobial activity against a wide range of gram positive and negative bacteria and is one of the most well-researched active components of essential oils by Burt (2004). Thyme (*Thymus* sp.) and oregano (*Origanum* sp.) oils contain large but variable quantities of thymol by Sivropoulou *et al.* (1996) and Burt (2004). Evans and Martin (2000) reported that thymol affected the energy metabolism of two relevant rumen bacteria grown in pure culture: Streptococcus bovis and Selenomonas ruminantium. It reduced methane and lactate concentrations, although at higher doses it also reduced overall nutrient digestion and total VFA production, a clear indication that microbial metabolism was inhibited. Compounds with phenolic structures, such as thymol, are more effective as antimicrobials in comparison with other non-phenolic secondary plant metabolites because of the presence of a hydroxyl group in the phenolic structure by Helander *et al.* (1998) and Ultee *et al.* (2002). Furthermore, the small molecular weight of thymol allows it to gain access to the cell membrane through the pores of the external wall. The strong and wide spectrum activity against gram positive

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and gram negative bacteria, the narrow margin of security between an optimal and a toxic dose and the effects reported, which were not always in the desired direction by Castillejos *et al*. (2006). Carvacrol [2-methyl-5-(1-methylethyl)phenol; C6H3CH3(OH)(C3H7)] is a phenolic compound similar to thymol found in oregano (*Origanum* sp.) and thyme (*Thymus* sp.) that has strong antimicrobial activity by Calsamiglia *et al*. (2007).The objective o this study were to evaluate thyme extract on the ruminal methane production in the *in vitro* condition.

MATERIALS AND METHODS

Plant materials and preparation of extracts: Dried *Zataria multiflora* (thyme) (Fig. 1) was buying from medical plant center of Tabriz. The plant extracts were prepared according to (Patra *et al*., 2006) with some modifications. The plant materials were dried at 50°C and ground in mills to pass a 1 mm sieve and 100 g placed in 1000 ml of distilled water solvent. The flasks of all the solvents were stopped and agitated with a magnetic stirrer for 24 h at room temperature. Then the solutions were centrifuged at 3000 g for 10 min. The residue was re-extracted with 500 ml of distilled water for 24 h stirring at room temperature and centrifuged again at 3000 g for 10 min. The plant extracts were combined. distilled water was evaporated from the solution at approximately 85°C by using a rotary-evaporator.

***In vitro* gas production:** The samples were incubated in the rumen fluid in serum bottles following the procedures of (Menke and Steingass, 1988) as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated serum bottles of 100 ml in the absence and presence of level 0.25 and 0.5 ml of thyme methanol extract. The serum bottles were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each serum bottles followed by incubation in a oven at 39°C. The serum bottles were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Methane production was measured as the volume of gas in the calibrated serum bottles and was recorded time of incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after incubation with GC (EX-TEC HS680). All samples were incubated in triplicate with three serum bottles containing only rumen fluid-buffer mixture (blank).

Fig. 1: Iranian *Zataria multiflora*

Statistical analysis: Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS (2000). Multiple comparison tests used Duncan's multiple-range test (1980).

RESULTS AND DISCUSSION

The effect of *in vitro* substrate incubation during various incubation hours with different levels of thyme methanol extract on methane production (in percentage) has been presented in Table 1.

After two hours incubation, 0.5 ml level of TME increased methane production significantly compared with control treatment, whereas at other hours incubation, both 0.25 and 0.5 ml TME, decreased methane production significantly compared with control treatment. This is probably due to thymol of thyme having strong antimicrobial effect against gram positive and gram negative bacteria. This has destroyed methane producing bacteria. Evans and Martin (2000) reported which was performed to examine the effects of thymol by 45, 90, 180 and 400 µ.g/ml consumed water on volatile fatty acids ratio, pH and methane concentration, indicated that thymol addition by the rate of 400 µ.g/ml consumed water changed volatile fatty acids concentration and increased final pH and acetate to propionate ratio, but methane, acetate, propionate and lactate concentrations were decreased which is in line with the present study.

Table 1: The effect of different levels of thyme methanol extract on methane production (in percentage) after incubation at various hours

		Incubation Times							
Diet	Thyme levels	2	4	12	16	24	48	72	96
100% Forage	0.00	1^{c}	3^{f}	8^{h}	9^{ji}	7^{i}	14^{fg}	9^{h}	11^{j}
	0.25	1^{c}	1^{h}	1^{i}	1^{k}	1^{j}	1^{i}	1^{i}	1^{k}
	0.50	2^{b}	2^{g}	2^{i}	1^{k}	1^{j}	1^{i}	1^{i}	2^{k}
p-values		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEM		0.0453	0.1083	0.4158	0.3617	0.3228	0.4386	0.7360	0.6267

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