

The study canola meal degradability with *Zataria Multiflora Extract* Using in Vitro Gas Production Technique

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Abstract: This experiment was conducted to survey effect of adding different levels (0, 0.15, 0.3 ml/30ml buffered rumen fluid) of *Zataria multiflora water extract* (ZMWE) on canola meal (CM) 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. Chemical composition for dry matter, crude protein, were and Non-Fibrous Carbohydrate 93.7, 35, and 21.46 percent, respectively. The results showed that gas volume at 24 h incubation (for 200 mg dry samples), were 47.32, 47.30 and 47.12 ml/200 mg DM for CM, levels 0.15 ZMWE and 0.3 ZMWE, respectively. the gas production from soluble fraction (a), the gas production from insoluble fraction (b), rate constant of gas production during incubation (c) and the potential gas production (a + b) contents of CM were 2.15 (ml/200 mg DM), 54.96 (ml/200 mg DM), 0.113 (ml/h) and 57.12 (ml/200mg DM), while for level 0.3 ZMWE were 1.73 (ml/200mg DM), 54.64 (ml/200 mg DM), 0.112 (ml/h) and 56.37 (ml/200 mg DM).

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Abbreviations: ZMWE, *Zataria multiflora water extract*; CM, canola meal; CP, crude protein; NFC, non-fibrous carbohydrate, DM, dry matter; ZM, *Zataria multiflora*; a, the gas production from soluble fraction (ml/200 mg 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/200 mg DM).

1. Introduction

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). Ruminant nutritionists have long been interested in modulating the competition among different microbial populations with the objective of improving the efficiency of energy and protein utilization in the rumen. This has been achieved through the optimization of diet formulations and the utilization of feed additives that modify the environment and enhance or inhibit specific microbial populations (Calsamiglia et al., 2006). Antibiotic ionophores have been very successful in

reducing these energy and protein losses in the rumen (Van Nevel and Demeyer, 1988).

The public concern over the routine use of antibiotics and growth promoters in livestock production has increased recently because of the risk of the antibiotic residues presence in milk and meat and its effect on human health. These led to its prohibition in the European Union in 2006 in animal feeding. Accordingly, there is greater interest in using plants and plant extracts as alternatives to feed antibiotics to manipulate ruminal fermentation, improve feed efficiency and animal productivity. Many plants produce secondary metabolites such as phenolic compounds, essential oils, and sarsaponins (Calsamiglia et al., 2007) that affect microbial activity. Although many plant extracts have been shown to affect microbial activity (Makkar., 2005), growth performance of growing lambs (Chaves et al., 2007) and on milk production (Benchaar et al., 2007).

Thymol, a phenolic molecule present as the main component in thyme oil, is highly active against *S. typhimurium* in vitro, presumably damaging the cytoplasmic membrane integrity of the pathogen (Si et al., 2006).

in vivo, *in situ* and *in vitro* methods have been used to evaluate the nutritive value of feedstuffs. The *in vitro* gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs, since it gives an estimate of the potential rate and extent of nutrient fermentation in the rumen. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein Mirzaei- (Aghsaghali et al., 2008a, 2008b); Maheri-Sis et al., 2007, 2008) kiyani et al., 2010).

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 level (0.15 and 0.3 mL: 200 mg sample) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.2. Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105 °C overnight and ash by igniting the samples in muffle furnace at 525 °C for 8h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as $N \times 6.25$ (Van Soest et al. 1991). Non-Fibrous Carbohydrate (NFC) is calculated using the equation of (NRC, 2001), $NFC = 100 - (NDF + CP + EE + Ash)$.

All chemical analyses were carried out in triplicate.

2.3. Procedure of plant extracts preparation

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 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.

2.4. Treatments and experimental design

The different levels of ZMWE were added to the diet sample. Three levels (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) of ZMWE were investigated as follow: (i) no additive; (ii) ZMWE0.15 and (iii) ZMWE0.3.

2.5. In vitro gas production

Fermentation of canola 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 0.15 and 0.3ml (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 canola 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, a the gas production from soluble fraction (ml/200 mg DM), b the gas production from insoluble fraction (ml/200 mg DM), c the gas production rate constant (ml/h), a + b the potential gas production (ml/200 mg 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. Chemical composition

The chemical composition of canola meal shown in Table 1.

Chemical composition including dry matter (DM), crude protein (CP), and Non-Fibrous Carbohydrate (NFC) were estimated; 93.77, 35, and 21.46 percent, respectively.

Table 1

Chemical composition of canola meal (%).	
dry matter (DM)	93.77
crude protein (CP)	35
non-fiber carbohydrate (NFC)	21.46

3.2. In vitro gas production

Gas production volumes (ml/200mg DM) (at different incubation times shown in Figure 1).

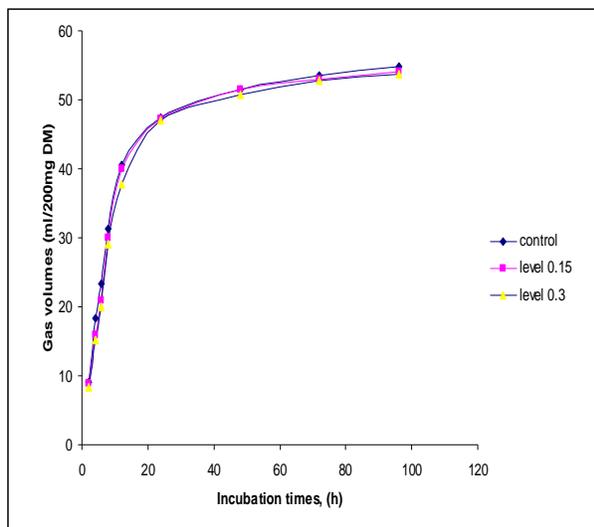


Fig. 1. In vitro gas production volume of canola meal at different incubation time.

Gas production parameters (a, b, c) and calculated amounts of CM, levels 0.15 and 0.3 ZMWE are presented in Table 2.

Gas volume at 24 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 CM were 47.32, 2.15, 54.96, 57.12 ml/200mg DM and 0.113 ml/h, while for 0.3 ZMWE were 47.12, 1.73, 54.64, 56.37 ml/200mg DM and 0.112 ml/h, respectively.

Gas volume at 48 and 72 h incubation (for 200 mg dry samples), of CM were 51.41 and 53.79 ml/200mg

DM, while for ZMWE_{0.3} were 50.77 and 52.82 ml/200mg DM, respectively.

Table 2. *In vitro* gas production volume (ml/200mg DM) and estimated parameters of canola meal at different incubation times.

Time (h)	treatment		
	i	ii	iii
2	9.05	9	8.36
4	18.42	18	17.24
6	23.33	23	22.42
8	31.32	31.02	21.96
12	40.49	39.99	37.83
24	47.32	47.30	47.12
48	51.41	51.41	50.77
72	53.79	52.90	52.82
96	54.78	54.07	53.74
Estimated parameters			
a (ml)	2.15	2.43	1.73
b (ml)	54.96	55.46	54.64
(a+b) (ml)	57.12	57.89	56.37
c (ml/h)	0.113	0.103	0.112

(i): no additive, (ii): ZMWE_{0.15}, (iii): ZMWE_{0.3}, a: the gas production from soluble fraction (ml/200 mg DM), b: the gas production from insoluble fraction (ml/200 mg DM), c: rate constant of gas production during incubation (ml/h), (a + b): the potential gas production (ml/200 mg DM).

4. Discussions

This study suggested that the ZMWE_{0.3} have the potential to affect ruminal fermentation efficiency, and be a promising methane mitigating agent.

(Patra et al., 2006) reported that extracts of plants in methanol and water had more soluble sugars than with ethanol. The seed pulp of Terminalia chebula extracted by methanol reduced methane emission by 95% with the lower dose (0.25 ml/30 ml incubation medium) and the inhibition was almost complete at the double level of extract.

Sommart et al. (2000) reported that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Gas volumes also have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993). The soluble fraction (a) makes it easily attachable by ruminal microorganisms and leads to much gas production (Blummel and Becker, 1997).

Nagy and Tengerdy (1968) found that addition of essential oils extracted from Sagebush (*Artemisa tridentata*) altered the composition of the

bacterial population during a 24 h incubation of rumen fluid *in vitro*.

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