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Full Length Research Paper

Potential nutritive value of some forage species used as ruminants feed in Iran

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A study was conducted to determine rumen degradability (*in sacco*) of dry matter and *in vitro* gas production of the most important forage species grown in Iran, to compare forage species according to calculated degradation and *in vitro* gas production parameters, and to establish prediction equations for relative feed value (RFV) from gas production parameters. Thus, six forage species consisting of *Lucerne*, *Eruca sativa*, *Crocus sativus*, *Cardaria draba*, *Setaria Spp*., and *Triticum aestivum* forages were evaluated. Crude protein (CP) contents in the forages ranged from 139.60 to 246.30 g kg^{-1} DM. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and organic matter (OM) were 273.30 to 596.60, 210.00 to 310.00, and 820.00 to 946.70 g kg^{-1} DM, respectively. The highest DM degradation and *in vitro* gas producton parameters were found for *E. sativa*. Also the relative feed value (RFV), relative forage quality (RFQ), dry mater intake and effective dry matter digestibility calculated for *E. sativa* was significantly ($P < 0.05$) higher than other forages. The variation of RFV explained by the gas production parameters ranged (R^2) from 0.023 to 0.846. The gas production at 6, 24 and 48 h incubation times explained 0.836, 0.800 and 0.805 of variation of RFV, respectively. There was a negative correlation between *in vitro* gas production in different time incubation with NDF, ADF and a positive correlation between gas production parameters and CP content of forage species. The study shows that these forages relatively had a good nutritive value in comparison of *Lucerne*, and therefore, may serve as potential supplements for ruminants in Iran, and it seems that RFV index of six forage species such as used in this present study may be predicted from *in vitro* gas production parameters.

Key words: Forage species, *in vitro* gas production, *in situ* degradability, relative feed value.

INTRODUCTION

Forage species are an important feed source for ruminants in the pasture of Iran, but there has not been research on their nutritive value (kazemi et al., 2009). Some forage species such as *Lucerne*, *Eruca sativa*, *Crocus sativus*, *Cardaria draba*, *Setaria Spp*., and *Triticum aestivum* are widely distributed in Iran (especially Razavi khorasan distinct), and constitute a significant part of the diet consumed by grazing ruminants. Determining the digestibility of feeds *in vivo* is laborious, expensive, requiring large quantities of feed, and is largely unsuitable for a single feedstuff, thereby making it unsuitable for routine feed evaluation. There are numbers of *in vitro* techniques available to evaluate the nutritive value of feeds at relatively low cost. Recently, the *in vitro* gas production and chemical composition of the uninvestigated forage have been widely used to evaluate the potential nutritive value of ruminant feedstuffs (Danesh Mesgaran et al., 2010; Chaji et al., 2010).

Also *in sacco* (*in situ*) analyses are the most frequently used methods for the determination of degradability

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Abbreviations: RFV, Relative feed value; **CP**, crude protein**; NDF**, neutral detergent fiber; ADF, acid detergent fiber; **OM**, organic matter; **RFQ**, relative forage quality.

parameters of DM, organic matter (OM), protein, fibre, minerals and other nutrients of feeds (Homolka et al., 2008; Jancik et al., 2008). Also recently, a series of experiments was informed about equations prediction from relationship between gas production and *in situ* degradability parameters or RFV index (Canbolat et al., 2006; Kamalak et al., 2005). These prediction equations are appropriate for institutions that lack experimental animals and equipment for *in vitro* digestibility analysis. The *in situ* nylon bag and *in vitro* gas production technique are well correlated with animal performance (Ørskov, 1989), food intake (Blummel and Ørskov, 1993), microbial protein synthesis (Krishnamoorthy et al., 1991) and *in vivo* digestibility (Khazaal et al., 1993).

The objectives of this study were to compare nutritive value of the most widely used forage species in Iran according to estimated parameters from *in vitro* gas production and *in situ* degradability, and to evaluate the regression equations for prediction of relative feed value (RFV) of forage species based on the gas production parameters. Following these results, determination coefficient (R square) between *in vitro* gas production and chemical composition of forage species was estimated.

MATERIALS AND METHODS

Forage samples and chemical analysis

Whole forage (that is, stem and leaf after harvest) from *Lucerne*, *E. sativa*, *C. sativus*, *C. draba*, *Setaria Spp*., and *T. aestivum* were harvested (early growth) in March 2010, from agriculture farm of Ferdowsi University of Mashhad, that is cited about 999.2 m above sea level, with rainfall mean of 262 mm per year, and semi-arid climate in Northeast of Iran. Forage sample were dried at 60°C in oven dryer for 48 h, and then milled with 2 mm mesh screen. The ash content was determined after 8 h oxidation at 525°C. Crude protein (CP) (Kjeltec 2300 Auotoanalyzer, Foss Tecator AB, Hoganas, Sweden) were analyzed by a standard Kjeldahl method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined, according to Van Soest et al. (1991). Fat content was determined by ether extraction (AOAC, 1995).

Ruminal degradability

The *in situ* dry matter (DM) degradation was carried out according to the procedure described by Mehrez and Ørskov (1977). Four (4) Balochi male sheep (40 ± 5.7 kg LW), fitted with ruminal fistula, from the flock of the research farm of the Agricultural College at Ferdowsi University, were used in this study. Sheep were fed with 1.5 Kg DM alfalfa and 0.4 kg DM concentrate (CP 165 g kg^{-1}) per head per day at 8.00 a.m. and 6.00 p.m. Forage samples (2 g DM with 2 mm screen) were weighed into nylon bags (6.5 × 14 cm) with a 52 µm pore size. Samples were incubated in the rumen of each sheep after feeding at 08:00 h, for 0 (bags were washed with cold tap water without incubation), 2, 4, 8, 16, 24, 48, 72 and 96 h. After each incubation time, bags were removed from the rumen and rinsed with cold tap water, until the water remained clear. The bags were dried at 60°C for 48 h in an oven then weighed, to determine DM disappearance. Ruminal disappearance at each incubation time was calculated as the difference between the residues and original samples. In each incubation time, six replications were used for each sample of forage.

In vitro gas production

Procedure of *in vitro* gas production was performed according to the method Menke and Steingass (1988). Rumen fluid was obtained from 4 fistulated Balochi male sheep (40 ± 5.7 kg LW) before morning feed, normally fed with 1.5 Kg DM alfalfa and 0.4 kg DM concentrate (165 g CP/kg DM) per head per day at 8.00 a.m. and 6.00 p.m., rumen fluid was strained through three layers of gauze. The laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Two hundred millgram (200 mg) of each sample (milled with 1 mm sieve) was introduced to each syringe (100 ml), and then it was filled with 30 ml of medium, consisting of 10 ml rumen fluid and 20 ml buffer solution, as described by Menke and Steingass (1988) and immediately transferred to a water bath at 39°C. Six replications were provided for each sample in each incubation time. Also, six syringes, with only buffered rumen fluid were incubated and considered as blank. The gas production was recorded after 2, 4, 6, 12, 16, 24, 48, 72 and 96 h of incubation. Total gas values were corrected for blank incubation.

Calculation and statistical analysis

The DM degradation data were fitted to the exponential equation $p = a + b (1 - e^{-ct})$ (Ørskov and McDonald, 1979); where p is the DM disappearance in rumen at time t; a is the rapidly soluble fraction; b is the insoluble but fermentable fraction and c is the constant rate of degradation of b (% h^{-1}). Effective DM degradability (EDMD) was calculated, applying the equation of Ørskov and McDonald (1979); EDMD = a + [bc / (c + k)], where k is the rumen outflow rate (at level of 2, 3 and 4% per h).

Cumulative gas production data were fitted to the exponential equation $y = b (1 - e^{-ct})$ (Osuji et al., 1993), where b is the gas production from the readily soluble fraction and the insoluble fraction (ml); c is the gas production rate constant (ml/h); t is the incubation time (h) and y is the gas production at time of t (ml).

The metabolizable energy (ME), and organic matter digestibility (OMD) were calculated using equations of Menke et al. (1979) as, ME (MJ/kg DM) = 2.20 + 0.136 × Gp + 0.057 × CP; OMD (g kg^{-1} DM) = (14.88 + 0.889 × Gp + 0.45 × CP + 0.0651 × XA) × 10, where, CP is the crude protein (% of DM); XA is the ash (% of DM) and Gp is the net gas production (ml) from 200 mg (DM of sample), after 24 h of incubation.

Short chain fatty acids (SCFA) was calculated, using the equation of Makkar (2005) as, SCFA (mmol) = 0.0222 × GP – 0.00425, where, GP is 24 h net gas production (ml/200 mg DM).

Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD, according to the method Czerkawski (1986).

RFV was calculated from the estimates of DDM and DMI (Rohweder et al., 1978) as, DDM (%) = 88.9 - (0.779 × % ADF); DMI (% of BW) = 120 / % NDF and RFV = (% DDM × % DMI) / 1.29. Where, DDM is the dry matter digestibility; ADF is the acid detergent fiber (% of DM); DMI is the dry matter intake (% of BW) and RFV is the relative feed value.

RFQ was estimated according the equation of Undersander (2003) as, RFQ = 1.1446 (RFV) – 32.224, where RFQ is the relative forage quality and RFV is the relative feed value.

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 Statistical Analysis System (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

Table 1. Mean chemical composition (g kg^{-1} DM) of six forage species.

Composition (g kg^{-1} DM)	Forage species							
	Lucerne	***Eruca sativa***	***Crocus sativus***	***Cardaria draba***	***Setaria Spp.***	***Triticum aestivum***	**SEM**	**p-value**
DM	235.00^{b}	151.40^{e}	266.40^{a}	221.10^{cd}	219.00^{d}	224.40^{c}	1.25	p <0.05
CP	185.00^{c}	246.30^{a}	139.60^{e}	229.00^{b}	181.50^{d}	226.80^{b}	0.82	p<0.05
NDF	353.10^{c}	273.30^{f}	336.60^{d}	286.60^{e}	596.60^{a}	573.30^{b}	1.37	p<0.05
ADF	295.00^{c}	210.00^{e}	303.30^{b}	260.00^{d}	310.00^{a}	305.00^{b}	1.48	p<0.05
OM	900.00^{b}	820.00^{f}	946.70^{a}	823.40^{f}	890^{c}	870.00^{d}	0.74	p<0.05
EE	19.50^{d}	25.60^{a}	21.00^{c}	23.40^{b}	14.90^{e}	15.30^{e}	0.18	p<0.05

$^{a, b, c, d, e, f}$ means in the same row with different superscript differ significantly (P<0.05). DM = Dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; OM = organic matter; EE = ether extract.

the analysis of variance. A simple correlation analysis (SAS, 9.1) was used to establish the relationship between chemical composition and *in vitro* gas production parameters. Regression analysis (simple and multiple regressions; SAS, 9.1) was used to establish the relationship between RFV and *in vitro* gas production.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of forages is presented in Table 1. There were observed variations in the chemical composition of the different forage species, with CP ranging from 139.6 to 246.3 g kg^{-1} DM, OM from 820 to 946.7 g kg^{-1} DM, NDF from 273.3 to 596.6 g kg^{-1} DM, ADF from 210 to 310 g kg^{-1} DM and EE from 14.90 to 25.60 g kg^{-1} DM. *E. sativa* recorded the highest CP 246.3g kg^{-1} DM and EE 25.60 g kg^{-1}DM ($P < 0.05$). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) value were highest ($P < 0.05$) for *Setaria Spp*., and the *C. sativus* ($P < 0.05$) had a highest dry matter than other forage species ($P < 0.05$). The obtained ADF and NDF values of *Lucerne* in this study were more than Kleinschmit et al. (2007) (447 and 326 g kg^{-1} DM) and Broderick et al. (2002) (435 and 347 g kg^{-1} DM); but the CP, NDF, ADF, OM and EE of *Lucerne* were consistent with the study of Biagi et al. (2005). The difference between chemical compositions may result from the variance in variety, climate condition, soil, cut and maturity.

The results of the current study indicate that these forage species are almost high in CP, and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in Iran. All treatments have higher (often), or relatively less values for chemical composition, in comparison of *Lucerne*. Some estimated parameters (such as DMI, DDM, RFV and RFQ) are shown in Table 2. Also, some standard value for forages was presented in Table 3. DMI, DDM, RFV and RFQ parameters for *E. sativa* were significantly higher than other forage species ($P < 0.05$). The decrease in DMD, DMI, RFV and gas production parameters are possibly associated with increased NDF and ADF contents (Wilson et al., 1991). RFV is intended to reflect how well an animal will eat and digest particular forage, if it is fed as the only source of energy.

In this study, RFV of *E. sativa*, *C. draba*, *C. sativus* and *Lucerne* was higher than 151 value quality standard (Table 3), so these forages can be cited in prime quality standard (high quality). Also, *Setaria Spp*. and *T. aestivem* can be cited in 2 and 3 quality standard (Table 3), respectively that represented a medium quality. RFQ is an estimate of how much available energy a non-lactating animal will obtain daily from a particular forage, if it is all that it is fed with.

The values of RFV and RFQ were significantly different (Table 2) between treatments. The RFQ emphasizes fiber digestibility, while RFV uses digestible dry matter intake. The difference is that the digestibility of the NDF is included in the equation. Therefore, the digestibility may be the reason cows produce differently on hays of similar RFV. Undersander and Moor (2004) reported that RFQ will become the standard test for evaluating forages throughout the country, and that it eventually will be used even more widely than RFV is today. Chemical composition, RFQ and RFV indexes should be taken into consideration when purchasing forages. Also Prediction of relative feed value from *in vitro* gas production is shown in Table 4. The RFV were well correlated with gas production p arameters, especially after

Table 2. Nutritive value of six forage species as animal forage source in Iran.

Estimated paramerer	Forage species							
	Lucerne	*Eruca sativa*	*Crocus sativus*	*Cardaria draba*	*Setaria Spp.*	*Triticum aestivum*	SEM	p-value
DMI[1]	3.40[d]	4.39[a]	3.56[c]	4.19[b]	2.01[f]	2.09[e]	0.01	p<0.05
DDM[2]	65.92[c]	72.54[a]	65.27[d]	68.65[b]	64.75[e]	65.14[d]	0.11	p<0.05
RFV[3]	173.63[d]	246.88[a]	180.35[c]	222.77[b]	100.94[f]	105.66[e]	0.8	p<0.05
RFQ[4]	166. 51[d]	250.36[a]	174.21[c]	222.76[b]	83.31[f]	88. 72[e]	0.91	p<0.05

[a, b, c, d, e, f] means in the same row with different superscript differ significantly (P < 0.05). [1]DMI = Dry matter intake; [2]DDM = Digestibility of dry matter; [3]RFV = Relative feed value; [4]RFQ = Relative forage. quality

Table 3. Legume, grass and legume-grass mixture quality standards.

Quality standard[1]	CP (% DM)	ADF (% DM)	NDF (% DM)	RFV[2]
Prime	>19	<31	<40	>151
1	17 - 19	31 - 40	40 - 46	151 - 125
2	14 - 16	36 - 40	47 - 53	124 -103
3	11 - 13	41 - 42	54 - 60	102 - 87
4	8 - 10	43 - 45	61 - 65	86 - 75
5	<8	>45	>65	<75

[1]Standard assigned by Hay Market Task Force of American Forage and Grassland Council. [2]Relative feed value (RFV): Reference hay of 100 RFV contains 41% ADF and 53% NDF. CP, Crude protein; ADF, acid detergent fiber; NDF, neutral detergent iber; RFV, relative feed value.

6, 24 and 48 h incubation. The estimated equation basis of b and c constants was a high determination coefficient (R^2), than other equation.

Gas production from the potential fraction (b) alone explained 2.3% of the variation of RFV, and this value for rate of gas production (c) was higher (76.9%). The inclusion of potentially gas production and the rate of gas production (c), in the regression equation, improved the accuracy of predicting RFV. This result is in agreement with the findings of Canbolat et al. (2006). Russell and Karsli (2003) observed that the water soluble fraction (a), determined by the *in sacco* method, was comparable with NDF as predictors of whole tract digestibility in cattle (R^2 of 0.83 and 0.85, respectively), and better than NDF for the prediction of voluntary feed intake (R^2 of 0.80 and 0.69, respectively).

In situ degradation

Dry matter (DM) disappearance of the forage species at different rumen incubation times is presented in Table 5. The disappearance of DM increased, with increasing time of incubation. The DM disappearance after 96 h incubation ranged between 870.00 and 918.33 g kg^{-1} DM. After 24 h incubation, the DM disappearance of *Setaria Spp*., was significantly lower (P < 0.05) than other forage. After 48 and 72 h incubation, the DM disappearance of *E. sativa* was significantly higher (P < 0.05) than other forage. Also after 96 h incubation, the DM disappearance of *C. sativus* and *Lucerne* was significantly less (P < 0.05) than other forages. The constant rate of DM degradability (fraction c) of *E. sativa* was significantly higher than other forages (P < 0.05). The insoluble but rumen degradable fraction (b)

Table 4. Prediction of relative feed value from in vitro gas production and estimated parameters within parentheses are standard error values.

Equations and parameters used	R^2	RMSE
$RFV = 74.671_{(14.499)} + 32.878_{(4.500)}\ gas_{\ 2\ h}$	0.61	34.889
$RFV = 24.427_{(15.565)} + 19.933_{(2.007)}\ gas_{\ 4\ h}$	0.744	28.321
$RFV = -12.33_{(14.484)} + 13.746_{(1.044)}\ gas_{\ 6\ h}$	0.836	22.655
$RFV = -211.281_{(36.432)} + 12.646_{(1.194)}\ gas_{\ 12\ h}$	0.767	26.97
$RFV = -425.567_{(66.548)} + 16.792_{(1.865)}\ gas_{\ 16\ h}$	0.704	30.408
$RFV = -875.540_{(89.726)} + 25.109_{(2.149)}\ gas_{\ 24\ h}$	0.8	24.975
$RFV = -1440.8_{(136.339)} + 33.516_{(2.832)}\ gas_{\ 48\ h}$	0.805	24.723
$RFV = -1724.060_{(483.023)} + 37.432_{(9.536)}\ gas_{\ 72\ h}$	0.312	46.397
$RFV = -1183.945_{(472.825)} + 26.567_{(9.264)}\ gas_{\ 96\ h}$	0.195	50.189
$RFV = -125.340_{(28.279)} + 4559.395_{(428.569)}\ c_{gas}$	0.769	26.882
$RFV = -336.11_{(560.621)} + 9.846_{(10.868)}\ b_{gas}$	0.023	55.267
$RFV = -1065.780_{(232.522)} + 17.979_{(4.423)}\ b_{gas} + 4761.245_{(358.549)}\ c_{gas}$	0.846	22.273

RFV: Relative feed value, c = The gas production rate constant for the insoluble fraction (b); a = The gas production from the immediately soluble fraction (ml); b = The gas production from the insoluble fraction (ml).

was least in *C. draba,* than other forages (P < 0.05). The immediately soluble fraction (a) of *C. draba* was highest (P < 0.05). The EDMD (out flow = 2, 3 and 4%) of *E. sativa* were higher than other forage (P < 0.05), and the maximum potential degradability (a + b) of *T. aestivum* was significantly highest (P < 0.05). There are no obvious data for dry matter degradability of *C. sativus*, *C. draba*, *T. aestivum* and *Setaria Spp*., but complete data exist for *Lucerne* forage about dry matter degradability (Nelson and Satter, 1992; Pawelek et al., 2007).

The obtained results for insoluble fraction was in consistent with the studies of Coblentz et al. (1998) (459 g kg^{-1} DM) and Pawelek et al. (2007) (430 g kg^{-1} DM). The higher value of immediately soluble fraction (a) for dry matter degradability in this experiment maybe was as a result of nylon bags pore size (52 µm) and early harvesting of forages. It seems in a similar growth phase, some forage species (such as *E. sativa*, *C. draba* and *T. aestivum*), in comparing with *Lucerne,* had an advantage for *in situ* rumen degradability.

This study reveals a general problem of overestimation of degradability by the nylon bag technique. Overestimation is especially noticeable at short incubation times. The ability to use *in vitro* gas production methods to study the kinetics of degradation of forage species, instead of the *in situ* technique, would have advantages, including avoiding the error associated with loss of small particles through the pores of the nylon bag.

In vitro gas production

Data on gas production, during the fermentation period, are given in Table 6. The cumulative volume of gas production increased with increasing time of incubation. Gas produced after 96 h incubation ranged between 50.17 and 51.92 mL per 0.2 g of substrate. At 24 and 48 h incubation times, the cumulative gas production of *E. sativa* was higher (P < 0.05) than other forage species. In 72 h incubation time, *E. sativa* and *C. draba,* respectively produced a higher gas than other (P < 0.05), but the cumulative gas production in time 96 h was highest for *E. sativa*, *C. draba* and *T. aestivum,* respectively than other forage (P < 0.05). Rate of gas production (constant c) of *E. sativa* was higher (P < 0.05) than other forages; whereas, the potential gas production (constant b) for *Lucerne* and *C. sativus* was lower (P < 0.05) than other forages. The *E. sativa* showed higher (P < 0.05) NE, SCFA, ME, OMD and MP, in comparison with alfalfa and other forages. The observed increase in OMD, ME, NE, SCFA, b constant, c constant and total cumulative gas production, in 96 h incubation of *E. sativa* (Table 6), were probably as a result of its high CP and low NDF, ADF and compounds concentrations.

Correlation coefficient (r) of the relationship between the chemical composition and *in vitro* gas production parameters are shown in Table 7. Some differences in the cumulative gas production between investigated forage species could be due to the extent of lignification of NDF (Van Soest, 1994), and gas production was negatively correlated with both NDF and ADF, but positively correlated with CP content. This is consistent with the results of Haddi et al. (2003) who reported that there were significant negative correlation between NDF and ADF, and the rate and extent of GP. Also, finding of this experiment is consistent with the findings of Khazaal et al. (1994), Tolera et al. (1997) and Abdulrazak et al. (2000). The negative effect of cell wall content on GP could be due to the reduction of the microbial activity, through increasing the adverse environmental conditions as incubation time progresses. Some variations between the studies (about estimated parameters) are probably

Table 5. In situ dry matter disappearance and estimated parameters of six forage species when incubated within rumen.

Degradation parameter	Forage species							
	Lucerne	***Eruca sativa***	***Crocus sativus***	***Cardaria draba***	***Setaria Spp.***	***Triticum aestivum***	**SEM**	**p-value**
a[1] (g kg^{-1} DM)	430.28^{d}	491.82^{b}	428.35^{d}	509.13^{a}	359.53^{e}	460.50^{c}	2.64	p<0.05
b[2] (g kg^{-1} DM)	431.32^{c}	418.88^{d}	425.68^{cd}	348.81^{e}	501.54^{a}	462.70^{b}	2.31	p<0.05
c[3] (% h^{-1})	0.092^{c}	0.14^{a}	0.07^{d}	0.10^{b}	0.06^{e}	0.06^{e}	0.002	p<0.05
a + b[4] (g kg^{-1} DM)	861.63^{c}	910.70^{b}	854.03^{d}	857.95^{cd}	861.07^{c}	923.20^{a}	1.4	p<0.05
Effective degradability (g kg^{-1} DM)								
K = 0.02	784.67^{d}	859.86^{a}	764.68^{e}	800.17^{c}	737.48^{f}	807.91^{b}	1.41	p<0.05
K = 0.03	755.67^{d}	838.80^{a}	732.77^{e}	777.91^{b}	691.43^{f}	769.44^{c}	1.54	p<0.05
K = 0.04	731.03^{c}	820.03^{a}	706.37^{d}	757.82^{b}	658.92^{e}	738.64^{c}	1.77	p<0.05
Disappearance (g kg^{-1} DM)								
24 h	838.07^{b}	887.50^{a}	807.50^{c}	809.15^{c}	758.33^{d}	882.50^{a}	4.2	p<0.05
48 h	846.52^{c}	915.53^{a}	836.60^{d}	832.25^{e}	831.10^{e}	899.97^{b}	1.49	p<0.05
72 h	848.50^{c}	916.93^{a}	843.33^{c}	833.33^{d}	843.30^{c}	907.50^{b}	2.08	p<0.05
96 h	855.13^{c}	918.33^{a}	853.33^{c}	916.67^{a}	870.00^{b}	915.83^{a}	2.18	p<0.05

$^{a, b, c, d, e, f}$ means in the same row with different superscript differ significantly (P < 0.05). [1]a = The rapidly soluble fraction; [2]b = The insoluble but fermentable fraction; [3]c = The constant rate of degradation of b; [4]a+b = Potential degradability of dry matter.

Table 6. *In vitro* gas production (mL) and estimated parameters of six forage species when incubated with buffered rumen liquid.

Estimated paramerer	Forage species							
	Lucerne	***Eruca sativa***	***Crocus sativus***	***Cardaria draba***	***Setaria Spp.***	***Triticum aestivum***	**SEM**	**p-value**
b[1]$_{gas}$ (ml/200 mg DM)	50.64^{c}	52.31^{a}	50.61^{c}	52.20^{ab}	51.59^{b}	52.10^{ab}	0.21	p<0.05
c[2]$_{gas}$ (ml/h/200 mg DM)	0.066^{c}	0.080^{a}	0.074^{b}	0.066^{c}	0.052^{d}	0.053^{d}	0.0009	p<0.05
Gas24 (ml/200 mg DM)	40.87^{c}	45.17^{a}	42.04^{b}	42.54^{b}	39.33^{d}	40.29^{c}	0.22	p<0.05
Gas48 (ml/200 mg DM)	47.25^{d}	50.33^{a}	48.25^{c}	49.42^{b}	46.83^{de}	46.58^{e}	0.21	p<0.05
Gas72 (ml/200 mg DM)	50.12^{c}	51.75^{a}	50.00^{c}	51.25^{ab}	50.00^{c}	50.75^{b}	0.2	p<0.05
Gas96 (ml/200 mg DM)	50.58^{bc}	51.92^{a}	50.17^{c}	51.83^{a}	50.50^{bc}	51.17^{ab}	0.27	p<0.05
OMD[3] (g kg^{-1} DM)	606.41^{d}	672.88^{a}	588.84^{e}	641.54^{b}	587.31^{e}	617.52^{c}	0.26	p<0.05
ME[4] (MJ kg^{-1} DM)	8.82^{d}	9.75^{a}	8.75^{d}	9.29^{b}	8.58^{e}	8.97^{c}	0.03	p<0.05
NE[5] (MJ kg^{-1} DM)	2.96^{d}	3.29^{a}	2.80^{f}	3.16^{b}	2.85^{e}	3.036^{c}	0.01	p<0.05
SCFA[6] (mmol)	0.90^{c}	1^{a}	0.93^{b}	0.94^{b}	0.87^{d}	0.89^{c}	0.005	p<0.05
Microbial protein yield (g kg^{-1} OMD)	73.15^{d}	81.17^{a}	71.03^{e}	77.39^{b}	70.84^{e}	74.49^{c}	0.31	p<0.05

$^{a, b, c, d, e}$ means in the same row with different superscript differ significantly (P < 0.05). [1]bgas = the gas production from the readily soluble fraction and the insoluble fraction (ml); [2]cgas = the gas production rate constant; [3]OMD = Organic matter digestability; [4]ME = Metabolizable energy; [5]NE = Net energy; SCFA = Short chain fatty acid.

Table 7. Correlation coefficients (r) of the relationship between the chemical composition and gas production or estimated parameters from different forage species.

In vitro	Chemical composition		
	CP[1]	ADF[2]	NDF[3]
2	0.464**	-0.693***	-0.747***
4	-0.030^{NS}	-0.597***	-0.918***
6	0.078^{NS}	-0.694***	-0.940***
12	0.110^{NS}	-0.729***	-0.851***
16	0.117^{NS}	-0.720***	-0.822***
24	0.428**	-0.887***	-0.802***
48	0.400**	-0.797***	-0.797***
72	0.725***	-0.745***	-0.395**
96	0.677***	-0.617***	-0.296^{NS}
b^4_{gas}	0.733***	-0.481**	0.051^{NS}
c^5_{gas}	0.045^{NS}	-0.695***	-0.884***

[1]CP = Crude protein (g kg^{-1} DM); [2]ADF = Acid detergent fiber (g kg^{-1} DM); [3]NDF = Neutral detergent fiber (g kg^{-1} DM); [4]bgas = The gas production from the readily soluble fraction and the insoluble fraction (ml); [5]cgas = The gas production rate constant (ml h^{-1}); ***P < 0.001; **P < 0.01; *P < 0.05; NS = Non significant.

not due to methodological differences, but could be from actual differences in the chemical composition of forage species. Makkar (2005) reported the close association between SCFA and the *in vitro* GP, and used the relationship between SCFA and GP to estimate the SCFA production from gas values, which is an indicator of energy availability to the animals. The lower SCFA predicted from GP for *Setaria Spp*., could be due to a lower absolute GP, which was most evident during the first 24 h of incubation (Table 6).

Conclusion

Forage quality can be defined in many ways. Forage quality is associated with nutrients, energy, protein, digestibility, fiber, mineral, vitamins and, occasionally with animal production; but it seems that the chemical composition analysis, *in vitro* gas production and *in situ* degradability can be considered as useful indicators, and low cost for the preliminary evaluation of the likely nutritive value of uninvestigated forage species. Although, there were significant variations in chemical composition, gas production, and *in situ* degradability characteristics of six forage species in this study but all forage species had a relatively high potential nutritive value.

This study shows that *E. sativa* forage presented the highest DM rumen degradation and gas production parameters of all evaluated forage species (in addition high nutritive value). Also, nutritive value can be expressed in standard units that can be applied also to the nutrient requirements of the animal. RFV and RFQ are indexes used to measure the quality of forage, and are determined by its content of ADF and NDF. Furthermore, the study confirmed that RFV in forage species can be predicted from chemical composition with satisfactory accuracy. It could be recommended that all prediction equations with $R^2 > 0.500$ can be applied in practice.

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