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Comparing fermentation kinetics and nutritional value of alfalfa hay using rumen and faeces liquor as inocula for *in vitro* gas production technique

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Abstract

This study was conducted to compare fermentation characteristics and nutritional value of alfalfa hay by *in vitro* gas production technique using rumen and faeces liquors as inocula. In the first experiment, rumen liquor was collected from three cannulated Ghezel rams. Thirty ml of the buffered rumen fluid were added to 100 ml syringes, containing 200 mg of alfalfa sample. The samples were incubated for 2, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 hours, after which net gas production was calculated. In the second experiment, fresh faeces samples were taken from rams and faeces suspension was prepared by adding artificial saliva. All incubation steps were performed similar to the gas production procedure with rumen liquor. Result indicated that, there were no significant differences between gas production volume, organic matter digestibility (OMD), short chain fatty acids (SCFA), metabolizable energy (ME) and net energy for lactation (NE_L) contents of alfalfa hay with rumen liquor and faeces suspension at different incubation times. The estimated values of ME, NE_L and OMD from gas production with rumen liquor were 10.32, 6.23 MJ/kg DM and 75.98 %, and for faeces liquor were 10.56, 6.36 MJ/kg DM and 77.66%, respectively. In an overall conclusion, it seems that the sheep faeces can be used instead of rumen liquor in adapted gas production method for feed evaluation. Development of the procedure can remove the need for fistulated animals.

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Introduction

In order to optimizing the use of feedstuffs, it is required to sufficient information regarding the requirements of the animals, nutritional value of feeds and availability of nutrients. Nutritional value of a feed can vary from region to other; these variations can limit the use of tabulated values in ration formulation. Ereifej and Haddad (2001) suggested that despite determining chemical analysis of feeds is time-consuming and expensive, it is necessary to determine the chemical composition of their, because the chemical composition of feedstuffs are influenced by various factors such as, climate, soil, variety, transport and storage.

There are three important methods of feed evaluation in ruminant include *in vivo*, *in situ* and *in vitro* (Mohamed and Chaudhry, 2008; Aghajanzadeh-Golshani *et al*., 2010). *In vitro* methods have several advantages over *in vivo* methods. The *in vitro* techniques are cheap, fast, simple, less time-consuming, more data obtained and small amounts of sample than that of *in vivo* methods (Bani *et al*., 1999; Getachew *et al*., 2002; Cone *et al*., 2009; Mirzaei-Aghsaghali *et al*, 2011). The gas production technique is one of the *in vitro* methods that used for nutritive value prediction of feedstuffs or ration by anaerobic fermentation (Chenost *et al*., 2001; Nezarati *et al*., 2014). Despite of the above mentioned advantages of the *in vitro* (gas production) method, there is a need for cannulated animals to take rumen liquor. Fistulated animals are having problems such as surgical operation, special care to prevent infection, expensiveness and ethical considerations of using these animals (Mauricio *et al*., 2001). To avoid the need for fistulated animal to provide rumen liquor, El Shaer *et al*., (1987) used sheep faeces as inocula for *in vitro* determination of digestibility. Recently, Laudadio *et al*., (2009) have used sheep, goat and camel faeces as inocula in the Daisy II incubator and no differences were found in dry matter digestibility of plant species when used sheep rumen liquor as microbial inoculum. Simultaneously, Parand and Taghizadeh (2009) obtained a high relationship between gas production of incubated barley grain using rumen liquor and faecal suspension in the *in vitro* gas production method described by Menke and Steingass (1988).

There is a little information in the term of comparing the nutritional value of feedstuff when using rumen liquor and faeces suspension as inocula in the adapted gas production method. The present study was designed to compare the estimated nutritional value of alfalfa hay using two sources of inocula in the adapted gas production technique.

Materials and methods

Chemical analysis

Alfalfa hay (Hamedani variety) samples were provided from Agricultural Research Centre of Islamic Azad University, Shabestar Branch, Shabestar, Iran, and were ground through a 1 mm sieve. To determine dry matter, samples were dried at 105°C overnight and ash content was determined by igniting the samples in muffle furnace at 525°C for 8 h. Ether extract (EE) and crude protein (CP) content of alfalfa hay were determined by soxhlet extraction and Kjeldahl method, respectively (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by procedures proposed by Van Soest *et al*., (1991). Following equation that proposed by NRC (2001), was used to calculate the non-fibrous carbohydrates (NFC): %NFC = 100 – (%NDF + %CP + % EE + %Ash). Mean chemical compositions of the tested alfalfa hay are presented in Table 1.

In vitro gas production using rumen liquor as inocula

The gas production was performed using the procedure describe by Menke and Steingass (1988). Rumen liquor was taken from three cannulated Ghezel rams with average weight about 55 kg before morning feeding. Obtained rumen liquor immediately was transferred to the pre-warmed flask. Rumen liquor was passed through two layers of cheese cloth and was kept at a temperature of 39 °C and under

flushing carbon dioxide. Required solutions were prepared using method proposed by Menke *et al.*, (1979). The samples were incubated in 100 ml syringes in a shacking incubator at 39 ° C in triplicate. The syringes contained approximately 200 mg samples (as DM basis) and 30 ml of buffered rumen fluid from the ratio of 2 parts (artificial saliva) to 1 part (rumen fluid). Three syringes without feed samples as blank (contain only 30 ml of buffered rumen fluid) for corrected of gas production from rumen fluid were incubated for each run. The gas production volumes were recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 h of incubation times and corrected for blank.

In vitro gas production using faeces suspension as inocula

Fresh faeces samples from rams mentioned above was taken before morning feeding and transferred to the pre-warmed flask. The faeces suspension was prepared using the method proposed by El Shaer *et al.*, (1987). The amount of 50 grams of faeces samples were collected and prepared Homogenous mixture by adding 50 ml of artificial saliva (Menke *et al.*, 1979) that saturated with CO_2. Then, added 250 ml of artificial saliva and the suspension strained through two layers of cheese cloth and pH was adjusted to 6.8. All incubation steps were performed similar to the procedure of conventional gas production with rumen liquor. The syringes contained approximately 200 mg samples (as DM basis) and 30 ml of faeces suspension. Three syringes only with 30 ml of faeces suspension (as blank) were incubated for each run.

Equations, calculations and statistical analyses

Net gas production data (both rumen liquor and faeces suspension) were fitted to the model outlined by Ørskov and McDonald (1979) and gas production parameters were estimated by the Fitcurve software version 6:

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

Where:

Y = amount of gas produced at time t

a = amount of gas production from the soluble fraction (ml/ 200 mg DM)

b = amount of gas production from the insoluble fraction (ml/ 200 mg DM)

c = the gas production rate constant for the insoluble fraction (/h)

a + b = total gas production from fermentable fraction (ml/ 200 mg DM)

t = incubation time (h)

e = 2.7182 (natural logarithm base)

The amount of organic matter digestibility (OMD), net energy for lactation (NE_L) and metabolizable energy (ME) were estimated using equations of Menke and Steingass (1988):

DOM (%) = 0.889 GP + 0.45 CP + 0.651 CA + 14.88

ME (MJ/kg DM) = 0.136 GP + 0.057 CP + 0.00286 EE^2 + 2.2

NE_L (MJ/kg DM) = 0.096 GP + 0.038 CP + 0.00173 EE^2 + 0. 54

Where, GP is gas production volume at 24 h of incubation time (ml/200mg DM), CP and EE are crude protein and ether extract percentage, respectively.

The estimated values of short chain fatty acids (SCFA) were obtained using equation of Makkar (2005): SCFA (mmol) = 0.0222 GP – 0.00425.

Statistical analysis of data from gas production both rumen liquor and faeces suspension (three replicates) was performed as a completely randomized design using SAS (2001, version 8.02) software. Means were compared by Duncan's multiple range tests. Regression equations between gas production using rumen liquor and faecal suspension were derived using SAS (2001, version 8.02) software.

Results and discussion

Cumulative gas production values at different incubation times of alfalfa hay, using two different sources of micro-organism (rumen liquor and faeces suspension) are shown in Table 2 and Fig. 1. There were no significant differences between gas production with rumen liquor and faeces suspension at different incubation times. As given in the Table, the gas production with rumen liquor at 24, 48 and 72h

of incubation time are 51.50, 57.83 and 58.67 (ml/200mg DM), respectively, which were higher than that of findings of Mansuri *et al.*, (2003; 46.95, 52.06 and 53.7 ml/200mg DM, respectively) and were in agreement with those reported by Taghizadeh *et al.*, (2008; 45.8-49, 54-58 and 57.6-62.4 ml/200mg DM, respectively) and were lower than that of obtained by Maheri-Sis *et al.*,(2007; 64.4, 69.8 and 72.2 ml/200mg DM, respectively). Generally, factors such as the source of rumen fluid, animal species, and time of the rumen fluid collecting as well as donor animal diet can affect the microbial activity in the rumen fluid, and subsequent gas production (Mansuri *et al.*, 2003; Rymer *et al* ., 2005; Mould *et al* ., 2005).

Table 1. Chemical composition of alfalfa hay on dry matter basis (%).

DM	Ash	CP	EE	NDF	ADF	NFC
91.78	10.29	19.14	3.01	44.17	34.02	23.39

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

Similar results obtained from the two sources of inocula are probably due to the similarities between the condition of rumen and hindgut. The similarities are bacterial action on, cellulase, protease, deaminase, and urease, fermentation products (VFA, NH_3, CH_4 and microbial cells) and bacterial concentrations (Gressley *et al.* 2011). Nevertheless, Bani *et al.*, (1999) found that when the alfalfa hay is incubated with faecal suspension, in comparison with rumen liquor, little gas is produced, and it is associated with low cellulolytic activity. They suggested that it can be used faecal suspension instead of rumen liquor, in particular to measure the total of gas production in incubations extended to 24-48 hours.

Table 2. Gas production volume (ml/200mg DM) at different incubation times of alfalfa hay using rumen liquor and faeces suspension.

Incubation time (h)	With rumen liquor	With faeces suspension	SEM	P value
2	5.78	7.22	1.2966	0.4757
4	11.78	13.28	1.7139	0.5691
6	19.78	20.50	1.8671	0.7984
8	28.20	27.33	2.3877	0.8106
12	40.14	38.25	3.2289	0.7007
16	45.83	46.17	3.1173	0.9433
24	51.50	52.83	3.2270	0.7844
36	55.50	56.78	3.3843	0.8021
48	57.83	59.20	3.6362	0.8038
60	58.50	61.25	3.6629	0.6232
72	58.67	62.42	3.6895	0.5116

There was a significant correlation between the gas production from both rumen liquor and faeces suspension. The equations offered in Table 3, predict the amount of gas production using rumen liquor from the faeces suspension for alfalfa hay.

The gas production parameters (*a, b, c*), OMD, SCFA, ME and NE_L contents of alfalfa hay using rumen

liquor and faeces suspension as inocula have been presented in Table 4. There are no significant differences between two sources of micro-organism in fermentation parameters and nutritional values except for "*a*" and "$|a|$".

The $|a|$, *b* and $|a|+b$ fractions in case of rumen liquor (8.13, 66.60 and 74.73 ml/200mg DM, respectively) were higher than that reported by Kamalak *et al.*, (2005a; 0.57, 59.32 and 59.89, ml/200mg DM, respectively).

Table 3. Regression equations for estimation of gas production with rumen liquor from gas production using faeces suspension as inocula for alfalfa hay in different incubation times.

No.	Equations	r^2	n
1	$Gas_R = 4.85 + 0.78\ Gas_F + 0.11\ t$	0.87	33
2	$Gas_R = 3.34 + 0.89\ Gas_F$	0.86	33

Gas_R: gas production using rumen liquor (ml); Gas_F: gas production using faeces suspension (ml), t: time of incubation.

The estimated values of ME, NE_L and OMD from gas production using rumen liquor were 10.32, 6.23 MJ/kg DM and 75.98 %, respectively, which were higher than the findings of Abas *et al.*, (2005), who reported 8.88, 5.2 MJ/kg DM and 66.32%, respectively. The ME and NE_L content of alfalfa hay were also higher than that of values of NRC (2007; 8.78 and 5.43 MJ/kg DM). Reported values for ME and OMD of alfalfa hays by Maheri-Sis *et al.*, (2007) were 10.96 MJ/kg DM and 71.2 %, respectively.

Table 4. comparison of gas production parameters, organic matter digestibility (OMD), short chain fatty acids (SCFA), metabolizable energy (ME) and net energy for lactation (NE_L) contents of alfalfa hay using different inocula

Items	Using rumen liquor	Using faeces suspension	SEM	P value
a (ml)	-8.13	-4.62	0.6455	0.0182
$\|a\|$ (ml)	8.13	4.62	0.6455	0.0182
b (ml)	66.60	66.02	3.2529	0.9055
c (/h)	0.0995	0.0849	0.0053	0.1251
a+*b* (ml)	58.47	61.40	3.5992	0.5949
$\|a\|+b$ (ml)	74.73	70.63	3.0070	0.3895
OMD (%)	75.98	77.66	2.9067	0.7024
SCFA (mmol)	1.1391	1.1687	0.0716	0.7844
ME (MJ/kg DM)	10.32	10.50	0.4389	0.7844
NE_L(MJ/kg DM)	6.23	6.36	0.3098	0.7844

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 (/h).

Differences in the results obtained from present research and other studies may be due to variation in the chemical composition of alfalfa such as CP, Ash, EE, NDF and NFC, which may affect gas production (Kamalak *et al.*, 2005b; Tang *et al.*, 2008; Bakhashwain *et al.*, 2010). On the other hand amount of gas production may affect inter-laboratory variation such as microbial origin and donor animals. Since, the amounts of OMD, ME and NE_L are calculated from the amount of gas production volume and some of chemical compositions (CP, EE and Ash); changes in any of these parameters can alter these values (Aghajanzadeh-Golshani *et al.*, 2014).

Measuring methods used for evaluation of energetic value of alfalfa can also be another source of variation.

According to the literature review, we can not found a published paper that calculated ME, NE_L and OMD values of alfalfa hay using gas production method with faecal suspension, so the comparison was made only with rumen liquor between current study and other researches.

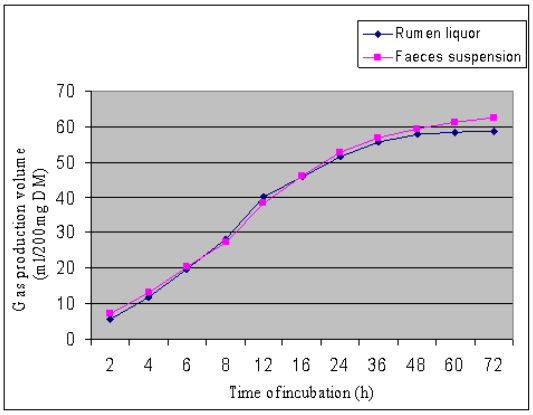

Fig. 1. Gas production volume at different incubation times of alfalfa hay using rumen and faeces liquor as inocula.

Conclusion

The results indicated that the sheep faeces is an appropriate alternative to rumen liquor in conventional gas production method for nutritive value evaluation of alfalfa hay. Development of this procedure can remove the need for cannulated animals and reduced additional costs. However, further research should be conducted on other feedstuffs.

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