The Journal of Animal & Plant Sciences, 21(2): 2011, Page:126-131
ISSN: 1018-7081

EFFECT OF MICROWAVE IRRADIATION ON NUTRITIVE VALUE OF SUNFLOWER MEAL FOR RUMINANTS USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT

This study was conducted to estimate nutritive value of sunflower meal (SFM) and evaluate the effect of microwave irradiation on the metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) production of sunflower meal using *in vitro* gas production technique. Sunflower meal was exposed to 1000W microwave irradiation for six minutes. The sun flower meal sample (1g) were incubated with rumen liquor taken from three fistulated Ghezel rams at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. Cumulative gas production volume at 24 h incubation, for treated SFM was significantly ($P < 0.001$) higher than that of untreated SFM (171.48 vs. 154.44 ml). Microwave treated SFM also had significantly higher ME (8.97 vs. 8.43 Mj Kg^{-1}DM), OMD (67.69 vs. 65.10 %) and SCFA (0.75 vs. 0.68 mmol) than control SFM ($P < 0.001$). Based on results of this study, it can be concluded that microwave irradiation may be a useful method to enhance nutritive value of sunflower meal.

Key words: sunflower meal, microwave, nutritive value, gas production, metabolizable energy.

INTRODUCTION

In arid and semi-arid areas of the world like Iran, the major limitation for animal production is low availability of energy and protein sources (Rowghani and Zamiri, 2007). Therefore, it is required to find new sources and technologies of feedstuffs to production systems. In recent years, sunflowers (*Helianthus annuus*), especially of the high oil varieties are being grown in Iran, primarily as a source of vegetable oils for the food industry. The sunflower meal (SFM) after extraction of the oil is available in increasing quantities for the feed industry. Sunflower meal can play an important role as alternative and cost effective source of nutrients for livestock. It is the fourth largest source of protein supplement after soybean, cottonseed, and canola meals in the world. The amount and composition of meal is affected by oil content of the seed, extent of hull removal, and efficiency of oil extraction. The amount of hulls or fiber in the meal is the major source of variation in nutrients (Hesley, 1994). Senkoylu and Dale (1999) reported that, unlike most other oilseed meals, SFM is not known to have anti-nutritional factors. Several researchers have studied protein characteristics of SFM in ruminant nutrition (Ratcliff, 1977; Schingoethe *et al*., 1977; Dinusson *et al*., 1981; Richardson *et al*., 1981; Hesley, 1994; Patterson, *et al*., 1999; Titi, 2003). However, little research has been reported on its digestibility and utilization as an energy source for ruminants. Ratcliff, (1977) reported that gross energy (GE) value of sunflower meal compares favorably with soybean meal and cottonseed meal; 4117 kcal/kg, vs. 4719 kcal/kg and 4540 kcal/kg, respectively. Nadeem, *et al* (2005) obtained that GE value of SFM equal to soybean meal (4501 vs. 4508 kcal/kg) and higher than that of cottonseed meal (4401 kcal/kg). These values for gross energy will vary due to the amount of residual oil and hulls after processing.

Different methods have been used for feed processing and technology. Microwave irradiation is one of the physical methods recently used in animal feed technology (Sadeghi and Shawrang, 2006a, Sadeghi and Shawrang, 2006b; Sadeghi and Shawrang, 2008; Pelletier *et al*., 2010). Microwave energy penetrates a food or feed material and produces a volumetrically distributed heat source, due to molecular friction, resulting from dipolar rotation of polar solvents and from conductive migration of dissolved ions. The dipolar rotation is caused by variations of the electrical and magnetic fields in the organic components (Alton, 1998). Water, the major constituent of most food and feed products, is the main source for microwave interactions due to its dipolar nature. Heat is generated throughout the material, leading to faster heating rates and shorter processing times compared to conventional heating, where heat is usually transferred from the surface to the interior (Fakhouri and Ramaswamy, 1993). Other advantages include space savings and energy efficiency, since most of the electro-magnetic energy is converted into heat (Mermelstein, 1997). Zhao *et al*., (2007) reviewed that microwave technology is increasingly playing an important role in drying in the food industry because of its rapid heating rate and ease of use. It also shows considerable potential for preventing mildew in food. In addition, this method of processing does not cause environmental pollution or introduce foreign chemical reagents. Farag *et al*., (1996) illustrated that microwave heating reduced the aflatoxin content considerably in contaminated material.

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 is beneficial assay to feed evaluation especially in developing countries because this method is capable of measuring rate and extend of nutrients degradation with less expenditure (Menke and Steingass, 1988; Getachew *et al*., 2004; Maheri-Sis *et al*., 2007). On the other hand the Hohenheim Gas test is one of the *in vitro* methods used for the estimation of organic matter digestibility and the energy content of feedstuffs for ruminants (Menke *et al*., 1979). Lee *et al*. (2000) and Şeker (2002) showed a close relationship between the energy values of forage calculated on *in vivo* digestion trail and *in vitro* gas production parameters. In addition gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs. This method relies on the measurement of total amount of gas production during fermentation of feed with buffered rumen fluid in calibrated glass syringes (Menke *et al*., 1979; Menke and Steingass, 1988; Makkar, 2003). The gas produced in the gas technique is the direct gas produced as a result of fermentation and the indirect gas produced from the buffering of short chain fatty acids (Makkar, 2003).

There was not found any study on the effect of microwave irradiation on ME, DOM and SCFA of sunflower meal. The aim of this study was to determine chemical composition and nutritive value of sunflower meal as well as evaluate effects of microwave processing on organic matter digestibility, metabolizable energy content and ruminal short chain fatty acids production of sunflower meal using *in vitro* gas production parameters.

MATERIALS AND METHODS

Animals and feeds: Three fistulated Gezel rams were used for rumen liquor collection in order to conduct in gas production technique. Three samples (500 g) were exposed to microwave irradiation (emitting a 2450MHz microwave frequency) at a power of 1000W (1.33 W/g) for 6 min under agitation. Sunflower meals milled through a 1 mm sieve in animal nutrition laboratories of Islamic Azad University, Shabestar Branch and gas production parameters were measured in laboratory of Tabriz University.

Chemical analysis: Sunflower meal from same source milled through a 1 mm sieve for chemical analysis and gas production procedure. Dry matter (DM) was determined by drying the samples at 105ºC overnight and ash by igniting the sample in muffle furnace at 550ºC for 8 h. Nitrogen (N) content was measured by the Kjeldal method. Crude protein (CP) was calculated as N×6.25 (AOAC, 1990). Neutral detergent fiber (NDF) was determined by procedures outlined by Van Soest *et al*., (1991).

***In vitro* gas production:** Rumen fluid was obtained from three fistulated Gezel rams (through suction tube before morning feeding) fed twice daily at the maintenance level with a diet containing alfalfa hay (60%) and concentrate (40%). The samples incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke *et al*. (1979). The 1g samples were weighed in triplicate into calibrated glass syringes of 100 mL. The syringes were prewarmed at 39ºC before the injection of 30ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39ºC. Readings of gas production were recorded before incubation (0) and 2, 4, 6,8,12,16,24,36 and 48 h after incubation. Total gas values were corrected for blank incubation and gas production from syringes contain rumen fluid.
Cumulative gas production data were fitted to the model of Orskov and McDonald (1979):

$Y = a+b\ (1-e^{-ct})$

Where:

a = the gas production from the immediately soluble fraction (mL).
b = the gas production from the insoluble fraction (mL).
c = the gas production rate constant for the insoluble fraction (b).
a + b = Potential gas production (mL).
t = incubation time (h).
Y = gas production at time t.

The Metabolizable energy (MJ per Kg DM) contents of sunflower meal were calculated using equations of Menke and Steingass (1988) as follows:

$ME\ (MJ/Kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP + 0.0029\ EE^2$

Where
GP = 24 h net gas production (ml 200 mg^{-1})
CP = crude protein (%)
EE = ether extract (%)

Organic mater digestibility (OMD) and short chain fatty acids (SCFA) of sunflower meal and its treatment were calculated using equations of Menke *et al*. (1979) and Makkar (2003), respectively as follows:

$OMD\% = 14.88+0.889GP+0.45CP+0.0651XA$

$SCFA\ (mmol) = 0.0222GP - 0.00425$

Where
GP = 24 h net gas production (ml 200 mg^{-1})
CP = crude protein (%)
XA = ash content (%)

Statistical analysis: All of the data were analyzed by using software of SAS (1991) and means of two sample groups were separated by independent-samples t-test (McDonald, 2008).

RESULTS AND DISCUSSION

Chemical composition of the SFM (not dehulled) is presented in Table 1. The DM, CP, CF, EE and CA content of SFM were 93, 30, 16, 5 and 5.5 %, respectively. Richardson *et al*. (1981) reported that DM, CP, CF, EE and CA content of SFM were 92.50, 33.67, 22.03, 2.67 and 6.30 %, respectively. Patterson *et al*. (1999) reported that DM, CP and ADF content of SFM were 87.8, 32.9 and 31.5 %, respectively. Pre-press solvent extraction of whole seeds with no dehulling produces meal with a crude protein content of 25 to 28 percent, partial dehulling yields 34 to 38 percent crude protein content, and completely dehulled SFM commonly yields 40 percent crude protein, but up to 50 percent crude protein has been observed (National Academy of Sciences, 1971). Generally, wide variation existed in the chemical composition of the meals between investigations, because chemical composition of them can be affected by many factor such as year, geographical origin, procedure of production or treatment and method of oil extraction (Khosravifar *et al*., 2008).

Gas production volume (mL/1g DM) at different incubation times and gas production parameters (a, b, a+b and c) and calculated amounts of OMD, SCFA and ME of untreated and microwave treated SFM are presented in tables 2 and 3. The gas volume for microwave treated SFM at different incubation times were significantly higher than that of untreated SFM (exception 2 and 4 h of incubation time). The immediately soluble fraction (a) were not significantly different between untreated and treated SFM (3.35 vs. 4.1 mL), while the gas production from the insoluble fraction (b) (40.15 vs. 46.3 mL) and the potential gas production (a+b), increased by microwave irradiation (43.5 vs. 50.43 mL). The gas production rate constant for the insoluble fraction (c) decreased by microwave irradiation (0.107 vs. 0.094 mL h^{-1}). Rodrigues *et al*. (2009) reported that estimated asymptotic gas production (α) and constant rate of gas production (c) of SFM were 170 ml/g OM and 0.142ml/h, respectively.

Eskicioglu *et al*. (2007) reported that, neither the chemical mechanism of microwave interaction with materials, nor microbial destruction (sterilization) mechanism of microwave in biological systems is fully understood. However, it was found that microwave irradiation resulted in more biogas production in anaerobic systems. This finding is in line with present results which indicate the increasing effect of microwave irradiation on gas production. Rossi *et al*., (2001) showed that *in vitro* CH_4 production (mM/g of dry matter) of SFM after 24 hours of incubation in the rumen was lower than that of soybean meal. Since rumen CH_4 production decreases the energetic efficiency of feed utilization by dairy cow and contributes to the global greenhouse effect, thus it can be concluded that SFM is energetically effective and environmentally cleaner than soybean meal as ruminant feed.

Metabolizable energy (ME), organic mater digestibility (OMD) and short chain fatty acids (SCFA) contents increased by microwave irradiation (8.43 vs. 8.97 Mj Kg^{-1}DM), (65.10 vs. 67.69 %) and (0.68 vs. 0.75 mmol), respectively ($p< 0.001$). Increased ME content of microwave treated SFM can be due to conversion of most of electro-magnetic energy into heat (Oliveira and Franca, 2002) or decreasing cell wall strength which leads to increase in microbial access to cell content. Doering and Hennessy (2008) found that, microwaves can enhance enzyme ability to hydrolyze cellulose. Partially, it can be explain that how microwave irradiation have been improved OMD of SFM in present study.

Menke *et al*. (1979) suggested that gas volume at 24 h after incubation has been relationship with metabolizable energy in feedstuffs. Additionally, *in vitro* dry matter and organic mater digestibility were shown to have high correlation with gas volume (Sommart *et al* 2000). The ME value for SFM in current study (8.43 Mj kg^{-1} DM) was in line with those reported by Abas *et al*., (2005: 6.69- 10.63 Mj kg^{-1} DM) and was lower than that of NRC (1996: 9.83 Mj kg^{-1} DM) and Ewing (1998: 9.50 Mj kg-1 DM). Tatli Seven *et al*., (2007) demonstrated that, estimated ME content of SFM can be varied by the evaluation method (enzyme technique: 1847.24±110.70 vs. gas technique: 2130±78.00 kcal/kg DM). Abas *et al*., (2005) reported gas production volume and OMD for SFM, 158.85 (mL) and 59.27 %, respectively. Different nutritive values obtained from various studies may be due to different chemical compositions, processing methods, sunflower varieties, hulls ratio, climate conditions, evaluation procedures and experimental diets and animals. The reason that, why energy content and organic matter digestibility of microwave treated SFM were higher than that of untreated (control) SFM, can be due to difference (changes) in chemical composition (especially soluble carbohydrates, crude protein, non fibrous carbohydrates, fat, acid detergent fibre and neutral detergent fibre) and volume of gas production (Menke and Steingass, 1988; Getachew *et al*., 2004; Aghajanzadeh-Golshani *et al*., 2010). Sadeghi and Shawrang (2008) concluded that different effects of microwave irradiation between various feedstuffs may be due to differences in their amylose and amylopectin content, crystallinity, particle size, structural carbohydrates and the proteins in the cell matrix. Blummel *et al*. (1999) stated that the gas volume in the bicarbonate buffered *in vitro* gas production test, reflect SCFA production very closely. Gas volumes were produced quantitatively and qualitatively as a result of SCFA production (the amount of fermentative CO_2 and CH_4 could be accurately calculated from the amount and proportion of acetate, propionate and butyrate present in the incubation medium). Thus, increase in amount of SCFA lead to increase in gas production which ultimately resulted in high digestibility and energetic value.

Table 1: Chemical composition of microwave treated sunflower meal (SFM) on dry matter basis (%).

	DM	CP	CF	EE	CA	NDF	NFC
Sunflower meal	93	30	16	5	5.5	50	4.5

EE: ether extract, NDF: neutral detergent fiber, CA: crude ash and NFC: non fiber carbohydrate.

Table 2: Cumulative gas production volume (mL per 1 g) at different incubation times for untreated and microwave treated sunflower meal.

Incubation time	Untreated	microwave treated	Significance	S.E.M
2	9.03	3.85	ns	1.53
4	61.29	57.59	ns	1.71
6	92.76	94.50	*	1.47
8	110.91	115.60	***	1.28
12	125.06	132.96	***	0.91
16	137.80	148.91	***	1.28
24	154.44	171.48	***	1.64
36	178.14	200.11	***	2.08
48	199.64	224.58	***	2.41

ns: Non significant , *: P<0.05 , *** P<0.001

Table 3: The gas production parameters, organic mater digestibility (OMD), short chain fatty acids (SCFA) and metabolizable energy (ME) contents of untreated and microwave treated sunflower meal.

Items	untreated	microwave treated	Significance	S.E.M
a(mL)	3.35	4.13	ns	0.469
b(mL)	40.15	46.30	***	0.620
a+b(mL)	43.50	50.43	***	0.963
c(mL h^{-1})	0.107	0.094	***	0.002
OMD (%)	65.10	67.69	***	1.250
SCFA (mmol)	0.68	0.75	***	0.680
ME(Mj/ Kg DM)	8.43	8.97	***	0.258

a: The gas production from the immediately soluble fraction (mL), b: The gas production from the insoluble fraction (mL), a+b: The potential gas production, c: The gas production rate constant for the insoluble fraction (b), ns: Non significant, *: P<0.05, *** P<0.001 and S.E.M: Standard Error of the Mean.

Conclusion: Based on the results of current study, it can be concluded that, microwave irradiation resulted in increase *in vitro* gas production parameters and improvement of nutritive value (metabolizable energy, organic matter digestibility and short chain fatty acids content) of sunflower meal. This method can be used as cost effective method for improvement of nutritional value of oil seed meals.

Acknowledgment: This article is part of M.Sc thesis in Animal Science, Islamic Azad University, Shabestar Branch. We would like to acknowledge Mr. A. Noshadi. Authors are also thankful to Animal Research Centre of Islamic Azad University, Shabestar Branch and Tabriz University, Iran.

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