Environmental Resources Research

Vol. 4, No. 2, 2016

GUASNR

Nutritive Value Assessment of Three Rangeland Species Using Sheep Rumen and Feces Fluid

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Received: March 2016 ; Accepted: May 2016

Abstract

This study was done to investigate the nutritive value of *Festocaovina*, *Agrostisgiganta* and *Avena sativa* using sheep rumen and feces liquor as sources of micro-organism through gas production method. Rumen and feces liquor were collected from three fistulated sheep. Rangeland species sampling was randomly conducted from different areas of Guilan province. All the collected samples were initially split and milled. To determine their chemical composition and digestibility, they were tested through Gas Production Process. The chemical composition in terms of ash, ether extract (EE), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were determined and gas production metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA), net energy for lactating (NEL), and digestibility dry matter intake (DDM) were predicted. CP ranged from 9.89% (*F. ovina*), 11.2% (*A. sativa*) to 11.84% (*A. giganta*) and ADF ranged from 28.37% (*F. ovina*), 42.07% (*A. giganta*) to 42.8% (*A. sativa*). The results showed that the amount of gas produced in species and different sources of micro-organism is significantly different ($p<0.05$). The results also indicated that the amount of gas production in rumen fluid and liquid stools increased with the time of incubation. The regression relationships between the amount of gas produced by the sheep rumen and feces fluid were significant ($p<0.05$, $R^2\geq 0.986$) and a curvilinear regression equation ($y = 19.33 \ln(x) - 6.074$)) was fitted to the data. The results also demonstrated that feces fluid can be used as a source of micro-organism for in-vitro gas production digestion of forage.

Keywords: Digestibility, Rangeland species, Gas production.

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1. Introduction

Three thousand million hectares of rangeland area (more than 25% of land surfaces) are the first and the most important resources of producing forage required by livestock. The world's grasslands feed approximately 1500 million livestock (including sheep, goat, cow, buffalo head, camel, etc) and provide more than 90% of food energy required by herbivores (Givens *et al.,* 1994). Livestock production has a vital role in social and economic welfare of the most rural and tribal societies and it is especially important in developing countries as a strategic supply in family stability and agricultural system (FAO, 1996). Today, development of reliable and acceptable laboratory methods for estimating forage quality is one of the problems in agricultural research (Akhter *et al.,* 1996). Therefore, identifying valuable rangeland plants in livestock breeding system in Mediterranean and Middle East countries have been considered by animal nutritionists (Golsen and Inal, 1995). Regarding the mentioned area (more than 90 million hectares), rangelands have high importance, for livestock forage in Iran, as we see a80% dependency of Iran's livestock, on these resources (Animal Department Report, 2002). In-vitro ruminal fermentation techniques have gained widespread acceptance as reliable approaches to predict digestibility of ruminant feed stuffs; however, these techniques require the use of rumen fluid as a source of microbial inoculums for fermentation of feed stuffs. Rumen fluid is either collected from animals fitted with rumen fistula, at slaughter (Cutrignelli *et al.,* 2005 and M. Hughes *et al.,* 2012) or via the oesophagus (Mould *et al.,* 2005). The need to identify an alternative to rumen fluid is mainly driven by ethical issues relating to surgical modification of an animal (Mould *et al.,* 2005 and Cutrignelli *et al.,* 2005). Other reasons that rumen fistulation requires special surgical skills, is that the fistulated animals need special care to ensure that the fistula is kept free of any infection, and a uniform diet must be fed. If the inoculums are to have constant activity and availability, skilled manpower for the surgical procedure, as well as drugs are required to maintain the fistula and these are the major challenges in less-developed countries (Akhter *et al.,* 1999; Mauricio *et al.,* 2001 and Williams, 2000). Thus, several studies have been carried out to test alternative inocula (Mauricio *et al.,* 1999; Cutrignelli *et al.,* 2005; Rymer *et al.,* 2005 and Mauricio *et al.,* 2001). Many researchers have recommended the use of feces as a potential alternative source of inoculums for in-vitro digestibility assay using the Tilley and Terry method (El Shaer*et al.,* 1987; Akhter *et al.,* 1999 and Cone *et al.,* 2002) and for the in-vitro gas production technique (Theodorou *et al.,* 1994; Harris *et al.,* 1995; Altaf *et al.,* 1998; Mauricio *et al.,* 1999; Zhao and Chen, 2004) to determine the digestibility of forages. Some limitations presented by fecal inocula are as follows: Omed *et al*. (2000) suggested that it may be necessary to increase the liquor sample ratio or increase the incubation time for forages with low digestibility. It has been made clear by Cutrignelli *et al*. (2005) that further

research on a wide range of feed stuff is needed to fully understand and develop digestibility techniques using Fecal microorganisms. Feces can, therefore, be a cheap, readily available source of micro-organisms which do not require the use of surgically prepared (cannulated) animals. It can be collected from any individual or several animals; therefore, minimizing the effects of animal to animal variation (Lowman *et al.,* 1999). Thus, feces may be an alternative to the rumen fluid inoculum techniques. There is a general agreement in the literature as to the potential of feces as a source of microbial inoculums in in-vitro ruminal fermentation systems. The objective of this experiment was to investigate whether Fecal microbial inocula have potential to be used as substitutes for investigating the nutritive value of three rangeland species (*Festocaovina*, *Agrostisgiganta* and *Avena sativa)* through using sheep rumen and feces fluid in in-vitro gas production method.

2. Materials and Methods

2.1. Plants

Three species of plants are naturally found in the rangelands of Iran, especially in the north of Iran (Guilan) which receive a total annual precipitation of 500–1100 mm. Forage samples from each herbaceous plants were randomly taken three times (each repeat was from five stands of a plant) and then pooled and dried at $60^{\circ C}$ in force draught oven for 48h. Dried samples were ground with a 1 mm screen and well mixed and stored frozen at ($-20^{\circ C}$) in sealed nylon bags for later analysis and evaluation. Chemical analysis of the forage samples was performed according to AOAC (2005) and contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined through Van Soest *et al's* (1991) method.

In-Vitro Procedure

Menke *et al.* method(1979) was used to determine the rate of gas production during 96h of incubation (0, 2, 4, 6, 8, 12, 24, 48, 72 and 96h) and digestion kinetics of feed samples over 96h according to the exponential equation $P= a+b\ (1- e^{-ct})$ of Ørskov and McDonald (1979) where P (mL) were defined as gas production at time (t), (a) in mL was the initial gas production, b in mL was the gas production during incubation, and (c) in mL/h was the fractional gas production. The forage samples (0.200 g dry weight) were incubated in triplicate in rumen fluid in calibrated 100 mL glass syringes at $39^{\circ C}$ following the procedure of Menke and Steingass (1988). The rumen fluid was collected from three rumen fistulated sheep (same age and weight (40-45kg)) before morning feeding (17h after the last feed) and was homogenized and strained through 100µm nylon cloth into a warm flask ($39^{\circ C}$) filled with CO_2. The fistulated sheep were fed twice daily with a diet containing hay (60%) and concentrate (40%). A total of 30mL of medium consisting of 10 mL of rumen fluid and 20 mL of bicarbonate-mineral-distilled

water mixture (1:1:2 by vol.) were pumped with an automatic pipette into the warmed syringes containing the samples (200 mg) and into the blank syringes. Feces were collected from the same animals with the same diet by collecting fresh feces directly from the rectum using palpation sleeves. The samples of ruminal contents (filtered through eight layers of gauze cloth) and feces were collected in thermos flasks and taken immediately to the laboratory where they were strained through various layers of cheese cloth and kept at 39°C under a CO_2 atmosphere until used (within approximately 20 min). The Fecal inoculums was prepared by homogenizing 40 g of feces with 360 ml of warm, distilled water for 2 min under CO_2, and then filtered through double-layered cheese cloth directly into the pre-warmed digestion vessels that contained 1.6 L of buffer solution, 400 ml of either rumen liquor or Fecal extract. The buffer solution consisted of 1.33 L buffer A (KH_2PO_4, 1.0 g/L; $MgSO_47H_2O$, 0.5 g/L; NaCl, 0.5 g/L; $CaCl_22H_2O$, 0.1 g/L; and urea, 0.5 g/L) and 266 ml of buffer B (Na_2CO_3, 15.0 g/L and Na_2S7H_2O, 1.0 g/L) mixed in each digestion vessel and the pH was adjusted to 6.8 (McDougall, 1948). Gas production from the forage sample was calculated by subtracting the volume of gas produced from the blank with or without the addition of forage depending on the treatment. The differences in gas production as a result of treatment were calculated according to the following equations (i.e., % increase).

DMD%= 83.54-0.824 (ADF %) + 2.626(N %) (Oddy*et al.,* 1983)

$NE_{(Mcal/lb)}$ = (2.20+ (.0272*Gas) +(.057*CP) +0.149*CF))/14.64 (Menke*et al.,* 1988)

OMD (%) = 0.9991 (G_{24h}) + 0.0595 (CP) + 0.0181 (CC) + 9 (Menke and Steingass, 1988)

$ME_{(MJ/kgDM)}$ = 0.157 (G_{24h}) + 0.0084 (CP) + 0.022 (EE) – 0.0081(CC) + 1.06 (Menke and Steingass, 1988).

$SCFA_{(mmol)}$ = 0.0222(G_{24h}) – 0.00425 (Makkar, 2005)

Where: G_{24h} is 24h net gas production (ml/g DM), CC, CP, EE and CF are crude ash, crude protein, ether extract and fat respectively (% of DM).

Design and Treatments

The experiment took the form of a factorial (2 ×3 (two liquor × three species)) arrangement of the treatments in a completely randomized design block. The effects of inoculums and substrate on IVOMD were analyzed through analysis of variance (ANOVA) and the contrast of means through Duncan test using the SPSS 13 software according to the following general linear model:

$X_{ijk}= \mu + a_{i(i=1-2)} + b_{j(j=1-3)} + c_{k(k=1-3)} + a_ib_j + e_{ijk}$

Where X_{ijk}= dependent variable, μ= overall mean, $a_{i=}$ effect of rumen and feces liquor, $b_{j=}$ effect of three species, ck= effect of block (each of sheep), a_ib_j= inter-active effect of liquor and species and e_{ijk}= random error

Correlation analysis was used to establish the relationship between rumen and feces liquor in in-vitro gas production (rumen liquor (Y) and feces liquor (X)). The level of significance was set at $P<0.05$.

3. Results

Chemical compositions of three plants collected during the experimental period are reported in Table 1. The CP content of plants ranged from 9.89% for F.*ovina* and 11.84% for *A. sativa*. The CF content ranged from (28.37-35.13) % for *F. ovina* and *A. sativa*. The NDF content varied between 61.5% in *A. sativa* and 67.67% for *F. ovina*. The ADF ranged from 28.37% for *F. ovina* to 42.8% in *A. sativa*. The results showed that the highest and lowest values of EE were 4.8% in *A. sativa* and 1.2% in *F. ovina, respectively*. Meanwhile, *A. giganta*had all values content between *F. ovina* and *A. sativa*.

Table 1. Chemical composition (% dry matter) of three plant species

Plant	%ADF	%NDF	%ASH	%CF	%EE	%CP
F. ovina	28.37	67.67	7.27	28.37	1.2	9.89
A. giganta	42.07	64.8	11.07	33.1	1.83	11.2
A. sativa	42.8	61.5	13.83	35.17	4.8	11.84

In-vitro gas production and gas produced parameters of rumen and Fecal liquor from the three range plants are presented in (Tables 2 and 3). Rate of gas production and parameters were not significantly different in each sheep (block). The rate of gas production (Ml/200mgDM) and parameters by rumen and Fecal liquor were significantly different ($p<0.05$) among rumen and Fecal liquor. The rate of gas production and value parameters of rumen liquor was significantly more than Fecal liquor ($p<0.05$). In addition, the rate of gas production which increased with increasing incubation time and potential gas production differed among three plants. There were significant differences ($p<0.05$) in GP (Ml/200 mgDM) and gas produced parameters among three plants and with duration of incubation. The highest and lowest rates of gas production were *A. sativa*, *F. ovina* and *A. giganta,* respectively (Table 2). Analysis of variance revealed that there was a high significant interaction between three rangeland plants and source of microorganism for each incubation time at the highest value. The rank order in terms of potential gas production performance was *A. sativa* with rumen liquor> *A. giganta* with rumen liquor >*F. ovina* with rumen liquor >*A. sativa* with Fecal liquor> *A. giganta* with Fecal liquor>*F. ovina* Fecal liquor (Table 2 and Figure 1). Generally, rate of gas production of three plants increased with increasing incubation and gas produced parameters a, b, and DMD were significantly ($p<0.001$) higher in *A. giganta* and parameters of (c), (OMD), (SCFA), (NEL), (ME) and (DOM) were significantly ($p<0.001$) higher in *A. sativa*. The higher and lower all gas produced parameters for accept (a) were in *A. sativa* with rumen liquor and *A. sativa* with Fecal liquor respectively (Table 3).

Table 2. Rumen and feces rate of gas production under different times

		Incubation time (h) (ml/200 mg DM)								
factor	Treatments	2	4	6	8	12	24	48	72	96
	Sheep1	3.5[a]	5.9[a]	7.5[b]	9.4[a]	14.8[a]	34.9[a]	44.9[b]	53.6[a]	61.6[a]
Block	Sheep2	4.2[a]	6.6[a]	8.3[a]	9.3[a]	14.8[a]	35.9[a]	47.3[a]	54.5[a]	60.6[a]
	Sheep3	3.9[a]	6.5[a]	8.2[a]	9.6[a]	14.5[a]	36.6[a]	47.2[a]	53.9[a]	60.2[a]
Sig		0.129	0.192	0.005	0.542	0.914	0.299	. . .	0.296	0.375
SEM		0.3	0.335	0.188	0.276	0.679	0.878	0.385	0.548	0.984
Liqour	Rumen	5.7[a]	10.3[a]	13.4[a]	16.1[a]	24.1[a]	50.6[a]	59.6[a]	65.8[a]	72.9[a]
	Feces	1.97[b]	2.4[b]	2.6[b]	2.8[b]	5.4[b]	20.8[b]	33.4[b]	42.3[b]	48.7[b]
Sig		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM		0.275	0.294	0.238	0.219	0.51	0.738	0.69	0.461	0.779
	F. ovina	3.7[a]	5.2[c]	6.4[c]	8.1[c]	12.3[b]	31.1[c]	42.9[c]	50.6[c]	57.4[b]
Plant	*A. giganta*	4.1[a]	6.1[b]	8.1[b]	9.1[b]	13.3[b]	34.2[b]	46.1[b]	53.7[b]	60.9[a]
	A. sativa	3.8[a]	7.9[a]	9.6[a]	11.2[a]	18.5[a]	41.8[a]	50.6[a]	57.7[a]	64.1[a]
Sig		0.568	0.001	0.00	0.002	0.004	0.003	0.00	0.001	0.00
SEM		0.337	0.36	0.292	0.268	0.625	0.904	0.845	0.564	0.954
	RL- *F. ovina*	5.45[a]	8.4[c]	10.7[c]	13.8[c]	20.2[c]	44.8[c]	54.9[c]	61.6[c]	68.8[c]
	RL- *A. giganta*	6.1[a]	9.8[b]	13.5[b]	15.5[b]	21.7[b]	49.5[b]	58.9[b]	65.4[b]	73.1[b]
Interaction	RL- *A. sativa*	5.63[a]	12.8[a]	16.1[a]	19.1[a]	30.2[a]	57.5[a]	64.8[a]	70.3[a]	76.8[a]
Effect	FL- *F. ovina*	1.8[b]	1.9[d]	2.1[d]	2.4[e]	4.5[e]	17.4[f]	30.8[f]	39.6[f]	46.1[e]
	FL- *A. giganta*	2.1[b]	2.3[d]	2.6[dc]	2.7[de]	4.9[e]	18.9[e]	33.1[e]	43[e]	48.8[d]
	FL- *A. sativa*	1.9[b]	3.1[d]	3.1[d]	3.3[d]	6.8[d]	26.1[d]	36.6[d]	45.2[d]	51.3[d]
Sig		0.00	0.00	0.00	0.00	0.00	0.003	0.00	0.001	0.00
SEM		0.476	0.511	0.412	0.379	0.884	1.279	1.195	0.798	1.349

*Columns having different superscripts are significantly different ($p< 0.05$) in each factor
(SEM = Standard error of the means), RL (Rumen Liqour), FL (Feces Liqour)

The results also showed that amount of gas production increased with time of incubation in used rumen fluid and liquid stools; however, in all species studied, this amount increased to 24 h of incubation with steep slopes, and then showed a slower growth rate (Figure 1).

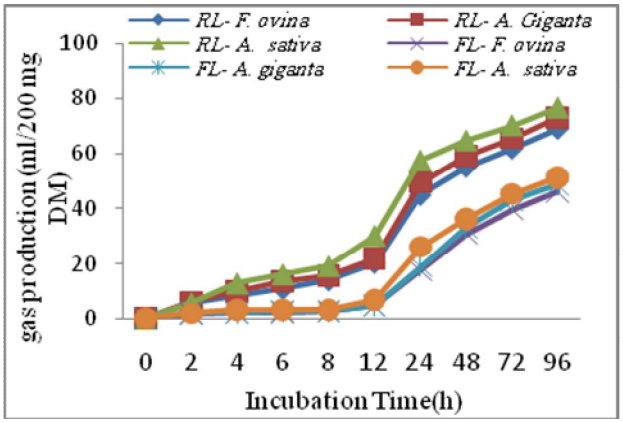

Figure 1. Cumulative gas production of three range plants in rumen and feces liquor (RL (Rumen Liqour), FL (Feces Liqour)

Table 3. Rumen and feces rate of gas production under different parameters

		Parameters of *in vitro* gas production(defined by the equation: $p = a + b(1-exp^{-ct})$								
factor	Treatments	a	b	c	OMD	DMD	DOM	SCFA	NEL	ME
Block	Sheep1	-2.17[a]	72.4[a]	0.0027[b]	[a]58.5	74.7[a]	48.7[a]	0.77[a]	3.9[a]	7[a]
	Sheep2	-2.49[a]	70.6[a]	0.0029[a]	[a]58.9	72.7[a]	49.5[a]	0.79[a]	4.1[a]	7.1[a]
	Sheep3	[a]-2.81[a]	69.6[a]	0.003[a]	[a]58.2	71.4[a]	49.9[a]	0.81[a]	4.1[a]	7.2[a]
Sig		0.057	0.383	0.032	0.762	0.253	0.36	0.299	0.342	0.345
SEM		0.229	1.917	0.001	0.884	1.882	0.799	0.019	0.084	0.119
Liqour	Rumen	-2.09[a]	74.49[a]	0.0413[a]	[a]71.79	77.76[a]	62.89[a]	1.12[a]	5.44[a]	9.13[a]
	Feces	-2.89[b]	67.25[b]	0.0162[b]	45.25[b]	63.07[b]	35.9[b]	0.46[b]	2.57[b]	5.08[b]
Sig		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SEM		0.227	1.573	0.001	0.677	1.609	0.460	0.061	0.070	0.099
Plant	*F. ovina*	-2.14[a]	69.76[a]	0.002[a]	51.71[c]	71.8[a]	45.13[c]	0.686[c]	3.56[c]	6.48[c]
	A. giganta	-1.99[a]	72.3[a]	0.0026[a]	57.51[b]	74.7[a]	48.03[b]	0.755[b]	3.86[b]	6.91[b]
	A. sativa	-3.34[b]	70.55[a]	0.0035[a]	66.33[a]	72.2[a]	55.02[a]	.923[a]	4.56[a]	7.94[a]
Sig		0.001	0.356	0.200	0.00	0.452	0.00	0.00	0.002	0.002
SEM		0.278	1.926	0.002	0.829	1.971	0.563	0.02	0.086	0.121
Interaction Effect	RL- *F. ovina*	-1.7[ab]	71.3[b]	0.003[b]	63.9[c]	74.5[ab]	57.53[c]	0.99[c]	4.9[c]	8.3[c]
	RL- *A. giganta*	-1.4[a]	74.3[ab]	0.004[b]	71.1[b]	78.1[a]	61.9[b]	1.1[b]	5.3[b]	8.9[b]
	RL- *A. sativa*	-3.2[c]	77.9[a]	0.005[a]	80.3[a]	80.7[a]	69.25[a]	1.3[a]	6.1[a]	10.1[a]
	FL- *F. ovina*	-2.5[bc]	68.3[bc]	0.001[d]	39.5[f]	69.2[bc]	32.73[e]	0.38[e]	2.2[e]	4.6[e]
	FL- *A. giganta*	-2.6[bc]	70.3[b]	0.001[d]	43.9[e]	71.4[b]	34.17[e]	0.42[e]	2.4[e]	4.8[e]
	FL- *A. sativa*	-3.5[c]	63.1[c]	0.002[c]	52.3[d]	63.7[c]	40.79[d]	0.57[d]	3.1[d]	5.8[d]
Sig		0.004	0.00	0.00	0.002	0.00	0.00	0.00	0.00	0.00
SEM		0.393	2.727	0.002	1.172	0.171	1.139	0.0028	0.1231	01716

*Columns having different superscripts are significantly different ($p < 0.05$) in each factor
(SEM = Standard error of the means), (SCFA (mmol/200 mg DM), Short Chain Fatty Acid), (NEL (MJ/kg DM), Net energy for Lactating), (ME (MJ/kg DM), Metabolism energy), (OMD (%), Organic Matter Digestible), (DMD(%), Digestibility Dry Matter), (DOM (%), Digestibility Organic Matter), (c = rate constant of gas production during incubation (ml h^{-1})), (a = gas produced from soluble fraction (ml)), (b = gas produced from insoluble but fermentable fraction (ml)), RL (Rumen Liqour), FL (Feces Liqour)

In the present study, the curvilinear relationship showed a correlation (R^2= 0.986, y=19.33 ln (x) -6.074) between rumen and Fecal liquor of gas production in three plants (Figure 2).

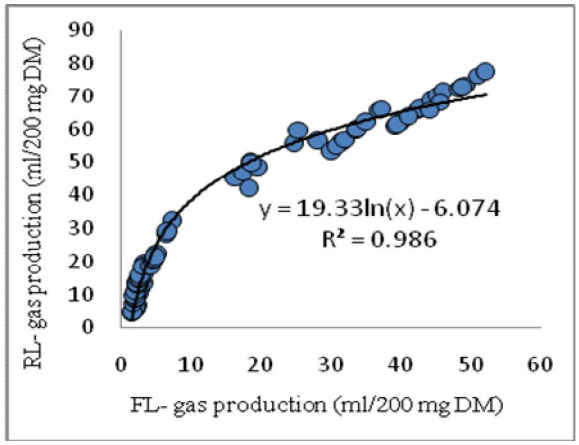

Figure 2. Relationship between rumen and Fecal liquor (RL, FL) of gas production in three plants

4. Discussion and Conclusion

The CP content of *F. ovina* (9.89%) and *A. sativa* (11.84%) and the CF content ranged from (28.37-35.13) % for *F. ovina* and *A. sativa*. The NDF content varied between 61.5% in *A. sativa* and 67.67% for *F. ovina*. The ADF ranged from 28.37% for *F. ovina* to 42.8% in *A. sativa*. Their botanical composition is mainly dependent on altitude, climate and adaphic factors (Tufarelli *et al.,* 2010). The rate of gas production and parameters were not different significantly in each sheep (block). The rate of gas production and value parameters of rumen liquor were significantly more than Fecal liquor ($p<0.05$). This agrees with the results of (Harris *et al.,* 1995; Mauricio *et al.,* 2001 and Akhter *et al.,* 1996). The lower rate of gas production and parameters values using feces liquor indicates that inoculum source have important effects on in-vitro results (Akhter *et al.,* 1996). Mauricio *et al*. (2001) and Gonçalves and Borba (1996) demonstrated that using feces liquor was associated with a longer lag phase. This is likely to be due to the fact that the Fecal micro-organisms originated mainly in the caecum/colon, where fermentation activity is lower than in the rumen. The lag phase is the period when the micro-organisms develops and is able to digest the substrate. The length of the lag time is longer with feces liquor than that of rumen liquor, because the micro-organism population is smaller, and they are in a 'state of suspended animation' from which it takes a longer period of time to begin growing and dividing. The different profiles may be due to the different bacterial populations in each inoculum (El-Meadaway*et al.,* 1998). The rate of gas production increased with increasing incubation time and potential gas production differed among three plants. There were significant differences ($p<0.05$) in GP (Ml/200mgDM) and parameters among three plants and with duration of incubation. The highest and lowest rate of gas production belonged toA. *sativa*, *F. ovina* and *A. giganta*, respectively (Table 2). This disagrees with the results of Tufarelli *et al*. (2010). Few soluble carbohydrates will be available; consequently, non-cellulolytic microbial growth will be inhibited until solubilisation occurs under the actions of the cellulolytic microbial population (Omed *et al.,* 2000). Although the hindgut of ruminants is regarded more as a fermentation chamber for structural carbohydrates escaping the rumen, it can be seen that the present microbes are very adaptable and able to degrade non-structural carbohydrates as well (Aiple *et al.,* 1992). The rank order in terms of gas production performance was *A. sativa* with rumen liquor> *A. giganta* with rumen liquor >*F. ovina* with rumen liquor >*A. sativa* with Fecal liquor> *A. giganta* with Fecal liquor>*F. ovina* Fecal liquor (Table 2 and figure 1). This is due to the fact that the Fecal micro-organisms fermentation activity is lower than that of the rumen (Mauricio *et al.,* 2001) and the order of increasing cell wall content (NDF) and (ADF) and the higher fiber content (Holden, 1999) or concentrates and protein supplements (Mabjeesh *et al.,* 2000). Generally, the rate of gas production of three plants increased with increasing incubation and parameters a, b, and DMD were

significantly ($p<0.001$) higher in *A. giganta* and parameters of (c), (OMD), (SCFA), (NEL), (ME), and (DOM) were significantly ($p<0.001$) higher in *A. sativa*. The highest and lowest gas production parameters (a) were in *A. sativa* with rumen liquor and *A. sativa* with Fecal liquor respectively (Table 2). This might be due to their content of ADF, NDF, and CP which are associated with feed degradability (Kamalak *et al.,* 2005). Therefore, the higher values obtained for the gas production and (c), (OMD), (SCFA), (NEL), (ME), and (DOM) in the *A. sativa* will indicate a better nutrient availability to rumen and feces microorganisms.It is possibility due to the order of the lower cell wall content (NDF) and the higher CP in the *A. sativa*. The results also showed that amount of gas production increased at the time of incubation in used rumen fluid and liquid stools However, in all species under study, this amount increased to 24 h of incubation with steep slopes and, then had a slower growth rate (Figure1). This is related to the ration and its constituents; as for more readily digestible carbohydrates, it takes 12-16 h and for less digestible carbohydrates it takes 24-96 h (Kinan and Krishnamoorthy, 2007; Vanic *et al.,* 2008). The results showed that feces could be a suitable source of microbial inoculum as microbial inoculum in gas production studies. This agrees with findings of Lowman et al. (1999) and El-Meadaway *et al.* (1998). In the present study, the curvilinear relationship showed a correlation ($R^2= 0.986$, $y = 19.33\ln(x) - 6.074$) between rumen and Fecal liquor of gas production in three plants (Figure 2). Nsahlai and Umunna (1996) found that the feces voided up to 2h prior to inoculum preparation, produced a significant correlation of in-vitro Fecal liquor digestibility ($r^2 =0.85$) with invitro rumen liquor. Akhter *et al*. (1996) reported $R^2=0.90\%$ between using rumen and Fecal liquor. The results revealed that the *A. sativa> A. giganta>F. ovina* are good sources of rangeland forage livestock, respectively. We also conclude that feces could be a suitable source of microbial inoculum as microbial inoculum in gas production studies. This outcome is in line with that of Lowman *et al*. (1999) and El-Meadaway *et al*. (1998).

Acknowledgements

The experiment was done at the Faculty of Agricultural Sciences and Animal Science. The experiment was financed by the Islamic Azad University, Rasht, Iran.

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