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Assessment of Nutritive Value of Four Dominant Weed Species in Range of Khorasan Distict of Iran by *in vitro* and *in situ* Techniques

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Abstract: *In vitro* gas production and *in situ* techniques were used to evaluate nutritional value of some alternative weed forages e.g., *Sorghum halepense*, *Salsola kali*, *Convolvulus arvensis* and *Portulaca oleracea*. Cumulative gas production was recorded at 2, 4, 8, 12, 24, 48, 72, 96 and 120 h of incubation also, *in situ* disappear of dry matter for these weed forage was measured at 0, 2, 4, 8, 16, 24, 48, 72 and 96 h of incubation and gas production constants (b and c) were described using the equation $y = b (1-e^{-ct})$. The most cumulative gas production was for *Sorghum halepense* in time of 120 h incubation (116.67 mL/300 mg of sample incubated) and *Portulaca oleracea* was the lowest cumulative gas production for 120 h incubation (66.35 mL/300 mg of sample incubated). The NDF, OM, Pr and DM were different among weed forages used in this study. The equation of $y = a+b (1-e^{-ct})$ was applied for degradability of DM. The constant (readily soluble fraction, 53.53%) of *Convolvulus arvensis* was higher but, the constant of *Salsola kali* was lowest (45.82%). The b constant (insoluble fraction but degradable in rumen) for *Sorghum halepense* (44.48%) was significantly higher than other treatments and the c constant (rate of degradation of b per hour) was significantly higher for *Portulaca oleracea* (0.089%). According to results from gas production and *in situ* techniques, it seems that the *Sorghum halepense* has a higher nutritive value than other treatments, but more experiments were required for accurate determination of nutritional values of these forages.

Key words: Gas production, *in vitro*, *in situ*, degradable, weed forage, incubation

INTRODUCTION

Weed forages are important feed resources for ruminants in the pasture of Iran, but there has been limited research on their nutritive value. The appellation; alternative feed resources has become commonly used to design those local feeds, which could replace partially or totally conventional feedstuffs either grass forages or concentrate feeds without reducing livestock performance but should decrease the feeding cost (Sallam, 2005). *Sorghum halepense*, *Salsola kali*, *Convolvulus arvensis* and *Portulaca oleracea* are four common weed forages that grow from March to November in pastures of Iran and offered to animals. Empirically, the nutritive value of these forages was confirmed by ranchers, however little information is known about their nutritive values scientifically thus, making it difficult to assess their potential contribution to sustain animal production. In addition, the determination of intake and digestibility of feedstuffs according *in vivo* is time consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large scale feed evaluation (Coelho *et al*., 1988). Therefore, *in vitro* gas production and *in situ* rumen degradability, both rapid and low-cost methods have been used to assess the degradation and nutritive value of feedstuffs (Sallam, 2005). The present study was carried out to determine the chemical composition, *in vitro* rumen fermentation and *in situ* degradation of four common weed forages in pastures of Iran.

MATERIALS AND METHODS

Forage samples: Several complete samples from *Sorghum halepense*, *Salsola kali*, *Convolvulus arvensis* and *Portulaca oleracea* were harvested in March 2008 from the city of Kashmar, in the east of Iran. The weather of this area is warm and dry. The mean annual rainfall and temperature are 170 mm and 25°C. Complete sample from stem and leaves of these forages were hand harvested from at least 15 different pastures.

Chemical analysis: Dry Matter (DM) was determined by drying the samples at 60°C for 48 h and also the ash content was determined after 5 h oxidation at 500°C. NDF

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and ADF contents were determined according to Van Soest *et al.* (1991). Nitrogen content was measured by the kjeldahl method and CP was calculated as N ×6.25. All chemical analysis was carried out in quadruplicate.

***In vitro* gas production:** Rumen fluid was obtained from three fistulated bull cows Holstein fed twice daily with a diet containing alfalfa hay, corn silage (60%) and concentrate (40%). The samples of 300 mg (1 mm screen) were incubated in the rumen fluid in calibrated glass syringes (100 mL) following the procedure of Menke and Steingass (1988) as follows. Three replication was applied for each treatment. The syringes were pre-warmed at 39°C before injecting 30 mL rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Total gas production for each treatment at 2, 4, 8, 12, 24, 48, 72, 96 and 120 h of incubation was recorded and then the gas production from previous stage was evacuated. Also, total gas production was corrected for blank incubation, which contained only rumen fluid. Cumulative gas production data were fitted to the model of Osuji *et al.* (1993):

$$y = b (1-e^{-ct})$$

Where:

b = The gas production from the readily soluble fraction and the insoluble fraction (mL)
c = The gas production rate constant
t = Incubation time (h)
y = Gas production at time of t

The OMD of forages was calculated using equation of Menke *et al.* (1979) as follows:

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

Where:

GP = Twenty four hours net gas production (mL per 300 mg)
CP = Crude protein (%)
XA = Ash content (%)

ME (MJ kg^{-1} DM) content of forages was calculated using equation of Menke *et al.* (1979) as follows:

$$ME (MJ\ kg^{-1}\ DM) = 2.20 + 0.136GP + 0.057CP + 0.0029CP^2$$

Where:

GP = Twenty four hours net gas production (mL per 300 mg)
CP = Crude Protein (%)

***In situ* degradation:** The nylon bag technique (Ørskov *et al.*, 1980) was used to study the ruminal degradation of DM for four weed forages. DM of each forage was determined on three bull cows fitted with ruminal fistula conducted on forest. Triplicate 5 g of samples were placed in nylon bags (9×17 cm, pore size 50 μm) and suspended in the rumen of animals before their departure to the forest. The bags were removed at 2, 4, 8, 16, 24, 48, 72 and 96 h of incubation. After withdrawal, bags were immediately washed until water was clear and immersed in ice water to stop microbial activity and transported to the laboratory. Bags were dried at 60°C, until constant weight and the remaining DM was determined. The equation was applied for degradability of DM as in Eq. 1:

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

Where:

a = The rapidly soluble fraction
b = The potentially degradable fraction
c = The constant rate of degradation of b (%/h)
y = DM disappearance in rumen at time t

Effective DM Degradability (EDMD) was calculated applying the equation of Ørskov and McDonald (1979):

$$EDMD = a + (bc/(c + k))$$

Where, a-c are the same as in Eq. 1 and k is the rumen outflow rate of 2-5%/h, which is at the maintenance level.

Statistical analysis: Data on *in situ* DM degradation and *in vitro* gas production were subjected to Analysis of Variance (ANOVA) in a completely randomized design using the SAS program General Linear Model procedure (SAS, 9.1). Significant means were compared using the Duncan's multiple range tests. Mean differences were considered significant at $p<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance. A correlation matrix of all coefficients for gas volume or DM degradation was obtained using SAS (9.1).

RESULTS AND DISCUSSION

Chemical composition of weed forages: The chemical composition of four weed forages was shown in Table 1. Although, no statistical analysis of forage composition was carried out, there was a considerable variation between forages in terms of chemical composition. The cell wall content (NDF and ADF), which shows the most

important fraction of dry matter for all forages, ranged from 25.50-55.50 and from 22.34-32.35, respectively. The crude protein and ash content for *Portulaca oleracea* was considerably higher than in the other forages. The organic matter for *Sorghum halepense* was higher than other treatment.

***In vitro* gas production:** The estimated parameters of four weed forages for gas production was shown in Table 2. The results from Table 2 shows that *Sorghum halepense* has a higher b_{gas}, ME and OMD contents than other forages and the c_{gas} for *Convolvulus arvensis* is highest ($p<0.05$). In the other hand, the cumulative gas production for the *Sorghum halepense* was significantly high in comparison to other weed forages. Sallam *et al.* (2007) reported that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. Despite of higher cell wall in *Sorghum halepense* in this study, the cumulative gas production was higher for it. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Wolin *et al*., 1960) and substantial changes in carbohydrate fractions were reflected by total gas produced (Deaville and Givens, 2001). Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while, contribution of fat to gas production is negligible. The predicted ME and OMD profile were widely varied in four weed forages particularly high in *Sorghum halepense*. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1988). Therefore, it seems that *Sorghum halepense* has a higher potential for gas production.

***In situ* degradation:** Degradation characteristics and disappearance of DM in four weed forages was shown in Table 3. The degradation of DM in the test weed forages differed significantly ($p<0.05$). The immediately soluble fraction (a) ranged from 45.82% in *Salsola kali* to 53.57% in *Convolvulus arvensis*. The insoluble but rumen degradable fraction (b) was least in *Convolvulus arvensis*. (28.54%). This is a reflection of the fact that its DM component was most readily soluble. And a slowest rate of degradation (c) per hour of the rumen degradable fraction in *Sorghum halepense* was considerable. *Sorghum halepense* was observed to contain the highest amount of potentially degradable DM with 91.26%. Effective Degradability (ED) of DM calculated at 2-5% outflow rates from the rumen showed *Sorghum halepense* had significantly highest values in 2% out flow rate, while the least value was recorded in *Salsola kali*. Also, in 3-5% out flow rate, the ED for *Portulaca oleracea* was highest. Effective DM degradability decreased with increase in outflow rates in this study. Mupangwa *et al.* (1997) observed ED of DM to decrease as the outflow rate increased. The disappearance of the DM contents in the leaves by the end of 48 h of incubation, generally considered to be equivalent to digestibility (Ehargava and Ørskov, 1987) and being the mean retention time of fibrous feeds in ruminants (Kimambo and Muya, 1991). For 24 h incubation, all the weed forages had DM disappearance values above 70%. The relatively high soluble DM values in these tree leaves, especially in *Convolvulus arvensis* and *Portulaca oleracea* reveals the potential of their being good sources of more nutrients for microbial growth (Djouvinov and Todorov, 1994) as Clark *et al.* (1992) and Gomes *et al.* (1994) reported a strong positive relationship between DM intake and microbial growth. The high

Table 1: Chemical composition of four common weed forages in east of Iran

	Weed forages			
Composition (%)	Sorghum halepense	Salsola kali	Convolvulus arvensis	Portulaca oleracea
DM	22.95±0.05	25.63±.0.10	22.64±0.04	11.94±0.07
CP	13.28±0.40	15.77±0.50	17.41±0.30	25.50±0.40
NDF	55.50±0.51	36.50±0.40	27.50±0.40	25.50±0.20
ADF	32.35±0.37	27.25±0.47	27.33±0.42	22.34±0.32
OM	87.00±0.10	72.50±0.20	82.50±0.10	72.00±0.10
ASH	13.00±0.10	27.50±0.20	17.50±0.10	28.00±0.10

Table 2: The estimated parameters of some weed forages when incubated with rumen fluid at different incubation times

	Weed forages				
Estimated paramerers	Sorghum halepense	Salsola kali	Convolvulus arvensis	Portulaca Oleracea	SEM
B_{gas} (mL/300 mg of sample)	116.670^{a}	77.9000^{c}	91.530^{b}	66.350^{d}	2.90
C_{gas} (mL/h/300 mg of sample)	0.059^{c}	0.0740^{bc}	0.114^{a}	0.109^{ab}	0.01
OMD (%)	116.010^{a}	106.5900^{a}	109.620^{a}	108.060^{a}	4.13
ME (MJ kg^{-1} DM)	16.190^{a}	12.5500^{b}	14.680^{ab}	13.750^{b}	0.67

^{a,b,c,d}Means along same rows bearing different superscripts are significantly different ($p<0.05$). B_{gas} = The gas production from the readily soluble fraction and the insoluble fraction (mL). C_{gas} = The gas production rate constant; OMD = Organic Matter Digestability; ME = Metabolisable Energy

Table 3: Degradation characteristics and disappearance of DM in four weed forages

	Weed forages				
Characteristics	Sorghum halepense	Salsola kali	Convolvulus arvensis	Portulaca oleracea	SEM
Degradation parameters					
a1 (%)	46.780^{b}	45.820^{b}	53.57^{a}	53.53^{a}	0.550
b2 (%)	44.480^{a}	39.110^{b}	28.54^{d}	30.68^{c}	0.370
c3/h	0.052^{c}	0.062^{bc}	0.075^{ab}	0.089^{a}	0.004
a+b4 %	91.260^{a}	84.940^{b}	82.12^{c}	84.21^{b}	0.320
Effective degradability (%)					
k = 0.02	79.01^{a}	75.49^{c}	76.18^{b}	78.61^{a}	0.160
k = 0.03	75.11^{b}	72.29^{d}	74.05^{c}	76.52^{a}	0.180
k = 0.04	72.05^{b}	69.72^{c}	72.29^{b}	74.75^{a}	0.190
k = 0.05	69.52^{c}	67.61^{d}	70.81^{b}	73.23^{a}	0.210
Disappearance (%)					
24 h	81.63^{a}	76.53^{b}	77.23^{b}	79.10^{b}	0.760
48 h	87.33^{a}	81.53^{b}	80.23^{b}	80.96^{b}	0.610
72 h	89.26^{a}	83.66^{b}	81.56^{c}	84.06^{b}	0.610
96 h	91.83^{a}	87.26^{b}	83.73^{c}	87.63^{b}	0.390

^{a,b,c,d}Means along same rows bearing different superscripts are significantly different ($p<0.05$). ^{1,2,3,4}Constants in the equation P = a+b(1-e-ct) where, P = level of degradation at time t; a = Readily soluble fraction; b = Insoluble fraction but degradable in rumen; c = Rate of degradation of b per hour; a+b = Potentially degradable fraction, ED (k = 0.02; 0.03; 0.04; 0.05) = Effective degradability calculated with outflow rates of 2-5%

potentially degradable DM fraction in the weed forages studied is of interest for the fact that this parameter measures the proportion that is fermentable if this component does not bypass the rumen.

CONCLUSION

The *in vitro* gas production and *in situ* techniques can be used to determine the nutritive value of the forages and to identify differences among their potential digestibility and energy contents. Chemical composition and *in vitro* digestibility can be considered useful indicators for the preliminary evaluation of the likely nutritive value of previously uninvestigated forages. There are considerable differences in the fermentability of carbohydrates between different forages. *Sorghum halepense*, *Salsola kali*, *Convolvulus arvensis* and *Portulaca oleracea* revealed that these weed forages could be interesting alternative animal feed sources and valuable in the ruminant feeding but it seems that *Sorghum halepense* have a higher nutritive value than other weed forages. Nevertheless, more experiments were required for better determination of these weed forages.

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