



Comparison of *In situ* Dry Matter Degradation with *In vitro* Gas Production of Oak Leaves Supplemented with or without Polyethylene Glycol (PEG)

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ABSTRACT : Dry matter (DM) degradation of leaves from *Quercus cercis*, *Quercus libani*, *Quercus branti*, and *Quercus coccifera* was determined using two different techniques: (i) *in vitro* gas production and (ii) the nylon bag degradability technique. *In vitro* gas production in the presence or absence of PEG and *in situ* DM disappearance were measured at 3, 6, 12, 24, 48, 72 and 96 h. *In situ* and *in vitro* DM degradation kinetics were described using the equation $y = a + b(1 - e^{-ct})$. At all incubation times leaves from *Quercus branti* incubated with or without PEG gave significantly higher gas production than the other oak leaves except for 3 and 6 h incubation when leaves from *Quercus branti* without PEG supplementation only gave higher gas production than *Quercus cercis* and *Quercus coccifera*. At all incubation times except at 3, 6 and 12 h the DM disappearance from *Quercus branti* was significantly higher than the other species. Generally, PEG supplementation considerably increased the gas production at all incubation times and estimated parameters such as gas production rate (c_{gas}), gas production (ml) from the quickly soluble fraction (a_{gas}), gas production (b) from the insoluble fraction, potential gas production (a+b). However, all oak leaves did not give the same response to the PEG supplementation. Although the increase in gas production at 96 h incubation time was 8.9 ml for *Quercus libani* the increase was 5.5 ml for *Quercus coccifera*. It was concluded that except at early incubation times the relationships between the two methodologies seem to be sufficiently strong to predict degradability parameters from gas production parameters obtained in the presence or absence of PEG. (**Key Words :** *In vitro* Gas Production, *In situ* Dry Matter Degradation, Oak Leaves, PEG, Tannin)

INTRODUCTION

The rate and extent of fermentation of DM in the rumen are very important determinants for the nutrients absorbed by ruminants. The nylon bag technique has been used for many years to provide estimate of both rate and extent of DM degradation of forages *in situ* (Mehrez and Orskov, 1977). On the other hand Menke et al. (1979) and Menke and Steingass (1988) developed the *in vitro* gas production technique to evaluate the nutritive value of forages and estimate the rate and extent of DM degradation indirectly using the gas production (CO₂) produced during fermentation. *In vitro* gas production technique was widely used to evaluate the nutritive value of legume forages (Evitayani et al., 2004) and tannin containing tree leaves (Rubanza et al., 2003).

The *in situ* nylon bag and *in vitro* gas production technique are well correlated with animal performance

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(Orskov, 1989), food intake (Blummel and Orskov, 1993; Kamalak et al., 2005a), microbial protein synthesis (Krishnamoorthy et al., 1991) and *in vivo* digestibility (Khazaal et al., 1993; Kamalak et al., 2005a). 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 productions parameters (Valentin et al., 1999).

More recently researches have been investigating relationship 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; Kamalak et al., 2005b). The use of browse species as fodder for ruminant animals is becoming important in many part of world. Oak leaves are an important source of forage for ruminants in most part of Turkey during critical periods of the year when quality and quantity of pasture herbage is limited. The presence of tannins and other phenolic compounds in a large number of

nutritionally important shrubs and tree leaves hampers their utilization as animal feed (Tolera et al., 1997). The potential negative effect of phenolic compounds on microbial fermentation is unlikely to be detected by *in situ* method. In this regard *in vitro* methods more reliable in detecting inhibitory compounds in feeds (Rubanza et al., 2003; Osuga et al., 2005) *In vitro* gas production method is a closed system with limited supply of rumen liquor where if there is any anti-nutritive compound, it is likely to affect the activity of rumen microbes. On the contrary, the *in situ* method is associated with a dilution effect, which results from an open system with a wider rumen environment and copious supply of rumen fluid to nylon bag contents (Apori et al., 1998). The relationship between two techniques might be increased when tannin containing tree leaves were incubated in the presence of polyethylene glycol (PEG) since there is a significant negative correlation between gas production and condensed tannin contents (Kamalak et al., 2004a, b; Kamalak et al., 2005c). Some studies also clearly showed that PEG supplementation increased the gas production (Getachew et al., 2001; Getachew et al., 2002; Seresinhe and Iben, 2003; Canbolat et al., 2005). However, the information available is limited.

The aim of this study was to (I) determine fermentation kinetics of oak leaves using the *in vitro* gas production in the presence or absence of PEG and *in situ* nylon bag technique and (II) to determine the effect of PEG supplementation on relationship between the fermentation kinetics obtained by *in vitro* gas production and *in situ* nylon bag techniques.

MATERIALS AND METHODS

Leave samples

Leaves from *Quercus cercis*, *Quercus libari*, *Quercus branti*, and *Quercus coccifera* were harvested in June, 2004 from city called Kahramanmaraş, in the south of Turkey. The area located at altitude of 630 m above sea level. The mean annual rainfall and temperature are 857.5 mm and 16.2°C respectively. Leaves were hand harvested from at least 10 different trees, then pooled and oven dried at 60°C at 48 h (Abdulrazak et al., 2000).

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 Kheldal method (AOAC, 1990). Crude protein was calculated as $N \times 6.25$. ADF content was determined using the method described by Van Soest (1991). Condensed tannin was determined by butanol-HCl method as described by Makkar et al. (1995). Mimosin tannin (MT; Hodgson, England) was used as an external standard. All chemical analyses were carried out in triplicate.

In vitro gas production

Rumen fluid was obtained from two fistulated Karaman male sheep (body weight 51.2±3.5 kg) fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). In the absence and presence of PEG the samples were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). 0.200 g dry matter of the sample was weighed 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. The PEG 8000 (Sigma Chemical Co, UK) was added in solution to the samples in the tubes immediately before addition of the rumen fluid/buffer to provide 80 mg PEG/tube. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. 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 and hay standards with known gas production. Two fermentation trials were conducted with duplicate samples. Cumulative gas production data were fitted to the exponential equation $p = a_{\text{gas}} + b_{\text{gas}} (1 - e^{-ct})$ (Orskov and McDonald, 1979), where p is the gas production at time t ; a_{gas} is the gas production from the immediately soluble fraction (ml), b_{gas} is the gas production from the insoluble fraction (ml), c_{gas} is the gas production rate constant, $(a+b)_{\text{gas}}$ = the potential gas production (ml), t = incubation time (h).

In situ dry matter degradation

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 3 mm were weighed into nylon bags with 35-40 µm pore size and incubated in two rumen fistulated sheep for 3, 6, 12, 24, 48, 72 and 96 h. The sheep were fed twice a day on a 60% alfalfa hay and 40% concentrate diet. On removal the nylon bags were thoroughly washed with running cold water until no further colored 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 $p = a_{\text{is}} + b_{\text{is}} (1 - e^{-ct})$ (Orskov and McDonald (1979), where p is DM disappearance in rumen at time t , a_{is} is the rapidly soluble fraction, b_{is} is the insoluble but fermentable fraction, c_{is} is the constant rate of degradation of b_{is} (percentage per h). Effective DM degradability (EDMD) was calculated applying the equation of Orskov and McDonald (1979), $EDMD = a_{\text{is}} + (b_{\text{is}} \times c_{\text{is}} / (c_{\text{is}} + k))$, where k is the rumen outflow rate of 2% per h which is at the maintenance level.

In the *in vitro* gas production studies, the average value of the two bottles from each incubation run was considered to be the experimental unit, and the experimental run was

Table 1. The chemical composition (g/kg DM) of oak leaves from different species

Chemical constituents	Oak species			
	<i>Quercus cercis</i>	<i>Quercus libari</i>	<i>Quercus branti</i>	<i>Quercus coccifera</i>
DM	946	956	956	957
ADF	360	298	342	408
CP	84	104	82	49
Ash	53	52	69	72
CT	20	13	5	20

DM: Dry matter, ADF: Acid detergent fiber.

CP: Crude protein, CT: Condensed tannin.

considered as a block. In the *in situ* studies the average value of the two bags from each incubation in the rumen of each animal was considered to be experimental unit and each animal was considered as a block.

Statistical analysis

Data were analyzed as a randomized complete block design using the GLM procedure of SAS (1989). The linear model used for each dependent variable was $Y_{ij} = \mu + P_i + T_j + \varepsilon_{ij}$, where, μ is the common mean, P_i , the block, T_j , the species and ε_{ij} , the random error. Significance between individual means was identified using the Tukey's multiply 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. A simple regression analysis was used to establish the relationship among chemical composition, *in situ* DM degradation and *in vitro* gas production.

RESULTS

Chemical composition

The chemical compositions of oak leaves are given in

Table 1. There was considerable variation among oak leaves in terms of chemical composition. Crude protein content of oak leaves ranged from 49 to 104 g/kg DM. Crude protein content of *Quercus cercis*, *Quercus libari* and *Quercus branti* was considerable higher than that of *Quercus coccifera*. Acid detergent fibre content of oak leaves ranged from 298 to 408 g/kg DM. *Quercus coccifera* had the highest ADF content. Tannin content of oak leaves ranged from 5 to 20 g/kg DM. *Quercus cercis* and *Quercus coccifera* had the highest CT contents.

Gas production

Data of *in vitro* gas production during the fermentation of leaves supplemented with or without PEG are given in Table 2. The cumulative volume (ml) of gas production increased with increasing time of incubation. PEG supplemented oak leaves gave considerable higher gas production than that for oak leaves without PEG supplementation. Gas production of leaves with or without PEG supplementation after 96 h incubation ranged from 66 to 75.6 and 60.5 to 67.0 ml per 0.200 g DM of substrate respectively.

At all incubation times leaves from *Quercus branti* incubated with or without PEG gave significantly higher gas production than the other oak leaves except 3 and 6 h incubation time.

The estimated parameters are given in Table 3. There were no significant ($p > 0.05$) differences among oak leaves in terms of gas production rate when oak leaves were incubated without PEG. On the other hand, there were significant differences ($p < 0.001$) among oak leaves in terms of oak leaves when oak leaves were incubated with PEG.

The PEG supplementation increased the gas production from quickly soluble fraction (a_{gas}). The a value for *Quercus*

Table 2. *In vitro* gas production (ml) of leaves from 4 different oak species incubated with rumen fluid in the presence (+PEG) or absence (-PEG)

IT	Treatment	Species				SEM	Sig.
		<i>Q. cercis</i>	<i>Q. libari</i>	<i>Q. branti</i>	<i>Q. coccifera</i>		
3	-PEG	21.2 ^a	23.7 ^c	22.2 ^{bc}	19.3 ^a	0.471	***
	+PEG	27.6 ^c	25.9 ^b	31.8 ^d	23.0 ^a	0.371	***
6	-PEG	30.0 ^{ab}	32.5 ^{bc}	34.8 ^c	28.2 ^a	0.634	***
	+PEG	36.4 ^b	37.9 ^b	41.1 ^c	33.6 ^a	0.462	***
12	-PEG	38.2 ^a	42.2 ^b	44.3 ^c	37.2 ^a	0.372	***
	+PEG	44.4 ^b	46.9 ^c	50.0 ^d	41.9 ^a	0.560	***
24	-PEG	46.2 ^a	47.2 ^a	50.8 ^b	46.4 ^a	0.389	***
	+PEG	53.6 ^b	56.4 ^c	58.0 ^d	50.1 ^a	0.291	***
48	-PEG	54.2 ^{ab}	55.2 ^b	58.5 ^c	53.8 ^a	0.289	***
	+PEG	60.7 ^b	62.0 ^b	66.7 ^c	58.6 ^a	0.373	***
72	-PEG	58.2 ^a	59.8 ^b	63.8 ^c	57.5 ^a	0.354	***
	+PEG	67.1 ^b	66.9 ^b	71.9 ^c	63.4 ^a	0.3307	***
96	-PEG	61.3 ^a	64.2 ^b	67.0 ^c	60.5 ^a	0.449	***
	+PEG	70.0 ^b	73.1 ^c	75.6 ^d	66.0 ^a	0.430	***

^{a, b, c} Row means with common superscripts do not differ ($p > 0.05$).

IT: Incubation time (h) SEM: Standard error mean, Sig.: Significance level, *** $p < 0.001$.

Table 3. Some estimated parameters of leaves from 4 different oak species incubated with rumen fluid in the presence (+PEG) or absence (-PEG)

IT	Treatment	Oak species				SEM	Sig.
		<i>Q. cercis</i>	<i>Q. libari</i>	<i>Q. branti</i>	<i>Q. coccifera</i>		
c _{gas}	-PEG	0.09	0.09	0.10	0.09	0.013	NS
	+PEG	0.10 ^a	0.11 ^{ab}	0.12 ^b	0.10 ^a	0.003	**
a _{gas}	-PEG	3.9 ^b	3.6 ^{ab}	2.9 ^a	3.4 ^{ab}	0.179	*
	+PEG	4.8 ^b	3.5 ^a	4.7 ^b	3.9 ^{ab}	0.232	**
b _{gas}	-PEG	53.1 ^a	54.6 ^b	58.7 ^c	53.4 ^{ab}	0.324	***
	+PEG	59.6 ^b	62.4 ^c	64.4 ^d	57.5 ^a	0.313	***
(a+b) _{gas}	-PEG	56.9 ^{ab}	58.1 ^b	61.6 ^c	56.7 ^a	0.306	***
	+PEG	63.4 ^b	65.8 ^b	69.1 ^c	61.5 ^a	0.352	***

^{a, b, c} Row means with common superscripts do not differ (p>0.05). SEM: Standard error mean, Sig.: Significance level, c_{gas}: Gas production rate, a_{gas}: gas production (ml) from quickly soluble fraction, b_{gas}: gas production (ml) from the insoluble fraction, (a+b)_{gas}: potential gas production, NS: Non significant. *** p<0.001, ** p<0.01, * p<0.05.

Table 4. *In situ* dry matter disappearance and some estimated parameters of leaves from four different oak species

IT	Species				SEM	Sig.
	<i>Q. cercis</i>	<i>Q. libari</i>	<i>Q. branti</i>	<i>Q. coccifera</i>		
3	23.6 ^a	27.5 ^b	28.6 ^b	24.8 ^a	0.335	***
6	28.6 ^a	32.6 ^b	33.9 ^b	29.2 ^a	0.403	***
12	36.7 ^{ab}	38.5 ^{bc}	40.0 ^c	35.5 ^a	0.697	**
24	51.3 ^a	49.8 ^a	54.2 ^c	50.3 ^a	0.462	***
48	60.4 ^a	61.5 ^a	64.8 ^b	58.8 ^a	0.935	**
72	64.5 ^{ab}	64.9 ^b	69.3 ^c	62.8 ^a	0.482	***
96	67.2 ^{ab}	68.2 ^b	71.8 ^c	65.7 ^a	0.432	***
Estimated parameters						
c _{is}	0.04	0.04	0.04	0.04	0.002	NS
a _{is}	17.9 ^a	20.0 ^b	21.7 ^c	18.4 ^a	0.356	***
b _{is}	48.5 ^{ab}	49.4 ^{bc}	51.7 ^c	46.0 ^a	0.673	***
EDMD	50.5 ^a	52.4 ^b	55.3 ^c	49.8 ^a	0.312	***

^{a, b, c} Row means with common superscripts do not differ (p>0.05), IT: Incubation time (h), SEM: Standard error mean, Sig.: Significance level, c_{is} = the constant rate of degradation of b (percentage per h), a_{is}: water soluble fraction, b_{is}: insoluble but fermentable fraction, EDMD: Effective dry matter degradability.

NS: Non significant, *** p<0.001, ** p<0.01.

branti without PEG supplementation was significantly lower than that of *Quercus cercis* whereas there were no significant differences between *Quercus branti* and *Quercus cercis* incubated with PEG supplementation. Gas production (b_{gas}) from insoluble fraction and potential gas production (a+b_{gas}) of leaves for *Quercus branti* with or without PEG were significantly higher than the others.

The gas production (b_{gas}, (a+b)_{gas}) of oak leaves for *Quercus cercis* and *Quercus coccifera* was similar when incubated without PEG supplementation. On the other hand whereas the gas production (b_{gas}, (a+b)_{gas}) of leaves with PEG supplementation was significantly higher for *Quercus cercis* than that for *Quercus coccifera*.

Dry matter degradation

Dry matter disappearance and estimated parameters are given in Table 4. At all incubation times DM disappearance of *Quercus branti* was significantly higher than the others except at 3, 6 and 12 h incubation times when DM disappearance of *Quercus branti* was significantly higher than those of *Quercus cercis* and *Quercus coccifera*. All the

estimated parameters except rate of DM disappearance for *Quercus branti* were significantly higher than the others.

DISCUSSION

The chemical compositions of oak leaves were in consistent with that observed by Kamalak et al. (2004b). The tannins from different sources affected the rate and extent of gas production (Makkar et al., 1995). One of the possible reasons for this could be that tannin decrease the attachment of micro-organisms to feed particles (Makkar, 2003). It has been well known that tannins bind to PEG. PEG is also considered to break already formed tannin-protein complexes since its affinity for tannins is higher than for proteins (Makkar, 2003). The PEG supplementation significantly affected the gas production from oak leaves when incubated with rumen fluid. However, PEG supplementation even at the same concentration produced effects of different magnitudes in the gas production of oak leaves possibly due to differences in chemical composition of tannins in different oak species. Although the increase in

Table 5. Prediction of *in situ* dry matter disappearance from *in vitro* gas production of oak leaves incubated with rumen fluid in the presence (+PEG) or absence (-PEG)

IT	Treatment	Equation	R ²	RSD	Sig.
3	-PEG	IS _{3 h} = 12.8+0.620GAS _{3 h}	28.3	1.872	*
	+PEG	IS _{3 h} = 17.8+0.308 GAS _{3 h}	23.2	1.937	NS
6	-PEG	IS _{6 h} = 9.03+0.702GAS _{6 h}	68.9	1.386	***
	+PEG	IS _{6 h} = 6.78+0.652GAS _{6 h}	62.6	1.521	***
12	-PEG	IS _{12 h} = 16.2+0.531GAS _{12 h}	55.9	1.506	***
	+PEG	IS _{12 h} = 17.2+0.358GAS _{12 h}	44.5	1.692	**
24	-PEG	IS _{24 h} = 16.9+0.723GAS _{24 h}	59.0	1.271	***
	+PEG	IS _{24 h} = 31.9+0.358GAS _{24 h}	40.1	1.396	**
48	-PEG	IS _{48 h} = 5.7+1.01GAS _{48 h}	49.8	2.062	***
	+PEG	IS _{48 h} = 16.4+0.723GAS _{48 h}	57.9	2.010	***
72	-PEG	IS _{72 h} = 14.2+0.855GAS _{72 h}	73.9	1.379	***
	+PEG	IS _{72 h} = 15.0+0.749 GAS _{72 h}	83.4	1.090	***
96	-PEG	IS _{96 h} = 20.5+0.754GAS _{96 h}	73.2	1.309	***
	+PEG	IS _{96 h} = 26.6+0.585 GAS _{96 h}	77.9	1.224	***

IT: Incubation times, IS: *In situ*, RSD: Residual standard deviation, Sig.: Significance level, *** p<0.001, ** p<0.01, * p<0.05.

Table 6. Prediction the estimated parameters of *in situ* dry matter disappearance from estimated parameters of *in vitro* gas production of oak leave

Treatment	Equations	R ²	RDS	Sig.
-PEG	c _{is} = 0.0437-0.0269c _{gas}	1.4	0.005	NS
+PEG	c _{is} = 0.060-0.190 c _{gas}	32.8	0.003	NS
-PEG	a _{is} = 25.5-1.72a _{gas}	26.2	1.475	NS
+PEG	a _{is} = 20-0.172 a _{gas}	0.50	0.701	NS
-PEG	b _{is} = 10.89+0.692b _{gas}	46.8	1.834	**
+PEG	b _{is} = 1.24+0.831 b _{gas}	88.7	0.851	***
-PEG	EDMD = 3.67+0.879b _{gas}	86.1	0.877	***
+PEG	EDMD = 6.25+0.748 b _{gas}	88.7	0.851	***
-PEG	EDMD = 8.06+1.03(a+b) _{gas}	89.6	0.760	***
+PEG	EDMD = 5.95+0.704(a+b) _{gas}	79.5	1.070	***
-PEG	EDMD = -9.50+3.24c _{gas} +1.19a _{gas} +1.04b _{gas}	89.7	0.817	***
+PEG	EDMD = 7.60+23.1c _{gas} +0.200a _{gas} +0.670b _{gas}	83.4	1.041	***
-PEG	EDMD = -9.63+1.18a _{gas} +1.05b _{gas}	89.6	0.789	***
+PEG	EDMD = 5.83+0.118a _{gas} +0.747b _{gas}	82.9	1.014	***
-PEG	EDMD = 3.86+2.46c _{gas} +0.872b _{gas}	86.2	0.907	***
+PEG	EDMD = 7.70+16.3c _{gas} +0.695b _{gas}	83.0	1.010	***

IS: *In situ*, gas: gas production (ml), RSD: Residual Standard deviation, Sig: Significance level. *** p<0.001, ** p<0.01.

gas production at 96 h incubation time was 8.9 ml for *Quercus libani* the increase was 5.5 ml for *Quercus coccifera*. This result is consistent with findings of Getachew et al. (2001), Getachew et al. (2002), Seresinhe and Iben, (2003). Although the increase in the gas production of *Acacia cyanophylla* was 10.3 ml, the increase in the gas production of *C. calothyrsus* was 22 ml when it was incubated in the presence of PEG (77 mg).

The mechanism of dietary effects of tannins may be understood by their ability to form complex with proteins. Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin can adversely affect the microbial and enzyme activities (Singleton, 1981; Lohan et al., 1983; Barry and Duncon, 1984; Makkar et al., 1989). In tree leaves tannins are present in NDF and ADF fractions in certain amounts

which are tightly bound to the cell wall and cell proteins and seem to be involved in decreasing digestibility (Reed et al., 1990). PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes (Makkar et al., 1995). PEG also can also liberate protein from the preformed tannin-protein complexes (Barry and Manley, 1986).

The regression equations showing the relationship between *in situ* DM disappearance and *in vitro* gas production are given in Table 5.

Although at early incubation times the relationship between gas production and DM disappearance were low the correlation coefficients (r) increased with increasing incubation time. At 3 h incubation time gas production accounted for only 23.2-28.3% of variation of DM disappearance whereas *in vitro* gas production at 96 h incubation explained 73.2-77.9% of variation of *in situ* DM

disappearance. This may be explained by the DM loss but which is not available to microbial fermentation during early incubation time. This study has revealed a general problem of overestimation of degradability by *in situ* nylon bag technique. Overestimation is especially noticeable at short incubation times (Willman, 1996).

The regression equations showing the relationship between *in situ* and *in vitro* parameters are given in Table 6. There were no significant relationship between gas production rate and rate of DM disappearance or a_{is} and a_{gas} when oak leaves incubated with rumen fluid in both the presence and absence of PEG. On the other hand, the relationship between b_{is} and b_{gas} was considerably increased. Gas production from insoluble fraction (b_{gas}) accounted for 46.8% of the variation of insoluble fraction (b_{is}) when oak leaves were incubated with rumen fluid in the absence of PEG whereas gas production from insoluble fraction (b_{gas}) accounted for 88.7% of the variation of insoluble fraction (b_{is}) when oak leaves were incubated with rumen fluid in the presence of PEG.

The results obtained in this experiment are in consistent with those reported by Khazaal et al. (1993), Blümmel and Orskov (1993) and Sileshi et al. (1996) who observed a correlation between DM disappearance and gas production, but did not find significant correlation between rate of DM degradation (c_{is}) and rate of gas production (c_{gas}). On the other hand, Piva et al. (1988) and Beuvink et al. (1993) who worked with corn silage and did not obtain significant ($p>0.05$) correlation for the same parameters. Valentin (1999) suggested that the consistency of the conclusions drawn by different authors may be due to number factors e.g. methodology used, the substrates and the types of animal used, the number of measurements and mathematical model used. *In vitro* gas production method indirectly evaluates the DM degradation through determining the gas yield whereas *in situ* technique determines the DM loss during incubation in the rumen through microbial degradation.

In situ effective DM degradability of oak leaves was highly related ($p<0.001$) to potential gas production. As can be seen from Table 6 the gas production (b_{gas}) from insoluble fraction explained 86.1% of the variation of EDMD when oak leaves were incubated with rumen fluid in the absence of PEG. On the other hand, the gas production (b_{gas}) from insoluble fraction explained 88.7% of variation of EDMD when oak leaves were incubated with rumen fluid in the presence of PEG. The inclusion of the other estimated parameters such as c_{gas} , a_{gas} and $(a+b)_{gas}$ to regression equation increased the percentage of variation of EDMD expressed by gas production parameters.

The results obtained in this experiment are in agreement with those reported by Khazaal et al. (1993), Blümmel and Orskov (1993) and Sileshi et al. (1996) who observed a significant correlation between effective DM degradability

(EDMD) and potential gas production $(a+b)_{gas}$.

CONCLUSION

It was concluded that except at early incubation times the relationships between the two methodologies seem to be sufficiently strong to predict degradability parameters from gas production parameters obtained in the presence or absence of PEG. The increase in gas production parameters due to PEG supplementation *in vitro* suggest a negative effect of tannin on digestibility, and represent recovered feed nutrients that were bound by tannins.

REFERENCES

- Abdulrazak, S. A., T. Fujihara, J. K. Ondilek and E. R. Orskov. 2000. Nutritive evaluation of some Acacia tree leaves from Kenya. Anim. Feed Sci. Technol. 85:89-98.
- AOAC. 1990. Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists. Washington, DC. USA.
- Apori, S. O., F. B. Castro, W. J. Shand and E. R. Orskov. 1998. Chemical composition, in Sacco degradation and *in vitro* gas production of some browse plants. Anim. Feed Sci. Technol. 76:129-137.
- Barry, T. N. and S. J. Duncan. 1984. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. I. Voluntary intake. J.A.O.A.C. 65:496-497.
- Barry, T. N. and T. R. Manley. 1986. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. 4. Site of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. Br. J. Nutr. 55:123-137.
- Beuvink, J. M. W., H. de Visser and A. Klop. 1993. *In vitro* gas production kinetics of different maize products: A comparison to nylon bag degradation kinetics. In: (Ed. J. M. W. Beuvink) Measuring and modeling *in vitro* gas production kinetics to evaluate ruminal fermentations of Feedstuffs. DLO-Research Institute for livestock feeding and nutrition report. pp. 41-51. Lelystad, The Netherlands.
- Blümmel, M. and E. R. Orskov. 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.
- Canbolat, O., A. Kamalak, E. Ozkose, C. O. Ozkan, M. Sahin and P. Karabay. 2005. Effect of polyethylene glycol on *in vitro* gas production, metabolizable energy and organic matter digestibility of *Quercus cerris* leaves. Livestock Research for Rural Development. Volume 17, Retrieved, August 5, 2005, from <http://www.cipav.org.co/lrrd/lrrd17/4/canb17042.htm>.
- Dewhurst, R. J., D. Hepper and A. J. F. Webster. 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.
- Evitayani, L. W., A. Fariani, T. Ichinohe, S. A. Abdulrazak and T. Fujihara. 2004. Comparative rumen degradability of some legume forages between wet and dry season in west Sumatra, Indonesia. Asian-Aust. J. Anim. Sci. 17(8):1107-1111.
- Getachew, G., H. P. S. Makkar and K. Becker. 2001. Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial

- protein synthesis from tannin-containing browses. Anim. Feed Sci. Technol. 92:51-57.
- Getachew, G., H. P. S. Makkar and K. Becker. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. J. Agric. Sci. 139:341-352.
- Kamalak, A., O. Canbolat and Y. Gurbuz. 2004a. Comparison between *in situ* dry matter degradation and *in vitro* gas production of tannin containing leaves from four tree species. S. A. J. Anim. Sci. 34(4):233-240.
- Kamalak, A., O. Canbolat, O. Ozay and S. Aktas. 2004b. Nutritive value of oak (*Quercus spp.*) leaves. Small Rum. Res. 53(1-2):161-165.
- Kamalak, A., O. Canbolat, Y. Gurbuz and O. Ozay. 2005a. Prediction of dry matter intake and dry matter digestibilities of some forages using gas production techniques in sheep. Turkish J. Vet. Anim. Sci. 29:517-523.
- Kamalak, A., O. Canbolat, Y. Gurbuz and O. Ozay. 2005b. Comparison of *in vitro* gas production technique with *in situ* nylon bag technique to estimate dry matter degradation. Czech J. Anim. Sci. 50(2):60-67.
- Kamalak, A., O. Canbolat, Y. Gurbuz, O. Ozay and E. Ozkose. 2005c. Chemical composition and its relationship to *in vitro* gas production of several tannin containing trees and shrub leaves. Asian-Aust. J. Anim. Sci. 18(2):203-208.
- Khazaal, K., X. Markantonatos, A. Nastis and E. R. Orskov. 1993. Changes with maturity in fibre composition and levels of extractable polyphenols in Greek browse: effect on *in vitro* gas production and *in sacco* dry matter degradation. J. Sci. Food Agric. 63:237-244.
- Krishnamoorthy, H., H. Steingass and K. H. Menke. 1991. Preliminary observations on the relationships between gas production and microbial protein synthesis *in vitro*. Arch. Fur Tier. 41:521-526.
- Kumar, R. and M. Sing. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447-453.
- Lohan, O. P., D. Lall, J. Vaid and S. S. Negi. 1983. Utilization of oak tree fodder in cattle ration and fate of oak leaf tannins in the ruminant system. Ind. J. Anim. Sci. 53:1057-1063.
- Makkar, H. P. S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rum. Res. 49:241-256.
- Makkar, H. P. S., M. Blümmel and K. Becker. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and their implication in gas production and true digestibility *in vitro* techniques. Br. J. Nutr. 73:897-913.
- Makkar, H. P. S., B. Singh and S. S. Negi. 1989. Relationship of rumen degradability with microbial colonization, cell wall constituents and tannin levels in some tree leaves. Anim. Prod. 49:299-303.
- Mehrez, A. Z. and E. R. Orskov. 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. and H. Steingass. 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., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. The estimation of the digestibility and metabolisable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93:217-222.
- Orskov, E. R. and P. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. 92:499-503.
- Orskov, E. R. 1989. Recent advances in evaluation of roughages as feeds for ruminants. In: Advances in animal nutrition (Ed. D. J. Farrell), pp. 102-108. University of New England Printery, Armidale.
- Osuga, I. M., S. A. Abdulrazak, T. Ichinohe and T. Fujihara. 2005. Chemical composition, degradation characteristics and effects of tannin on digestibility of some brose species from Kenya harvested during wet season. Asian-Aust. J. Anim. Sci. 18(1):54-60.
- Pearse, E. S and H. O. Hartley. 1966. Biometrika tables for statisticians. Vol.1. pp. 1-270. Cambridge University Press. UK.
- Piva, G., E. Santi, S. Belladonna and O. Curto. 1988. Kinetics of *in vitro* fermentation of forages. J. Alim. Nutr. Herb. 28:101-102.
- Reed, J. D., H. Soller and A. Wood. 1990. Fodder tree and straw diets for sheep: intake, growth, digestibility and the effect of phenolics on nitrogen utilization. Anim. Feed Sci. Technol. 30: 39-50.
- Rubanza, C. D. K., M. N. Shem, R. Otsyina, T. Ichinohe and T. Fujihara. 2003. Nutritive evaluation of some browse tree legume foliages native to semi arid areas in western Tanzania. Asian-Aust. J. Anim. Sci. 16(10):1429-1437.
- SAS. 1989. SAS/STAT User's Guide, Version 6, 4th Edition, Vol. 2. p. 846. SAS Institute Inc., Cary, NC.
- Seresinhe, T. and C. Iben. 2003. *In vitro* quality assessment of two tropical shrub legumes in relation to their extractable tannins content. J. Anim. Phys. Anim. Nutr. 87:109-115.
- Sileshi, Z., E. Owen, M. S. Dhanona and M. K. Theodorou. 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.
- Singleton, V. L. 1981. Naturally occurring food toxicants: Phenolic substances of plant origin common in foods. Adv. Food Res. 27:149-242.
- Tolera, A., K. Khazaal and E. R. Orskov. 1997. Nutritive evaluation of some browses species. Anim. Feed Sci. Technol. 67:181-195.
- Valentin, S. F., P. E. V. Williams, J. M. Forbes and D. Sauvant. 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.
- Van Soest, P. J. 1963. The use of detergents in the analysis of fibre feeds. II. A rapid method of determination of fibre and lignin. J. AOAC International. 46:829-835.
- Willman, D., G. R. Foulkes and D. Given. 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.