Effects of Zataria Multiflora Water Extract on Rumen Fermentation Using *in Vitro* Gas Production Technique

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Abstract: The aim of the present study was conducted to survey effect of adding different levels (0, 1 ml/30 ml buffered rumen fluid) of *Zataria multiflora water extract* (ZMWE) on soybean meal (SBM) degradability were studied by in *vitro* gas producing techniques. Gas production test with mixtures of filtered rumen liquid of three Taleshi native male cattle rumen in times of 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were performed. The results showed that gas volume at 12 h incubation (for 200 mg dry samples), were 46.23 and 40.9 ml/200mg DM for soybean meal and *Zataria multiflora water extract* (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 24 h incubation (for 200 mg dry samples), were 56.38 and 43.8 ml/200mg DM for soybean meal and *Zataria multiflora water extract* (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 48 h incubation (for 200 mg dry samples), were 62.12 and 45.95 ml/200mg DM for soybean meal and *Zataria multiflora water extract* (1 ml/30 ml buffered rumen fluid), respectively.
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Abbreviations: ZMWE, *Zataria multiflora water extract*; SBM, soybean meal; CP, crude protein; NFC, non-fibrous carbohydrate; ZM, Zataria multiflora; a, the gas production from soluble fraction (ml/200mg DM); b, the gas production from insoluble fraction (ml/200mg DM); c, rate constant of gas production during incubation (ml/h); a + b, the potential gas production (ml/200mg DM).

1. Introduction

Global warming due to increases in the atmospheric concentrations of greenhouse gases such as carbon dioxide and methane is an important issue. Generation of methane from livestock, particularly from ruminants, represents 2 to 12% of gross energy intake (Johnson and Johnson, 1995; Bunthoeun, 2007).

A growing concern about global climate change has increased attention on ways to abate ruminal methanogenesis (Bunthoeun, 2007). Therefore, current interest is focused on use of safe natural products, vs. chemical compounds, to beneficially manipulate ruminal fermentation. Antimicrobial compounds are routinely incorporated into ruminant diets to improve feed efficiency, suppress methanogenesis and reduce excretion of N in urine and feces. In recent years, there has been increased concern regarding use of in feed antibiotics in ruminants due to the progressive increase of antibiotic resistance among pathogenic microorganisms (Carro et al., 2003; Bunthoeun, 2007).

Several methods such as *in vivo, in situ* and *in vitro* techniques have been used in order to evaluate the nutritive value of feedstuffs (Maheri Sis et al., 2008). The *in vitro* gas production technique has proved to be a potentially useful technique for feed evaluation (Menke and Steingass, 1988; Getachew et al., 2004; Maheri-Sis et al., 2008), as it is capable of measuring rate and extent of nutrient degradation. In addition, in vitro gas production technique provide less expensive, easy to determine (Getachew et al., 2004; Maheri-Sis et al., 2008) and suitable for use in developing countries (Chumpawadee et al., 2005; Maheri-Sis et al., 2007; Maheri-Sis et al., 2008). The objective of this study was to evaluate the potential of natural plant extracts as fermentation pattern *in vitro* gas production characteristics, by *in vitro* gas production technique.

2. Material and Methods

2.1. zataria multiflora Samples:

During summer season ZM samples were collected from different parts of Esfahan province. Next, there were drying for one week, and homogeneous mixture were papered for nutritive chemical analyzes. For determination of (zataria multiflora) effects, we added zataria multiflora

extracts with two levels (1 mL: 200 mg sample) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.3. Procedure of plant extracts preparation

The plant extracts were prepared according to (Patra et al., 2006; Sallam et al., 2009) 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 stoppered 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 (Patra et al., 2006; Sallam et al., 2009).

2.4 Treatments and experimental design

The different levels of ZMWE were added to the diet sample. Three levels (0 and 1 ml/30 ml buffered rumen fluid) of ZMWE were investigated as follow: (i) no additive and (ii) ZMWE (1 ml/30 ml buffered rumen fluid).

2.5. *In vitro* gas production

Fermentation of soybean meal samples were carried out with rumen fluid was obtained from three fistulated Taleshi native male cattle fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of (Menke and Steingass, 1988, 1979) as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence and presence of level 1 ml (ZMWE).

The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for soybean meal samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model of (Ørskov and McDonald 1979).

$$P = a + b (1-e-ct)$$

Where P is the gas production at time t, the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble fraction (ml/200mg DM), c the gas production rate constant (ml/h), a + b the potential gas production (ml/200mg DM) and t is the incubation time (h).

2.6. 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).

Significance between individual means was identified using the Duncan's multiple range tests. Mean differences were considered significant at ($P<0.05$). Standard errors of means were calculated from the residual mean square in the analysis of variance. All data obtained from three replicates n=3.

3. Results

3.1. *In vitro* gas production

Gas production volumes (ml/200mg DM) at differents incubation times were shown in Figure1 and 2.

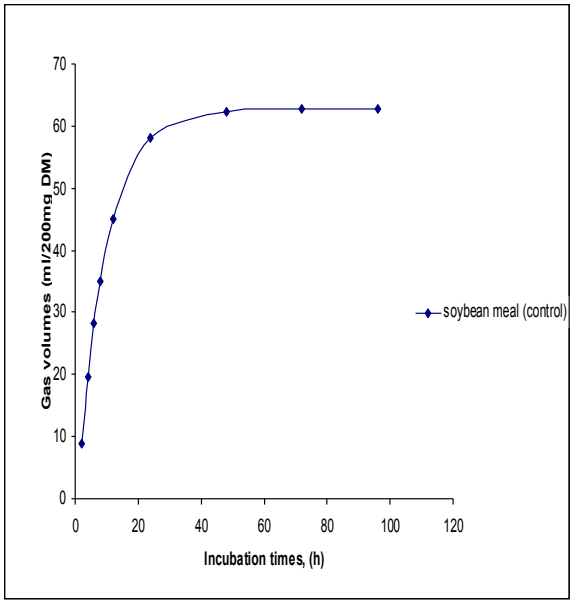

Fig.1. *In vitro* gas production volume of soybean meal was at different incubation times.

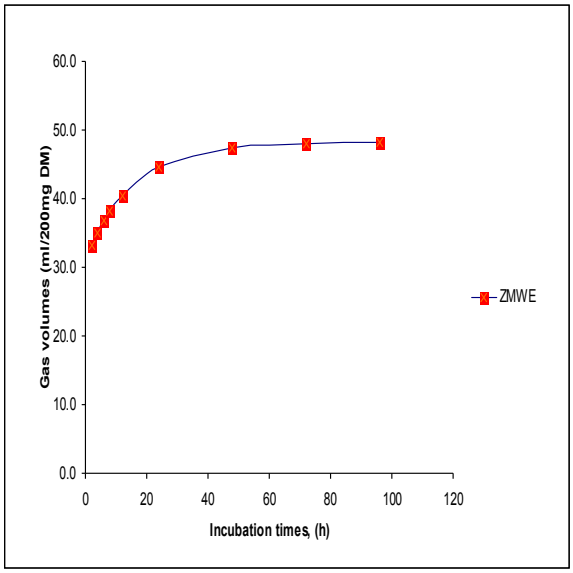

Fig.1. *In vitro* gas production volume of Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid) was at different incubation times.

In vitro gas production volume of Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid) and soybean meal was at different incubation times are presented in Table 1.

The results showed that gas volume at 2 h incubation (for 200 mg dry samples), were 9.31 and 31.64 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 4 h incubation (for 200 mg dry samples), were 19.96 and 33.56 ml/200 mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 6 h incubation (for 200 mg dry samples), were 27.61 and 31.50 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 8 h incubation (for 200 mg dry samples), were 37.25 and 38.85 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 12 h incubation (for 200 mg dry samples), were 46.23 and 40.9 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 24 h incubation (for 200 mg dry samples), were 56.38 and 43.8 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively. Gas volume at 48 h incubation (for 200 mg dry samples), were 62.12 and 45.95 ml/200mg DM for soybean meal and Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid), respectively.

Table 1: *In vitro* gas production volume of Zataria multiflora water extract (1 ml/30 ml buffered rumen fluid) and soybean meal was at different incubation times

Incubation time (h)	Control	ZMWE $_{1\ ml}$
2	9.31	31.64
4	19.96	33.56
6	27.61	36.50
8	37.25	38.85
12	46.23	40.9
24	56.38	43.8
48	62.12	45.95
72	62.76	48.3
96	63.61	49.43

4. Discussions

Salamat Azar et al (2011) estimation effect of tree doses thyme methanolic extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on degradability kinetics, of sunflower meal and report gas volume at 48 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of sunflower meal were 44.99, 3.60, 49.32, 52.92 ml/200 mg DM and 0.135 ml/h, gas volume at 48 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) were 29.91, 0.53, 36.25, 36.79 ml/200 mg DM and 0.049 ml/h, respectively.

Cardozo et al. [38], in a continuous culture experiment, were the first to suggest that cinnamon oil 0.22 mg/L of rumen fluid) modified the N metabolism of rumen microorganisms by inhibiting peptidolysis**,** but the effects on VFA concentration were negligible (Calsamiglia et al., 2006; Calsamiglia et al., 2007)**.** Rezaei et al (2011) evaluation effect of tree doses *clove methanolic extract* (0, 0.5 and 1 ml/30 ml buffered rumen fluid) on degradability, of soybean meal and report gas volume at 48 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of soybean meal were 71.240, 1.767, 70.880, 72.647 ml/200 mg DM and 0.100 ml/h, gas volume at 48 h incubation (for 200 mg dry samples),

soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of *clove methanolic extract* (1 ml/30 ml buffered rumen fluid) were 22.717, 8.914, 19.516, 28.429 ml/ 200 mg DM and 0.051 ml/h, respectively. Gas volume at 72 and 96 h incubation (for 200 mg dry samples), of soybean meal were 72.24 and 74.360 ml/200 mg DM, while for *clove methanolic extract* (1 ml/30ml buffered rumen fluid) were 25.383 and 29.130 ml/200 mg DM, respectively.

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