Comparison of *in vitro* gas production technique with *in situ* nylon bag technique to estimate dry matter degradation

A. Kamalak [1], O. Canbolat [2], Y. Gurbuz[1], O. Ozay[1]

[1]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Science, Kahrmanmaras, Turkey

[2]Bursa Uludag University, Faculty of Agriculture, Department of Animal Science, Bursa, Turkey

ABSTRACT: Dry matter (DM) degradation of wheat straw (WS), barley straw (BS), lucerne hay (LH) and maize silage (MS) was determined using two different techniques: (*i*) *in vitro* gas production and (*ii*) nylon bag degradability technique. *In vitro* gas production and *in situ* DM disappearance were measured after 3, 6, 12, 24, 48, 72 and 96 hours of incubation. *In situ* and *in vitro* DM degradation kinetics was described using the equation $y = a + b\ (1 - e^{ct})$. In all incubations there were significant ($P < 0.001$) correlations between gas production and *in situ* DM disappearance or estimated parameters ($(a + b)_{gas}$ and $(a + b)_{is}$ or $(a + b)_{gas}$ and $EDMD_{is}$) whereas there were no significant ($P > 0.05$) correlations between c_{gas} and c_{is} or b_{gas} and b_{is}. Gas production from the insoluble fraction (b) alone explained 98.3% of the variation of EDMD. The inclusion of gas production from the quickly soluble fraction (a) and rate constant (c) of gas production in the regression equation improved the accuracy of EDMD prediction. The correlations between the results of both methodologies seem to be sufficiently strong to predict degradability parameters from gas production parameters. It was concluded that the *in vitro* gas production technique has good potentiality to predict *in situ* DM disappearance and some DM degradation parameters.

Keywords: *in vitro* gas production; *in situ* dry matter degradability; forage

The rate and extent of DM fermentation in the rumen are very important determinants for the nutrients absorbed by ruminants. The nylon bag technique has been used for many years to estimate both the rate and the extent of DM degradation in forages *in situ* (Mehrez and Orskov, 1977). Although this method is widely used, it is laborious, time consuming and expensive (Cone *et al*., 2002). Therefore, several other techniques have been developed. Menke *et al*. (1979), Menke and Steingass (1988) developed the *in vitro* gas production technique to evaluate the nutritive value of forages and to estimate the rate and extent of DM degradation indirectly using the gas production (CO_2) during fermentation. Compared to the *in situ* degradability technique, gas production methods are less animal dependent, more appropriate for characterizing soluble or small particulate feeds and they can be automated thus reducing the labour input (Adesogan, 2002). The *in situ* nylon bag and *in vitro* gas production technique are well correlated with animal performance (Orskov, 1989), food intake (Blummel and Orskov, 1993), microbial protein synthesis (Krishnamoorthy *et al*., 1991) and *in vivo* digestibility (Khazaal *et al*., 1993). Considering the advantages of gas production technique with its simplicity of use and the possibility of processing a large number of samples in a short time it will be important to find significant and valid correlations between *in situ* DM degradability and *in vitro* gas production parameters (Valentin *et al*., 1999).

More recently, researchers have investigated relationships between fermentation kinetics obtained by *in situ* nylon bag technique and *in vitro* gas production technique (Blummel and Orskov, 1993; Khazaal *et al*., 1993; Dewhurst *et al*., 1995; Lopez

et al., 1999; Cone *et al.*, 1999, 1998, 2002). However, the information available can be considered limited and results somewhat inconsistent.

The aim of this study was (I) to determine fermentation kinetics using *in vitro* gas production and the *in situ* nylon bag technique and (II) to evaluate the correlations between the fermentation kinetics obtained by two different techniques.

MATERIAL AND METHODS

Forages and chemical analysis

Commercially available and widely used four forages consisting of wheat straw, barley straw, lucerne hay and maize silage were used in this experiment. After drying forage samples were milled through a 1-mm sieve for chemical analysis. DM was determined by drying the samples at 105°C overnight and ashing the samples in a muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined by the method of AOAC (1990). Starch content was determined by the method according to MacRea and Armstrong (1968).

In vitro gas production

Forage samples milled through a 1-mm sieve were incubated *in vitro* in rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing lucerne hay (60%) and concentrate (40%). Digestion medium was prepared mixing 500 ml of distilled water, 0.1 ml micro-mineral solution, 200 ml buffer solution, 200 ml macro-mineral solution, and 1 ml Resazurin solution. CO_2 gas was bubbled through the solution until the colour turned pink per purple or for 3 hours. 0.200-gram dry weight of samples was weighed into calibrated glass syringes of 100 ml. The syringes were prewarmed at 39°C before the injection of 30 ml of rumen fluid-buffer mixture consisting of 10 ml rumen fluid and 20 ml digestion medium into each syringe followed by incubation in a water bath at 39°C. Triplicates of each sample were used in two separate runs. Readings of gas production were recorded before incubation (0) and after 3, 6, 12, 24, 48, 72 and 96 h of incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979):

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

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
$a + b$ = the potential gas production (ml)
t = the incubation time (h)
y = the gas produced at the time t

The fermentation kinetics was estimated using a computer package programme called Fig P (Biosoft, Cambridge, UK).

In situ DM disappearance

The *in situ* DM degradation analysis was carried out according to the procedure described by Mehrez and Orskov (1977). 5-gram samples dried and milled through a 3-mm sieve were weighed into nylon bags and incubated in three rumen fistulated sheep for 3, 6, 12, 24, 48, 72 and 96 h. The sheep were fed twice a day on 60% lucerne hay and 40% concentrate diet. On removal the nylon bags were thoroughly washed with cold running water until no further coloured liquid could be extruded, and dried at 60°C for 48 h. DM losses for each incubation time were determined. The DM degradation data were fitted to the exponential equation:

$$Y = a + b\,(1 - e^{-ct}) \quad (2)$$

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

The degradation kinetics was estimated using a computer package programme called Fig P (Biosoft, Cambridge, UK). Effective DM Degradability (EDMD) was calculated applying the equation of Orskov and McDonald (1979):

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

Where *a, b* and c are the same as in (2) and k is the rumen outflow rate of 2% per h which is at the maintenance level.

Statistical analysis

Data on *in situ* DM degradation and *in vitro* gas production were subjected to standard analysis of variance using the general linear model (GLM) of Statistica for Windows (Stastica, 1993). Significance between individual means was identified using Tukey's multiple range test (Pearse and Hartley, 1966). 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 *in situ* DM degradation and *in vitro* gas production parameters were obtained by simple linear regression analysis.

RESULTS AND DISCUSSION

The chemical compositions of forages are presented in Table 1. Although no statistical analysis of forage composition was carried out, there was a considerable variation between forages in terms of chemical composition. The cell wall content (NDF and ADF), which represents the most important fraction of dry matter for all forages, ranged from 75.56 to 42.40 and from 54.33 to 24.10, respectively. The crude protein of lucerne hay was considerably higher than in the other forages. Maize silage is the only forage containing starch in this experiment. Chemical compositions of forages used in this experiment were consistent with Khazaal *et al.* (1993), Valentin *et al*. (1999), Giger-Riverdin *et al*. (2000).

Cumulative gas production profiles from the *in vi*tro fermentation of some forages are shown in Figure 1 and the estimated parameters are given

Table 1. Chemical composition of some forages

Constituents (%)	Forages			
	wheat straw	BS	LH	MS
DM	92.18	91.78	91.52	25.25
NDF	75.56	72.73	42.40	44.83
ADF	54.33	53.23	27.36	24.10
CP	3.14	4.22	18.37	7.86
Starch	ND	ND	ND	23.40
Ash	5.83	7.44	10.73	5.63

DM = dry matter, NDF = neutral detergent fibre, ADF = acid detergent fibre, CP = crude protein, ND = non-detectable

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

	Forages				SEM	Sig.
	wheat straw	barley straw	lucerne hay	maize silage		
c_{gas}	0.078[a]	0.076[a]	0.113[c]	0.095[c]	0.003	***
a_{gas}	2.23[b]	2.57[b]	0.571[a]	4.19[c]	0.267	***
b_{gas}	40.71[a]	41.22[a]	59.32[b]	68.80[c]	0.372	***
$(a + b)_{gas}$	42.94[a]	43.79[a]	59.89[b]	73.00[c]	0.363	***

Means within the same row with various superscripts are significantly different, c_{gas} = gas production rate, a_{gas} = gas production (ml) from quickly soluble fraction, b_{gas} = gas production (ml) from insoluble fraction, $(a + b)_{gas}$ = potential gas production, ***$P < 0.001$, SEM = Standard Error Mean, Sig. = significance level

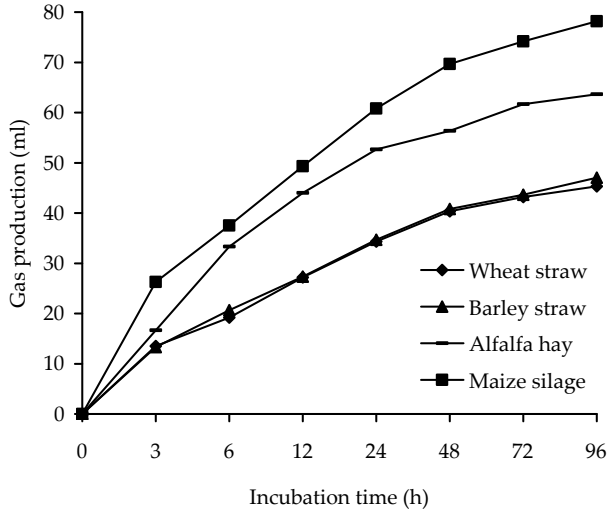

Figure 1. Gas production of some forages when incubated with rumen fluid at different incubation times

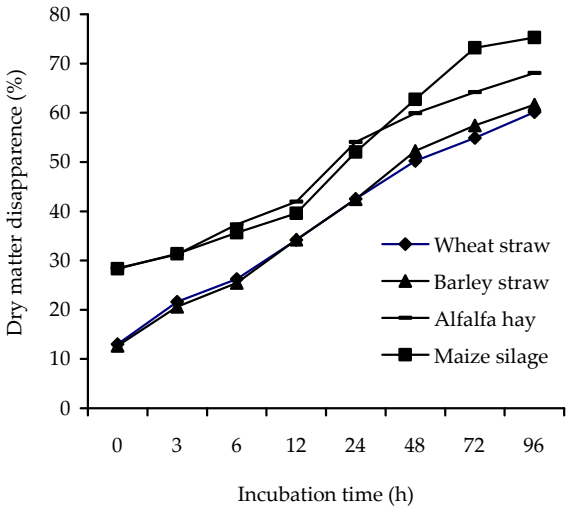

Figure 2. Dry matter disapparence of some forages when incubated in the rumen

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. Cumulative gas production and estimated parameters were comparable to those reported by Filya *et al.* (2002). At all incubation times cumulative gas productions (ml) of maize silage were significantly ($P < 0.001$) higher than those of lucerne, wheat straw and barley straw. Therefore the estimated parameters (*c*, *a*, *b* and *a* + *b*) of maize silage were significantly higher than the others. The cell wall content (NDF and ADF) was negatively correlated with gas production at all incubation times and estimated parameters except gas production constant *a*. The negative correlation between gas production and the cell wall content may be a result of the reduction of microbial activity, from increasingly adverse environmental conditions as the incubation time progresses. This result is consistent with findings of Abdulrazak *et al.* (2000) and Ndlovu and Nherera (1997).

DM disappearance of some forages from bags at different rumen incubation times is given in Figure 2 and the estimated digestion kinetics is presented in Table 3. There was an increase in DM disappearance associated with the increasing time of incubation. At all incubation times DM disappearances for lucerne hay and maize silage were significantly ($P < 0.001$) higher than those of wheat straw and barley straw. Although no significant ($P > 0.05$) differences between lucerne hay and maize silage in terms of DM disappearance were found out at early incubation times, there were significant ($P < 0.001$) differences after 72 and 96 h of incubation.

Table 3. Dry matter disappearance and estimated parameters of some forages when incubated in the rumen

	Forages				SEM	Sig.
	wheat straw	barley straw	lucerne hay	maize silage		
c_{is}	0.042[a]	0.040[a]	0.035[a]	0.022[b]	0.002	***
a_{is}	15.39[a]	14.57[a]	27.85[b]	28.03[b]	0.324	***
b_{is}	42.76[ab]	46.13[bc]	39.78[a]	54.25[d]	1.037	***
$EDMD_{is}$	44.35[a]	45.34[a]	53.25[b]	56.45[c]	0.279	***

Means within the same row with various superscripts are significantly different, c_{is} = rate of dry matter degradation, a_{is} = quickly soluble fraction, b_{is} = insoluble but fermentable fraction, EDMD = effective dry matter degradability, ***$P < 0.001$, SEM = Standard Error Mean, Sig. = significance level

Although rapidly soluble DM fractions (a) of lucerne hay and maize silage were significantly ($P < 0.001$) higher than those of wheat and barley straw, the potentially fermentable fraction (b) of maize silage was significantly ($P < 0.001$) higher than the others. Therefore EDMD of maize silage was significantly ($P < 0.001$) higher than in the other forages although maize silage had a lower degradation rate (c) than the others. DM disappearance at all incubation times and estimated parameters were comparable to those reported by Filya *et al.* (2002).

As can be seen from Table 4, at all incubations there were significant correlations between gas production and *in situ* DM disappearance or estimated parameters $(a + b)_{gas}$ and $(a + b)_{is}$ or $(a + b)_{gas}$ and $EDMD_{is}$ whereas there was no significant ($P > 0.05$) correlation between c_{gas} and c_{is} or b_{gas} and b_{is}. The results obtained in this experiment are in agreement with those reported by Khazaal *et al.* (1993), Blummel and Orskov (1993) and Sileshi *et al.* (1996), who observed a correlation between DM disappearance and gas production but they did not find a significant correlation between the rate of DM degradation (c_{is}) and the rate of gas production (c_{gas}). On the other hand, Piva *et al.* (1988), who worked with maize silage, did not obtain a significant ($P > 0.05$) correlation for the same parameters. Valentin *et al.* (1999) suggested that differences between the conclusions drawn by different authors might be due to a number of factors e.g. methodology, substrates and types of animals, number of measurements and mathematical model. The *in vitro* gas production method indirectly evaluates DM degradation by determining the gas yield whereas the *in situ* technique determines DM loss during incubation in the rumen through microbial degradation.

Although the correlation coefficients (r) between gas production and DM disappearance were low at early incubation times, the correlation coefficients (r) increased with increasing incubation time. This may be explained by the DM loss during early incubation times but which is not available to microbial fermentation. This study has revealed a general problem of overestimation of degradability by the *in situ* nylon bag technique. Overestimation is especially noticeable at short incubation times (Willman *et al.*, 1996). Gas production as a measure

Table 4. Correlation coefficients (r) of the relationship between *in vitro* and *in situ* dry matter degradation of some forages

In situ	*In vitro*						
	3_{gas}	6_{gas}	12_{gas}	24_{gas}	48_{gas}	72_{gas}	96_{gas}
3_{is}	0.761***	0.948***	0.954***	0.947***	0.898***	0.919***	0.845***
6_{is}	0.684**	0.926***	0.935***	0.924***	0.857***	0.883***	0.861***
12_{iss}	0.611*	0.869***	0.873***	0.861***	0.786***	0.815***	0.797***
24_{is}	0.663**	0.914***	0.915***	0.904***	0.841***	0.871***	0.848***
48_{is}	0.848***	0.867***	0.960***	0.965***	0.950***	0.962***	0.954***
72_{is}	0.949***	0.946***	0.944***	0.941***	0.980***	0.976***	0.985***
96_{is}	0.944***	0.953***	0.952***	0.965***	0.985***	0.982***	0.986***

Estimated parameters	c_{gas}	a_{gas}	b_{gas}	$(a + b)_{gas}$
c_{is}	–0.483^{NS}	–0.481^{NS}	–0.875***	–0.898***
a_{is}	0.879***	0.011^{NS}	0.957***	0.924***
b_{is}	–0.156^{NS}	0.867***	0.482^{NS}	0.560^{NS}
$(a + b)_{is}$	0.510^{NS}	0.504^{NS}	0.940***	0.963***
EDMD	0.748***	0.219^{NS}	0.991***	0.982***

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; NS: = non-significant ($P > 0.05$); IS = *in situ*; Gas: gas production, c_{is} = rate of dry matter degradation, a_{is} = quickly soluble fraction, b_{is} = insoluble but fermentable fraction, EDMD = effective dry matter degradability, c_{gas} = gas production rate, a_{gas} = gas production (ml) from quickly soluble fraction, b_{gas} = gas production (ml) from insoluble fraction, $(a + b)_{gas}$ = potential gas production

Table 5. Prediction of *in situ* dry matter disappearance and estimated parameters from *in vitro* gas production and estimated parameters

Y	Equation and factors used	R^2	RSD	Sig.
3_{is}	$Y = 13.1 + 0.749gas_{3h}$	58.0	3.719	***
6_{is}	$Y = 13.8 + 0.629gas_{6h}$	85.7	2.238	***
12_{is}	$Y = 26.6 + 0.288gas_{12h}$	76.2	1.754	***
24_{is}	$Y = 28.3 + 0.426\ gas_{24h}$	81.7	2.538	***
48_{is}	$Y = 34.8 + 0.414gas_{48h}$	90.2	1.823	***
72_{is}	$Y = 32.8 + 0.533\ gas_{72h}$	95.2	1.714	***
96_{is}	$Y = 40.3 + 0.443gas_{96h}$	97.2	1.110	***
c_{is}	$Y = 0.0590 + 0.2638c_{gas}$	23.4	0.008	NS
a_{is}	$Y = 21.6 - 0.05a_{gas}$	0.00	7.125	NS
b_{is}	$Y = 33.9 + 0.224b_{gas}$	23.3	5.377	NS
EDMD	$Y = 47.8 + 0.84\ a_{gas}$	4.8	5.509	NS
EDMD	$Y = 27.6 + 0.424b_{gas}$	98.3	0.742	***
EDMD	$Y = 27.0 + 252c_{gas}$	55.9	3.748	***
EDMD	$Y = 27.7 - 0.145a_{gas} + 0.428b_{gas}$	98.4	0.751	***
EDMD	$Y = 31.2 - 0.71a_{gas} + 0.510b_{gas} - 71c_{gas}$	98.5	0.784	***
EDMD	$Y = 24.7 + 0.358\ (a + b)_{gas} - 60.3c_{gas}$	98.3	0.780	***
EDMD	$Y = 26.9 + 0.409\ b_{gas} + 16.1c_{gas}$	98.4	0.758	***
EDMD	$Y = 27.6 + 0.407\ (a + b)_{gas}$	96.5	1.063	***

c_{is} =rate of dry matter degradation, a_{is} = quickly soluble fraction, b_{is} = insoluble but fermentable fraction, EDMD: effective dry matter degradability, c_{gas} = gas production rate, a_{gas} = gas production (ml) from quickly soluble fraction, b_{gas} = gas production (ml) from insoluble fraction, $(a + b)_{gas}$ = potential gas production, Sig. = significance level

of organic matter degradation was often observed as promising when compared with the *in situ* nylon bag technique (Khazaal *et al.*, 1993; Cone *et al.*, 1998; Rymer and Givens, 2002).

As can be seen from Table 5, gas production explained 58–97.2% of variation of dry matter disappearance. Gas production from the insoluble fraction (*b*) alone explained 98.3% of the variation of EDMD. The inclusion of gas production from the quickly soluble fraction (*a*) and rate constant (*c*) of gas production in the regression equation improved the accuracy of EDMD prediction. Cone *et al.* (1998) showed in grass samples fertilized with different N-levels and harvested from late spring to late summer that both the rate and the extent of OM degradation determined *in situ* could be predicted by gas production parameters. Cone *et al.* (1999) also showed that there was a close relationship between *in situ* degradation characteristics and gas production parameters for grass silage samples differing in maturity.

The highly significant relationship between gas production and dry matter disappearance data suggests that the extent of dry matter degradation can be predicted from *in vitro* gas production.

A possibility of applying *in vitro* gas production methods to study the kinetics of forage DM degradation instead of the *in situ* technique will have advantages, including avoiding the error associated with the loss of small particles through the pores of nylon bag and reducing the cost.

CONCLUSION

The correlations between the results of both methodologies seem to be sufficiently strong to predict degradability parameters from gas production parameters. It was concluded that the *in vitro* gas production technique had good potentiality to predict DM disappearance and some DM degradation parameters.

REFERENCES

Abdulrazak S.A., Fujihara T., Ondilek J.K., Orskov E.R. (2000): Nutritive evaluation of some Acacia tree leaves from Kenya. Anim. Feed Sci. Technol., *85*, 89–98.

Adesogan A.T. (2002): What are feeds worth? A critical evaluation of selected nutritive value methods. In: Proceedings 13th Annual Florida Ruminant Nutrition Symposium, 33–47.

AOAC – Association of Official Analytical Chemists (1990): Official Method of Analysis. 15th ed. Washington, DC., USA, 66–88.

Blummel M., Orskov E.R. (1993): Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting of food intake in cattle. Anim. Feed. Sci. Technol., *40*, 109–119.

Cone J.W., Van Gelder A.H., Valk H. (1998): Prediction of nylon bag degradation characteristics of grass samples with the gas production technique. J. Sci. Food Agric., *77*, 421–426.

Cone J.W., Van Gelder A.H., Soliman I.A., De Visser H., Van Vuuren A.M. (1999): Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. J. Dairy Sci., *82*, 957–966.

Cone J.W., Van Gelder A.H., Bachmann H., Hindle V.A. (2002): Comparison of organic matter degradation in several feedstuffs in the rumen as determined with the nylon bag and gas production techniques. Anim. Feed Sci. Technol., *96*, 55–57.

Dewhurst R.J., Hepper D., Webster A.J.F. (1995): Comparison *in sacco* and *in vitro* techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Technol., *51*, 211–229.

Filya I., Karabulut A., Canbolat O., Degirmencioglu T., Kalkan H. (2002): Investigations on determination of nutritive values and optimum evaluation conditions by animal organisms of the foodstuffs produced at Bursa province by *in vivo* and *in vitro* methods. Uludag Universitesi Ziraat Fakultesi Bilimsel Arastirmalar ve Incelemeler Serisi, No. *25*, Bursa, 1–16.

Giger-Riverdin S. (2000): Characterisation of feedstuffs for ruminants using some physical parameters. Anim. Feed Sci. Technol., *86*, 53–69.

Khazaal K., Markantonatos X., Nastis A., Orskov E.R. (1993): Changes with maturity in fibre composition and levels of extractable polyphenols in Greek browse: effect *in vitro* gas production and *in sacco* dry matter degradation. J. Sci. Food Agric., *63*, 237–244.

Krishnamoorthy H., Steingass H., Menke K.H. (1991): Preliminary observations on the relationships between gas production and microbial protein synthesis *in vitro*. Arch. Tierernahr., *41*, 521–526.

Lopez S., Carro, M.D., Gonzales J.S., Ovejero F.J. (1999): Comparison of different *in vitro* and *in situ* methods to estimate the extent and rate of degradation of hays in the rumen. Anim. Feed Sci. Technol., *73*, 99–113

MacRea J.C., Armstrong D.G. (1968): Enzyme method for detection of α-linked glucose polymers in biological materials. J. Sci. Food Agric., *19*, 578–581.

Mehrez A.Z., Orskov E.R. (1977): A study of artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci., *88*, 645–650.

Menke H.H., Steingass H. (1988): Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev., *28*, 7–55.

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W. (1979): The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci., *93*, 217–222.

Ndlovu L.R., Nherera F.V. (1997): Chemical composition and relationship to *in vitro* gas production of Zimbabwean browsable indigenous tree species. Anim. Feed Sci. Technol., *69*, 121–129.

Orskov E.R. (1989): Recent advances in evaluation of roughages as feeds for ruminants. In: Farell D.J. (ed): Advances in animal nutrition. University of New England Printery, Armidale, 102–108.

Orskov E.R., McDonald P.(1979): The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci., *92*, 499–503.

Pearse E.S., Hartley H.O. (1966): Biometrika tables for statisticians. Vol. 1. Cambridge University Press.

Piva G., Santi E., Belladona S., Curto O. (1988): Kinetics of *in vitro* fermentation of forages. J. Alim. Nutr. Herbivors., *28*, 101–102.

Rymer R., Givens D.I. (2002): Relationship between patterns of rumen fermentation measured in sheep and *in situ* degradability and the *in vitro* gas production profile of diet. Anim. Feed Sci. Technol., *101*, 31–44.

Sileshi Z., Owen E., Dhanona M.S., Theodorou M.K. (1996): Prediction of *in situ* rumen dry matter disappearance of Ethiopian forages from an *in vitro* gas production technique using a pressure transducer, chemical analyses or *in vitro* digestibility. Anim. Feed Sci. Technol., *61*, 73–87.

Stastica (1993): Stastica for Windows release 4.3, StatSoft, Inc.,Tulsa, OK.

Valentin S.F., Williams P.E.V., Forbes J.M., Sauvant D. (1999): Comparison of the *in vitro* gas production technique and the nylon bag degradability technique to measure short and long term processes of degradation

of maize silage in dairy cows. Anim. Feed Sci. Technol., *78*, 81–99.

Willman D., Foulkes G.R., Given D. (1996): A comparison of four methods of estimating the rate and extent of cell wall degradation in grass silages. Anim. Feed Sci. Technol., 63, 99–109.

Received: 03–12–08
Accepted after corrections: 04–11–14

Corresponding Author

Assistant Prof. Dr. Adem Kamalak, Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Science, Kahrmananmaras, Turkey
Tel. +90 344 221 05 68, fax +90 344 223 00 48, e-mail: akamalak@ksu.edu.tr