Zataria Multiflora Extract could affect Metobolizable Energy and Organic Matter Digestibility of Canola Meal?

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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 ether extract , ash, neutral detergent fiber and acid detergent fiber were 3.4, 7.14, 33 and 22 percent, respectively. The results showed The organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) contents of CM were 79.46 g/kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM, while for level 0.3 ZMWE were 41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM.
[Mohammad Salamat Azar, Ramin Salamat Doust-Nobar, Yahya Asadi, Morteza Kiani Nahand, Saeid Najafyar, Bakhshali Khodaparast, Hamed Aminipur. Zataria Multiflora Extract could affect Metobolizable Energy and Organic Matter Digestibility of Canola Meal?. Journal of American Science 2011;7(7):131-134]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Zataria multiflora; Canola Meal; gas production technique; neutral detergent fiber; organic matter digestibility

Abbreviations: ZMWE, *Zataria multiflora water extract*; CM, canola meal; EE, ether extract; ZM, *Zataria multiflora*; OMD, organic matter digestibility; ME, metabolizable energy, SCFA, short chain fatty acid; NE_l, net energy for lactation; NDF, neutral detergent fiber; ADF, acid detergent fiber

1. Introduction

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).

Many plants produce secondary metabolites, a group of chemicals that are not involved in the primary biochemical processes of plant growth and reproduction but are important to protect the plants from insect predation or grazing by herbivores. Several thousand plant secondary metabolites have been reported (Kamra et al., 2006).

Accordingly, it has been suggested that secondary metabolites could be used as alternatives to antibiotics in ruminant feeds (Greathead, 2003), as they may modify ruminal fermentation thereby enhancing the efficiency of utilization of feed energy while decreasing methane emissions (Garcia-Gonzalez et al., 2006).

Ruminal microbial activity is essential for the use of structural carbohydrates and synthesis of high quality protein in ruminants. However, microbial fermentation in the rumen may result in considerable energy and protein losses as methane and ammonia (NRC, 2001).

Methane emission and excretion of N from ruminant livestock substantially implicate global warming and N pollution. Recently, there has been an increased interest in saponins or saponin-containing plants for modifying ruminal fermentation. Saponins are phytochemical compounds composed of a steroid or triterpernoid sapogenin linked to one or more sugar chains (Cheeke, 1999).

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., 2006), that affect microbial

activity. Although many plant extracts have been shown to affect microbial activity (Patra et al., 2006), gro wth performance of growing lambs (Chaves et al., 2007), and on milk production (Benchaar et al., 2007).

The objective of this study was to evaluate the potential of natural plant extracts as fermentation pattern in vitro gas production characteristics, organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation (NE_l) 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

Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were determined by procedures outlined by (Van Soest et al. 1991). with modifications described by (Van Soest et al. 1991). 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 wer 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) 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).

$$P = a + b\,(1\text{-}e^{-ct})$$

Where P is the gas production at time t, a 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).

The metabolizable energy (MJ/kg DM) content of canola meal was calculated using equations of (McDonald et al. 1995), (Menke and Steingass 1988) and (Menke et al. 1979) as follows:

for all feeds,

ME (MJ/kg DM) = 0.016 DOMD

for forage feeds,

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CF^2

for concentrate feeds,

ME (MJ/kg DM) = 1.06 + 0.157 GP + 0.084 CP + 0.22 CF -0.081 CA

Where:

GP = The 24 h net gas production (ml/200 mg^{-1}), CP = Crude protein

Short chain fatty acids (SCFA) is calculated using the equation of (Makkar 2005) and (Maheri-Sis 2007, 2008).

Where, Gas is 24 h net gas production (ml/200mg DM).

SCFA (mmol) = 0.0222 × GP – 0.00425

The organic matter digestibility was calculated using equations of (Menke et al.1979) as follows:
OMD (g/kg DM) = (%)14.88 + 0.889 GP + 0.45 CP +XA
Where:
GP = About 24 h net gas production (ml /200 mg^{-1})
CP = Crude protein (%)
XA = Ash content (%)
NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36 (Abas et al., 2005).

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.
The chemical composition of canola meal shown in Table 1. Chemical composition including ether extract (EE) , crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF) were estimated; 3.4, 7.14, 33 and 22 percent, respectively.

Table 1. Chemical composition of canola meal (%).

neutral detergent fiber (NDF)	33
acid detergent fiber (ADF)	22
Ash	7.14
ether extract (EE)	3.4

3.2. In vitro gas production

calculated amounts of organic matter digestibility, metabolizable energy, short chain fatty acid, net energy for lactation of canola meal are presented in Table 2.

Calculated amounts of organic matter digestibility, metabolizable energy, short chain fatty acid, net energy for lactation of canola meal (79.46 g/kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM, respectively) were high as compared to 0.3 ZMWE (41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively).

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

	Estimated parameters			
	OMD	ME	SCFA	NE_l
i	79.46	10.27	1.046	5.28
ii	73.16	9.16	1.05	6.09
iii	41.85	3.63	1.047	1.22

(i): no additive, (ii): $ZMWE_{0.15}$, (iii): $ZMWE_{0.3}$,
OMD: organic matter digestibility (g/kg DM),
ME: metabolisable energy (MJ/kg DM),
SCFA: short chain fatty acid (mmol),
NE_l: net energy lactation (MJ/kg 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.

In general, rumen microbial activity was affected by the use of plant extracts and secondary plant metabolites. It is interesting to point out that when supplied at high levels (3,000 mg/L) most essential oils and their secondary constituents reduced the total VFA concentration compared with control, which is consistent with their antimicrobial activity (Davidson and Naidu, 2000).

Menke and Steingass (1988) suggested that gas volume at 24 h after incubation has been relationship with metabolisable energy in feedstuffs.

In vitro dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart et al., 2000).

Bravo and Doane (2008) recently presented results from a meta-analysis of lactating dairy cow trials involving essential oils comprised of cinnamaldehyde and eugenol (Pancosma, Geneva, Switzerland); 16 trials and 33 treatment comparisons. essential oils increased DMI and milk yield by 1.5 kg/d ($P < 0.001$) and 1.1 kg/d ($P <0.001$), respectively. It was reported that diet nutrient composition, greater NEL, NFC or NDFD, improved the milk yield response to essential oils (Bravo and Doane, 2008).

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6/2/2011