Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value

Frutos, P.[1]; Hervás, G.; Ramos, G.; Giráldez, F.J. and Mantecón, A.R.
Estación Agrícola Experimental. CSIC. Apdo 788. 24080-León (Spain)

[1] Corresponding author. Fax: + 34 987 317161; e-mail: p.frutos@eae.csic.es

Abstract

The condensed tannin (CT) content of eight shrub species (*Cytisus purgans, Cytisus scoparius, Genista florida, Genista occidentalis, Calluna vulgaris, Erica arborea, Erica australis* and *Juniperus communis*) from a grazing mountain area of northern Spain was analysed and related to various indicators of nutritive value. Chemical composition, *in vitro* gas production, OM degradation and true DM digestibility were evaluated in samples collected in January and June. With the exception of two samples comprised of considerable amounts of flowers, the shrub legumes examined had low contents of CT (less than 6.51 g quebracho tannin equivalents/kg DM) which would generally be considered unlikely to affect digestion of nutrients in the gastrointestinal tract of animals. However, *Ericaceae* species and *J. communis*, which are evergreen species, showed a high CT content (higher than 176 g quebracho tannin equivalents/kg DM) throughout the year. CT were negatively correlated ($P<0.05$) with OM degradation and cumulative gas production, and positively correlated with lag time, which is consistent with the extensively reported suppressive effect of condensed tannins on rumen degradation and on the interference of these compounds with microbial attachment to feeds. The positive correlation between these plant secondary compounds and the partitioning factor (OM degradation/total gas production) indicates that the effect of CT is more strongly reflected in the reduction of gas production than in the reduction of OM degradation.

Keywords

Browse species, condensed tannins, *in vitro* degradation, gas production

1.- Introduction

The hill areas of northern Spain have traditionally been used to graze sheep, goats, cattle and horses in a transhumance pastoral system (i.e. seasonal movement of livestock to another region), which is being increasingly supported by the European Union (Zorita, 1994; Frutos et al., 1998). Pasture production in these mountain areas is restricted by low temperatures in winter and by high temperatures and lack of rainfall in summer (Alonso, 1994). Under such conditions, animals consume significant amounts of shrubs to meet their nutrient requirements.

Despite their potential as feeds, most shrubs also contain large amounts of tannins, which have most likely been evolved by plants as a defence mechanism against being eaten by herbivores. Tannins are conventionally classified into hydrolyzable and condensed tannins (proanthocyanidins; CT), the latter being more widely distributed in nature (McLeod, 1974). Recent studies on CT (Aerts et al., 1999; Barry and McNabb, 1999) point out the importance of considering their dosage-dependent effect. Thus, while species containing moderate concentrations of CT (2 to 4% of DM) can exert beneficial effects on protein metabolism in ruminants, decreasing the rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine of the animal, high dietary CT concentrations (6 to 12% of DM) can depress voluntary feed intake, digestive efficiency and animal productivity.

The aim of the present experiment was to analyse the CT content of several shrubs from a grazing mountain area of northern Spain and examine its relationship with traditional indicators of the nutritive value of those shrubs (i.e., chemical composition and *in vitro* rumen degradation and true digestibility).

2. Materials and Methods

2.1. Shrub species and preparation

New shoots of less than 6.0 mm in diameter were harvested from eight shrub species in 1998. They were collected first in January and then again in June to cover a wide range of phenological states of the plants at the moment of harvest. The eight species, according to families, were as follows:

LEGUMINOSEAE:	*Cytisus purgans*
	Cytisus scoparius
	Genista florida
	Genista occidentalis
ERICACEAE	*Calluna vulgaris*
	Erica arborea
	Erica australis
CUPRESACEAE	*Juniperus communis*

From the January harvest, winter-dormant shoots did not contain leaves in samples from *C. purgans* and *C. scoparius*. Shoots sampled from *Genista* spp. included some green leaves. *C. vulgaris*, *E. arborea*, *E. australis* and *J. communis* are evergreen forms.

In the June harvest, all samples comprised the current season's shoots. Samples collected from *C. scoparius* and *G. florida* consisted of flowering shoots and samples from *G. occidentalis* and *J. communis* of very young growing shoots. Samples from *C. purgans* and *E. australis* contained some flowers and some fruits and that from *E. arborea* some flowers.

Samples were collected from several shrubs of each species (≈ 200 g), then pooled and freeze-dried at -30ºC. Dried samples were then ground to pass a 1 mm screen and stored until required for chemical analysis and *in vitro* incubations.

2.2. Site

Samples were collected from different locations at "La Liviada" (Latitude 43º 04' 10'', longitude 5º 21' 10''), an area of about 100 ha, at a mean altitude of 1500 m, in the north of the province of León (Spain). The zone has a typical climate of a mountainous area with Atlantic influences.

2.3. Chemical analysis

Procedures described by AOAC (1999) were used to determine ash (AOAC official method 942.05) and Kjeldahl nitrogen (N; AOAC official method 976.06). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) were determined by the method of Goering and Van Soest (1970), adding sodium sulphite to the solution. NDF was assayed without alpha amylase and expressed with residual ash.

Condensed tannins fractions (free, protein-bound and fibre-bound) were sequentially extracted, in duplicate, following the method of Terrill et al. (1992), with modifications described by Perez-Maldonado and Norton (1996). Analyses of the free, protein-bound and fibre-bound CT were conducted, in triplicate, according to the butanol-HCl method of Porter et al. (1986) with modifications of Terrill et al. (1992), using purified CT from quebracho as the reference standard. In the results, CT contents are therefore reported in units of quebracho tannins equivalents (QT equivalents). The purification was performed with Sephadex LH-20 as described by Asquith and Butler (1985) with modifications by Hagerman (1991).

2.4. <u>In vitro</u> gas production and OM degradation

In vitro gas production characteristics of the shrubs were studied using a modification of the gas production technique described by Theodorou et al. (1994) and modified by Mauricio et al. (1999).

Three replicates of each substrate (≈ 0.5 g milled to pass a 1 mm screen) were incubated in 125 ml serum flasks at 39ºC with 10 ml strained rumen fluid and 40 ml medium (1:4, v:v), in order to determine the rate and extent of gas production (at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, 96 and 120 h) as well as substrate disappearance at 120 h. The rumen fluid inoculum was obtained before the morning feeding from three ruminal-cannulated Merino ewes (mean live weight 52.2 kg ± 1.57), fitted with ruminal cannulae and fed grass hay, in two equal meals, at 1.2 times their estimated maintenance energy requirements (AFRC, 1993).

Pressure values, corrected for the quantity of substrate organic matter (OM) incubated and gas released from controls (i.e., rumen fluid plus buffer medium, without substrate; 9 flasks in total), were used to generate gas volume estimates using a predictive equation derived from earlier simultaneous pressure and volume measurements:

$$V\ (ml) = 5.3407 \times P\ (psi)$$

where, V represents head-space gas volume (ml) and P pressure transducer reading (psi) ($n = 17{,}790$; $r^2 = 0.999$; $P < 0.001$).

Substrate disappearance after 120 h incubation was estimated by filtering residues using sintered glass crucibles. Organic matter degradation (OMD) was determined by ashing at 550ºC for 6 h.

2.5. In vitro true digestibility

A modification of the Tilley-Terry method (Tilley and Terry, 1963) with NDF as the final digestion step instead of pepsin was used to estimate *in vitro* true digestibility (TD; Robinson et al., 1999).

Duplicate samples of ≈ 0.5 g DM from each shrub species were weighed into Ankom filter bags and incubated in a Daisy^{II} incubator (Ankom Technology, Fairport, NY, USA) in a combination of buffer solution (80%) and pre-feeding rumen inoculum (20%). After incubation for 48 h, bags were treated with a neutral detergent solution using an Ankom^{200} fibre analyser (Ankom Technology, Fairport, NY, USA). They were then washed, dried and weighed to determine DM losses.

2.6. Calculations and statistical analysis

Gas production data were fitted to the model of France et al (1993) to provide parameters describing cumulative gas release in terms of potential gas production (*A*, ml/g OM), lag time (*L*, h), time to half-asymptote (*T/2*, h) and fractional rate of gas production at *T/2* (μ, h^{-1}).

An estimate of the efficiency of fermentation (partitioning factor; PF) was calculated by relating OM degradation to total gas production at 120 h (i.e., OM disappearance/total gas production; Blümmel et al., 1997).

Gas production data were fitted using the DUD method for interactive, nonlinear regressions using the SAS package. ANOVA and correlation analysis were also conducted using the procedures of the Statistical Analysis Systems (SAS, 1989).

3.- Results

Chemical composition of the eight shrub species is shown in Table 1 and condensed tannin content in Table 2. There was a wide range of CT contents for shrubs sampled both in January and June. The shrub legumes examined had low contents of CT (less than 6.51 g QT equivalents/kg DM), with the exception of samples collected in June from *C. scoparius* and *G. florida*. However, *Ericaceae* species and *J. communis* showed a high CT content (higher than 176 g QT equivalents/kg DM) throughout the year. Samples

collected in January from species belonging to the *Leguminosaea* family, presented a high proportions of bound tannins (PCT + FCT)/ TCT).

Differences among shrub species occurred for the values related to end-point (120 h) OM degradation and true DM digestibility. Legume shrub species were degraded to the greatest extent and *Erica* species to the least (P<0.001). True DM digestibility values were highly variable among shrub species and samplings and did not follow a regular pattern.

Cumulative gas production profiles from the *in vitro* fermentation of the shrub species are in Figure 1 and parameters describing the cumulative gas production in Table 3. All parameters, but *T/2*, showed significant differences among species and among samplings (P<0.001), although change from January to June was different among species (species x sampling time interaction: P<0.001). Potential gas production (*A*) ranged from a minimum value of 43 ml/g OM in *E. australis* to maximum values (up to 199 ml/g OM) in legume shrub species (P<0.001) and was higher in samples harvested in June (P<0.001). Lag time (*L*; h) and fractional rate of gas production at *T/2* (μ, h^{-1}) were also higher in samples collected in June (P<0.001). However, time to half-asymptote (*T/2*; h) did not differ between samplings (P>0.05).

Correlation coefficients between condensed tannin contents and true DM digestibility, OM disappearance, gas production parameters and chemical composition of the shrub species are given in Table 4. Tannin fractions were correlated (P<0.05) with each other, with coefficients ranging from 0.54 between free-CT and FCT, to 0.98 between free-CT and TCT. Total condensed tannins, and most of their fractions, were negatively correlated with OMD and cumulative gas production (*A*), and positively with lag time (*L*) and the partitioning factor (PF). Only fibre-bound condensed tannins (FCT) were significantly related to fractional rate of gas production at *T/2* (μ). No correlations (P>0.05) were

observed between these secondary compounds and “true digestibility”. In addition, condensed tannins were negatively correlated with CP, NDF and ADF but there was no significant correlation with ADL and ash.

4.- Discussion

The shrub legumes examined had low contents of CT (less than 6.51 g QT equivalents/kg DM) which would generally be considered unlikely to significantly affect digestion of nutrients in ruminants. There were, however, two exceptions to this in samples collected in June from legumes *C. scoparius* and *G. florida*, which showed high CT contents. These two samples were comprised of high amounts of flowers, especially that from *C. scoparius*, whose reproductive tissue is particularly abundant. Influorescences are very valuable tissues for the plant, representing a large investment of resources in reproduction, and it is therefore accepted that they can be heavily defended against herbivory by the accumulation of plant secondary compounds such as tannins (Roberts and Olson, 1999).

No increases in CT contents were observed in samples collected in June from the other two legume species (*C. purgans* and *G. occidentalis*), even though samples consisted of young current season twigs which would also be expected to contain higher concentrations of condensed tannins acting as feeding deterrents to ruminants (Provenza et al., 1990). It is probable that shrub legumes only defend the reproductive tissue, confined to a very short period, by producing secondary metabolites, due to the accompanying high production costs for the plant (Coley, 1986). Alternatively, legume shrub species may have other means of defending less valuable tissues from herbivory, such as rapid regrowth due to their dense and deep root system, a high canopy height (e.g., *C. scoparius*), or physical defences such as thorns (e.g., *G. occidentalis*).

Ericaceae species and *J. communis* showed CT contents (higher than 176 g QT equivalents/kg DM, see Table 2) clearly superior to those considered to be potentially detrimental for herbivores. In these species, the highest contents were observed in samples containing reproductive tissues: flowers and fruits, which corresponded to samples from *E. arborea* and *E. australis* collected in June.

The mountain area where the shrubs were sampled has traditionally been used to graze sheep, goats, cattle and horses. These species are grazers that only consume considerable amounts of shrub species when the herbage allowance is reduced, which in the study area occurs both in winter, because of low temperatures, and in late summer, because of high temperatures and lack of rainfall. In the present study, the low herbage allowance occurred at the January sampling. At that time, the high canopy height of *C. purgans*, *C. scoparius* and *G. florida*, the thorns of *G. occidentalis*, along with the scarce presence of leaves in these species, would most likely have protected them from consumption by herbivores. On the other hand, the presence of evergreen leaves in *Ericaceae* species and *J. communis*, would make them more susceptible to consumption throughout the year, which is consistent with their high content of CT throughout the year (Perevolotsky, 1994).

CT contents of *Calluna vulgaris*, close to those found in this study, were reported by Duncan et al. (1994) as 181.7 and 186.0 g QT equivalents/kg DM. However, to our knowledge, there are no many other comparable results in the literature; comparisons with other published CT concentrations in similar species are very complicated due to variations in methods, procedures and standards used for the analysis.

The pattern of distribution of tannins in free-unbound, protein-bound and fibre-bound fractions is known to be dependent on several factors, such as the total content of condensed tannins, the age of the plant, and certain conditions of climatic or nutritive

stress during growth of the plant (Barry and Manley, 1986; Iason et al., 1995). In the present study, following the general pattern, species with a high TCT content had a higher proportion of free and lower proportion of bound tannin fractions. In contrast, species with low TCT contents had a lower proportion as free, and a higher proportion as bound. Although bound and free tannins have been reported, respectively, as indices of nutritionally beneficial and detrimental effects of condensed tannins, Perez-Maldonado and Norton (1996) proved that interchange between these fractions in different parts of the digestive tract was actually complex, which make it unlikely that the nutritional effects of CT could be predicted from the distribution of those fractions in the plant itself.

Condensed tannins were not correlated ($P>0.05$) with true digestibility. These results provide confirmation that the detergent system is not applicable for tannin-rich feeds (Makkar et al., 1995), since most of CT and CT-complexes are soluble in neutral detergent solutions (Van Soest, 1994), but are indigestible.

The *in vitro* gas production technique has been widely used for tannin-containing forage evaluation (e.g. Makkar et al., 1995; Getachew et al., 2000). The negative correlations between CT and cumulative gas production and lag time are consistent with the extensively reported suppressive effect of condensed tannins on rumen degradation and on the interference of these compounds with microbial attachment to feeds (McLeod, 1974; McAllister et al., 1994; Aharoni et al., 1998). Consistently with these results, FCT were negatively correlated with μ.

Condensed tannins were also negatively correlated with OM disappearance. The significant positive correlation with the partitioning factor (PF: OM disappearance/total gas production) indicates that the effect of CT is more strongly reflected in the reduction of gas production than in the reduction of OMD.

Although CT have been reported to evolve close to cell wall components, basically lignin (Barry and Manley, 1986; Van Soest, 1994), data of correlations between these compounds available in the literature are highly variable and difficult to compare. The same occurs for correlations with fermentation parameters. There may be several explanations for this variability. On the one hand, techniques generally used to measure tannins do not reliably reflect the activity of the tannins in reducing digestibility. On the other hand, in the present study, there may be a multiplicity of factors that counterbalance their effects. Thus, for example, flowering shoots presented lower cell wall contents together with higher CT contents.

Further research is needed to assess the nutritive value of the shrub species studied in this trial and particularly the role of the tannins. In order to progress in this field, it would be necessary to analyse total phenolics and total tannins content of the different plant tissues, detect other inhibitory compounds and carry out bioassays in the presence and absence of tannin-complexing agents, such as PEG, to estimate tannin activity. It will also be important to conduct *in vivo* studies to determine the actual biological effects of tannins on the performance of ruminants.

Acknowledgements

The authors wish to thank Dr. B. A. Williams (University of Wageningen, The Netherlands) for helpful comments and revision of the manuscript. G. Hervás gratefully acknowledges receipt of a fellowship from the Ministerio de Educación y Cultura (MEC, Spain). This work was supported by the Junta de Castilla y León (Projects CSI 3/99).

References

Aerts, R.J., Barry, T.N., McNabb, W.C., 1999. Polyphenols and agriculture, beneficial effects of proanthocyanidins in forages. Agr. Ecosyst. Environ. 75, 1-12.

AFRC, 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on responses to nutrients. CAB International, Wallingford, UK.

Aharoni, Y., Gilboa, N., Silanikove, N., 1998. Models of suppressive effect of tannins. Analysis of the suppressive effect of tannins on ruminal degradation by compartmental models. Anim. Feed Sci. Technol. 71, 251-267.

Alonso, I., 1994. Estudio ecológico y valoración de un sistema pastoral de la montaña leonesa. Ph.D. Thesis, University of León. Spain.

AOAC, 1999. Official methods of analysis of the Association of Official Agricultural Chemists. 16th edition (5th revision). AOAC International, Gaithersburg, MD, USA.

Asquith, T.N., Butler, L.G., 1985. Use of dye-labeled protein as spectrophotometric assay for protein precipitans such as tannin. J. Chem. Ecol. 11, 1535-1544.

Barry, T.N., Manley, T.R., 1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus* spp., their possible consequences in ruminant nutrition. J. Sci. Food Agric. 37, 248-254.

Barry, T.N., McNabb, W.C., 1999. The implications of condensed tannins on the nutritional value of temperate forages fed to ruminants. Br. J. Nutr. 81, 263-272.

Blümmel, M., Makkar, H.P.S., Becker, K., 1997. *In vitro* gas production: a technique revisited. J. Anim. Physiol. Anim. Nutr. 77, 24-34.

Coley, P.D., 1986. Cost and benefits of defense by tannins in a neotropical tree. Oecologia 70, 238-271.

Duncan, A.J., Hartley, S.E., Iason, G.R., 1994. Fine-scale discrimination of forage quality by sheep offered a soyabean meal or barley supplement while grazing a nitrogen-fertilized heather (*Calluna vulgaris*) mosaic. J. Agric. Sci., Camb. 123, 363-370.

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R., Isac, D., 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. J. Theor. Biol. 163, 99-111.

Frutos, P., Wright, I.J., Giráldez, F.J., Mantecón, A.R., Iason, G., 1998. Seasonal variation in nutrition and supplementation in sheep extensive system in North-Central Spain. In: Goddard, P. (Ed.), Extensification implications for beef cattle and sheep health and welfare. Aberdeen, UK, pp. 17-23.

Getachew, G., Makkar, H. P. S., Becker, K., 2000. Effect of polyethylene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. Brit. J. Nutr. 84, 73-83.

Goering, M.K., Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook, No. 379. Washington, USA: Agricultural Research Service, USDA.

Hagerman, A.E., 1991. Tannin Analysis. Miami University, Oxford, OH, USA.

Iason, G.R., Hodgson, J., Barry, T.N., 1995. Variation in condensed tannin concentration of a temperate grass (*Holcus lanatus*) in relation to season and reproductive development. J. Chem. Ecol. 21, 1103-1112.

Makkar, H.P.S., Blümmel, M., Becker, K., 1995. Formation of complexes between polyvinyl pyrrolidone or polyethylene glycol and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. Brit. J. Nutr. 73, 897-913.

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S., Theodorou, M.K., 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Technol. 79, 321-330.

McAllister, T.A., Bae, H.D., Jones, G.A., Cheng, K.J., 1994. Microbial attachment and feed digestion in the rumen. J. Anim. Sci. 72, 3004-3018.

McLeod, M.N., 1974. Plants tannins - their role in forage quality. Nutr. Abstr. Rev. 44, 803-815.

Perevolotsky, A., 1994. Tannins in Mediterranean woodlands species, lack of response to browsing and thinning. Oikos 71, 333-340.

Perez-Maldonado, R.A., Norton, B.W., 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. Br. J. Nutr. 76, 515-533.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochem. 25, 223-230.

Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B., Distel, R.A., 1990. Conditioned flavor aversion, a mechanism for goats to avoid condensed tannins in blackbrush. Am. Nat. 136, 810-828.

Roberts, J.L., Olson, B.E., 1999. Effect of *Euphorbia esula* on sheep rumen microbial activity and mass *in vitro*. J. Chem. Ecol. 25, 297-314.

Robinson, P.H., Campbell Mathews, M., Fadel, J.G. 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fibre at 48 h, and comparison to 48 h in sacco neutral detergent fibre digestion. Anim. Feed Sci. Technol. 80, 257-266.

SAS, 1989. SAS/STAT® User´s guide Int (Version 6, 4th ed.) Stastistical Analysis Systems Institute Inc., Cary, NC, USA.

Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agric. 58, 321-329.

Theodorou, M.K., Willians, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185-197.

Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18, 104-111.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, USA.

Zorita, E., 1994. Los sistemas pastorales y la conservación de la naturaleza en la España peninsular. Una perspectiva histórica. In: Sistemas extensivos de producción de rumiantes en zonas de montaña. Ciencias Veterinarias XIII, 13-49.

FIGURE CAPTION

Figure 1. Cumulative gas production profiles from the fermentation of the shrub species —●— *C. purgans,* —○— *C. scoparius,* —■— *G. florida,* —□— *G. occidentalis,* —△— *E. australis,* —▲— *E. arborea,* —×— *C. vulgaris* and —▬— *J. communis,* (a) in January sampling (s.e.d. for the interaction incubation time x species = 1.56) and (b) in June sampling (s.e.d. for the interaction incubation time x species = 4.10)

Table 1. Chemical composition (g/kg DM) of the shrub species[1].

	CP		NDF		ADF		ADL		Ash	
	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun
LEGUMINOSEAE										
Cytisus purgans	105	95	506	583	365	457	147	209	21	19
Cytisus scoparius	116	189	531	401	385	285	110	89	18	41
Genista florida	124	154	555	535	412	340	148	100	20	26
Genista occidentalis	67	118	600	454	450	322	165	103	28	35
ERICACEAE										
Calluna vulgaris	56	55	530	460	370	291	149	149	27	29
Erica arborea	55	91	453	347	333	261	173	132	19	20
Erica australis	74	47	458	489	327	367	165	198	30	15
CUPRESACEAE										
Juniperus communis	51	80	423	371	335	273	147	109	67	45

CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin
Jan = January sampling; Jun = June sampling.
[1] Values represent single samples analysed in duplicate

Table 2. Condensed tannins content (g QT equivalents / kg DM) of the shrub species[1].

	Free CT		PCT		FCT		TCT	
	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun
LEGUMINOSEAE								
Cytisus purgans	1.9	2.1	3.4	3.3	1.2	0.8	6.5	6.2
Cytisus scoparius	1.8	220.3	3.3	31.2	0.8	1.6	5.9	253.1
Genista florida	1.8	74.3	3.2	23.3	0.9	2.0	5.8	99.7
Genista occidentalis	1.3	2.5	3.1	1.8	0.7	1.0	5.0	5.4
ERICACEAE								
Calluna vulgaris	134.0	167.8	35.7	25.5	8.0	10.3	177.8	203.6
Erica arborea	229.1	227.0	36.5	67.5	5.7	7.6	271.4	302.1
Erica australis	117.9	237.6	85.9	60.4	11.6	5.7	215.5	303.7
CUPRESACEAE								
Juniperus communis	183.9	207.7	8.6	18.5	4.5	4.2	197.0	230.4

Free CT = free condensed tannins; PCT = protein-bound condensed tannins; FCT = fibre-bound condensed tannins; TCT = total condensed tannins.
Jan = January sampling; Jun = June sampling; QT = quebracho tannins.
[1] Values represent single samples analysed in duplicate

Table 3. *In vitro* organic matter disappearance (OMD; %), true digestibility (TD; %), gas production parameters (*A*, *T/2*, μ and *L*) and partitioning factor (PF) of the shrub species.

	OMD		TD		*A*		*T/2*		μ		*L*		PF[1]	
	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun	Jan	Jun
LEGUMINOSEAE														
Cytisus purgans	58.0^{a}	58.3^{c}	65.7^{a}	58.9^{d}	184^{a}	197^{a}	23.2^{c}	20.3^{bc}	0.029^{b}	0.036^{bc}	0.09^{c}	0.79^{b}	3.290^{de}	2.995^{b}
Cytisus scoparius	54.3^{b}	73.5^{a}	52.8^{e}	69.8^{a}	154^{b}	197^{a}	17.2^{e}	14.7^{c}	0.036^{a}	0.048^{a}	0.67^{b}	1.18^{b}	3.549^{c}	3.686^{b}
Genista florida	59.1^{a}	66.9^{b}	62.1^{bc}	63.8^{bc}	183^{a}	199^{a}	20.4^{d}	18.4^{bc}	0.035^{a}	0.037^{bc}	0.34^{c}	1.03^{b}	3.285^{de}	3.376^{b}
Genista occidentalis	54.2^{b}	65.8^{b}	53.6^{e}	68.7^{a}	182^{a}	199^{a}	20.2^{d}	16.8^{bc}	0.035^{a}	0.043^{ab}	0.22^{c}	1.21^{b}	3.009^{f}	3.307^{b}
ERICACEAE														
Calluna vulgaris	41.6^{d}	49.6^{e}	57.8^{d}	61.8^{cd}	130^{c}	167^{b}	28.4^{a}	32.2^{a}	0.024^{c}	0.024^{d}	0.00^{c}	1.48^{b}	3.426^{cd}	3.310^{b}
Erica arborea	34.1^{e}	35.8^{f}	59.1^{cd}	60.9^{cd}	76^{e}	55^{c}	21.2^{cd}	22.0^{b}	0.030^{b}	0.040^{abc}	0.93^{ab}	3.90^{a}	4.581^{a}	6.603^{a}
Erica australis	33.9^{e}	27.3^{g}	54.2^{e}	50.9^{e}	84^{d}	43^{c}	20.5^{d}	20.6^{bc}	0.031^{b}	0.039^{abc}	1.07^{a}	1.28^{b}	4.058^{b}	6.860^{a}
CUPRESACEAE														
Juniperus communis	48.2^{c}	55.7^{d}	64.0^{ab}	68.1^{ab}	156^{b}	173^{b}	25.8^{b}	22.7^{b}	0.028^{b}	0.033^{c}	1.23^{a}	1.44^{b}	3.167^{e}	3.306^{b}
SEM	0.01	0.01	0.02	0.04	6.7	2.8	1.48	11.31	<0.0001	<0.0001	0.033	0.258	0.0069	1.0820
SIGNIFICANCE LEVEL:														
Species	***		***		***		***		***		***		***	
Sampling time	***		***		***		ns		***		***		**	
Species × Sampling time	***		***		***		ns		ns		***		**	

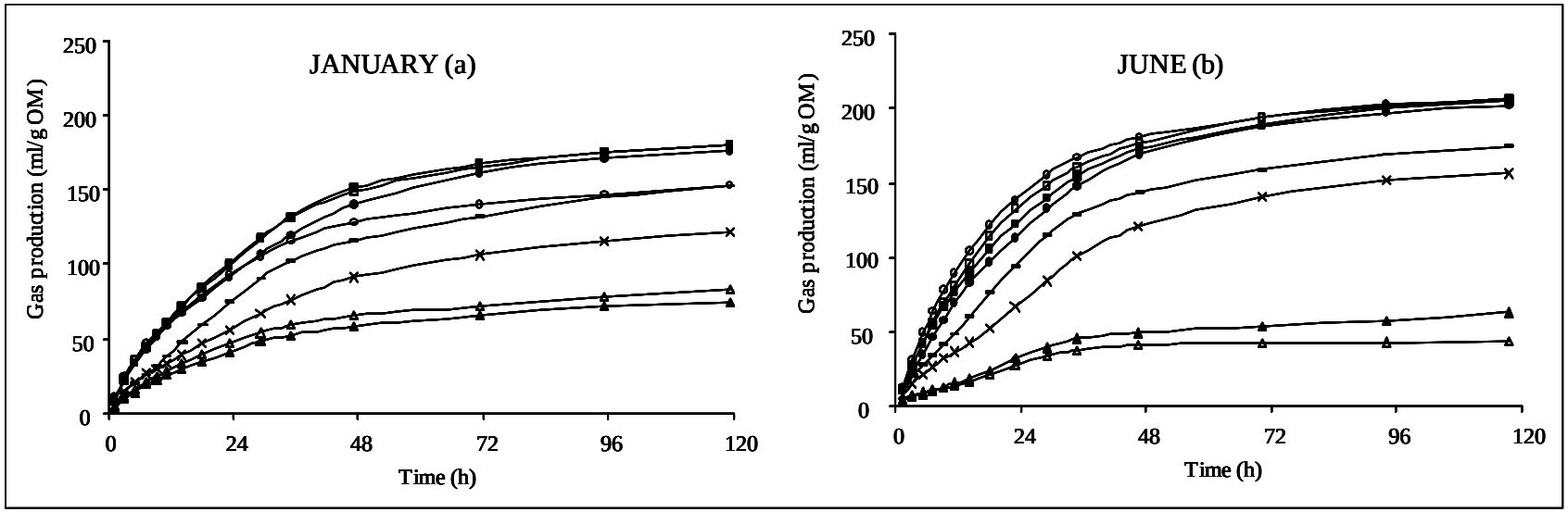
A = cumulative gas production (ml/g OM); *T/2* = time to half-asymptote (h); μ = fractional rate of gas production at *T/2* (h^{-1}); *L* = lag time (h).
Jan = January sampling; Jun = June sampling.
[a, b, c, d, e, f, g] Means in a column with a different superscript differ significantly. SEM = standard error of the means. ns: non significant (P>0.05); **: P<0.01; ***: P<0.001.
[1] Partitioning factor is an estimate of the efficiency of fermentation (i.e., OM degradation relative to total gas production)

Table 4. Correlation coefficients between condensed tannins contents and true digestibility, gas production parameters, *in vitro* OM disappearance, partitioning factor and chemical composition of the shrub species.

	TD	*A*	*T/2*	μ	*L*	OMD	PF	CP	NDF	ADF	ADL	Ash	Free CT	PCT	FCT
Free CT	0.08	-0.61	0.24	-0.00	0.56	-0.50	0.59	-0.28	-0.75	-0.70	-0.00	0.28			
	ns	*	ns	ns	*	ns	*	ns	***	**	ns	ns			
PCT	-0.34	-0.82	0.11	0.02	0.51	-0.70	0.72	-0.25	-0.45	-0.46	0.20	-0.18	0.64		
	ns	***	ns	ns	*	**	**	ns	ns	ns	ns	ns	**		
FCT	-0.19	-0.57	0.53	-0.52	0.36	-0.63	0.27	-0.51	-0.43	-0.51	0.17	0.08	0.54	0.73	
	ns	*	ns	*	ns	**	ns	*	ns	*	ns	ns	*	**	
TCT	-0.02	-0.72	0.23	-0.00	0.57	-0.58	0.69	-0.30	-0.73	-0.69	0.05	0.19	0.98	0.77	0.64
	ns	**	ns	ns	*	*	**	ns	**	**	ns	ns	***	***	*

Free CT = free condensed tannins; PCT = protein-bound condensed tannins; FCT = fibre-bound condensed tannins; TCT = total condensed tannins; TD = true digestibility; *A* = cumulative gas production; *T/2* = time to half-asymptote; μ = fractional rate of gas production at *T/2*; *L* = lag time; OMD = organic matter (OM) disappearance, PF = partitioning factor.
ns: non significant (P>0.05); *: P<0.05; **: P<0.01; ***: P<0.001.

Figure 1


JANUARY (a)
JUNE (b)
Gas production (ml/g OM)
Time (h)
0
24
48
72
96
120
50
100
150
200
250