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In Vitro Fermentation Characteristics and Nutritive Value of Iranian Oil Seed Meals for Ruminants

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ABSTRACT

This study was conducted to determine the chemical composition and estimating nutritive value of soybean meal, sunflower meal, rapeseed meal and cotton- seed meal using *in vitro* gas production technique. The experimental samples (200 mg DM from each) were incubated *in vitro* with rumen liquor taken from three canulated rams at 2, 4, 6, 8, 12, 24, 36, 48 and 72 h in three replicates. The results showed that there are significant differences between gas production values of samples at all incubation times ($p<0.0001$). Sunflower meal and soybean meal had the lowest and the highest gas production at 24 h incubation, respectively. There were significant differences in metabolizable energy (ME), net energy for lactation (NEL), organic matter digestibility (OMD) and short chain fatty acid (SCFA) of samples ($p<0.0001$). The sunflower meal has the lowest and soybean meal has the highest ME, NEL, OMD and SCFA compared with those of the others (8.69 MJ/kg DM, 5.48 MJ/kg DM, 55.14%, 0.60 mmol for sunflower meal and 12.98 MJ/kg DM, 9.03 MJ/kg DM, 85.03%, 1.13 mmol for soybean meal). In conclusion, it can be said that comparative nutritive value of Iranian oil seed meals which are tested in current study as below: soybean meal> rapeseed meal> cotton- seed meal> sunflower seed meal.

INTRODUCTION

Shortage and expensiveness of conventional animal feeds in arid and semi arid countries such as Iran make the animal nutritionists to effectively use agro-industrial by-products in animal nutrition (Rowghani et al., 2008; Maheri-Sis et al., 2011). Developing food industrial units have led to produced huge amounts of wastes and by-products which can play an important role in livestock nutrition. Local availability, nutritional value and low cost of such by-products in animal nutrition can overtop the feed shortage and expensiveness (Mirzaei-Aghsaghali and Maheri-Sis, 2008a; Mirzaei-Aghsaghali et al., 2008b). Banning the use of animal origin protein sources in livestock animal nutrition in order to assure consumer safety on animal products by European Commission (2001) led to worldwide attention of animal nutritionists to using plant protein sources. Oilseed meals can be regarded as the important alternative protein sources from plant origin. Soybean and soybean products are used widely in human and animal nutrition. Soybean meal is generally regarded as the best plant protein source in livestock and poultry feeding. Also, this valuable by-product has a complementary relationship with cereal grains in meeting the amino acids requirements of farm animals. Consequently, it is the standard to which other plant protein sources are compared. Moreover, the proteins of this by-product are low degradable in the rumen and well proportioned to the non structural carbohydrates (NSC). Also, its energy content is usually higher than that of other oilseed meals (Barros et al., 2011; Cutrignelli et al., 2011). In spite of higher nutritional value of soybean meal in livestock nutrition, higher price and importing problems as well as public caution of consuming genetically modified products limits its availability and utilization in animal nutrition, in particular in Iran. Most utilized alternative plant origin protein sources in animal nutrition in Iran are sunflower seed meal, rapeseed meal and cotton seed meal.

There are three common methods (i.e. *in vivo*, *in situ* and *in vitro)* to evaluate the nutritive value of various feedstuffs in livestock nutrition. Although theoretically *in vivo* methods are ideal for measuring feed digestibility in the animal, nowadays, *in vitro* gas production technique is used as potentially useful technique to estimate feed intake, organic matter and dry matter digestibility, microbial nitrogen supply, amount of short chain fatty acids, carbon dioxides and metabolizable energy of feeds for ruminants. Moreover, gas production is an indirect measure of substrate degradation in the rumen (Liu et al., 2002; Getachew et al., 2004; Maheri-Sis et al., 2008) . This method , also due to low cost and low time consuming, is suitable for developing countries such as Iran (Maheri-Sis et al., 2008).

Due to lack of sufficient information about the nutritive value of Iranian oil seed meals, data published in NRC (2001) are usually, used in feed formulation for ruminants, in Iran. It is clear that those data may not accurate in all conditions and countries. Thus, it needs to evaluate the nutritive value of feedstuffs when used in diets of the animals. Therefore, this study was conducted to evaluate and compare the nutritive value of Iranian soybean meal, sunflower meal, rapeseed meal and cotton seed meal including chemical composition, *in vitro* gas production characteristics, organic matter digestibility (OMD), metabolisable energy (ME), net energy for lactation (NE_L) and short chain fatty acids (SCFA) using *in vitro* gas production technique.

MATERIALS AND METHODS

Samples collection and treatments

Soybean meal, sunflower meal, rapeseed meal and cotton seed meal samples were obtained from commercial sources in Tabriz, Iran. Collected samples were milled through a 1 mm sieve for chemical analysis and gas production procedure.

Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105 °C for 24 h, ash content by ashing in a muffle furnace at 550 °C for 8 h and nitrogen (N) content was estimated using the Kjeldahl method (Association of Official Analytical Chemists, 1990). Neutral detergent fiber (NDF) and acid detergent fibre (ADF) determinations were based on the method of Van Soest et al. (1991). Crude protein (CP) was calculated as N×6.25 (Association of Official Analytical Chemists, 1990). Non-fibrous carbohydrate (NFC) was calculated using the equation outlined by NRC (2001): NFC=100 (NDF + CP + EE + Ash). All chemical analyses were carried out in triplicate.

***In vitro* gas production**:

Rumen fluid was obtained from three fistulated Ghezel rams fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Rumen fluid was collected before the morning feeding. Approximately 200 mg dry weight of samples (soybean meal, sunflower meal, rapeseed meal and cotton- seed meal) was weighed in triplicate into 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1988). The syringes were pre-warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in an incubator at 39 °C for 72 h. To prevent the gas volume in the syringes from exceeding 60 ml, the pistons were moved back to the ml piston after 12 hours of fermentation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions of samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model proposed by Ørskov and McDonald (1979):

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

Where Y is the gas production at time t, a is the gas production from soluble fraction (ml/200mg DM), b is the gas production from insoluble but fermentable fraction (ml/200mg DM), c the gas production rate constant for the insoluble fraction (ml/h), $a + b$ the potential gas production (ml/200mg DM) and t is the incubation time (h).

Metabolisable energy (ME), organic matter digestibility (OMD) and net energy for lactation (NEL) were calculated using the equations of Menke and Steingass (1988) as:

ME (MJ/kg DM) = 0.157 × GP + 0.084 CP + 0.22 EE – 0.081 CA + 1.06

NEL (MJ/kg DM) = 0.115 GP + 0.054 CP + 0.14 EE – 0.054 CA – 0.36

OMD (g/kg DM) = 0.9991 GP + 0.595 CP + 0.181 CA + 9

Where, GP is 24 h net gas production volume (ml/200mg DM), and CP, EE, CA are crude protein, ether extract and crude ash (g/kg DM), respectively.

Short chain fatty acids (SCFA) were calculated by equation of Makkar (2005):

SCFA (mmol) = 0.0222 GP – 0.00425

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

Statistical analyses

Data from *in vitro* gas production test were subjected to analysis of variance as a completely randomized design (CRD) with four treatments including soybean meal, rapeseed meal, cotton seed meal, sunflower seed meal (three replicates for each treatment) using general linear model (GLM) procedure of SAS (2001). Means compared by Duncan multiple range tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The chemical compositions of soybean meal, sunflower meal, rapeseed meal and cotton seed meal are presented in table 1. Dry matter was ranged from 90.5% (cotton seed meal) to 94.56% (sunflower meal). The crude protein contents were found in the range of 30% to 40%; soybean meal had the higher crude protein than those of the others. Ether extract content of the samples ranged from 3.2% to 5.67% and rapeseed meal had the lowest EE level compared with those of the others. Maximum NDF contents were found in sunflower meal (43.2%), followed by cotton seed meal (36%), rapeseed meal (32.3%), soybean meal (17.8%). The ADF contents were found in the range of 7.5% to 31.2%; sunflower meal had the highest ADF level compared with those of the others. The NFC was greatest for the soybean meal (30.83%) and lowest from the sunflower meal (15.83%). The ash was ranged from 5.2% (cotton seed meal) to 7.12% (rapeseed meal). There is some variation between chemical compositions of oil seed meals reported by other Iranian researchers (Maheri-Sis et al., 2011; Salamatazar et al., 2011; Salamat Azar et al., 2011; Salamatazar et al., 2012; Faramarzi-Garmroodi et al., 2014). These variations in chemical composition of by-products may be due to different original materials, growing conditions (such as geographic, seasonal variations, climatic conditions and soil characteristics), and extent of foreign materials, impurities, varieties, different processing and measuring methods. It is clear that, any variation in chemical composition can be resulted in different nutritive value; because chemical composition is one of the most important indices of nutritive value of feedstuffs (Maheri-Sis et al., 2007, 2008; Aghjanzadeh-Golshani et al., 2010).

Table 1: Chemical composition of the Iranian oilseed meals (%)

Feedstuff	DM	CP	EE	Ash	NDF	ADF	NFC
Soybean meal	92.78	40	4.87	6.5	17.8	7.5	30.83
Sunflower meal	94.56	30	5.67	5.3	43.2	31.2	15.83
Rapeseed meal	92.36	35	3.2	7.12	32.3	21	22.38
Cotton seed meal	90.5	37	5.5	5.2	36	22	16.30

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: non- fibrous carbohydrate.

Amounts of gas produced (ml/200 mg DM) in different incubation times are illustrated in table 2. The results of in vitro gas production showed that there are significant differences between gas production volume of samples at all incubation times ($p<0.0001$). The gas production volume at 2 h incubation ranged from 4.41 to 12.73

ml/200mg DM. The gas production volume at 24 h incubation was ranged from 27.36 to 51.10 ml/200 mg DM. Sunflower meal and soybean meal had the lowest and the highest gas production at 24 h incubation, respectively. Amount of gas production at 24 h incubation is important because of its high positive correlation by energetic value of feedstuffs (Getachew et al., 2004; Maheri-Sis et al., 2008).

Table 2: *In vitro* gas production volume (ml/200mg DM) of Iranian oilseed meals at different incubation times

Incubation times (h)	Soybean meal	Sunflower meal	Rapeseed meal	Cotton seed meal	*P* value	S.E.M
2	11.79^{b}	4.41^{d}	12.73^{a}	8.49^{c}	0.0001	0.1794
4	18.71^{b}	8.17^{d}	20.59^{a}	13.04^{c}	0.0001	0.3294
6	24.85^{b}	15.25^{c}	26.88^{a}	15.09^{c}	0.0001	0.4164
8	31.13^{b}	17.45^{c}	32.38^{a}	16.98^{c}	0.0001	0.3352
12	39.46^{a}	20.44^{c}	38.83^{a}	21.38^{b}	0.0001	0.2529
24	51.10^{a}	27.36^{d}	47.32^{b}	30.04^{c}	0.0001	0.2764
36	51.42^{a}	28.30^{c}	51.40^{a}	34.91^{b}	0.0001	0.3647
48	52.68^{b}	29.72^{d}	53.45^{a}	37.26^{c}	0.0001	0.2394
72	53.94^{a}	30.66^{c}	54.07^{a}	38.37^{b}	0.0001	0.3000

a-d: Means with different superscripts within a row differ significantly.
a: the gas production from soluble fraction (ml/200mg DM), *b*: the gas production from insoluble but fermentable fraction (ml/200mg DM), c: rate constant of gas production during incubation (/h), *a+ b*: the potential gas production (ml/200mg DM).

Gas production parameters (*a, b, c*) and estimated values of OMD, ME, NEL and SCFA of samples are presented in table 3. There are significant differences about soluble fraction (*a*), insoluble but fermentable fraction (*b*), potential gas production (*a+b*) and rate constant of gas production (*c*) of treatments ($p<0.0001$). Blummel and Becker (1997) stated that the soluble fraction (*a*) of feed makes it easily attachable by rumen microorganisms and leads to much gas production. The soluble fraction in cotton seed meal (5.50 ml/200 mg DM) was highest and sunflower meal (0.003) was lowest. In other hand, the gas volume at asymptote (*b*) is an important index for predicting feed intake. Fermentable fraction (*b*), was ranged from 31.76 to 52.80 ml/200 mg DM. The cotton seed meal had the lowest rate constant of gas production (*c*) level compared with those of the others. Maximum organic matter digestibility (ME), was found in soybean meal (12.98 ml/200 mg DM), followed by rapeseed meal (11.55 ml/200 mg DM), cotton seed meal (9.67 ml/200 mg DM), sunflower meal (8.69 ml/200 mg DM). The sunflower meal had the lowest NEL and soybean meal had the highest NEL compared with those of the others. OMD was ranged from 55.14 ml/200 mg DM (sunflower meal) to 85.03 ml/200 mg DM (soybean meal). The SCFA contents were found in the range of 0.60 mmol (sunflower meal) to 1.13 mmol (soybean meal). Faramarzi-Garmroodi et al (2014) also, found that, gas production parameters and nutritive value of Iranian oilseed meals including soybean meal, rapeseed meal, cotton seed meal, and sunflower seed meal are different. The estimated nutritive value of oil seed meals in current study approximately in line with those reported by Maheri-Sis et al. (2011), Salamatazar et al. (2011), Salamat Azar et al. (2011) and Salamatazar et al. (2012). In current study as well as our previous works (Maheri-Sis et al., 2007, 2008; Aghajanzadeh-Golshani et al., 2010), it is obvious that there is a positive inter-relationship among gas production at 24h incubation, OMD, ME, NEL and SCFA. Also gas production positively and negatively correlated by NFC and NDF content of feeds, respectively. Therefore it was predictable that soybean meal with lower NDF and higher NFC than that of other oilseed meals, must has the highest nutritive value.

Table 3: *In vitro* gas production parameters and estimated metabolisable energy (ME), net energy for lactation (NEL), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of Iranian oilseed meals

Items	Soybean meal	Sunflower meal	Rapeseed meal	Cotton seed meal	*P* value	S.E.M
a (ml)	0.70^{c}	-2.00^{d}	4.3^{b}	5.50^{a}	0.0001	0.3647
b (ml)	52.80^{a}	31.76^{d}	48.95^{b}	33.92^{c}	0.0001	0.5470
a + b (ml)	53.57^{a}	33.76^{c}	53.26^{a}	39.43^{b}	0.0001	0.3623
c (ml)	0.10^{a}	0.11^{a}	0.10^{a}	0.05^{b}	0.0001	0.0041
ME (MJ/ Kg DM)	12.98^{a}	8.69^{d}	11.55^{b}	9.67^{c}	0.0001	0.0433
NE_L (MJ/ Kg DM)	9.03a	5.48^{d}	7.80^{b}	6.14^{c}	0.0001	0.0317
OMD (%)	85.03^{a}	55.14^{d}	78.39^{b}	61.96^{c}	0.0001	0.2705
SCFA (m mol)	1.13^{a}	0.60^{d}	1.04^{b}	0.66^{c}	0.0001	0.0058

a-d: Means with different superscripts within a row differ significantly.

a: the gas production from soluble fraction (ml/200mg DM), *b*: the gas production from insoluble but fermentable fraction (ml/200mg DM), *c*: rate constant of gas production during incubation (/h), *a+ b*: the potential gas production (ml/200mg DM).

CONCLUSION

Based on chemical composition and gas production characteristics, comparative nutritive value (ME, NE_L and OMD) of Iranian oil seed meals which are tested in current study as below: soybean meal> rapeseed meal> cotton seed meal> sunflower seed meal.

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