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SUMMARY – Differences in the fermentative activity of ruminant species were examined *in vitro* in batch cultures inoculated with rumen fluid obtained from either sheep or goats fed on the same diet (alfalfa hay). *In vitro* dry matter digestibility (IVD) and gas production kinetics were determined for foliage of four indigenous shrub species: *Erica australis* (Spanish heather), *Cistus laurifolius* (laurel-leaved rock-rose), *Quercus pyrenaica* (hoary oak) and *Rosa canina* (wild dog rose), collected in two growing seasons (spring and autumn) from uplands in the province of León (northwestern Spain). IVD was measured by a two stage technique with a first incubation in buffered rumen fluid (48 h) and extraction with neutral detergent solution for 1 h at 100°C. Samples were also incubated *in vitro* with buffered rumen liquid in bottles, and volumes of gas produced were measured at 15 different incubation times (till 144 h). The estimated extent of degradation in the rumen, the gas production at 24 h of incubation and the fractional rate of gas production were estimated. There were no significant differences between sheep and goat rumen fluid for the IVD of any of the shrubs. Gas production after 24 h was higher in sheep rumen fluid ($P < 0.05$) for *E. australis* and young leaves of *C. laurifolius*, and higher in goat rumen fluid ($P < 0.05$) for young leaves of *Q. pyrenaica*. Gas production rate was significantly faster ($P < 0.05$) with goat rumen fluid for *Q. pyrenaica* and young leaves of *R. canina*, with lower ($P < 0.05$) values with goat rumen fluid for the young leaves of *E. australis* and *C. laurifolius*. The extent of degradation of *R. canina* and young leaves of *Q. pyrenaica* was higher ($P < 0.05$) with goat than with sheep rumen fluid, with no significant differences between ruminant species for the other shrub species. These results suggest some interspecies differences in the *in vitro* ruminal fermentative activity between sheep and goats that could determine their ability to digest the foliage of different shrub species.

Key words: Rumen, sheep, goat, *in vitro* digestibility, gas production, shrub.

RESUME – *"Effet de la source d'inoculum (jus de rumen de mouton ou de chèvre) sur la digestibilité et la cinétique de production de gaz* in vitro *du feuillage de quelques arbustes espagnols". L'effet de la source d'inoculum (jus de rumen de mouton ou de chèvre) sur la digestibilité* in vitro *(DIV) et la cinétique de production de gaz à partir du feuillage de quatre arbustes espagnols a été étudié. Il s'agit de* Erica australis*,* Cistus laurifolius*,* Quercus pyrenaica *et* Rosa canina*. Des échantillons des parties consommables de ces arbustes ont été prélevés pendant deux stades de développement (printemps et automne) dans la montagne de la province de León au Nord-Ouest de l'Espagne. La DIV a été mesurée par une technique à deux phases. La première phase consiste en une incubation dans le jus du rumen tamponné (48 h) et dans la seconde phase, une extraction avec une solution de détergent neutre pendant une heure à 100°C est réalisée. Les échantillons ont été aussi incubés dans du jus de rumen dans des bouteilles à sérum et les volumes de gaz produits ont été enregistrés après 15 différentes durées d'incubation (jusqu'à 144 h). La dégradation théorique dans le jus de rumen, la production de gaz après 24 h d'incubation et la vitesse de production de gaz ont été mesurées. La DIV de toutes les espèces étudiées n'a pas varié avec la source d'inoculum. La production de gaz après 24 h d'incubation a été largement supérieure dans le jus de rumen de mouton ($P < 0,05$) pour* E. australis *et les jeunes feuilles de* C. laurifolius*, alors qu'elle a été plus importante dans le jus de rumen de chèvre ($P < 0,05$) pour les jeunes feuilles de* Q. pyrenaica*. Par rapport au jus de rumen de mouton, la vitesse de production de gaz dans le jus de rumen de chèvre a été plus rapide ($P < 0,05$) pour* Q. pyrenaica *et les jeunes feuilles de* R. canina *et nettement faible ($P < 0,05$) pour les jeunes feuilles de* E. australis *et* C. laurifolius*. La dégradation théorique de* R. canina *et des jeunes feuilles de* Q. pyrenaica *a été plus élevée ($P < 0,05$) dans le jus de rumen de chèvre que dans celui de mouton. En revanche, pour les autres espèces arbustives il n'y a eu aucune différence significative entre les deux types de jus de rumen. Ces résultats reflètent des différences entre les ovins et les caprins dans l'activité ruminale* in vitro*, qui pourraient expliquer la variation de leur capacité à digérer du feuillage des différentes espèces arbustives.*

Mots-clés : *Jus de rumen, ovins, caprins, digestibilité* in vitro*, production de gaz, arbustes.*

Introduction

In the extensive livestock production systems of the Mediterranean countries, browse plants are considered important alternative resources for grazing ruminants, especially during critical periods of grass shortage. Many of these plants contain relatively high levels of tannins, which may have beneficial and detrimental nutritional effects. Potential benefits include protein sparing in the rumen and anthelminthic effects, whereas some of the potential adverse effects are microbial inhibition and decreased performance. Ruminant species have been classified as grazers or browsers according to their ability to select their diet and to browse shrub and tree foliage. Therefore, it is possible that the different species of ruminants are not equally vulnerable to tannins. Experiments conducted under field conditions (Narjisse and Bare, 1986) and *in vivo* (El Hag, 1976) have demonstrated that goats are more tolerant than sheep to the high tannin concentrations present in browse plants. The main objective of the present study was to detect any interspecies differences between sheep and goat rumen fluid in its fermentative activity when foliage from different browse plants is incubated *in vitro* to measure dry matter digestibility and gas production kinetics.

Material and methods

Browse samples

Samples from four Spanish indigenous browse species were collected from uplands in the province of León (Norwest Spain) at different growing seasons in spring and autumn. The shrub species were *Erica australis* (Spanish heath), *Cistus laurifolius* (laurel-leaved rock-rose), *Quercus pyrenaica* (hoary oak) and *Rosa canina* (wild dog rose). The plants were clipped with scissors and a mixture of leaves and thin stems were collected. In the laboratory, leaves were manually separated from the original samples, immediately freeze-dried and ground in a hammer mill and passed through a 1-mm sieve.

Animals and extraction of rumen fluid

Four Merino sheep and four Alpine goats housed in individual cages, fitted with rumen cannulae and fed 1 kg alfalfa hay daily were used. Rumen fluid was withdrawn before the morning meal, collected in thermos flasks and taken immediately to the laboratory where it was strained through four layers of cheesecloth and kept at 39°C under a CO_2 atmosphere.

In vitro digestibility

The two-stage procedure for *in vitro* digestibility described by Goering and van Soest (1970), with the modifications proposed by the ANKOM-DAISY technique (ANKOM, 1998), was used. In this method, polyester bags instead of flasks and crucibles were used. Samples (250 mg) were weighed into polyester bags (size 5 cm $\times$ 5 cm; pore size 20 μm), which were sealed with a heater. Bags were placed in 5-l fermentation glass jars that can be closed with a plastic lid provided with a single-way valve that avoids the accumulation of fermentation gases. Two jars were used, and 18 bags (two per sample plus two empty bags) were placed in each jar. Buffered rumen fluid (RF) was prepared according to Goering and van Soest (1970) by diluting fresh RF in the culture medium (20% RF and 80% medium) at 39°C and under a CO_2 atmosphere. A special "complete" medium was formulated to provide any essential factor for microbial growth, with the aim that none of the micro-organisms existing in the rumen contents of sheep or goats could undergo any growth inhibition or limitation for a deficit in a nutrient or a growth factor. Thus the basal medium of Goering and van Soest (1970) containing buffer, macro- and micro-mineral and reductive solutions was enriched with trypticase, yeast, haemin, branched fatty acids, Co-M and a mixture of sheep and goat clarified rumen fluid. For each incubation run, fresh RF from sheep and goats was collected on the same day and at the same time, and then each buffered RF was prepared separately but at the same time. The jars containing the bags were filled with 2 l of buffered RF, one with sheep and the other with goat buffered RF. Then the jars were placed in a special incubator (DAISY, ANKOM) at 39°C with continuous rotation to facilitate the effective immersion of the bags. After 48 h of incubation, the jars were opened and the

bags were gently rinsed first under cold tap water and then in a washing machine (short washing cycle – 10 min – with cold water). Bags were dried at 60°C for 48 h and then treated with a neutral detergent solution at 100°C for 1 h. The dry residue was weighed and considered as the truly indigestible matter to calculate the IVD (Goering and van Soest, 1970). Two runs of incubation were performed in two consecutive weeks (four replicates per treatment).

In vitro gas production kinetics

The method used for the gas production measurements was as described by Theodorou *et al*. (1994). About 500 mg of each sample were weighed into serum bottles kept at approximately 39°C and flushed with CO_2 before use. Four bottles were used for each substrate in each incubation run, two for each inoculum source (sheep or goat RF). Fifty ml of buffered rumen fluid (in the proportion of 20% RF + 80% medium) prepared as described before were anaerobically dispensed in each bottle at 39°C. The culture medium used was the same enriched medium described above, formulated with the aim to provide any essential factor for microbial growth. All the bottles were crimped, placed in the incubator at 39°C, and shaken at regular times. The volume of gas produced in each bottle was recorded using a pressure transducer at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 y 144 h after start of the inoculation (Theodorou *et al*., 1994). At the end of the incubation period, the contents of each serum bottle were filtered using sintered glass crucibles to calculate DM loss after 144 h incubation. Cumulative gas production data were fitted to an exponential model to estimate kinetic parameters, and then to calculate the extent of degradation of feeds in the rumen (France *et al*., 2000). Two runs of incubations were performed in different weeks.

By using the same enriched non-limiting medium, it is expected that any difference between sheep and goat RF in microbial population and degradation activity can be exhibited, and thus within each substrate the differences between experimental treatment be attributed to the effects of inoculum source. Differences among sheep and goat RF were examined by Student's *t*-test for samples with equal variance.

Results and discussion

Chemical composition and the concentration of phenolic compounds and tannins in the foliage of the browse plant species have been reported in previous studies (Ammar *et al*., 2000 and this volume). Foliage of *E. australis* is characterised by low crude protein (CP) and high lignin contents. Leaves of *R. canina* had high CP and low fibre contents. *Q. pyrenaica* had high CP and fibre contents and *C. laurifolius* had intermediate values. In spring, tannin concentration in the shrubs ranged between 90 and 125 g/kg, whereas in autumn the differences were enlarged with tannin concentrations in *E. australis* and *R. canina* foliage of about 130 g/kg, being significantly lower in *Q. pyrenaica* and *C. laurifolius* with values of 45 g/kg. In spite of these general trends, it is noteworthy that in all plants there were large differences in the chemical composition between sampling seasons (Ammar *et al*., 2000).

As stated in Table 1, there were no significant differences ($P > 0.05$) in the IVD between RF of sheep and goats. These results are in agreement with those observed by Molina *et al*. (1997). The extraction with neutral detergent solution can reduce the interspecies differences (van Soest, 1994). In fact, our IVD values can be considered somewhat higher than those reported in the literature for feeds of similar composition when a different technique was used to measure IVD. The extraction with neutral detergent solution was proposed to remove microbial contamination of the residue that could result in an overestimation of the undigested residue, thus resulting in higher values of IVD than other commonly used techniques.

The gas production technique has received much attention as a means of evaluating the nutritional quality of feedstuffs. In the present study, the technique has been used to detect differences between the rumen liquor of sheep and goats in their fermentative activity. This technique can be considered more sensitive to detect such differences than IVD. Table 2 shows the fractional rate of gas production (c), the cumulative gas produced after 24 h of incubation (G24) and the estimated extent of degradation (dg) in the rumen. Gas production rates (c values) were significantly higher ($P < 0.05$) in cultures inoculated with RF of sheep for young leaves of *E. australis* and *C. laurifolius*, and higher

with goat RF for *Q. pyrenaica* and young leaves of *R. canina*. Young leaves of *Q. pyrenaica* and *R. canina* showed the fastest gas production rates, what can be indicative of a high microbial activity, probably because these were the substrates with higher protein contents.

Table 1. Effect of rumen fluid source (sheep or goats) on *in vitro* dry matter digestibility (%) of the foliage of some browse plants

Species	Season	Source of rumen liquor		sed†
		Sheep	Goat	
E. australis	Spring	65.9	64.3	1.57
	Autumn	60.0	59.8	0.63
C. laurifolius	Spring	70.6	69.9	0.73
	Autumn	71.8	69.9	1.91
Q. pyrenaica	Spring	73.2	73.6	0.83
	Autumn	59.1	58.1	0.57
R. canina	Spring	87.5	86.9	0.81
	Autumn	84.3	85.6	0.46

†sed = standard error of difference.

Table 2. Effect of rumen fluid source (sheep or goats) on *in vitro* gas production kinetic parameters of the foliage of some browse plants

Species	Parameter	Spring			Autumn		
		Sheep	Goats	sed†	Sheep	Goats	sed†
E. australis	c (%/h)	5.1[a]	3.9[b]	0.27	4.7	4.4	0.33
	dg (%)	28.9	28.4	1.16	26.2	25.5	1.11
	G24 (ml)	94[a]	76[b]	5.4	76[a]	58[b]	5.2
C. laurifolius	c (%/h)	5.2[a]	3.7[b]	0.07	5.1	4.1	0.47
	dg (%)	23.5	22.4	0.58	33.1	31.3	1.02
	G24 (ml)	102[a]	86[b]	2.4	116	134	11.0
Q. pyrenaica	c (%/h)	6.0[b]	6.8[a]	0.14	3.6[b]	4.0[a]	0.15
	dg (%)	36.6[b]	41.9[a]	0.87	25.1	26.9	0.76
	G24 (ml)	158[b]	174[a]	3.2	80	80	0.8
R. canina	c (%/h)	6.3[b]	7.2[a]	0.12	4.4	4.2	0.14
	dg (%)	49.1[b]	55.1[a]	1.37	43.0[b]	45.6[a]	0.84
	G24 (ml)	208	218	3.4	164	162	1.4

†sed = standard error of difference.
[a,b]Within the same row and sampling season, values with different superscripts represent significant differences ($P < 0.05$).

As depicted in Table 2, there was a significant effect ($P < 0.05$) of inoculum source on G24 for *E. australis* and young leaves of *C. laurifolius* in favour of sheep RF, whereas G24 was significantly greater with goat RF only for young leaves of *Q. pyrenaica*. Likewise, interspecies differences in dg were not significant ($P > 0.05$) for *E. australis*, *C. laurifolius* and mature leaves of *Q. pyrenaica*, although there was a tendency for increased values for the sheep RF. However, dg values were greater ($P < 0.05$) for goat than for sheep RF for *R. canina* and young leaves of *Q. pyrenaica*, species with lower cell wall (Ammar *et al*., 2000) than the other ones.

It is noteworthy that the evolution of the phenological stage of browse species may account for the decline in IVD, G24 and dg values observed in the foliage collected in autumn in comparison with the one harvested in spring.

Comparative studies between sheep and goats in digestibility and degradability conducted by other authors (El Hag, 1976; Narjisse and Bare, 1986; Isac *et al*., 1994; Molina *et al*., 2000) are not comparable to the results reported herein obtained in batch cultures *in vitro*. In earlier studies, the observed differences between sheep and goats were not the same when studied *in vivo* or *in situ*, and were highly dependent on the quality of the feed used for the comparison. The level of intake can also be an important factor in the interspecies comparisons, as differences can be larger when animals are able to select their diet from the feed on offer. Donor animals used in this trial were fed at maintenance level and confined in individual cages, with minimal differences in selective behaviour between animals.

González-López *et al*. (1990) pointed out that in batch cultures *in vitro*, sheep RF was affected to a greater extent than goat RF in response to the incubation of low quality feeds. Differences in gas production kinetic parameters may reflect some differences in microbial activity in sheep and goat RF that may result in differences in the digestion of the foliage of browse species. Under field conditions, goats are better adapted than sheep to browse shrub and tree foliage and to select the more digestible parts of the plant material on offer. Furthermore, goats seem to be more efficient in the digestion of plant cell walls (Tisserand *et al*., 1991). On the other hand, it is commonly accepted that goats are more tolerant to the presence of tannins in the diet owing, in part, to adaptive mechanisms developed by the microbial population in the rumen (Brooker *et al*., 1994) or the possible production of an active tannase enzyme (Begovic *et al*., 1978).

Conclusion

Our results provide evidence that microbial population in the rumen liquid of Merino sheep and Alpine goats can exhibit a different capability to digest the foliage of some browse species. This trend can be due to differences in the degradation of the cell wall polysaccharides and in the tolerance to the presence of tannins in the foliage. Therefore, the digestibility of some browse species can be higher in sheep, whereas the foliage of other species can be more digestible in goats, depending upon the cell wall content and the level of phenolic compounds and tannins in the roughage.

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References

Ammar, H., Ranilla, M.J., Tejido, M.L., González, J.S. and López, S. (2000). Variations saisonnières de la composition chimique des feuilles d'arbustes de la montagne de León (Nord Ouest de l'Espagne). In: *Quality and Valorization of Animal Products in Mountains, XIth Meeting of the Mountain Grassland Sub-network of the FAO/CIHEAM Inter-regional Development Network on Pastures and Fodder Crops,* Lux St. Sauveur (France), 13-17 September 2000, 9 pp.

ANKOM (1998). Procedures for fibre and *in vitro* analysis. URL Address: www. ankom.com.

Begovic, S., Duzic, E., Sacirbrebegovic, A. and Tafro, A. (1978). Examination of variations of tannase activity in ruminal content and mucosa of goats on oak leaf diet and during intraruminal administration of 3 to 10% tanninc acid. *Veterinarya Sarajevo,* 27: 445-457.

Brooker, J.D., O'Donovan, L.A., Skene, I., Clarke, K., Blackall, L. and Muslera, P. (1994). *Streptococcus caprinus* sp. nov. A tannin resistant ruminal bacterium from feral goats. *Lett. Appl. Microbiol*., 18: 313-318.

El Hag, C.A. (1976). A comparative study between desert goats and sheep on efficiency of feed utilization. *World Rev. Anim. Prod*., 12: 43-48.

France, J., Dijkstra, J., Dhanoa, M.S., López, S. and Bannink, A. (2000). Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: Derivation of models and other mathematical considerations. *Br. J. Nutr*., 83: 143-150.

Goering, M.K. and van Soest, P.J. (1970). *Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications)*. Agricultural Handbook, No. 379, USDA, Washington.

González-López, J., Salmerón, V., Ramos-Cormenzana, A., Silva-Colmer, J. and Boza, J. (1990). Influence of several feeds on bacteria in sheep and goat rumen liquor *in vitro. Microbios*, 62: 75-81.

Isac, M.D., García, M.A., Aguilera, J.F. and Molina, E. (1994). A comparative study of nutrient digestibility, kinetics of digestion and passage and rumen fermentation pattern in goats and sheep offered medium quality forages at the maintenance level of feeding. *Arch. Anim. Nutr*., 46: 37-50.

Molina, M.E., García, M.A. and Aguilera, J.F. (1997). The *in vitro* digestibility of pastures from semi-arid Spanish lands and its use as predictor of degradability. *Options Méditerranéennes*, *Series A*, 34: 27-31.

Molina, M.E., Martín, A.I. and Aguilera, J.F. (2000). A comparative study of nutrient digestibility, kinetics of degradation and passage and rumen fermentation pattern in goats and sheep offered good quality diets. *Livest. Prod. Sci.*, 64: 215-223.

Narjisse, H. and El Bare, B. (1986). Seasonal changes in the botanical composition of sheep and goats grazing in an oak forest. In: *Rangelands: A Resource under Siege,* Ross, P.J., Lynch, P.W. and Williams, O.B. (eds). Australian Academy of Science, Camberra, pp. 369-371.

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

Tisserand, J.L., Hadjipanayiotou, M. and Gihad, E.A. (1991). Digestion in goats. In: *Goat Nutrition,* Morand-Fehr, P. (ed). Pudoc, Wageningen, pp. 46-60.

van Soest, P.J. (1994). *Nutritional Ecology of the Ruminant,* 2nd edn. Cornell Univ. Press, Ithaca, NY.