Evaluation of nutritional properties of alfalfa and sainfoin forages by gas production techniques

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Abstract Two forage species alfalfa (*Medicago sativa*), and sainfoin *(Onobrychis viciifolia* Scop.), at two consecutive growing season (spring and summer), were studied for their chemical composition and *in vitro* gas production characteristics. Data on cumulative gas production (mL gas/g DM) were fitted to the non-sigmoidal model, and for evaluation of model, the concordance correlation coefficient (CCC) was used to assess the agreement between predicted and observed data. Chemical analysis showed that nutrient composition was affected by the forage type. Concentration of fiber components (NDF, ADF, cellulose and crude fiber) were higher in alfalfa compared to sainfoin ($P < 0.05$). Concentration of fiber components was higher in the first cut compared to the second cut ($P < 0.05$) in both forages, but cellulose content was not affected by the growing season ($P > 0.05$). The results showed that there is a great potential for improving the analytical capacity of the technique, by reducing the length of incubation from 48 to 24 h for studies on high quality forages. This study showed that 24 h incubation provided informative results with high reproducibility of the measurements, clear relationship and high correlations between different parameters and the relative feed value (RFV), and also reliable models for prediction of metabolizable energy with high values for the coefficients of determination. The results indicated that the logistic model can be used to describe the *in vitro* gas production kinetics (CCC = 0.992). It was concluded that, in addition to chemical analysis, the *in vitro* gas production is a useful and simple technique for determination of the relative feeding value of these forage species.

Keywords: alfalfa, sainfoin, gas production, logistic model, concordance correlation

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Introduction

For confined animals with greater concentrate supplementation, forages have been used as a source of fiber to maintain rumen activity, while for grazing animals forages may be the only source of nutrient supply. The validity of a particular nutritive value entity is highly dependable on the forage use.

Plant development is a major factor affecting forage quality; as plants change from the vegetative to reproductive stages, forage quality generally decreases. As plants mature, fiber content generally increases, and organic matter (OM) digestibility decreases, and consequently feed intake will be lowered by the livestock. The rate and timing of reproductive development is determined by species, day length (photoperiod) and temperature (Parsons and Chapman, 2000). The decrease in digestibility with increased maturity is mainly due to increased stem/leaf or flower/leaf ratio, and less because of decreased digestibility of the individual stem and leaf fractions (Søegaard and Weisbjerg, 2007).

Alfalfa is the primary forage fed to lactating dairy cows; however, there has been renewed interest in utilization of other forages in the diet of lactating dairy cows, particularly because of farm nutrient management issues.

Sainfoin *(Onobrychis viciifolia* Scop.) is a legume, widely grown for forage in Europe and Asia. It has many advantages as a forage legume. Sainfoin is a non-bloating legume (Reid et al., 1975) that is both drought and winter hardy, as well as resistant to alfalfa weevil (Ditterline and Cooper, 1975). Its positive effects in ruminants, tend to be due to high levels of condensed tannins. Condensed tannins bind to proteins in sainfoin; thereby protecting the protein from being hydrolyzed in the rumen. Jensen et al. (1968) reported that weight gain, feed consumption, feed efficiency, and digestibility were similar in beef cattle fed on alfalfa or sainfoin hay. Smoliak and Hanna (1975) repor-

ted no apparent differences in palatability for sheep given a choice of grazing alfalfa, sainfoin or cicer milkvetch with or without crested wheatgrass, although sheep grazed sainfoin first.

The *in vitro* gas production technique is widely used to evaluate the nutritive value of feedstuff; it is also more efficient than other *in vitro* techniques in determining the nutritive value of tanning-containing feeds (Getachew et al., 2002).

The aim of this study was to: (i) determine the effect of two consecutive cuts on the chemical composition, relative feed value (RFV), and *in vitro* gas production of alfalfa hay (*Medicago sativa*), and sainfoin hay *(Onobrychis viciifolia* Scop.); (ii) evaluate the potential of the gas production technique to appraise nutritional properties of the forages; and (iii) evaluate the performance of logistic model in fitting the cumulative gas production.

Materials and methods

Forage

This study was carried out under irrigation system in Shahrekord, Iran, (32º 20' N, 50º 51' E, 2061 m altitude) during spring and summer 2009, using sainfoin and alfalfa seeds originating from the west of Iran. Annual mean minimum and maximum temperature was -7.1°C and 33.2°C, respectively.

The seeds were sown in silty-clay-loam soils with organic matter around 1.0%. Plants were established in May 2009 within a 1 × 0.5 m frame. Farming conditions were the same for all plots. Sainfoin and alfalfa were grown in adjacent plots and the forages were harvested when alfalfa was in 1/10 bloom. The interval between the first and second cuts was 21 days.

The clipped sample consisted of a 50-cm mower strip through the center of each experimental unit, when the mean plant height was 15 centimeters. Weight of green materials was recorded, and a 500-g sample was taken from each experimental unit and oven-dried at 60°C. After drying, samples were ground through a 0.9-mm screen, and analyzed for chemical composition and *in vitro* gas production.

Chemical analysis

Soluble crude protein (CP) was extracted by mixing the forage sample with deionized water at 39°C (100 mL per gram air-dried forage). The samples were filtered through tared Gooch crucibles (porosity 40 to 60 mm) under light vacuum. The soluble fraction, extracted from the forages, was subjected to protein determination using Kjeldahl method. Concentration of N in crude protein was determined using the copper catalyst Kjeldahl method (ID 984.13), and fat content (solvent extraction method ID 991.36) according to AOAC, (1997). Neutral detergent fiber (NDF) was determined

Table 1. Analysis of the forages in different cuts (dry matter basis).

Composition (%)	Alfalfa		Sainfoin		SE	Probability		
	Cut 1	Cut 2	Cut 1	Cut 2		Forage (F)	Cut (C)	F × C
DM	73.57	79.22	78.47	78.95	0.643	0.0348	0.0098	0.0219
NDF	46.49	48.01	39.70	40.64	0.361	<0.0001	0.0426	0.5854
ADF	31.35	33.35	29.71	30.60	0.317	0.0012	0.0122	0.2488
Cellulose	23.44	23.06	22.11	20.45	0.350	0.0041	0.0734	0.2323
CF	24.53	26.13	23.22	23.93	0.254	0.0012	0.0121	0.2489
ADL	7.91	10.29	7.60	10.14	0.178	0.3958	<0.0001	0.7567
ADL/NDF	0.17	0.21	0.19	0.25	0.004	0.0006	<0.0001	0.2133
ASH	10.30	10.94	9.11	10.00	0.181	0.0031	0.0172	0.6320
ICP	15.83	13.41	15.43	15.77	0.557	0.2481	0.2241	0.1176
SCP	6.04	10.32	4.62	7.17	0.431	0.0056	0.0005	0.1935
CP	21.87	23.72	20.04	22.94	0.839	0.3031	0.0802	0.6748
EE	2.15	2.19	1.87	1.94	0.042	0.0020	0.4294	0.7609
NFC	19.19	15.14	29.28	24.49	0.933	<0.0001	0.0101	0.7850
RFV	138.6	111.2	124.4	121.5	2.736	0.6288	0.0045	0.0132
ME(MJ/kg)	9.90	9.51	8.59	8.85	0.109	0.0002	0.6977	0.0669

DM = dry matter; ICP = insoluble crude protein; SCP = soluble crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; CF = crude fiber; ADL = acid detergent lignin; CP = crude protein; EE = ether extract; NFC = non-fiber carbohydrate; RFV = relative feed value; ME = metabolizable energy.
SE = Standard error.

using a heat stable amylase and as ash free according to Mertens (2002), and acid detergent fiber (ADF) and Lignin (ADL) according to ISO method 13908 (2008). Ash was determined by ignition at 525°C. Harvest times and chemical composition for forage samples are shown in Table 1.

Gas production test

Buffer. Buffer composition was: 9.8 g $NaHCO_3$, 4.62 g Na_2HPO_4, 0.46 g NaCl, 0.57 g KCl, 0.04 g $CaCl_2$ and 0.06 g $MgCl_2$ per 1000 mL.

Preparation of inoculum media. Fresh feces was collected directly from the rectum of the dairy cows and placed in a pre-warmed thermos flask. The fresh feces, 20 g, was diluted in 140 mL of buffer and stirred with a magnet stirrer. The resulting suspension was strained through four layers of gauze, and the remaining solids were re-suspended in 140 mL of buffer and homogenized. The homogenate was strained through two layers of gauze, mixed with the first strained solution and kept at 39°C. All manipulations with each bottle took only a few minutes.

Gas measurements. Duplicate samples of the unfractionated forages were incubated in test tubes with a nominal volume of 13 mL (Moharrery and Hvelplund, 2008). The tubes contained 200 mg of air-dried sample, 1 mL of inoculum media, 5 mL of buffer, and were mixed by vortexing. The tubes were flushed with carbon dioxide for 3 min and sealed by rubber stoppers. Pressure readings in tubes were recorded 2 hourly during the first 24 h and repeated 4 hourly till the end of gas production test. Three blank tubes (*i.e.*, inoculum media + buffer) were incubated for each batch. Mean gas production data from blanks were subtracted from the recorded gas production of the standards on all substrates to calculate the net gas production values.

Calculations and Statistical analysis

Using proximate composition and *in vitro* net gas production (i.e. corrected for blanks) at 24 h incubation, metabolizable energy (ME) was calculated using the equation proposed by Menke and Steingass (1988).

The RFV was calculated from the estimates of dry matter intake (DMI) (Rohweder et al., 1978). Dry matter digestibility (DDM%) was estimated as follows (Moharrery, unpublished data):

$$DDM\ \% = a + b\ (lnx)^2 + c/lnx + d/x \quad (1)$$

(a = -5208; b = 57.985; c = 20615; d = -45423; x = gas production at 24 h; $R^2 = 0.90$; $P < 0.01$)

$$DMI\ (\%body\ weight) = 120/\%NDF \quad (2)$$

$$RFV = (\%DDM \times \%DMI)/1.29 \quad (3)$$

Data for cumulative gas production (mL gas/g DM) were fitted to the logistic model using Sigmaplot (Version 9.0, 2004).

For evaluation of the logistic model, the concordance correlation coefficient (CCC) was used to assess the agreement between predicted and observed data (Lin, 1989). As there is, as yet, no literature providing a descriptive scale for the degree of agreement based on CCC, the Landis and Koch (1977) scale was used to describe the degree of concordance, with: 0.21–0.40 being "Fair"; 0.41–0.60 being "Moderate"; 0.61–0.80 being "Substantial"; and 0.81–1.00 being "Almost perfect".

A completely randomized model in factorial arrangement 2 × 2 (forages and cuts) was used to analyze the data for chemical composition and gas production (SAS, 2003). All measurements were performed in at least duplicate. Correlation coefficients among parameters were determined, and tested using a *t*-test.

Results

Chemical compositions of forages are presented in Table 1. Concentration of fiber components (NDF, ADF, cellulose and crude fiber) was higher in alfalfa compared to sainfoin, and in the first cut compared to the second cut ($P < 0.05$) in both forages. Cellulose content was not affected by the growing season ($P > 0.05$). Results showed higher soluble crude protein (SCP), crude protein (CP), lignin (ADL), ash, the ratio of ADL to NDF and non-fiber carbohydrates (NFC), and lower insoluble crude protein (ICP) concentration

Table 2. Effect of forage cut on *in vitro* gas production (mL/g DM).

Incubation time (h)	Alfalfa		Sainfoin		SE	Probability		
	Cut 1	Cut 2	Cut 1	Cut 2		Forage (F)	Cut (C)	F × C
6 h	31.93	28.89	28.71	25.47	1.436	0.1409	0.1608	0.9611
24 h	173.03	146.28	135.38	135.47	3.262	0.0008	0.0202	0.0197
6-24 h	141.10	117.39	106.68	110.00	2.725	0.0006	0.0295	0.0080
48 h	311.01	267.38	263.78	272.61	6.210	0.0438	0.0829	0.0174
6-48 h	279.08	238.49	235.07	247.14	5.319	0.0466	0.0946	0.0081

SE: standard error.

Table 3. Gas production responses forage cuts during fermentation.

Variable[1]	Alfalfa		Sainfoin		SE	Probability		
	Cut 1	Cut 2	Cut 1	Cut 2		Forage (F)	Cut (C)	F × C
$T_{1/2}$	23.54	23.87	27.98	28.08	0.492	0.0003	0.7627	0.8703
T_{max}	13.69	12.82	13.14	15.41	0.197	0.0708	0.1922	0.0127
R_{max}	9.61	8.07	7.28	7.64	0.347	0.0011	0.0670	0.0091

[1]$T_{1/2}$ = half time of asymptotic gas production (h); R_{max} = maximal rate of gas production (mL/h); T_{max} = time of occurrence of R_{max} (h).
SE: standard error.

in second cut compared to first cut forages.

Because of the higher fiber content of the second cuts of forages compared to the first cut at similar stages of maturity, the relative feed value (RFV) as a forage quality index was lower for the second cut (P = 0.0045). ME concentration, using *in vitro* net gas production after 24 h incubation, was significantly lower in sainfoin (mean value 11.2% MJ/kg DM lower) than in alfalfa (P = 0.0002), but no significant difference was observed between the two consecutive cuts (P = 0.6288).

A significant forage × cut interaction was observed, indicating that variations in chemical composition can occur when different forage cuts are analyzed.

In vitro gas production from different cuts and after various incubation periods (h) (mL/g DM) is presented in Table 2. Gas production was 24 mL/g DM higher for alfalfa than for sainfoin after 24 h of incubation (P = 0.0008). This superiority was maintained (21 mL/g DM) up to 48 h of incubation, but the ratio of gas production (alfalfa/sainfoin) was 1.12 after 6 h of incubation and gradually increased to time points of 24 h of incubation, which was the highest (1.18); from this point, the ratio gradually decreased until the end of incubation. The first alfalfa cut was superior to the second cut throughout the incubation times. In the case of sainfoin, gas production values at 24 h incubation were similar in the first and second cuts, after which the second cut produced more gas until the end of incubation period. A significant interaction between forage × cut (P < 0.01) was observed for gas production and for all time points after 6 h incubation.

Data for cumulative gas production was fitted to the monophasic logistic model described by Groot et al. (1996). The gas production kinetics, adapted during 96 h of incubation, is shown in Figure 1. The components of the model are as follows:

$$G = A/[1 + (C/t)^B] \quad (4)$$

in this equation, G = total gas produced (mL), A = asymptotic gas production, B = switching characteristic of the curve, C = time at which one-half of the asymptote had been reached ($T_{1/2}$), and t = time (h). Maximum rate of gas production (R_{max}) and the time at which it occurred (T_{max}) were calculated according to the following equations (Bauer et al., 2001):

$$R_{max}=\{A\times C^B)\times B\times[T_{max}^{(-B-1)}]\}/\{1+(C^B)\times[T_{max}^{(-B)}]\}^2 \quad (5)$$

and,

$$T_{max} = C \times \{[(B - 1)/(B + 1)]^{(1/B)}\} \quad (6)$$

The mean values for fitted gas production variables are shown in Table 3. Forages differed in $T_{1/2}$, T_{max}, and R_{max} values. However, $T_{1/2}$, T_{max}, and R_{max} were not different between the two consecutive cuts. Alfalfa showed a faster fermentation rate with greater R_{max} (P = 0.0011) and earlier $T_{1/2}$ (P = 0.0003) and T_{max} (P = 0.0708) whereas sainfoin had a slower fermentation rate with lower R_{max} and slower $T_{1/2}$ and T_{max} (Table 3). The rate of gas production at different time points is shown in Figure 2. Rate of gas production for forages decreased to less than one at approximately 87, 83, 79, and 84 h for alfalfa (first and second cuts) and sainfoin (first and second cuts), respectively.

The concordance correlation coefficient (CCC) was used to determine whether logistic model for fitting curves on gas volume measurements could reproduce suitable results. The CCC is calculated as: $\rho c = \rho \times C_b$ with ρc being the concordance correlation coefficient, ρ Pearson's correlation coefficient; C_b the bias correction factor, which is calculated as: $C_b = 2\sigma_o\ \sigma_p/(\sigma^2_o + \sigma^2_p + (\mu_o - \mu_p)^2)$ with σ_o, μ_o, σ_p and μ_p the S.D. and mea-

Table 4. Accuracy, bias and correlation measures for the assessment of the reproducibility of the logistic model using cumulative gas production (mL/g DM), with the evaluation performed on observation.

Gas production	CCC	ρ	C_b	Shift	
				Scale	Location
Alfalfa					
Cut 1	0.996	0.996	1.000	0.981	0.014
Cut 2	0.989	0.994	0.996	0.910	.004
Sainfoin					
Cut 1	0.995	0.995	1.000	0.986	0.012
Cut 2	0.988	0.990	0.998	1.063	0.023

CCC = concordance correlation coefficient, ρ = Pearson's correlation coefficient and C_b the bias correction factor.

n of the values determined by gas volumes and calculated from the logistic model, respectively. Pear son's correlation coefficient reflects the precision, and the bias correction factor reflects accuracy. The bias correction factor consists of a scale shift (= σ_o / σ_p) and a location shift relative to the scale (= $[\mu_o - \mu_p]/[\sigma_o \times \sigma_p]^{1/2}$) as per Lin (1989) and St-Pierre (2003).Accuracy, bias and correlation measures for the assessment of the reproducibility of the logistic model are presented in Table 4. The location shift diminished to zero, indicating that the observed gas volumes generally are very close to the predicted volumes, which are presented by the logistic model (for both forages and cuts).

Correlation coefficients of the cumulative gas production in various times with RFV and ME, and among each other are shown in Table 5. The cumulative gas produced at different time points showed a positive relationship ($P < 0.05$) with RFV. The coefficient of correlation was more or less similar for gas production at different time points and ME ($P < 0.05$). The correlation coefficient was positive and strong for all time points, RFV, and ME, except for gas production at 6 h. Results showed no significant correlation between time points at 6 h incubation and gas production from 6 to 24 h of incubation ($P > 0.05$).

Discussion

Large variations in fiber concentration and gas production were recorded due to forage type, and clipping time. Changes in chemical composition were pronounced from first to second cuts. Increases from first to second cuts were 42.8 and 25.5 g/kg ($P = 0.0005$) for SCP and 15.2 and 9.4 g/kg ($P = 0.0426$) for NDF content for alfalfa, and sainfoin, respectively. Similar trend was recorded for lignin and fiber, with a higher increase from first to second cut. Generally, the changes in CP and fiber concentrations were much higher, in alfalfa than sainfoin. The soluble protein concentration was the main source of variation in CP content between two consecutive cuts in alfalfa. Differences in gas production between the two consecutive alfalfa cuts were partly due to alfalfa phonological characteristics. This is in agreement with Moharrery et al. (2009), who reported that with increasing stage of forage maturity, proportion of cell wall components of the forages (*i.e.*, cellulose, hemicellulose, lignin) increased and, as a consequence, proportion of cell contents decreased. Because of changes in the ratio of cell wall to cell contents, the digestibility of forage is highest in the early vegetative stage with a high content of cell solubles (Groot et al., 1999). At advanced stages of maturity, an indigestible fraction related to the content of cell walls increases (Moharrery et al., 2009), as also found in the present study for alfalfa.

Analysis of forages for chemical composition may be useful, but for comparison of forages for quality rank it can be confusing. Incorporating biological parameters (such as degradation and gas production) into a forage quality prediction system is a suitable first step in improving forage quality prediction systems (Hackmann, 2008). In the present study, with respect to chemical composition of sainfoin, it could have been reasonable to expect that, gas production rates were higher than for alfalfa, or at least were of the same magnitude. But results showed that alfalfa produced a greater gas volume during incubation. One would expect the anti-nutritional factors such as tannins affected the gas production in sainfoin. These observations provide a basis for theorizing how the *in vitro* gas method is also expected to be better than chemical methods for quantification of anti-nutritional factors (Getachew et al., 1998). Generally, chemical methods measure anti-nutritional factors related to one or another standard. The nature of the standard and, hence, their biological effects could be different from the anti-nutritional factors present in feeds. This is particularly true for heterogeneous classes of anti-nutritional compounds such as tannins, saponins, alkaloids, etc. In addition, chemical

Table 5. Correlation coefficients among several parameters using pooled data.

	RFV	ME	At 6 h[1]	At 24 h[1]	6-24 h[1]	At 48 h[1]	6-48 h[1]
RFV	1	0.435^{NS}	0.613*	0.727**	0.681**	0.863***	0.848***
ME		1	0.571*	0.898***	0.888***	0.760**	0.741**
At 6 h			1	0.666*	0.513^{NS}	0.663*	0.556^{NS}
At 24 h				1	0.982***	0.912***	0.893***
6-24 h					1	0.881***	0.886***
At 48 h						1	0.991***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = non-significant. RFV = Relative feed value; ME = metabolizable energy (MJ/kg DM).

[1]Gas production (mL/g DM) at different incubation times.

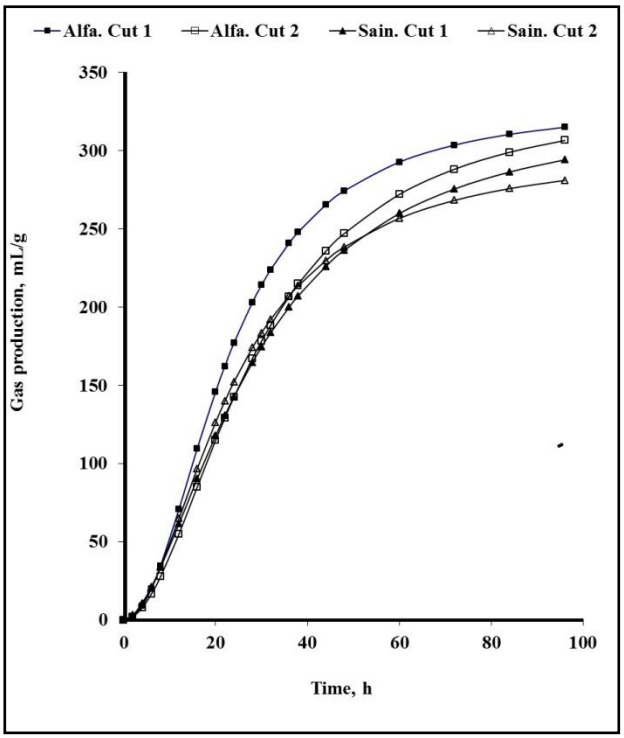

Figure 1. Gas production kinetics as affected by forage type and cut.

assays do not indicate the possible interaction of different anti-nutritional factors that take place during fermentation. Longland et al. (1995) reported a significant inverse relationship between gas accumulation at different time of incubation and tannin contents of feedsamples. In this regards, RFV is an index, which combines biological and chemical properties of feeds into one number. The RFV ranks forages relative to full bloom alfalfa (full bloom alfalfa is considered to have an RFV equal to 100). For example, mean value of sainfoin in the present study with an RFV of 123 (Table 1) contains 23 percent more energy than mature alfalfa. The RFV sharply decreased in alfalfa from first to second cuts, but similar value was recorded for sainfoin cuts. In this regard, the second cut of sainfoin showed different pattern of gas production compared to the second cut of alfalfa (Figures 1 and 2). With second cut of sainfoin, a slower gas production was observed, because of the smaller amount of highly fermentable cell contents (NFC), as the fermentation continued steadily as the cellulose continued to ferment by providing high soluble CP, at an appreciable rate. At 24 h incubation, similar volume of gas was produced. At this time point, one would expect that a higher CP concentration (Table 2) could provide a better condition for microbial activity in the inoculum's media for higher degradation of the cell wall, and consequently higher gas production.

Metabolizable energy content in both forages was estimated from gas production after 24 h incubation. Based on extensive studies, Menke et al. (1979) concluded that prediction of metabolizable energy is more accurate when based on gas and chemical constituents constituents only. Other workers (Chenost et al., 1997; data as compared to calculations based on chemical Romney et al., 1997) also reported significant correlation

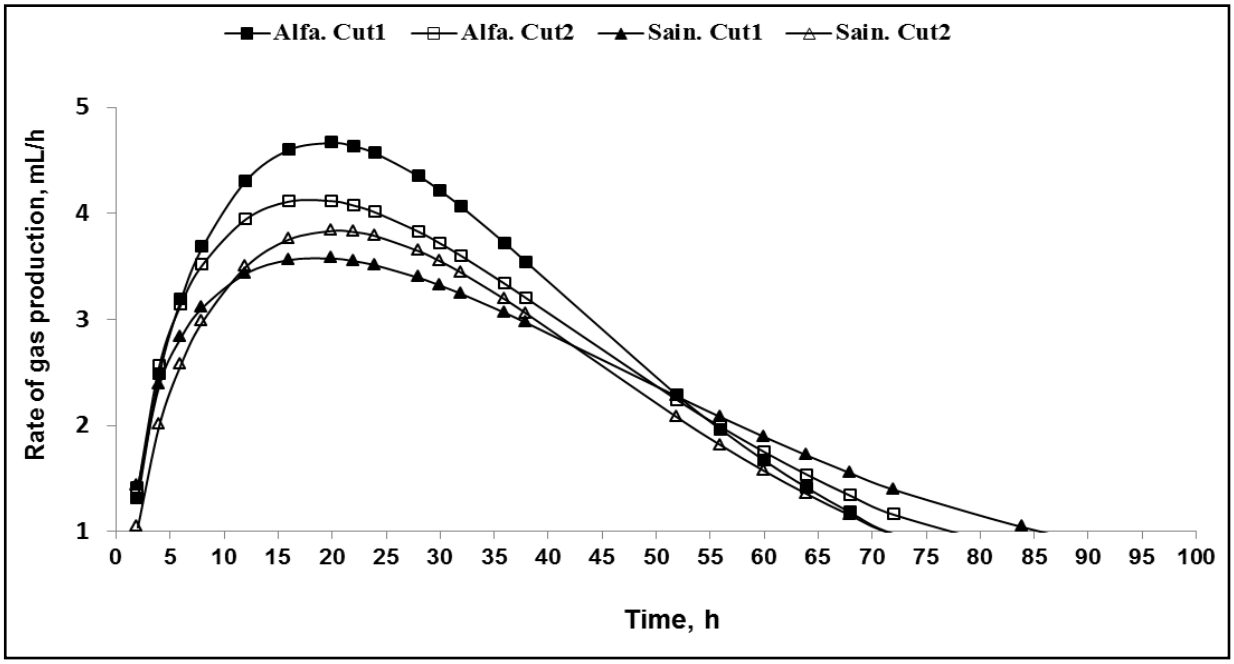

Figure 2. Rate of gas production as affected by forage type and cut.

tion between *in vitro* gas measurement and *in vivo* digestibility.

Kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degradable, and undegradable particles in feedstuff. Mathematical descriptions of gas production profiles allow analysis of data, evaluation of substrate, and fermentability of soluble and slowly fermentable components of feeds. Various models have been used to describe gas production profiles. The exponential model (Ørskov and McDonald, 1979) is widely used in ruminant feed evaluation to describe degradation kinetics, as measured by the nylon bag technique, but the model has also been used to describe kinetics of gas production data (Siaw et al., 1993; Khazaal et al., 1993). This model is based on first-order kinetics, assuming constant fractional rate fermentation (Groot et al., 1996). Since feed particles may ferment at different rates, the assumption in exponential model is not universally valid. In the present study, we used sigmoidal (logistic) models for fitting the cumulative gas production curve. In agreement with present results, Beuvink and Kogut (1993) evaluated various curve fitting models and reported that the exponential model resulted in larger residual mean squares as compared to sigmoidal models. On the other hands, Non-sigmoidal shapes indicated that rate of gas production decreased continually, while sigmoidal shapes indicated that rate of gas production increased initially, reached a maximum value and then decreased, which might suggest a close relationship with increased microbial activities during the early stages of incubation. France et al. (1993, 2000) reported that sigmoidal shapes reflected increased substrate accessibility, which might be caused by increased hydration of particles, microbial attachment and microbial numbers at the beginning of incubation. Groot et al. (1996) also proposed that sigmoidal shape was likely, because the microbial population had to multiply and colonize the substrate to form a 'biofilm' before reaching maximum rate of fermentation. Based on results from CCC evaluation, it can be indicated that logistic model and measured gas volume are concordant, with high precision (Table 4). It is clear that this approach allows a high accuracy and precision to fitting logistic curve to gas production values. This is in agreement with Wang et al. (2010) who reported that logistic models can be use as alternatives for description of the *in vitro* gas production kinetics.

Calculated RFV and ME showed positive and significant correlation with different incubation time points of forages (Table 5; $P < 0.05$). These results are in agreement with Hackmann (2008) who reported that the extension of DM degradation of forages was highly correlated with RFV scores. In contrast, Weiss (2002) reported only moderate to poor correlations between RFV and degradation characteristics. However, it must be emphasized that degradation parameter values were poorly correlated to RFV. Given that, degradation parameters are often linked to DMI and DDM (Mertens, 1973), because calculation of RFV was based on both DMI and DDM.

The correlation coefficient between RFV and gas production at 6 h of incubation was not strong ($r = 0.61$; $P < 0.05$), because RFV is essentially a re-expression of NDF (Weiss, 2002), and gas production at 6 h of incubation is mostly related to the extent of fermentation of NFC. In addition, higher correlations were found after 24 h incubation, which indicated that 24 h incubation provides more information compared to 48 h incubation. The coefficients of determination (r^2) calculated between RFV and ME, and between RFV and gas production at 24 h of incubation was 0.19 and 0.53, respectively, indicating that 19 and 53% of the total variation in RFV score can be explained by the correlation between RFV and the magnitude of ME and gas production at 24 h of incubation, respectively.

Conclusion

Alfalfa and sainfoin differed significantly with respect to chemical composition, *in vitro* gas production, RFV and ME. This study showed that 24 h incubation provided more informative data with high reproducibility, clear relationships between different parameters and REV, and could produce more reliable models for prediction of ME.

Theoretical investigation indicated that, compared to the non-sigmoidal models, the logistic model made more biological sense in describing *in vitro* gas curves. The logistic models improved the fit to curve, and led high accuracy and precision. However, more studies, including more incubation points, different gas measurement systems and wider ranges of feedstuffs are needed to fully investigate the performance of logistic model.

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