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Variation In Metabolizable Energy Content of Forages Estimated Using *in vitro* Gas Production Technique

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Abstract: The metabolizable energy contents of wheat straw (WS), barley straw (BS), alfalfa hay (AH), alfalfa silage (AS) and maize silage (MS) were predicted by the *in vitro* gas production technique using different regression equations obtained from previous experiments. Gas productions were measured at 3, 6, 12, 24, 48, 72 and 96 and gas production constants (c, a, b) were described using the equation $y = a+b\ (1-e^{ct})$. Metabolizable Energy (ME) contents of forages were determined using different regression equations obtained previous experiments. There were significant ($P<0.001$) differences between forages in terms of ME content. There were also significant difference ($P<0.001$) between ME contents of each forage predicted using different equations. The regression equation for prediction of ME had a significant effect on ME of forages. This resulted in different rank orders of forages based on ME. Therefore ME content of forages predicted by gas production method using different regression equations obtained from previous experiments cannot be considered absolute. The care that must be taken when comparing results obtained using different regression equations.

Key words: Gas production, metabolizable energy

INTRODUCTION

Forages, fed either as hay or silage, represent a major component of ruminant diets[1]. The nutrient composition including the metabolizable energy (ME) value of forage varies widely according to different plant genetics, environment and management, i.e. growing season, fertilizer, irrigation and harvesting stage[2]. Estimation of ME value of forages *in vivo* using animal tedious and requires labour and facilities. *In vitro* methods less expensive, less time consuming and allow incubation conditions to be maintained more precisely than *in vivo*[3]. Lately a lot of researches have been carried out to determine the ME value of foodstuffs such as straws[4], agro-industrial by products[5], compound food[6] and various tropical feeds[7] using the *in vitro* gas production technique[8]. The ME value was predicted from 24 h *in vitro* gas production and chemical composition of foods using regression equation obtained previous experiment. Getachew *et al*.[3] found that there was a significant variation between laboratories using the gas production technique of Menke and Steingass[8]. The gas production and ME values significantly ($P<0.001$) different from each other.

In addition to variation of *in vitro* gas production and calculated ME values the regression equations which are used to estimate ME value may have an effect on the ME values of forages. There were a lot of regression equations which are used to estimate the ME value of forages[8-12].

The aim of this study was to determine the ME value of different forages using the gas production technique of Menke and Staingass[8] and assess the variability of ME values estimated by different regression equations.

MATERIALS AND METHODS

Samples: Commercially available and widely used five roughages consisting of wheat straw, barley straw, alfalfa hay, alfalfa silage and maize silage were used in this experiment. The experiment was carried out in the laboratory of Faculty of Agriculture, Department of Animal Science, in Kahramanmaras, Turkey in September, 2003.

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

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the samples at 105°C overnight and ash igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kheldal method[13]. Crude protein (CP) was calculated as N X 6.25. Fat content, Neutral detergent fibre (NDF) and acid detergent fibre (ADF) content were determined by the method of AOAC[13]. Starch content was determined by the method of MacRea and Armstrong[14].

***In vitro* gas production:** Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass[8]. 0.200 g dry weight of the sample was 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 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[15].

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

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’

ME contents of forages were estimated using the equation in Table 1.

Table 1: Regression equations used to determine ME value of foods

Item	Equations for prediction of ME contents of forages
ME1	$0.145Gb+0.0412CP+0.00650CP^2+0.0206fat+1.54$[9]
ME2	$3.16+0.0695Gb+0.000073Gb^2+0.00732CP+0.0178Fat$
ME3	$1.56+0.1390Gb+0.0074CP+0.01780\,Fat$[11]
ME4	$1.20+0.1456Gb+0.000765CP+0.01642Fat$[12]
ME5	$2.20+0.136Gb+0.057\,CP+0.0029\,CP^2$[8]

Gb = Gas production at 24 h incubation (ml/200 mg DM)
CP : Crude protein (%) or (g/kg DM)
Fat: Ether Extracts (%) or (g/kg DM)

Statistical analysis: Data were subjected to standard analysis of variance using General Linear Model (GLM) of Statistica for windows[16]. Significance between individual means were identified using the Tukey’s multiple range test[17]. 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.

RESULTS AND DISCUSSION

There was considerable variation between forages in terms of chemical composition. Cell wall (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 (Table 2). The crude protein contents of alfalfa hay and silage were considerable higher than the others. Maize silage is the only forage containing starch in this experiment. Chemical compositions of forages used in this experiment were consistent with those reported by Khazaal *et al.*[18] and Giger-Riverdin[20].

Data of gas production during the fermentation period are given in Table 3. 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. Cumulative gas production and estimated parameters were comparable to those reported by Filya *et al.*[21].

At all incubation time cumulative gas productions (ml) of alfalfa hay, alfalfa silage and maize silage were significantly ($P<0.001$) higher than those of wheat and barley straw. Therefore the estimated parameters (c, a, b and a+b) of alfalfa hay, alfalfa silage and maize silage were significantly ($P<0.001$) higher than those of wheat and barley straw due to low cell wall content. Cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters except gas production constant (a). 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 progress. This result is consistent with findings of Abdulrazak *et al.*[22] and Ndlovu and Nherera[23].

ME contents of forages are given in Table 4. There were significant ($P<0.001$) differences between forages in terms of ME content. The equation for prediction of ME significantly ($P<0.001$) affected the ME content of forages (Table 4).

ME energy content of alfalfa hay, alfalfa silage and maize silage were significantly ($P<0.001$) higher than those of wheat and barley straw irrespective of the equation for prediction of ME content forages. ME content of forages except barley straw were significantly ($P<0.001$) lower than those obtained using the other equations when equation 2 was used for prediction of ME.

ME value of maize silage in this experiment ranged from 9.20 to 11.59. This range is comparable to those

Table 2: Chemical composition (%) of forages

Constituents (%)	Forages				
	WS	BS	AH	AS	MS
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
Oil	1.31	1.23	2.52	3.42	2.61
Starch	ND	ND	ND	ND	23.40
Ash	5.83	7.44	10.73	10.82	5.63

ND: Not detected

Table 3: Gas production (ml) and estimated parameters of some forages when incubated with rumen fluid at different incubation times

Time(h)	Forages						
	WS	BS	AH	AS	MS	SEM	Sig.
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	0.07a	0.076a	0.113c	0.113c	0.09b	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	***

Means within same row with differing superscript are significantly different. *** P<0.001, SEM: Standard Error Mean, Sig: Significance Level

Table 4: Metabolizable energy content of some forages

	Metabolizable Energy (MJ / kg DM)						
	ME1	ME2	ME3	ME4	ME5	SEM	Sig.
WS	6.62bc(a)	6.13a(a)	6.80c(a)	6.41b(a)	7.07d(a)	0.057	***
BS	6.72bc(a)	6.22a(a)	6.91c(a)	6.46ab(a)	7.19d(a)	0.057	***
AH	11.49e©	8.88a(b)	10.69c(b)	9.39b(b)	11.18d(b)	0.043	***
AS	11.59d©	9.20a©	11.21c©	10.05b©	11.41cd©	0.078	***
MS	10.84cd(b)	8.77a(b)	11.07d(b)	10.51b(d)	11.06d(b)	0.053	***
SEM	0.066	0.033	0.063	0.0647	0.062		
Sig.	***	***	***	***	***		

Means in the same columns with different letters (in bracket) differ significantly
Means in the same rows with different letters (in bracket) differ significantly
*** P<0.001
SEM: Standard Error Mean. Sig: Significance Level

reported by Getachew *et al.*[3] who found that ME value of maize silage ranged from 8.45 to 9.35 and 8.35 to 10.04 obtained by Lee *et al.*[2]. It can be seen that the upper level obtained in this study is considerable higher than those found by Getachew *et al.*[3] and Lee *et al.* [2]. ME value of alfalfa hay in this experiment ranged from 8.88 to 11.49. This is also comparable to those reported by Getachew *et al.*[3] who found that ME value of alfalfa hay ranged from 8.80 to 10.10 and Lee *et al.*[2] who found that ME value of alfalfa hay ranged from 7.11 and 9.54.

Regression equations used ME content of forage were obtained from different laboratories and different time. This may effect the regression equation for prediction of ME contents of forages since rumen fluid or physiological state animal has a significant effect on gas production. Bonsi *et al.*[24] studied the effect of donor animal diets on *in vitro* gas production and found that rumen fluid from animals on different diets resulted in different gas values at different incubation times. For example rumen fluid from animals fed teff straw resulted in lower gas values compared to those fed either Sesbania or Leucaena. Trei *et al.*[25] also found that gas production of concentrate was significantly different when incubated with rumen fluid from two animals fed the same diet. Nagadi *et al.*[26] reported that there was also interaction between diet of the donor animal and type of feed incubated. Therefore Getachew *et al.*[3] reported that *in vitro* biological assay (gas production) can only be standardized to a limited extent. They showed that seven different laboratories used the same procedure but obtained different gas production(ml) at 24 h incubation. They concluded that ME value predicted using gas production method of Menke and Stengass[8] by laboratories in different parts of the world cannot be considered absolute since ME value predicted using gas production method of Menke and Stengass[8] is affected many factors[3].

This experiment clearly showed that the regression equations used for ME prediction of forages obtained in different condition and times resulted in differences in ME contents of forages. Variation of ME is as high as 2.29-2.61 MJ kg^{-1} DM for alfalfa hay, alfalfa silage and maize silage as found in this experiment. This variation in ME content was comparable with findings of Getachew *et al.*[3] who found 3 MJ kg^{-1} DM variation. The amount of variation as high as 3 MJ kg^{-1} DM will be deemed unacceptable by feed traders (feed companies, forage brokers, nutritionists, farmers and forage producers).

Based on ME content of forages different ranking orders were obtained. When regression equation 1 was used the rank order was wheat straw = barley straw<maize silage<alfalfa hay<alfalfa silage, when regression equations 2, 3 and 5 were used the rank order was wheat straw = barley straw<maize silage = alfalfa hay<alfalfa silage. When regression equation 4 was used the rank order is wheat straw = barley straw<alfalfa hay<alfalfa silage<maize silage. This result also clearly showed that the rank orders of forages were significantly affected by the equations which were used to predict the ME content of forages. This highlights the care that must be taken when comparing results obtained using different regression equations.

The regression equation for prediction of ME had a significant effect on ME contents of forages. This resulted in different rank orders of forages based on ME content. Therefore ME contents of forages predicted by gas production method using different regression equations obtained from previous experiments cannot be considered absolute. The care that must be taken when comparing results obtained using different regression equations.

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