The study *Zataria Multiflora* Water Extract effects On the Short Chain Fatty Acid, Net Energy, Metobolizable Energy and Organic Matter Digestibility Of Sunflower Meal Using *In Vitro* Gas Production Technique

Mohammad Salamat Azar, Ramin Salamat Doust-Nobar, Yahya Asadi, Morteza Kiani Nahand, Saeid Najafyar, Bakhshali Khodaparast, Hamed Aminipour

Department of Animal Science, Shabestar branch, Islamic Azad University, Shabestar, Iran
m.salamatazar@gmail.com

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 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 ether extract , ash, neutral detergent fiber and acid detergent fiber were 5.97, 5.5, 45.8 and 30.6 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 SM were 8.36 MJ/kg DM, 0.937 mmol and 4.533 MJ/kg DM, while for level 0.3 ZMWE were 64.76 g/kg DM, 8.04 MJ/kg DM, 0.895 mmol and 4.664 MJ/kg DM.
[Mohammad Salamat Azar, Ramin Salamat Doust-Nobar, Yahya Asadi, Morteza Kiani Nahand, Saeid Najafyar, Bakhshali Khodaparast, Hamed Aminipur. The study Zataria Multiflora Water Extract effects On the Short Chain Fatty Acid, Net Energy, Metobolizable Energy and Organic Matter Digestibility Of Sunflower Meal Using In Vitro Gas Production Technique. Journal of American Science 2011;7(7):127-130]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: metabolizable energy; *zataria multiflora*; sunflower meal; gas production technique; neutral detergent fiber; organic matter digestibility

Abbreviations: ZMWE, *Zataria multiflora water extract*; SM, sunflower meal; EE, ether extract; ZM, Zataria multiflora; OMD, organic matter digestibility; ME, metabolizable energy, SCFA, short chain fatty acid; CP, crude protein; NE_l, net energy for lactation.

1. Introduction

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).

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, 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 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) 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).

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

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).

The metabolizable energy (MJ/kg DM) content of sunflower meal was calculated using equations of (McDonald et al., 1995), (Menke and Steingass1988) 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 sunflower meal shown in Table 1.

The chemical composition of sunflower meal shown in Table 1. Chemical composition including ether extract (EE) , crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF) were estimated; 5.97, 5.5, 45.8 and 30, 6 percent, respectively.

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

neutral detergent fiber (NDF)	45.8
acid detergent fiber (ADF)	30.6
Ash	5.5
ether extract (EE)	5.97

3.2. In vitro gas production

calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NEl) of sunflower meal (SM) are presented in Table 2.

Calculated amounts of organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NEl) of SM (66.43 g/kg DM, 8.36 MJ/kg DM, 0.937 mmol and 4.53 MJ/kg DM, respectively) were high as compared to 0.3 Zataria multiflora water extract (ZMWE) (64.76 g/kg DM, 8.04 MJ/kg DM, 0.895 mmol and 4.664 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	66.43	8.36	0.937	4.533
ii	65.56	8.20	0.915	4.669
iii	64.76	8.04	0.895	4.664

(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

Effects of essential oil on rumen microbial fermentation in vitro or in situ are well established (Calsamiglia et al., 2007).

The potential of plant secondary metabolites to reduce enteric CH4 production has been recognized and very extensive screening of a large range of plants and their secondary compounds (patra et al., 2006).

There was a positive correlation between NFC content of feeds and gas production, but feed CP, NH_3-N and NDF levels were negatively correlated with gas production (Getachew et al., 2004; Maheri-Sis et al., 2007). Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of *in vitro* gas production volume (Maheri-Sis et al., 2008).

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.

Corresponding Author:
Mohammad salamatazar
Department of Animal Science, Shabestar branch, Iran
E-mail: m.salamatazar@gmail.com

References

1. Abas, I., Ozpinar, H., Can-Kutay, H., Kahraman, R., 2005. Determination of the metabolizable energy (ME) and net energy actation (NEL) contents of some feeds in the marmara region by in vitro gas technique. Turk. J. Vet. Anim. Sci. 29, 751-757.
2. Calsamiglia S., M. Busquet , P.W. Cardozo, L. Castillejos and A. Ferret. 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J Dairy Sci 90:2580–2595.
3. Cheeke, P.R., 1999. Actual and potential application of Yucca schidigera and Quillaja saponaria saponins in human and animal nutrition. Proc. Am. Soc. Anim. Sci. 1-10.
4. Kiyani Nahand M, Salamat Doust-Nobar R, Maheri-Sis N, Global Veternaria., 2010, 4: 587-591.
5. Getachew, G., Robinson, P.H,, DePeters, E.J., Taylor, S.J., 2004. Relation-ships between chemical compositions, dry matter degradation and in vitro gas production of several ruminant feeds. Anim. Feed Sci. Technol. 111, 57-71.
6. McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan,. 1995. Animal Nutrition. Long Man Company, London.
7. Maheri-Sis N, Chamani M, Sadeghi Mirza-Aghazadeh A.A., Abolfazl A.G, Afr. J.Biotechnol, 2008. (7) 16: 2946-2951.
8. Maheri-Sis, N., Chamani, M., Sadeghi, A.A., Mirza-Aghazadeh, A., Safaei, A.A., 2007. Nutritional evaluation of chickpea wastes for ruminants using in vitro gas production technique. J. Anim. Vet. Adv. 6(12), 1453- 1457.

9. Makkar, H.P.S. Anim. Feed Sci. Technol., 2005.123-124: 291-302.
10. Menke, K.H. and H. Steingass,. Anim. Res. Dev., 1988. 28: 7-55.
11. Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider,. J.Agric. Sci., 1979. 93: 217-222.
12. Mirzaei-Aghsaghali, A., Maheri-Sis, N., 2008a. Nutritive value of some agro-industrial by-products for ruminants - A review. World J. Zool. 3 (2), 40-46.
13. Mirzaei-Aghsaghali, A., Maheri-Sis, N., Mirza-Aghazadeh, A., Safaei, A.R., Aghajanzadeh-Golshani, A., 2008b. Nutritive value of alfalfa varieties for ruminants with emphasis of different measuring methods: A review. Res. J. Biol. Sci. 3(10), 1227-1241.
14. NRC, 2001. Nutrient requirements of dairy cattle. (7threw.ed.) National Research Council, National Academy Press, Washington, DC.
15. Ørskov ER, McDonald I, J. Agric. Sc.i, 1979, 92- 499.
16. Patra A.K., Kamra D.N. and Agarwal N., Anim. Feed Sci. Technol., 2006. 128. p. 276-291.
17. SAS, 2000. SAS Users Guide. Cary, USA: Statistical Analysis Systems Institute.
18. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Iowa State University Press, Ames, IA, USA.
19. Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

6/1/2011