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Research Article

Prediction of Dry Matter Intake and Dry Matter Digestibilities of Some Forages Using the Gas Production Technique in Sheep

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Abstract: Wheat straw, barley straw, alfalfa hay, alfalfa silage and maize silage were offered ad libitum to three male sheep, and daily dry matter intake (DMI) and apparent dry matter digestibility (DMD) were measured. Samples of forages were incubated with rumen fluid to determine gas production. Gas productions were measured at 3, 6, 12, 24, 48, 72 and 96, and gas production constants (a, b, c,) were described using the equation $y = a + b\ (1 - e^{-ct})$. Gas production at all incubation times and gas production constants (c, b and a+b) were significantly ($P < 0.001$) correlated to DMD or DMI.

Although the gas production constant c explained 74% of the variation of DMD, the gas production constant b explained 92% of the variation of DMD. The gas production constants c and (a+b) in combination explained 96% of the variation of DMD. The gas production constants b and c alone explained 70% and 78% of the variation of DMI respectively. The gas production constants in combination explained 84-90% of the variation of DMI.

It was concluded that the accuracy of predicting DMD or DMI can be increased when gas production constants are used in combination, and the *in vitro* gas production technique has good potentiality to predict DMD and DMI.

Key Words: Digestibility, food intake, gas production, sheep

Koyunlarda, Bazı Kaba Yemlere Ait Kuru Madde Tüketim Düzeyi ve Kuru Madde Sindirilebilirliklerin Gaz Üretim Tekniği ile Tahmini

Özet: Serbest yemlemeye tabi tutulan erkek toklularda buğday samanı, arpa samanı, yonca samanı, yonca silajı ve mısır silajına ait yem tüketimi ve sindirim derecesi tespit edilmiştir. Aynı yemlere ait örnekler, zamana bağlı gaz üretim değerlerini tespit etmek için, rumen sıvısıyla inkübasyona tabi tutulmuştur. Gaz ölçümleri 3, 6, 12, 24, 48, 72 ve 96 saatler için yapılmıştır. Gaz üretimine ait a, b ve c değerleri $y = a + b\ (1 - e^{-ct})$ fonksiyonu kullanılarak tespit edilmiştir. Bütün inkübasyon sürelerinde elde edilen gaz üretim değerleri (ml) ile yem tüketimi ve sindirim dereceleri arasında önemli ($P < 0.001$) korelasyon bulunmuştur.

Sabit c değeri, kuru madde sindirim derecesindeki varyasyonun % 74'lük kısmını açıklamasına rağmen, sabit b değeri % 92'lik kısmını açıklamıştır. Sabit c ve (a+b)'nin birlikte kullanılması durumunda sindirim derecesinde görülen varyasyonun % 96'lık kısmı açıklanmıştır. Sabit c değeri, yem tüketimindeki varyasyonun % 78'lik kısmını açıklarken, sabit b değeri ise ancak % 70'lik kısmını açıklamıştır. Gaz üretimine ait parametrelerin birlikte kullanılması durumunda, yem tüketimindeki varyasyonun % 84-90'lık kısmı açıklanmıştır.

Sonuç olarak, kuru madde sindirim derecesi ve yem tüketimini tahmin etmedeki isabet derecesi, gaz üretimine ait parametrelerin birlikte kullanılmasıyla artmaktadır. Gaz üretim tekniği, yem tüketimi ve yemin sindirim derecesini tahmin etmede iyi bir potansiyele sahiptir.

Anahtar Sözcükler: Sindirim derecesi, yem tüketimi, gaz üretimi, koyun

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Introduction

Forages, fed either as hay or silage, represent a major component of ruminant diets. Nutritive value of forages depends on their dry matter digestibility and voluntary dry matter intake. Predictions of voluntary intake and digestibility of forages by ruminant animals have long been a research priority in animal nutrition. Historically, intake and digestibility have been predicted by simple and multiple regression equation based on the chemical composition. Although these equations may allow prediction with acceptable precision, other simple methods of feed evaluation have been proposed (1). Simple and rapid procedures to predict feed intake are essential to allow correct ration formulation and adequately predict their performance (2).

Many studies have been carried out to investigate the relationship between voluntary food intake and degradation characteristics obtained using the nylon bag technique (3,4). However, as this technique is gravimetric, the extent and rate of digestion are obtained assuming that dry matter loss equals fermentation, which is not normally the case because considerable substrate is lost from nylon bags (2). Pearce et al. (5) have found quite different levels of fermentability of the soluble fraction of wheat straw, a fraction which would be lost from nylon bag or upon filtration and so overestimating digestibility. Gas production techniques to study kinetics of rumen digestion have made it possible to overcome this problem, since gas production derives from fermentable fraction of feeds and washing losses are non-existent (2). More recently, researchers have been investigating the relationship between voluntary food intake or dry matter digestibility and in vitro gas production kinetics (2,6). The information available can be considered limited and the results somewhat inconsistent.

The objective of this study was to evaluate the potential of gas production technique to predict voluntary DMI and in vivo DMD of some forages widely used in ruminant diets.

Materials and Methods

Forages

Five commercially available and widely used roughages consisting of wheat straw, barley straw, alfalfa hay, alfalfa silage and maize silage were used in this experiment.

Wheat and barley straw were obtained as crop residues after harvesting for grain. First cut alfalfa (*Medicago sativa* L) harvested at the beginning of flowering (~ 10%) was used for silage and hay production. The cut alfalfa was chopped to approximately 1.5 cm particle length and ensiled without additives. Some of the alfalfa was dried under shade for hay production. Whole crop maize was harvested at the milky stage with a chop harvester and ensiled without additives.

Chemical Analysis

After drying forage samples were milled through a 1 mm sieve for chemical analysis and gas production. Dry matter (DM) was determined by drying the samples at 105 °C overnight and ash by igniting the samples in muffle furnace at 525 °C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (7). Crude protein (CP) was calculated as N X 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) content were determined by the method of AOAC (7). Starch content was determined by the method of MacRea and Armstrong (8).

Voluntary Food Intake and Digestibility Trials

Each of the forages was offered ad libitum to three male sheep kept in metabolism cages to enable accurate determination of feed intake and allow easy collection of faeces. They were given free access to fresh water and mineral salt licks. The animals were adapted to each forage for two weeks, followed by balance trials of seven days, in which daily measurement of food intake and faecal excretion were made. The faeces were collected in a canvass bag secured by means of harness over body of sheep. Sub-samples of forages were taken and data on their daily intake (g DM per kg $LW^{0.75}$) and digestibility in vivo were obtained.

In vitro gas production

Rumen fluid was obtained from two fistulated sheep fed twice daily at the maintenance level with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in in vitro rumen fluid in calibrated glass syringes following the procedures of Menke et al. (9). 0.200 g samples were weighed in triplicate into calibrated glass syringes of 100 ml. The syringes were prewarmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39 °C. Readings of gas production were recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to the model of Orskov and McDonald (10)

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

where

a = the gas production from the immediately soluble fraction (ml)

b = the gas production from the insoluble fraction (ml)

c = the gas production rate constant for the insoluble fraction (b)

a+b = potential gas production (ml)

t = incubation time (h)

y = gas produced at time‘t’

Statistical Analysis

Data were subjected to standard analysis of variance using General Linear Model (GLM) of Statistica for Windows (11). Significance between individual means was identified using Tukey’s multiple range test (12). 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. The relationships between different characteristics of roughages and DMD, DMI gas production and gas production constants were obtained by simple linear and multiple regression analysis

Results

The chemical compositions of forages are presented in Table 1. There was considerable variation between forages in terms of chemical composition. Cell wall contents (NDF and ADF), which represent the most important fraction of dry matter for all forages, ranged from 75.56 to 42.40, 54.33 to 24.10, respectively. The CP contents of alfalfa hay and silage were considerable higher than the others. Maize silage is the only forage containing starch in this experiment.

DMD (%) and DMI (g per kg LW $^{0.75}$) of forages are given in Table 2. There were highly significant ($P < 0.001$) differences between forages in terms of DMD and DMI. DMD and DMI of alfalfa hay, alfalfa silage and maize silage was significantly ($P < 0.001$) higher than those of wheat and barley straw.

Data of gas production during the fermentation period are given in Table 2. The cumulative volume of gas production increased with increasing time of incubation. Gas produced after 96 h incubation ranged between 45.33 and 78.17 ml per 0.200 g of dry matter.

At all incubation times, cumulative gas productions (ml) of alfalfa hay, alfalfa silage and maize silage were significantly ($P < 0.001$) higher than those of wheat and barley straws. Therefore the estimated parameters (a, b, c and a+b) of alfalfa hay, alfalfa silage and maize silage were significantly ($P < 0.001$) higher than those of wheat and barley straws due to low cell wall content.

Table 1. Chemical composition of forages as to dry matter basis.

Constituents (%)	Forages				
	Wheat Straw	Barley Straw	Alfalfa Hay	Alfalfa Silage	Maize Silage
DM	92.18	91.78	91.52	26.50	25.25
NDF	75.56	72.73	42.40	43.72	44.83
ADF	54.33	53.23	27.36	33.44	24.10
CP	3.14	4.22	18.37	16.41	7.86
Starch	ND	ND	ND	ND	23.40
Ash	5.83	7.44	10.73	10.82	5.63

DM: Dry matter; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; CP: Crude protein; ND: Non-detected

Table 2. Dry matter digestibility, dry matter intake, gas production (ml) and estimated parameters of some forages at different incubation times.

Time(h)	Forages					SEM	P
	Wheat Straw	Barley Straw	Alfalfa Hay	Alfalfa Silage	Maize Silage		
3	13.50a	13.33a	16.67b	20.97c	26.33c	0.383	***
6	19.17a	20.67a	33.33b	33.83b	37.50c	0.582	***
12	27.17a	27.33a	44.00b	48.17c	49.33c	0.623	***
24	34.33a	34.67a	52.67b	56.33c	60.83d	0.459	***
48	40.33a	40.83a	56.33b	61.17c	69.17d	0.542	***
72	43.17a	43.67a	61.67b	65.17c	74.17d	0.542	***
96	45.33a	47.03a	63.67b	68.17c	78.17d	0.599	***
Estimated Parameters							
c	0.078a	0.076a	0.113c	0.113c	0.095b	0.002	***
a	2.23bc	2.57c	0.57a	1.33ab	4.19d	0.250	***
b	40.71a	41.22a	59.32b	62.88c	68.80c	0.387	***
(a+b)	42.94a	43.79a	59.89b	64.21c	73.00d	0.349	***
DMD	44.67a	41.53a	64.09b	64.34b	65.43b	0.737	***
DMI	28.90a	28.32a	60.98b	50.46c	50.00c	0.859	***

Means within same row with differing superscript are significantly different. a: the gas production from the immediately soluble fraction (ml); b: the gas production from the insoluble fraction (ml); c: the gas production rate constant for the insoluble fraction (b); (a+b): potential gas production (ml); DMD: Dry matter digestibility (%), DMI: Dry matter intake (g DM per kg LW0.75); ***: P < 0.001, SEM: Standard Error Mean

Discussion

Chemical compositions of forages used in this experiment were consistent with findings by Khazaal et al. (6), Giger-Riverdin (13) and Valentine et al. (14). The reason why alfalfa hay, alfalfa silage and maize silage had significantly higher DMD and DMI than those of wheat and barley straw is low cell wall content (NDF and ADF). High DMD resulted in high DMI of forages. The often stated relationship that "increased digestibility results in increased intake" is influenced by the residence time of forage in the rumen (15). Analysing data from thirteen different legume silages Wilkins et al. (16) concluded there was a significant and positive correlation between voluntary intake and in vivo digestibility. DMD and DMI of forages obtained in this study were consistent with those reported by Rodrigeus et al. (2), Khazaal et al. (6) and De Boever et al. (17).

Cumulative gas production and estimated parameters were comparable to those reported by Filya et al. (18).

Cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters except gas production constant a. Negative correlations between gas production and NDF or ADF may be a result of the reduction of microbial activity, from increasingly adverse environmental conditions as incubation time progresses. This result is consistent with the findings of Abdulrazak et al. (19) and Ndlovu and Nherera (20).

As can be seen from the Figure, volume of gas production was always significantly related (P < 0.001, r=0.796 to 0.975) to DMD at all incubation periods.

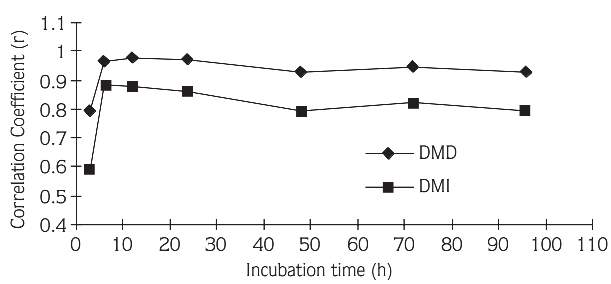

Figure. Correlation coeffecient (r) of the relationship between volume of gas (ml) and DMD or DMI.

Volume of gas production was also significantly related ($P < 0.001$, r=0.582 to 0.888) to DMI at all incubation periods.

The relationship between DMI and gas production was weakest after 3 h incubation (r=0.582, $P < 0.001$). After this point correlation coefficients between gas production and DMD or DMI remained constant. This result is not in agreement with findings of Rodrigeus et al. (2), who found that after 10 h incubation the correlation values decreased by about 20 h and has fallen to near 0. In vitro gas production at 8 h explained 48% of the variation of DMI. In this study gas production after 3 h incubation explained more than 80% of the variation observed for DMD and DMI. This result is consistent with the findings of Khazaal et al. (6), who found a significant correlation between gas production and DMD or DMI at all incubation times. There is no obvious explanation for these anomalies. Differences between the results by the different authors may be due to a number of factors: e.g., methodology and the substrates used. DMI was calculated as g DM per kg $LW^{0.75}$ in the study carried out by Khazaal et al. (6) whereas DMI was calculated as g DM per kg LW in the study carried out by Rodrigues et al. (2)

The rate constant (c) of gas production alone was significantly ($P < 0.001$) correlated to DMI or in vivo DMD. This is not consistent with the findings of Khazaal et al. (6) but in agreement with the findings of Rodrigues et al. (2).

As can be seen from Table 3, equations predicting DMD and DMI from in vitro gas production constant showed that constants of the exponential equation alone or in various combinations were well correlated with DMD and DMI.

Although the gas production constant c explained 74% of the variation of DMD, the gas production constant b explained 92% of variation of DMD. The gas production constants c and (a+b) in combination explained 96% of the variation of DMD. This result is in agreement with findings of Khazaal et al. (6), who found that the gas production constants (c, a, b, a+b) in combination explained 58-78.4% of the variation of DMD, and the findings of Blummel and Orskov (21), who found that the gas production constants (c, a, b) alone or in combination explained 86-87% of the variation of DMD of ten different forages.

Table 3. Prediction of dry matter digestibility and dry matter intake.

Y	Equation and factors	R^2	P	MSE
DMD	y = 2.58+560 c	0.74	***	5.66
	y = 7.56 + 0.888b	0.92	***	3.12
	y = 7.93 + 0.847(a+b)	0.86	***	4.08
	y = 6.6 + 76 c + 1.94b - 1.12 (a+b)	0.96	***	2.18
	y = - 3.12 + 275 c + 0.579 (a+b)	0.96	***	2.14
DMI	y = -23.5 + 705 c	0.78	***	6.22
	y = -8.68 + 0.960 b	0.70	***	7.32
	y = -6.7 + 0.888 (a+b)	0.65	***	8.35
	y = 44.4 - 959 c + 14.3 b - 12.2 (a+b)	0.90	***	4.182
	y = -27.4 + 515 c + 0.387 (a+b)	0.84	***	5.34

DMD: Dry matter digestibility (%), DMI: Dry matter intake (g DM per kg LW0.75); a, b and c are constants in the exponential equation $p= a +b (1-e^{ct})$; a: the gas production from the immediately soluble fraction (ml); b: the gas production from the insoluble fraction (ml); c: the gas production rate constant for the insoluble fraction b; (a+b) = potential gas production (ml). MSE: Error Mean Square; ***: $P < 0.001$

The gas production constants alone or in various combinations explained 65-90% of the variation of DMI. This result was not consistent with the findings of Rodrigues at al. (2), who showed that gas production constants of the alone, or in various combinations, were not correlated with DMI when exponential equation ($y= a + b(1-e^{ct})$) (10) was used to estimate the gas production constants. However, Rodrigues et al. (2) showed that DMI was best predicted by stepwise regression analysis when the biphasic Michaelis-Menten equation was used to determine the gas production constants. The gas production constants alone or in various combinations explained 39-94% of the variation of DMI.

The inclusion of rate constant (c) of gas production in the regression equation improved the accuracy of predicting DMD or DMI, and results were supported by the high correlation observed between gas production constants (c, b, (a+b)) and DMD or DMI, whereas Blummel and Orskov (21) found that the inclusion of rate constant (c) of gas production in the regression equation for DMI did not improve the precision of the prediction. In addition, it was determined that gas production constant a alone explained 75% of the variation of DMI.

The high correlation coefficient (r) between DMD or DMI and gas production or gas production constants provides validation of gas production technique as an appropriate in vitro method for evaluating forages widely used in ruminant diets.

In conclusion, results obtained from the present study indicated that prediction of voluntary DMI and DMD from in vitro gas production constants is possible. The accuracy of predicting DMD or DMI can be increased when gas production constants are used in combination.

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