

### Effect of *Zataria Multiflora Extract* on Degradability Kinetics, of Sunflower Meal

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**Abstract:** The aim of the present study 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 sunflower meal (SM) 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, and Non-Fibrous Carbohydrate 95.88, 30, and 12.73 percent, respectively. The results showed that gas volume at 24 h incubation (for 200 mg dry samples), were 42.40, 41.41 and 40.52 ml/200 mg DM for SM, 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 SM were 3.607 (ml/200 mg DM), 49.32 (ml/200 mg DM), 0.135 (ml/h) and 52.92 (ml/200 mg DM), while for level 0.3 ZMWE were 4.655 (ml/200mg DM), 48.66 (ml/200 mg DM), 0.134 (ml/h) and 53.321 (ml/200mg DM).

[Mohammad Salamat Azar, Ramin Salamat Doust-Nobar, Yahya Asadi, Morteza Kiani Nahand, Saeid Najafyar, Bakhshali Khodaparast, Hamed Aminipour. Effect of *Zataria Multiflora Extract* on Degradability Kinetics, of Sunflower Meal. Journal of American Science 2011;7(7):119-122]. (ISSN: 1545-1003).  
<http://www.americanscience.org>.

**Keywords:** Non-Fibrous Carbohydrate; *zataria multiflora*; sunflower meal; gas production technique; crude protein; Taleshi native male cattle

**Abbreviations:** ZMWE, *Zataria multiflora water extract*; SM, sunflower meal; CP, crude protein; NFC, non-fibrous carbohydrate; ZM, *Zataria multiflora*; (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).

#### 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).

A growing concern about global climate change has increased attention on ways to abate ruminal methanogenesis. 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).

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 Nessel and Demeyer, 1988).

These losses not only reduce production performance, but also contribute to the release of pollutants to the environment (Tammimga, 1996).

*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* extract 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 sunflower 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 sunflower 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 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 sunflower meal shown in Table 1.

The chemical composition of sunflower meal shown in Table 1. Chemical composition including dry matter) DM, crude protein (CP), and Non-Fibrous Carbohydrate (NFC) were estimated; 95.88, 30, and 12.73 percent, respectively.

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

Table 1

Chemical composition of sunflower meal (%).

|                              |       |
|------------------------------|-------|
| dry matter (DM)              | 95.88 |
| crude protein (CP)           | 30    |
| non-fiber carbohydrate (NFC) | 12.73 |

## 3.2. In vitro gas production

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

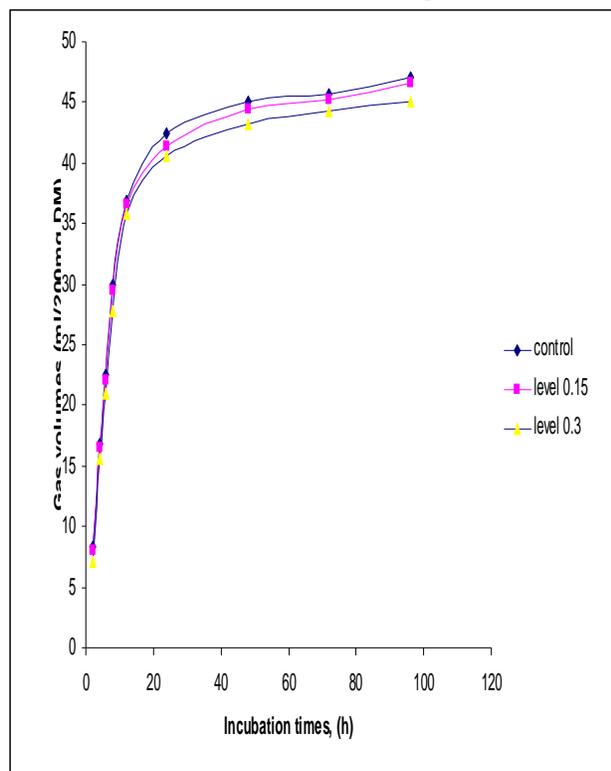


Fig. 1. In vitro gas production volume of sunflower meal at different incubation time.

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 SM were 42.40, 3.607, 49.32, 52.92 ml/200 mg DM and 0.135 ml/h, for ZMWE<sub>0.3</sub> 41.41, 3.921, 49.08, 53.004 and 0.135 while for ZMWE<sub>0.3</sub> were 40.52, 4.655, 48.66, 53.321 ml/200mg DM and 0.134 ml/h, respectively.

Gas volume at 48 h incubation (for 200 mg dry samples), of SM, ZMWE<sub>0.15</sub> and ZMWE<sub>0.3</sub> were 44.99, 44.49 and 43.27 (ml/200 mg DM), respectively. Gas volume at 72 h incubation (for 200 mg dry samples), of SM, ZMWE<sub>0.15</sub> and ZMWE<sub>0.3</sub> were 45.75, 45.23 and 44.25 respectively.

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

| Time (h)             | treatment |        |        |
|----------------------|-----------|--------|--------|
|                      | i         | ii     | iii    |
| 2                    | 8.34      | 97.95  | 7.17   |
| 4                    | 16.86     | 16.57  | 15.51  |
| 6                    | 22.54     | 22.04  | 21.01  |
| 8                    | 29.88     | 29.49  | 27.84  |
| 12                   | 36.89     | 36.62  | 35.85  |
| 24                   | 42.40     | 41.41  | 40.52  |
| 48                   | 44.99     | 44.49  | 43.27  |
| 72                   | 45.75     | 45.23  | 44.25  |
| 96                   | 47        | 46.65  | 45.10  |
| Estimated parameters |           |        |        |
| a (ml)               | 3.607     | 3.921  | 4.655  |
| b (ml)               | 49.32     | 49.08  | 48.66  |
| (a+b) (ml)           | 52.92     | 53.004 | 53.321 |
| c (ml/h)             | 0.135     | 0.135  | 0.134  |

(i): no additive, (ii): ZMWE<sub>0.15</sub>, (iii): ZMWE<sub>0.3</sub>, 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<sub>0.3</sub> have the potential to affect ruminal fermentation efficiency, and be a promising methane mitigating agent.

Newbold et al. (2006) and Calsamiglia et al. (2007) described essential oils (EO) as follows: volatile aromatic compounds with an oily appearance extracted from plant materials typically by steam distillation; alcohol, ester or aldehyde derivatives of phenylpropanoids and terpenoids; some of the more common EO compounds available include thymol (thyme and oregano), eugenol (clove), pinene (Juniper), limonene (dill), cinnamaldehyde (cinnamon), capsaicin (hot peppers), terpinene (tea tree), allicin (garlic), anethol (anise), etc.; antimicrobial activity; modify rumen microbial fermentation.

(Patra et al., 2006) reported that extracts of plants in methanol and water had more soluble sugars than with ethanol. The cumulative volume of gas production increased with increasing time of incubation. Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented.

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5/25/2011