Chemical Composition and Its Relationship to *In vitro* Gas Production of Several Tannin Containing Trees and Shrub Leaves

Adem Kamalak*, Onder Canbolat[1], Yavuz Gurbuz, Osman Ozay and Emin Ozkose

Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Nutrition, Kahrmanmaras, Turkey

ABSTRACT : The aim of this experiment was to determine the chemical composition of six commonly utilized fodder trees and shrub species in Turkey, namely *Glycrrhiza glabra* L, *Arbutus andrachne*, *Carpinus betilus*, *Juniperus communis*, *Quercus libari* L and *Pistica lentiscus* and its relationship with gas production and estimated parameters when incubated with rumen fluid *in vitro*. There were significant ($p<0.001$) differences between leaves in terms of crude protein (CP), acid detergent fiber (ADF), total condensed tannin (TCT), bound condensed tannin (BCT) and soluble condensed tannin (SCT). Crude protein contents ranged from 5.74% (*Juniperus communis*) to 12.59% (*Glycrrhiza glabra* L). Acid detergent fiber contents ranged from 25.14% (*Glycrrhiza glabra* L) to 39.23% (*Juniperus communis*). Total condensed tannin (TCT) contents of leaves ranged from 4.34% (*Quercus libari* L) to 20.34% (*Juniperus communis*). Acid detergent fiber (ADF) and total condensed tannin contents of leaves were negatively correlated with gas productions and some estimated parameters. Potential gas productions (*A*) of *Glycrrhiza glabra* L, *Arbutus andrachne*, *Quercus libari* L and *Pistica lentiscus* were significantly ($p<0.001$) higher than those of *Carpinus betilus* and *Juniperus communis* whereas gas production rate (μ_{24}) of *Pistica lentiscus* was significantly ($p<0.001$) higher than the others. Time (h) to produce 50% of total gas pool size (T_{50}) of *Juniperus communis* was significantly ($p<0.001$) lower than that of *Carpinus betilus* whereas time (h) to produce 90% of total gas pool size (T_{90}) of *Juniperus communis* was significantly lower than the others except for *Pistica lentiscus*. The metabolizable energy (ME) contents of leaves ranged from 8.86 to 10.39 MJ kg^{-1} DM. The results obtained in this study suggested that browse species had a significant effect on chemical composition, gas production and estimated parameters of leaves. Leaves from *Glycrrhiza glabra* L with a considerable amount of CP had a high rank value in terms of ME. Therefore leaves from *Glycrrhiza glabra* L may have a high potential value for small ruminant animals in terms of rumen and whole digestibility. Leaves from other species studied require protein supplementation when they are the only feed consumed by ruminant animals. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2 : 203-208)***

Key Words : Tree Leaves, Chemical Composition, Condensed Tannin, Gas Production, Metabolizable Energy

INTRODUCTION

Inadequate nutrients become a major constraint to generating more income from ruminants due to low level of energy intake (Jones, 1988). Trees and shrubs have been used for generations as multipurpose resources in many parts of world (Smith, 1992). Tree leaves are an important component of goats and sheep (Holechek, 1984; Papachristou and Nastis, 1996) and play an important role in the nutrition of grazing animals in areas where few or no alternatives are available (Meuret et al., 1990). However the use of tree and shrub leaves by herbivores are restricted by defending or deterring mechanisms related to high tannin content (Provenza, 1995; Rubanza et al., 2003; Bakshi et al., 2004).

Although tree and shrub leaves are an important source of forage for small ruminants in most parts of Turkey during the critical periods of year when quality and quantity of pasture herbages are limited, there is little information on the nutritive value of tree and shrub leaves.

The aim of this experiment was to determine the chemical composition including condensed tannin content and its relationship with *in vitro* gas production of leaves from some trees and shrubs widely used for small ruminant animals in Turkey.

* Corresponding Author: Adem Kamalak. Tel: +90-344-2237666 (324), Fax: +90-344-2230048, E-mail: akamalak@ksu.edu.tr

[1] Bursa Uludag University, Faculty of Agriculture, Department of Animal Nutrition, Bursa, Turkey.

Received March 4, 2004; Accepted August 3, 2004

MATERIALS AND METHOD

Leaves

Leaves from a shrub and five trees; *Glycrrhiza glabra* L, *Arbutus andrachne*, *Carpinus betilus*, *Juniperus communis*, *Quercus libari* L and *Pistica lentiscus*, were harvested in dry period (August, September and October) in 2003 from the city of Kahramanmaras, in southern Turkey. The area is located at an altitude of 630 m above sea level. The mean annual rainfall and temperature were 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 for 48 h (Abdulrazak et al., 2000).

Chemical analysis

All chemical analyses were carried out in triplicate. Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in a muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude

Table 1. Chemical composition (%) of shrub and tree leaves

Species	Nutrients (%)					
	DM	CP	ADF	TCT	BCT	SCT
Glycrrhiza glabra L	92.91	12.59^{d}	25.15^{a}	12.66^{b}	8.55^{b}	4.10^{ab}
Arbutus andrachne	95.93	7.25^{ab}	31.97^{bc}	12.04^{b}	3.00^{a}	9.30^{bc}
Carpinus betilus	94.82	10.73^{cd}	33.25^{bc}	19.70^{c}	17.00^{c}	2.69^{ab}
Juniperus communis	95.64	5.74^{a}	39.23^{d}	20.34^{c}	3.87^{a}	16.46^{d}
Quercus libari L	95.23	8.91^{bc}	27.19^{ab}	4.34^{a}	1.60^{a}	2.32^{a}
Pistica lentiscus	95.64	9.50^{bc}	30.58^{abc}	15.68^{bc}	2.35^{a}	13.09^{cd}
SEM	0.814	0.548	1.159	1.320	0.919	1.443
Significance level	NS	***	***	***	***	***

Means within the same column with differing superscripts are significantly different.
SEM: Standard error mean. *** $p<0.001$.
NS: non-significant. DM: dry matter, CP: crude protein. ADF: acid detergent fiber.
TCT: total condensed tannin. BCT: bound condensed tannin, SCT: soluble condensed tannin.

protein was calculated as N×6.25. ADF content of leaves was determined using the method described by Van Soest (1963).

Total condensed tannin (TCT), bound condensed tannin (BCT) and soluble condensed tannin (SCT) were determined by the butanol-HCl method as described by Makkar et al. (1995). Mimosa tannin (MT; Hodgson, England) was used as an external standard.

***In vitro* gas production**

Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet containing tumbleweeds hay (60%) and concentrate (40%). The samples were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Dry sample (0.200 g) 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. 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. Cumulative gas production data were fitted to the model of France et al. (1993) using the MLP (Most Likelihood Program), (Ross, 1987):

$$y = A\{1-\exp[-b(t-T)-c(\sqrt{t}-\sqrt{T})]\}$$

where y represents the cumulative gas production (ml), t the incubation time (h), A the asymptote (total gas, ml) T the lag time (h), b and c are the rate constants (h^{-1}) and ($h^{-1/2}$). Estimated values of four parameters, A, T, b and c were determined from time course experiment of 96 h incubation.

The model postulates that the fractional degradation rate (μ, h^{-1}) is not constant, but it varies with time along the fermentation period:

$$\mu = b+c/(2\sqrt{t});\ t\geq T$$

Metabolizable energy (MJ/kg DM) contents of forages were calculated using equations of Menke et al. (1979) as follows:

$$\text{ME (MJ/kg DM)} = 2.20+0.136\ \text{GP}+0.057\ \text{CP}$$
$$R^2 = 0.94$$

GP is 24 h net gas production (ml/200 mg).

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to compare chemical composition, *in vitro* gas production and estimated parameters with species as the main factor using General Linear Model (GLM) of Statistica for windows (1993). Significance between individual means was identified using the 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. A simple correlation analysis was used to establish the relationship between chemical composition and gas production or estimated parameters.

RESULT AND DISCUSSION

Chemical composition

Chemical compositions of shrub and tree leaves used in this experiment were given in Table 1. Generally there were considerable variations between the tree and shrub leaves in terms of chemical compositions. Crude protein contents of leaves ranged from 5.74% in *Juniperus communis* to 12.59% in *Glycrrhiza glabra* L. Feedstuffs containing less than 8% crude protein can not provide the minimum ammonia levels required by rumen microorganisms to

Table 2. Gas production (ml) of different silages when incubated with rumen buffered liquid *in vitro*

Species	Incubation time (h)						
	3	6	12	24	48	72	96
Glycrrhiza glabra L	21.83^{c}	35.17^{c}	45.22^{c}	55.00^{c}	64.89^{c}	69.94^{c}	73.55^{b}
Arbutus andrachne	20.28^{cd}	30.72^{bc}	41.89^{bc}	52.89^{bc}	59.89^{b}	65.25^{abc}	69.83^{b}
Carpinus betilus	15.39^{ab}	26.61^{ab}	36.56^{a}	45.44^{a}	55.06^{a}	60.05^{a}	62.33^{a}
Juniperus communis	15.06^{a}	25.00^{a}	36.28^{a}	46.56^{a}	56.50^{ab}	61.41^{ab}	61.84^{a}
Quercus libari L	19.22^{bcd}	30.44^{b}	38.56^{ab}	50.95^{b}	59.00^{ab}	66.67^{bc}	70.05^{b}
Pistica lentiscus	17.95^{abc}	29.72^{b}	42.72^{bc}	55.00^{c}	65.44^{c}	69.89^{c}	72.33^{b}
SEM	0.812	0.940	1.072	0.715	0.843	1.116	1.137
Significance level	***	***	***	***	***	***	***

Means within the same row with differing superscripts are significantly different.
SEM: standard error mean. *** $p<0.001$.

support optimum activity (Norton, 2003).

The CP contents of leaves of *Arbutus andrachne* and *Juniperus communis* were lower than that required by micro-organisms in the rumen to support optimum activity. Therefore these leaves require the protein supplementation when they are the only feed consumed by ruminant animals. Acid detergent fiber contents ranged from 25.14% in *Glycrrhiza glabra* L to 39.23% in *Juniperus communis*. Ash contents of leaves ranged from 4.76% in *Quercus libari* L to 7.89% in *Glycrrhiza glabra* L. Total condensed tannin, BCT and SCT contents of trees were given in Table 1. There were wide variation between tree and shrub leaves in terms of TCT, BCT and SCT. Total condensed tannin contents of leaves ranged from 4.34% in *Quercus libari* L to 20.34% in *Juniperus communis*. The TCT content of *Juniperus communis* except for *Carpinus betilus* and *Pistica lentiscus* was significantly ($p<0.001$) higher than the other tree leaves. Bound condensed tannin content of *Carpinus betilus* was significantly ($p<0.001$) higher than the others whereas SCT content of *Juniperus communis* except for *Pistica lentiscus* was significantly ($p<0.001$) higher than the other leaves.

Average chemical compositions of the studied samples fitted well with published literature, even considering differences in location and growth conditions. The chemical composition of *Pistacia lentiscus was* similar to that reported by Decandia et al. (2000). The chemical composition of *Carpinus betilus* was similar to that of *Carpinus orientalis* M reported by Papachristou (1996). Total condensed tannin content of *Pistacia lentiscus* was lower than those reported by Decandia et al. (2000) and Silanikove et al. (1996). They found that TCT contents of *Pistacia lentiscus* were 21.7% and 20.5% respectively. A possible reason could be the differences in the methods and climatic conditions where leaves grow. Quebracho condensed tannin was used as an external standard in the experiment carried out by Silanikove et al. (1996) whereas Mimosa tannin was used in the current experiment. The external standard has a significant effect on the tannin quantification (Schofield et al., 2001). The TCT content of *Quercus libari* L was similar to that of *Quercus semecarpifolia* reported by Singh et al. (1998).

Total condensed tannin contents of forages in the range of 60-100 g/kg^{-1} DM depresses intake and growth of animals (Barry et al., 1984). The tannin content of *Quercus libari* L obtained in this experiment fell towards the lower end of this range whereas TCT contents of leaves of *Glycrrhiza glabra* L, *Arbutus andrachne*, *Carpinus betilus*, *Juniperus communis* and *Pistacia lentiscus* L higher than the upper level of this range. Therefore, supplementation of polyetyhylene glycol (PEG) can be recommended to reduce the detrimental effect of tannin in leaves. Pritchard et al. (1988) showed that feeding of PEG to sheep fed mulga markedly increased feed intake, weight gain and wool growth.

Gas production and estimated parameters

Data of gas production during the fermentation period were given in Table 2. The cumulative volume of gas production increased with increasing time of incubation. Gas produced after 96 h incubation ranged between 61.84 and 73.55 ml per 0.200 g of substrate.

At 3 h incubation the cumulative gas production of *Glycrrhiza glabra* L was significantly ($p<0.001$) higher than those of *Carpinus betilus*, *Juniperus communis* and *Pistica lentiscus* whereas the cumulative gas production of *Glycrrhiza glabra* L at 6 h incubation was significantly ($p<0.001$) higher than the others except *Arbutus andrachne*.

Although at 12 and 24 h incubation cumulative gas productions of *Glycrrhiza glabra* L were significantly ($p<0.001$) higher than those of *Carpinus betilus*, *Juniperus communis* and *Quercus libari* L at 72 and 96 h incubation cumulative gas productions of *Glycrrhiza glabra* L were significantly ($p<0.001$) higher than those of *Carpinus betilus* and *Juniperus communis*.

As can be seen from Table 2, there were significant differences ($p<0.001$) between leaves in term of gas production at different incubation times possibly due to the amount of substrate fermented and volatile fatty acid production during fermentation. Gas production is associated with volatile fatty acid (VFA) production following fermentation of substrate so the more fermentation of a substrate the greater the gas production,

Table 3. Estimated parameters of different leaves when incubated with rumen buffered liquid *in vitro*

Leaves	Estimated parameters				
	A	μ_{24}	T_{50}	T_{90}	ME
Glycrrhiza glabra L	75.19^{b}	0.031^{a}	8.73^{ab}	91.49^{d}	10.39^{d}
Arbutus andrachne	73.65^{b}	0.033^{ab}	8.61^{ab}	89.62^{d}	9.80^{bc}
Carpinus betilus	59.59^{a}	0.036^{bc}	9.57^{b}	69.93^{bc}	8.99^{a}
Juniperus communis	60.13^{a}	0.035^{bc}	7.72^{a}	48.87^{a}	8.86^{a}
Quercus libari L	71.89^{b}	0.037^{c}	9.63^{b}	84.29^{cd}	9.63^{b}
Pistica lentiscus	71.78^{b}	0.056^{d}	9.36^{b}	54.20^{a}	10.22^{cd}
SEM	0.795	0.001	0.236	4.067	0.097
Significance level	***	***	***	***	***

Means within the same column with differing superscripts are significantly different.

A: total gas production, μ_{24}: gas production rate at 24 h, T_{50}: time (h) to produce 50% of total gas pool size.

T_{90}: time (h) to produce 90% of total gas pool size, ME: metabolizable energy, *** $p<0.001$.

Table 4. Correlation coefficient (r) of the relationship between the chemical composition and gas production from the shrub and tree leaves

Nutrients	Incubation time (h)						
	3	6	12	24	48	72	96
DM	-0.416	-0.456	-0.353	-0.238	-0.325	-0.395	-0.359
Ash	0.141	0.124	0.302	0.119	0.266	0.047	0.103
CP	0.445	0.650**	0.475*	0.361	0.437	0.459*	0.484*
ADF	-0.768***	-0.775***	-0.663**	-0.638**	-0.610**	-0.669**	-0.747***
TCT	-0.551*	-0.507*	-0.274	-0.468*	-0.243	-0.455	-0.555*
BCT	-0.264	-0.114	-0.240	-0.469*	-0.376	-0.426	-0.392
SCT	-0.304	-0.398	-0.044	-0.017	0.109	-0.060	-0.189

*** $p<0.001$, ** $p<0.01$, * $p<0.05$.

although the fermentation end products do influence more closely with gas production (Blummel and Orskov, 1993). Differences between total gas productions could be explained by the differences in total VFA production and molar proportion of VFA (Bevunik and Spoelstra, 1992). Doane et al. (1997) found a significant correlation between gas production and VFA production.

Data of the estimated parameters were given Table 3. There were significant ($p<0.001$) differences between tree species in terms of the estimated parameters. The lag times for all leaves were very low and very close to zero. Therefore, lag times were ignored and not included in Table 3. Potential gas production (A) of *Glycrrhiza glabra* L, *Arbutus andrachne*, *Quercus libari* L and *Pistica lentiscus* were significantly ($p<0.001$) higher than those of *Carpinus betilus* whereas gas production rate (μ_{24}) of *Pistica lentiscus* was significantly ($p<0.001$) higher than the others.

Time (h) to produce 50% of total gas pool size (T_{50}) of *Juniperus communis* was significantly ($p<0.001$) lower than that of *Carpinus betilus* whereas time (h) to produce 90% of total gas pool size (T_{90}) of *Juniperus communis* was significantly lower than the others except *Pistica lentiscus*. Time (h) to produce 50% of total gas pool size and T_{90} are very important digestibility parameters. A substrate which degraded more effectively results in a shorter time to produce 50 and 90% of maximum gas production. It will remain in the rumen for a shorter period and supply more nutrients to the animals. The maximum gas production (A) must be taken into consideration when the samples were compared in terms of T_{50} and T_{90}. Low total gas production (A) may result in the lower T_{50} and T_{90} values as in *Carpinus betilus* and *Juniperus communis*.

There were also significant ($p<0.001$) differences between leaves in terms of ME values. The ME value of *Glycrrhiza glabra* L was significantly higher than the other except for *Pistica lentiscus*.

The ME contents of tree and shrub leaves studied in this experiment were comparable to *Machilus gamlei, Garuga pinnata, Ficus nemoalis, Litsea polyantha, Terminalia alata* and *Bredelia retusa* reported by Khazaal et al. (2001) but the ME contents of both tree and shrub leaves studied in this experiment were lower than those of *Acacia brevispica, Acacia nubica* and *Acacia mellifera* reported by Abdulrazak et al. (2000) although the gas productions (ml/200 mg) of acacia leaves were considerably low when compared with gas production obtained in this experiment. The protein contents ranged from 21.3% in *Acacia brevispica* to 19.4% in *Acacia mellifera*. Gas productions at 24 h ranged from 19.5 ml in *Acacia brevispica* to 21.6 ml in *Acacia mellifera*. Therefore high protein contents of Acacia tree leaves are partly responsible for the high ME contents of Acacia tree leaves.

Correlation coefficients (r) of the relationship between the chemical composition and the gas production from the shrub and tree leaves were given in Table 4. There were significant ($p<0.001$) negative correlations between ADF content and gas production at all incubation times. This result is consistent with Abdulrazak et al. (2000). Negative

Table 5. Correlation coefficient (r) of the relationship between the chemical composition and the estimated parameters of gas production from the shrub and tree leaves

Nutrients (%)	Estimated parameters				
	A	μ_{24}	T_{50}	T_{90}	ME
DM	-0.207	0.254	-0.050	-0.297	-0.335
Ash	0.110	0.068	0.053	0.089	0.234
CP	0.331	-0.078	0.462	0.406	0.530*
ADF	-0.758***	0.026	-0.488*	0.700***	-0.739***
TCT	-0.651**	0.096	-0.351	-0.613**	-0.454*
BCT	-0.484*	-0.298	0.277	0.093	-0.312
SCT	-0.188	0.326	-0.644**	-0.698***	-0.155

A: total gas production, μ_{24}= gas production rate at 24 h, T_{50}: time (h) to produce 50% of total gas pool size.
T_{90}: time (h) to produce 90% of total gas pool size, ME: metabolizable energy.
*** p<0.001, ** p<0.01, * p<0.05.

correlation between ADF and gas production may be a result of reduction of microbial activity, from increasingly adverse environmental conditions as incubation times progress.

There were also significant correlations ($p<0.05$) between TCT and gas production at all incubation times except 12, 48 and 72 h incubations. This result is not in agreement with findings of Abdulrazak et al. (2000) but in agreement with findings of Khazaal and Orskov (1994) and Tolera et al. (1997). A possible reason could be the differences in nature of tannins between the browse species (Jackson et al., 1996). The findings in this experiment supported the fact that the anti-nutritive factors like tannins may also contribute to reduction of microbial activity.

Correlation coefficients (r) of the relationship between the chemical composition and the estimated parameters of gas production from leaves were given in Table 5. There were significant correlations between ADF content and estimated parameters except gas production rate at 24 h incubation (μ_{24}). Total condensed tannin contents of leaves were well correlated with the estimated parameters except μ_{24} and T_{50}. Bound condensed tannin contents were negatively ($p<0.05$) correlated with maximum gas production (A) whereas SCT contents were negatively correlated with T_{50} ($p<0.01$) and T_{90} ($p<0.001$).

Microbial activity was negatively affected by the BCT content of leaves possibly due to reduced organic matter available for micro-organisms. Therefore maximum gas production (A) decreased when the BCT contents of leaves increased. On the other hand, time (h) to produce 50% and 90% of total gas production decreased with increased SCT contents of leaves.

IMPLICATIONS

The results obtained in this study suggested that browse species had a significant effect on chemical composition, gas production and estimated parameters of leaves. The ADF and total condensed tannin in leaves were negatively correlated with gas production and some estimated parameters.

Leaves from *Glycrrhiza glabra* L with a considerable amount of CP had a high rank value in terms of ME. Therefore leaves from *Glycrrhiza glabra* L may have a high potential value for small ruminant animals in terms of rumen and whole digestibility. Leaves, except from *Glycrrhiza glabra* L, require the protein supplementation when they are the only feed consumed by ruminant animals.

REFERENCES

Abdulrazak, S. A., T. Fujihara, J. K. Ondiek and E. Orskov. 2000. Nutritive evaluation of some Acacia tree leaves from Kenya. Anim. Feed Sci. Technol. 85:89-98.

AOAC. 1990. Official Method of Analysis. 15th. edn. Association of Official Analytical Chemist, Washington, DC. USA.

Bakshi, M. P. S and M. Wadhwa. 2004. Evaluation of forest tree leaves of semi-hilly arid region as livestock feed. Asian-Aust. J. Anim. Sci. 17:777-783.

Barry, T. N., T. R. Manley and J. S. Duncan. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep intake. Br. J. Nutr. 51:485-491.

Beuvink, J. M. W. and S. F. Spoelstra. 1992. Interactions between substrate, fermentation end products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen micro-organisms *in vitro*. Appl. Microbiol. Biotechnol. 37:505-509.

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

Decandia, M., M. Sitzia, A. Cabiddu, D. Kababya and G. Molle. 2000. The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in goats fed woody species. Small Rum. Res. 38:157-164.

Doane, P. H., P. Schofield and A. N. Pell. 1997. Neutral detergent fibre disappearance, gas and volatile fatty acids production during the *in vitro* fermentation of six forages. J. Anim. Sci. 75:3342-3352.

France, J., M. S. Daharona, M. K. Theodorou, S. J. Lister, D. R. Davies and D. Isaac. 1993. Model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feed. J. Theo. Bio. 163:99-111.

Holecheck, J. L. 1984. Comparative contribution of grasses, forbs, and shrubs to the nutrition range ungulates. Rangelands. 6:261-263.

Jackson, F. S., T. N. Barry, C. Lascona and B. Palmer. 1996. The extractable and bound condensed tannin content of leaves from tropical tree. J. Sci. Food Agric. 71:103-110.

Jones, R. J. 1988. The future of the grazing herbivore. Harry Stobbbs memorial lecture for 1987. Trop. Grassland. 22:97-115.

Khazaal, K and E. R. Orskov. 1994. The *in vitro* gas production technique: an investigation on its potential use with insoluble polyvinylpolypyrrolidone for the assessment of phenolics-related antinutritive factors in browse species. Anim. Feed Sci. Technol. 47:305-320.

Khazaal, R. C. and D. B. Subba. 2001. Nutritional evaluation of leaves from some major fodder trees cultivated in the hills of Nepal. Anim. Feed Sci. Technol. 92:17-32.

Makkar, H. P. S., M. Blümmel and K. Becker. 1995. Formation of complexes between polyvinily pyrorolidones or polyethylene glycols and their implication in gas production and true digestibility *in vitro* techniques. Br. J. Nutr. 73:897-913.

Menke, K. H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they incubated with rumen liquor *in vitro*. J. Agric. Sci. Camb. 92:217-222.

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.

Meuret, M., J. Boza, N. Narjisse and A. Nastis. 1990. Evaluation and utilization of rangeland feeds by goats. In: Goat Nutrition (Ed. P. Morand Fehr). Wageningen, The Netherlands, pp. 161-170.

Norton, B. W. 2003. The Nutritive value of tree legumes. In: Forage Tree Legumes in Tropical Agriculture (Ed. R. C. Gutteridge and H. M. Shelton). http://www.fao.org/ ag/AGP/ AGPC /doc/Publicat/Gutt-shel/x5556e0j.htm.

Papachristou, T. G. and A. S. Nastis. 1996. Influence of deciduas broadleaved woody species in goat nutrition during the dry season in Northern Greece. Small Rum. Res. 20:15-22.

Papachristou, T. G. 1996. Intake, digestibility and nutrient utilization of oriental hornbeam and manna ash browse by goats and sheep. Small Rum. Res. 23:91-98.

Pearse, E. S and H. O. Hartley. 1966. Biometrika tables for statisticians. Camb. University Press. UK. Vol. 1:1-270.

Pritchard, D. A., D. C. Stocks, B. M. O'Sullivan, P. R. Martin, I. S. Hurwood and P. K. O'Rourke. 1988. The effect of polyethylene glycol (PEG) on wool growth and live weight of sheep consuming a mulga (Acacia aneura) diet. Proc. Austr. Soc. Anim. Prod. 17:290-293.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food selection and intake in ruminants. J. Range Manage. 48:2-17.

Ross, G. J. S. 1987. Maximum likelihood program. Rothamstad Experimental Station. Harpenden, UK.

Rubanza, C. D., M. N. Shem, R. Otsyina, T. Ichinohe and T. Fujihara. 2003. Nutritive evaluation of some browse tree legumes foliages native to semi arid areas in western Tanzania. Asian-Aust. J. Anim. Sci.16:1429-1437.

Schofield, P., D. M. Mbugua and A. N. Pell. 2001. Analysis of condensed tannins: A Review. Anim. Feed Sci. Technol. 91:21-40.

Silanikove, N., N. Gilboa, A. Perevolotsky and Z. Nitsan. 1996. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Rum. Res. 21:195-201.

Singh, P., A. K. Verma, N. N. Pathak and J. Biswas. 1998. Nutritive value of oak (*Quercus semecarpifolia*) leaves in Pashmina kids. Anim. Feed Sci. Technol. 72:183-187.

Smith, O. B. 1992. Fodder trees and fodder shrubs in range and farming systems in tropical humid Africa. In: Legume trees and other fodder trees as protein sources for livestock (Ed. A. Speedy and P. L. Pugliese). FAO Animal Production and Health Paper 102:43-60.

Statistica. 1993. Statistica for windows release 4.3, StatSoft, Inc. Tulsa, OK.

Tolera, A., K. Khazaal and E. R. Orskov. 1997. Nutritive evaluation of some browses species. Anim. Feed Sci. Technol. 67:181-195.

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. 46:829-835.