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Nutritive value assessment of three range plants by chemical and *in vitro* gas production techniques

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This study was conducted in order to measure the nutritional value, as ruminant food, of three range plants (*Dactylic glomerata, Onobrychis sativa* and *Setaria galauca)*, that were collected completely random from north of Iran (Guilan). Chemical analysis and *in vitro* gas production technique were used as the base for that evaluation. The chemical composition in term of ash, ether extract (EE), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), nitrogen free extract (NFE), crude fiber (CF) and organic material (OM) have significant differences ($p < 0.05$) among *D. glomerata, O. sativa* and *S. galauca*. CP were ranged from 8 (*S. galauca*) to 24% (*O. sativa*), and ADF from 37.8 (*O. sativa*) to 67.45% (*S. galauca*). Amount of fermentable fraction (b) were ranged from *D. glomerata* (68.53 ml), *O. sativa (*66.09 ml) and *S. galauca* (49.5 ml). Potential gas production (a+b) were ranged from *D. glomerata* (63.89 ml), *O. sativa (*63.39 ml) and *S. galauca* (47.4 ml). This is due to their high content of CP and low content of ADF. Therefore, according to the potential gas production performance, *D. glomerata* was ranked higher than the two other plants. The higher values obtained for the potential gas production in the *D. glomerata* and *O. sativa* indicate a better nutrient availability for rumen microorganisms. Most of chemical compounds have significant correlation with gas production factors such as fermentable part fraction (b) and potential gas production (a+b).

Key words: Range plant, nutritive value, gas production, chemical, correlation.

INTRODUCTION

More than 90 of 164 billion ha of Iran are allocated to rangelands area as an important part of livestock feed (above 80%) (Arzani et al., 2004). It is well known that forages have an important role in ruminant animal in terms of providing energy, protein and minerals as well as fibre for chewing and rumination (Kamlak, 2010). *Dactylic glomerata* and *Setaria galauca* belong to the Gramineae family and *Onobrychis sativa* belongs to the Leguminosae (Fabaceae) family. They are widely spread in rangelands of Iran and are grazed well by ruminants especially by small ruminants. Holchek et al. (1986) results showed that the amounts of crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) for *O. sativa* are 21.2, 52.3 and 47%, respectively. Jancik et al. (2010) results showed that the amounts of CP, NDF and ADF for *D. glomerata*

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are 13.1, 56.3 and 31.4%, respectively. Recently, some researchers have used the *in vitro* gas production technique to evaluate the fermentation kinetics of ruminant feedstuff (Mesgaran and Mohammadabadi, 2010; Chaji et al., 2010; Kamlak, 2010). In the gas method, kinetics of fermentation can be studied by simply reading the increase in gas production at a series of chosen time intervals and using the exponential equation $P = a+b\ (1 - e^{-ct})$ (Ørskov and McDonald, 1979). The present study was done to evaluate the nutritive value, by the use of *in vitro* incubation techniques with rumen fluid (gas production) and chemical analysis, for *D. glomerata, O. sativa* and *S. galauca* range plants.

MATERIALS AND METHODS

Three species of range plants, were grown naturally on the rangeland of Iran, especially north of Iran (Guilan), which receive a total annual precipitation of 500 to 1100 mm (Moghaddam, 2009). Forage samples from each herbaceous range plants were randomly taken, in three repeats (each replication were a compound of 5 samples of each species) and dried at room temperature (25 to 30°C) for 3 weeks, ground to pass through a 1 mm sieve, well mixed and stored frozen at (-20°C) in sealed nylon bags for later analysis and evaluation. Chemical analysis of forage samples were performed according to AOAC (2005), and contents of NDF and ADF were determined by the method of Van Soest et al. (1991).

The method of Menke et al. (1979) was used to determine the rate of gas production during 96 h incubation (0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h). Digestion kinetics were calculated 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 (ml) was the initial gas production, b (ml) was the gas production during incubation, a+b (ml) was the potential gas production and (c) (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°C following the procedure of Menke and Steingass (1988).

The rumen fluid was collected from three rumen fistulated sheep's (same age and weight) before morning feeding (17 h after the last feed) and was homogenized and strained through 100 µm nylon cloth into a warm flask (39°C) filled with CO_2. The fistulated sheeps were fed twice daily with a diet containing hay (60%) and concentrate (40%). A total of 30 ml of medium, consisting of 10 ml of rumen fluid and 20 ml of bicarbonate-mineral-distilled water mixture (1:1:2 by vol.), was pumped with an automatic pipette into the warmed syringes containing the samples (200 mg) and into the blank syringes. 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 treatment. The difference in gas production as a result of treatment was calculated and expressed as a proportion of that for the untreated sample (that is, % increase).

$$DMD\% = 83.54 - 0.824(ADF\%) + 2.626(N\%) \text{ (Oddy et al., 1983)}$$

$$NE_{(Mcal/lb)} = [2.20 + (0.0272 * Gas) + (0.057*CP) + 0.149 * CF)] / 14.64 \text{ (Menke et al., 1988)}$$

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

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

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

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

Means of the studied parameters were subjected to an analysis of variance (ANOVA) test, and Duncan test at the 95% confidence level by SPSS soft. Correlation analysis was used to establish the relationship between chemical composition and *in vitro* gas production parameters.

RESULTS

The chemical composition percentage as DM bases and correlation of chemical parameters for three range plants are presented in Tables 1 and 2, respectively. Ash, EE, CP, ADF, NDF, non fibrous carbohydrates (NFC), nitrogen free extract (NFE), crude fiber (CF) and organic material (OM) has significant differences ($p \leq 0.05$) among *D. glomerata, O. sativa* and *S. galauca*. CP was ranged from 8 (*S. galauca*) to 24% (*O. sativa*) and ADF from 37.8 (*O. sativa*) to 45.67% (*S. galauca*).

In vitro gas production of rumen gas from the three range plants is presented in Tables 3 and 4. Amount of gas production in all treatments has on an uptrend and it increases. Amount of produced gas (Ml/200 mgDM) in three species at all times of incubation has significant differences ($P \leq 0.05$). The rate of fermentation fraction (c) was significantly ($p < 0.01$) higher in *S. galauca* than in *O. sativa* and *D. glomerata*, but fraction (b) and fraction (a+b) were significantly ($p < 0.01$) higher in *O. sativa* and *D. glomerata* than in *S. galauca* (Table 3).

The rank order in terms of potential gas production performance in 0 to 24 h of incubation are *S. galauca* > *O. sativa* > *D. glomerata* and in 24 to 96 h time of incubation is *D. glomerata* > *O. sativa* > *S. galauca* (Table 4 and Figure 1).

There were significant correlations between the fermentation parameters and the chemical composition of three species (Table 5). Significant correlations were found for all gas parameters, except NE, SCFA and ME with ash. No significant correlation was found, except OMD and NE, with CF content. Negative correlations were detected between all gas parameters, except SCFA

Table 1. Chemical composition of three range plants as percent DM bases.

Treatment	CP	EE	CF	ASH	ADF	NDF	DMD	NFE	NFC	TDN	OM
S. galauca	7.95^{c}	4.06^{a}	31.00^{a}	4.23^{b}	45.67^{a}	73.93^{a}	49.25^{c}	49.7^{a}	38.09^{a}	54.93^{c}	95.77^{a}
D. glomerata	12.16^{b}	3.47^{a}	28.13^{b}	6.09^{ab}	41.81^{b}	72.78^{b}	54.19^{b}	45.24^{b}	35.56^{a}	55.71^{b}	93.00^{b}
O. sativa	23.95^{a}	2.51^{b}	12.43^{c}	7.00^{b}	37.78^{c}	53.84^{c}	62.47^{a}	51.16^{a}	29.667^{b}	68.59^{a}	93.91^{ab}

Columns having different superscripts are significantly different ($p < 0.05$).

Table 2. Correlation coefficient (r) between chemical composition parameters in three range plants.

Pearson correlation	CP	EE	CF	ASH	ADF	NDF	DMD	NFE	NFC	TDN	OM
CP	1.00										
EE	-0.87**	1.00									
CF	-0.99**	0.81**	1.00								
ASH	0.37^{ns}	-0.44^{ns}	-0.29^{ns}	1.00							
ADF	-0.96**	0.86**	0.92**	-0.49^{ns}	1.00						
NDF	-0.97**	0.82**	0.99**	-0.18^{ns}	0.89**	1.00					
DMD	0.991**	-0.871**	-0.97**	0.42^{ns}	-0.99**	-0.95**	1.00				
NFE	0.49^{ns}	-0.40^{ns}	-0.58^{ns}	-0.56^{ns}	-0.31^{ns}	-0.65^{ns}	0.41	1.00			
NFC	-0.95**	0.798**	0.936**	-0.56^{ns}	0.90**	0.89**	-0.93**	-0.31^{ns}	1.00		
TDN	0.97**	-0.82**	-0.99**	0.18^{ns}	-0.896**	-1.00**	0.945**	0.65^{ns}	-0.89**	1.00	
OM	-0.37^{ns}	0.44^{ns}	0.29^{ns}	-1.00**	0.49^{ns}	0.18^{ns}	-0.43^{ns}	0.56^{ns}	0.56^{ns}	-0.18^{ns}	1.00

*$p < 0.05$; **$p < 0.01$; ns = Non significant.

Table 3. Parameters of *in vitro* gas production in three range plants (defined by the equation: $p = a + b\ (1 - \exp^{-c\ t})$.

Treatment	Incubation time								
	a	b	c	a+b	DMD	OMD	SCFA	ME	NE
S. galauca	-2.08^{b}	49.5^{b}	0.09^{a}	47.42^{b}	55.20^{b}	55.43^{b}	9.38^{b}	7.55^{b}	6.11^{a}
D. glomerata	-4.68^{a}	68.53^{a}	0.04^{b}	63.85^{a}	69.24^{a}	64.3^{a}	10.65^{a}	8.52^{a}	6.3^{a}
O. sativa	-2.71^{b}	66.09^{a}	0.04^{b}	63.39^{a}	69.06^{a}	65.92^{a}	9.3^{b}	7.6^{b}	4.62^{b}
SEM	0.2	0.5	0.27	0.34	0.39	1.36	0.21	0.21	0.12
Significant	**	**	**	**	**	**	**	*	**

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); a+b = potential gas production (ml); Columns having different superscripts significantly ($p < 0.01$).

and ME, with ADF content ($P < 0.01$) (Table 5).

DISCUSSION

The results showed that there were significant variations in chemical composition and gas production characteristics of three range plants. Amount of CP in *S. galauca, D. glomerata* and *O. sativa* is 7.65, 12.1 and 23.95%, respectively. Theminimal CP content of DM for maintenance of sheep has been indicated by Milford and Haydock (1965) to be 7.2%. However, it was suggested to be at least 8.9% CP in plant material. The CP value in the present study plants were mostly well above the recommended levels by Milford and Haydock (1965) and NRC (1990), suggesting that they might maintain animals. On the other hand, the CP of *S. galauca* is fitted for sustain sheep if used as the only sources of feed. Range land forages are composed of structural and non-structural constituents. Rezayi (2004) reported that amount of CP belonged to theleaf:stem ratio and causes an increase in plant protein thus this reason amount of CP in *O. sativa* is more than that of *S. galauca* and *D. glomerata*. The leaves of *O. sativa* is phyllde form, but *S. galauca* and *D. glomerata* do not have phyllde leaves (Gramine family) and the leaf:stem ratio is low.

The surface of a leaf will be increased growing rate, DM and protein content as a result of increasing number of photosynthetic organ per unit of leaf surface (Hattab and Harb, 1990). The results showed that the ADF

Table 4. Rumen gas production (ml/200 mg DM) from three range plants under different times.

Treatment	Incubation time								
	2	4	6	8	12	24	48	72	96
S. galauca	3.74^{a}	9.67^{a}	18.12^{a}	24.38^{a}	32.83^{a}	39.00^{b}	44.69^{c}	47.94^{c}	49.08^{c}
D. glomerata	2.12^{b}	4.16^{c}	7.10^{c}	10.69^{c}	21.46^{b}	44.31^{a}	56.40^{a}	59.91^{a}	60.98^{b}
O. sativa	2.44^{b}	5.11^{b}	8.53^{b}	12.91^{b}	20.63^{c}	38.67^{b}	53.45^{b}	57.68^{b}	61.42^{a}
SEM	0.21	0.27	0.36	0.03	0.42	0.73	0.89	0.4	0.24
Significant	**	**	**	**	**	**	**	**	**

Columns having different superscripts are significantly different ($p < 0.05$).

Table 5. The correlation coefficients (r) between the chemical composition and gas production parameters.

Pearson correlation	CP	EE	CF	ASH	ADF	NDF
a	0.03^{ns}	0.09^{ns}	-0.14^{ns}	-0.67*	0.22^{ns}	-0.27^{ns}
b	0.61^{ns}	-0.62^{ns}	-0.52^{ns}	-0.74*	-0.79*	-0.44^{ns}
c	-0.70*	0.69*	0.61^{ns}	-0.72*	-0.86**	0.54^{ns}
a+b	0.68*	-0.67*	-0.59^{ns}	0.72*	-0.85**	0.54^{ns}
OMD	0.79*	-0.75*	-0.72*	0.68*	-0.92**	-0.66^{ns}
DMD	0.69*	-0.68*	-0.60^{ns}	0.72*	-0.85**	-0.53^{ns}
SCFA	-0.31^{ns}	0.16^{ns}	0.42^{ns}	0.54^{ns}	0.07^{ns}	0.50^{ns}
ME	-0.22^{ns}	0.08^{ns}	0.33^{ns}	0.59^{ns}	-0.03^{ns}	0.41^{ns}
NE	-0.93**	0.76**	0.96**	-0.07$^{\mathbf{ns}}$	0.82**	0.99**

c = Rate constant of gas production during incubation (% h^{-1}); a = gas produced from soluble fraction (ml/0.200 g OM); b = gas produced from insoluble but fermentable fraction (ml/0.2 g OM); a+b = potential gas production (ml/0.200 g OM); *P < 0.05, **P < 0.01***P < 0.001; ns = non significant.

content was ranged from 37.8 (*O. sativa*) to 67.45% (*S. galauca*). The leaf:stem ratio decreased in the fibre content and an increased level of protein in the plants (Aaron et al., 2005). Amount of CP, NDF and ADF of *O. sativa* is 25, 53.8 and 37%, respectively. This result is in agreement with findings by Holchek et al. (1986) who reported 21.2, 52.3 and 47%, respectively. Arzani et al. (2006) reported that CP of legume family is more than gramine family and ADF is lower. However, in contrast to our results, Kaplan (2011) showed lesser CP (16.9%) and same ADF and NDF according to chemical compounds for the *O. sativa.* Jancik et al. (2010) detected that amount of CP (13.1%),

NDF (56.3%) and ADF (31.4%) contents are in agreement with present findings in the *D. glomerata*. Results of Bostan et al. (2010), in *D. glomerata* showed that the amount of ADF (31%) is lesser than that of present findings, CP (18%) is more than that of present findings and NDF (71%) is similar with that of present findings. The highest amount of NDF and ADF are caused to decreasing dry matter digestibility (DMD) and increasing plant fiber (van Soest et al., 1991; Mohanty et al., 2000; Beakou et al., 2008). The result showed that the highest level of gas production occurred after 16 to 24 h incubation. This stage of incubation in ruminants showed that fermentation of forage is maximal. It related to the ration and its constituents, and for more readily digestible carbohydrates is 12 to 16 h and for less digestible carbohydrates is 24 to 96 h (Kinan and Krishnamoorthy, 2007; Vanic et al., 2008).

The most of fermentable fraction (b) and the potential gas production (a+b) in 0 to 20 h of incubation time belonged to *S. galauca* and after 20 h of incubation time, belonged to *O. sativa, D. glomerata* and *S. galauca,* respectively. It may be due to their content of ADF, NDF and protein, whereas the potential gas production (a+b) is associated with degradability of feed (Kamalak et al., 2005). Therefore, the higher values obtained for the potential gas production in the *O. sativa* indicated a better nutrient availability for rumen microorganisms. ADF and EE correlation with fraction (b) and (a+b) were negative but ash and CP correlation with fraction (b) and (a+b) were positive. This result is consistent with findings of Frutos et al. (2002). ADF were negatively correlated with most of the estimated parameters. This result is in agreement with the findings of Abdulrazak et al. (2000) and Kamalak et al. (2004).

The negative correlation between potential gas production and ADF may be due to the reduction of microbial activity from increasingly adverse environmental conditions as incubation time progress. CP was positively correlated with the rate of fermentation

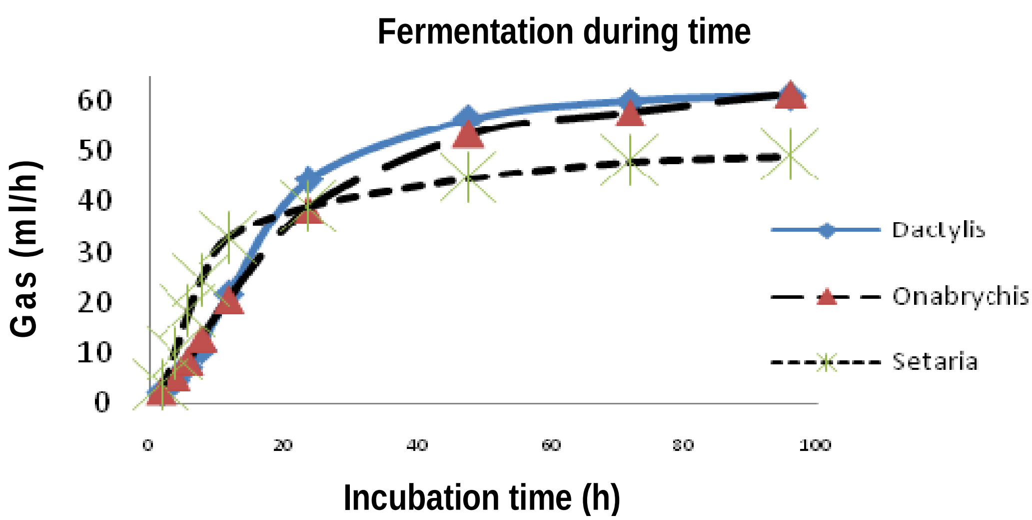

Figure 1. Cumulative gas production of three range plants.

fraction (c), fraction (b) and fraction (a+b). These findings were supported by Kamalak et al. (2004). Correlation relationship of chemical composition with gas production kinetics and some estimated parameters are in agreement with findings of Kaplan (2011). The chemical characteristics correlation showed that CP was negatively correlated with EE, ADF, CF, and NDF. The high amount of a leaf could be increased growing rate, DM and protein content and decreased ADF, CF, and NDF as a result of increasing number of photosynthetic organ per unit of leaf (Hattab and Harb, 1990).

Conclusion

The results revealed that the *O. sativa, D. glomerata* and *S. galauca* could be used such as forage for livestock, respectively.

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