Effect of the administration of quebracho extract on rumen fermentation and diet digestibility in sheep

G. Hervás*, P. Frutos, A. R. Mantecón and F. J. Giráldez

Estación Agrícola Experimental (CSIC). Apdo. 788. 24080 León. Spain

Abstract

This work was carried out with the aim of studying the effect of the administration of commercial quebracho extract (76% condensed tannins, CT) to sheep, for 70 days. Ten ruminally cannulated ewes were distributed into two experimental groups (control and quebracho). Zero (placebo) or 0.75 g of quebracho tannins extract per kg of live weight and day were intra-ruminally administered to the animals. The nylon bag technique was used to examine alfalfa hay *in situ* dry matter (DM), nitrogen (N) and neutral-detergent fiber (NDF) disappearances. *In vivo* digestibility, pH and ammonia-N and volatile fatty acid (VFA) concentrations were also measured. The daily ruminal administration of quebracho extract did not affect rumen fermentation parameters such as pH and ammonia-N and VFA concentrations, but reduced significantly the alfalfa hay DM potentially degradable fraction and the fractional rate of N degradation. Only on day 8 of the experiment were the DM and NDF disappearance values, after 24 h of *in situ* incubation, significantly lower in the animals treated with quebracho. No differences were observed on any other day of the experiment. Furthermore, the quebracho CT extract significantly decreased the *in vivo* digestibility of the following diet components: DM, crude protein and NDF.

Key words: ruminal degradation, *in vivo* digestibility, condensed tannins.

Resumen

Efecto de la administración de extracto de quebracho sobre la fermentación ruminal y la digestibilidad de la dieta en ovejas

Este trabajo se realizó con el objetivo de estudiar el efecto de la administración intrarruminal a ovejas de extracto de quebracho (76% de taninos condensados), durante un período de tiempo relativamente largo (70 días). Para ello, se utilizaron 10 ovejas de raza Merina, canuladas en el rumen, distribuidas en dos grupos experimentales (control y quebracho). A todos los animales se les administró diariamente en el rumen una solución que contenía 0 (placebo) ó 0,75 g de extracto de quebracho por kg de peso vivo. Se estudiaron diferentes parámetros indicativos de la actividad degradativa del rumen (pH, concentraciones de N-amoniacal y ácidos grasos volátiles), la degradación ruminal en bolsas de nailon y la digestibilidad de la dieta. En general, la administración de quebracho no afectó a los parámetros de la fermentación ruminal pero redujo significativamente tanto la fracción potencialmente degradable de la materia seca (MS) como el ritmo fraccional de degradación del N del heno de alfalfa. Únicamente la desaparición de materia seca y de fibra neutro detergente del heno de alfalfa, tras 24 h de incubación ruminal *in situ*, fueron significativamente menores el día 8 del experimento en los animales tratados con quebracho, y no se observaron diferencias significativas el resto de los días de incubación. Por otra parte, el extracto de quebracho redujo la digestibilidad de la MS, la proteína bruta y la fibra neutro detergente del heno de alfalfa que constituía la dieta de los animales.

Palabras clave: degradación ruminal, digestibilidad *in vivo*, taninos condensados.

Introduction

Condensed tannins (CT), named after the French word *tan*, which refers to the bark of oak and other trees and that was used as a tanner, are phenolic compounds that developed in the biochemical evolution of plants as a defense mechanism to prevent them from being eaten by herbivores. CT, or proanthocyanidines, are widely found in nature and appear in several ruminant feeds (McLeod, 1974). Tannins are highly reactive due to their great number of hydroxyl groups that furnish them with many sites for the formation of hydrogen bonds with other molecules,

mainly proteins (Mueller-Harvey and McAllan, 1992), although complexes can also be formed through other types of bond (hydrophobic, ionic or covalent).

Several authors have suggested that CT could be used as chemical additives to reduce ruminal degradation of dietary protein, owing to the pH-dependent behavior of these compounds (Schwab, 1995; Frutos *et al.*, 2000). However, the effects CT have on animals is highly variable and depend on the type and the amount ingested. In fact, CT intake can have no effect or reduce voluntary intake (Barry and McNabb, 1999), reduce, not affect or even increase ruminal degradation (Miller *et al.*, 1995; Hervás *et al.*, 2003), improve or reduce diet digestibility (Barry *et al.*, 1986; Waghorn *et al.*, 1994; Komolong *et al.*, 2001; McSweeney *et al.*, 2001), etc.

The CT extracted from quebracho (*Schinopsis* spp.) are prophysethinidines. These have less hydroxyl groups than other types of condensed tannins and a compact structure, which means less reactivity (Mueller-Harvey and McAllan, 1992). Because quebracho CT can be found on the market in large amounts, it has often been used as a model in research (*e.g.*, by Dawson *et al*., 1999; Komolong *et al*., 2001), in spite of the fact that the results obtained with a certain kind of CT cannot be applied to other tannins owing to the wide diversity of these phenolic compounds (Nelson, 1996).

The aim of this experiment was to study the effect of intraruminal administration of quebracho extract (76% of CT) to sheep (0.75 g kg^{-1} live weight per day), for a *long* time period (70 days), on different parameters that reflect rumen degradation activity and diet digestibility.

Material and Methods

Animals and experimental diet

A total of 10 ruminally cannulated Merino ewes, with a live weight (LW) of 54.6 ± 2.87 kg were used. The animals were fed 1.2 times their maintenance energy requirements (AFRC, 1993) with alfalfa hay [dry weight (DW) = 926 g kg^{-1}; crude protein (CP) = 169 g kg^{-1} DM; neutral-detergent fiber (NDF) = 417 g kg^{-1} MS; acid detergent fiber (ADF) = 315 g kg^{-1} MS; ash = 114 g kg^{-1} DM]. The hay was daily administered in two equal feeds at approximately 9:30 h

and 18:00 h. All animals had *ad libitum* access to fresh water and to a vitamin-mineral block throughout the pre-experimental (15 days) and experimental (70 days) periods.

Experimental treatments

Animals were allocated to two groups of five sheep each, balanced for live weight. All animals received their daily experimental treatment before administration of the morning feed, for a period of 70 days. This involved dissolving the powdered commercial quebracho extract in 200 ml of an aqueous solution containing 0.08% methanol and administering it to sheep through the ruminal cannula. The amounts of quebracho administered daily were 0 (i.e., it only contained the aqueous solution: placebo) and 0.75 g kg^{-1} LW for control (C) and quebracho (Q) treatments, respectively.

The commercial quebracho used (Roy Wilson Dickson Ltd., UK) is a complex mixture of phenolic compounds extracted from quebracho (*Schinopsis* spp.). It is mainly comprised of condensed tannins (760 g kg^{-1} DM), and the remaining compounds correspond to simple phenols, ash, etc.

In situ rumen degradation

During weeks 5 (from day 29 to 37 of the experiment) and 10 (from day 62 to 70), two rumen degradation kinetics of alfalfa hay (DM = 915 g kg^{-1}; CP = 151 g kg^{-1} DM; NDF = 431 g kg^{-1} DM; ash = 78 g kg^{-1} DM) were carried out by *in situ* methods, and the following incubation series: 0, 3, 6, 12, 24, 48 and 72 h. Incubations were performed in duplicate in each animal.

Nylon bags (165×105 mm size and 45 mm pore diameter; Maissa®, Spain) were filled with approximately 4 g of alfalfa hay ground to 2 mm and introduced in the rumen 15 min after administration of the quebracho extract. After removal from the rumen, bags were washed briefly by hand under running water and frozen at –30ºC. They were thawed 24 h later and washed in an automatic washing machine on a cold programme for approximately 20 min. Bags were dried in a forced air oven at 45-50ºC to constant weight and weighed to determine the DM content. The residues of two bags (duplicate), for each incubation time and

animal, were mixed and ground to 1 mm, to be then analyzed for N and NDF. To estimate zero-time disappearances, three more bags were used that were washed, dried and weighed in a similar way as those removed from the rumen.

In addition to the degradation kinetics, the effect of quebracho administration on ruminal degradation was studied by performing a series of 24 h *in situ* incubations, with the same hay, on days 0, 8, 15, 23, 36, 50 and 64 of the experiment. All the incubations were performed in duplicate. As for the degradation kinetics, the residues of two bags for each animal were mixed and ground to 1 mm diameter for laboratory analysis (N and NDF).

Parameters indicative of ruminal fermentation

On days 0, 9, 25, 37, 51 and 64 of the experiment, samples of rumen fluid from each of the animals used in the experiment were taken to study a series of parameters indicative of ruminal fermentation: pH, ammonia-N and volatile fatty acids (VFA). These samples were collected, through the ruminal cannula, 0, 1, 3, 6 and 9 h after administering the morning feed.

The rumen fluid was filtered through two layers of cheesecloth. Immediately after obtaining the filtered sample its pH was measured. Ten ml of rumen fluid were acidified with 10 ml of 0.2 M HCl solution, and frozen at –30ºC until the ammonia-N concentration analysis. Similarly, 0.8 ml were added to 0.5 ml of a deproteinizing solution of metaphosphoric acid (20%, wt v^{-1}), that contained crotonic acid (0.4%, wt v^{-1}) as internal standard, and frozen at –30ºC until the VFA determination.

In vivo digestibility

During weeks 5 (days 29-37) and 10 (days 62-70) of the experiment, two *in vivo* digestibility trials were carried out. To do this, animals were kept in individual metabolic cages from which, after a preliminary 3-day adaptation period, animal faeces were collected and weighed daily over six consecutive days. From the total daily collection, two aliquots (approx. 10%) were taken and frozen at –30ºC. All the aliquots for each sheep were then bulked, and the pooled sample was thawed and dried in a forced air oven at 70-75ºC until

constant weight. Dried samples were ground to 1 mm before analysis (N, NDF and ADF).

When there were feed refusals, these were collected each morning and weighed. Around 10% of the total was kept for later analysis of the DM content.

Chemical analysis

Analysis of DM, ash and Kjeldahl N was conducted according to procedures described by AOAC (1999). The NDF and ADF contents were determined using an Ankom^{220} fiber analyzer, following the basic principles of the Goering and Van Soest technique (1970). Analysis of ammonia-N was performed by spectrophotometry (Weatherburn, 1967) and VFA concentration by gas chromatography (Carro *et al.*, 1999).

Calculations and statistical analysis

Data on ruminal disappearance of DM, N and NDF of alfalfa hay obtained on week 5 and 10 of the experiment were adjusted by the NLIN procedure (Nonlinear Regression) of the SAS statistical programme (SAS, 1989) to the model proposed by France *et al.* (1993) to obtain the immediately degradable fraction (a, g g^{-1}), the insoluble but potentially degradable fraction (b, g g^{-1}), the time to half-asymptote ($T/2$, h), the fractional rate of degradation in $T/2$ (μ, h^{-1}) and the lag time (L, h).

All the data (kinetic parameters of ruminal degradation, *in vivo* digestibility, *in situ* ruminal disappearance and parameters indicative of ruminal fermentation) were analysed by repeated measurements, using the MIXED procedure of the SAS statistical programme. When the interaction effect «treatment × day» was significant, means were compared using a Student's t (P < 0.05).

Results

Rumen degradation

Ruminal administration of the quebracho extract significantly reduced (P < 0.05) the insoluble but potentially degradable fraction (b) of the alfalfa hay DM (Table 1) and tended to reduce (P < 0.10) the fractional rate of degradation in $T/2$ (μ). Hence, in relation

Table 1. Effect of quebracho administration on the kinetic parameters of *in situ* rumen degradation *(a, b, μ, T/2* and *L)* of the alfalfa hay DM, N and NDF, in weeks 5 and 10 of the experiment

	a			*b*			*μ*			*T/2*			*L*		
	C	Q	sed	C	Q	sed	C	Q	sed	C	Q	sed	C	Q	sed
DM															
Week 5	0.2615	0.2567	0.00483	0.4049	0.3931	0.00891	0.1343	0.1084	0.01252	5.28	6.46	0.689	n.d.	n.d.	—
Week 10	0.2610	0.2642	0.00483	0.4164	0.3965	0.00891	0.1237	0.1069	0.01252	5.85	6.58	0.689	n.d.	n.d.	—
Signif. level (P)															
Treat.		NS			*			†			NS			—	
Week		NS			NS			NS			NS			—	
Treat. × Week		NS			NS			NS			NS			—	
N															
Week 5	0.1982	0.1990	0.00797	0.6807	0.6803	0.01500	0.1733	0.1333	0.01881	4.06	5.32	0.521	0.00	0.00	0.044
Week 10	0.2092	0.2169	0.00845	0.6723	0.6645	0.01500	0.1837	0.1445	0.01881	3.91	5.15	0.551	0.00	0.08	0.047
Signif. level (P)															
Treat.		NS			NS			*			*			NS	
Week		*			NS			NS			NS			NS	
Treat. × Week		NS			NS			NS			NS			NS	
NDF															
Week 5	0.0028	0.0790	0.05264	0.3532	0.2443	0.06524	0.0800	0.1105	0.03430	13.04	8.93	4.426	2.88	1.76	1.108
Week 10	0.0045	0.0000	0.05992	0.4028	0.4780	0.07389	0.0984	0.0457	0.03836	14.71	20.92	5.039	1.86	3.87	1.206
Signif. level (P)															
Treat.		NS			NS			NS			NS			NS	
Week		NS			*			NS			†			NS	
Treat. × Week		NS			NS			NS			NS			NS	

a: immediately degradable fraction (g g⁻¹). *b:* insoluble but potentially degradable fraction (g g⁻¹). *T/2:* time to half-asymptote (h). *μ:* fractional rate of degradation in *T/2* (h⁻¹).*L:*lag time (h). C: control treatment. Q: quebracho treatment. sed: standard error of difference. n.d.: not detected. Treat.: treatment. NS: $P > 0.10$. †: $P < 0.10$. *: $P < 0.05$.

to the parameters of N degradation (Table 1), the immediately degradable fraction (*a*) varied with the study week ($P < 0.05$), but no statistically significant differences were found ($P > 0.10$) related to the quebracho treatment. However, this did cause a significant reduction ($P < 0.05$) in *μ* value and increased the *T/2* time. The *b* fraction of the NDF and the *T/2* value varied with the study week ($P < 0.10$), but were not affected by ruminal administration of quebracho extract (Table 1).

Table 2 shows the mean values of disappearance of DM (DMD), N (ND) and NDF (NDFD) from the alfalfa hay after 24 h of *in situ* ruminal incubation. DMD and NDFD were only lower on day 8 of the experiment ($P < 0.05$) in the animals treated with quebracho (Q), and were not significantly different on the other incubation days. Ruminal disappearance varied with incubation day in all cases ($P < 0.05$).

Parameters of ruminal fermentation

Only for the sampling conducted before administration of the morning feed (hour 0), the effect of treatment (control *vs*. quebracho), both for pH and ammonia-N, varied with the study day (interaction treatment x day: $P < 0.05$).

Six and 9 h after morning feeding (see Fig. 1), the animals that received the quebracho extract presented significantly higher levels of ammonia-N ($P < 0.05$) than those of control animals (255 *vs*. 205 and 227 *vs*. 185 mg L⁻¹ for 6 and 9 h post-feeding, respectively).

For the VFA concentration, however (Fig. 1), treatment with quebracho did not have any significant effect ($P > 0.05$). Changes due to «day» were observed but the interaction «treatment x day» was not significant ($P > 0.10$).

Table 2. Effect of administration of quebracho on the rumen disappearance (g g^{-1}) of alfalfa hay DM (DMD), N (ND) and NDF (NDFD), after 24 h *in situ* incubation, on days 0, 8, 15, 23, 36, 50 and 64 of the experiment

Day	DMD			ND			NDFD		
	C	Q	sed	C	Q	sed	C	Q	sed
0	0.6706	0.6544	0.00885	0.8791	0.8597	0.01379	0.3569	0.3499	0.01918
8	0.6394[a]	0.5997[b]	0.00885	0.8519	0.8309	0.01379	0.3202[a]	0.2654[b]	0.02088
15	0.6296	0.6237	0.00885	0.8464	0.8464	0.01456	0.2821	0.2697	0.01787
23	0.6245	0.6312	0.00885	0.8525	0.8474	0.01456	0.2542	0.2879	0.01776
36	0.6347	0.6251	0.00885	0.8382	0.8380	0.01379	0.2707	0.2532	0.01709
50	0.6510	0.6342	0.00885	0.8599	0.8383	0.01379	0.3078	0.2912	0.01709
64	0.6464	0.6292	0.00885	0.8583	0.8403	0.01456	0.2911	0.2762	0.01790
Significance level (P)									
Treat.		*			NS			NS	
Day		***			*			***	
Treat. × Day		**			NS			*	

C: control treatment. Q: quebracho treatment. sed: standard error of the difference. Treat.: treatment. NS: $P > 0.10$. *: $P < 0.05$. **: $P < 0.01$. ***: $P < 0.001$. [a,b] For each parameter, means with different super indices in the same row differ significantly ($P < 0.05$).
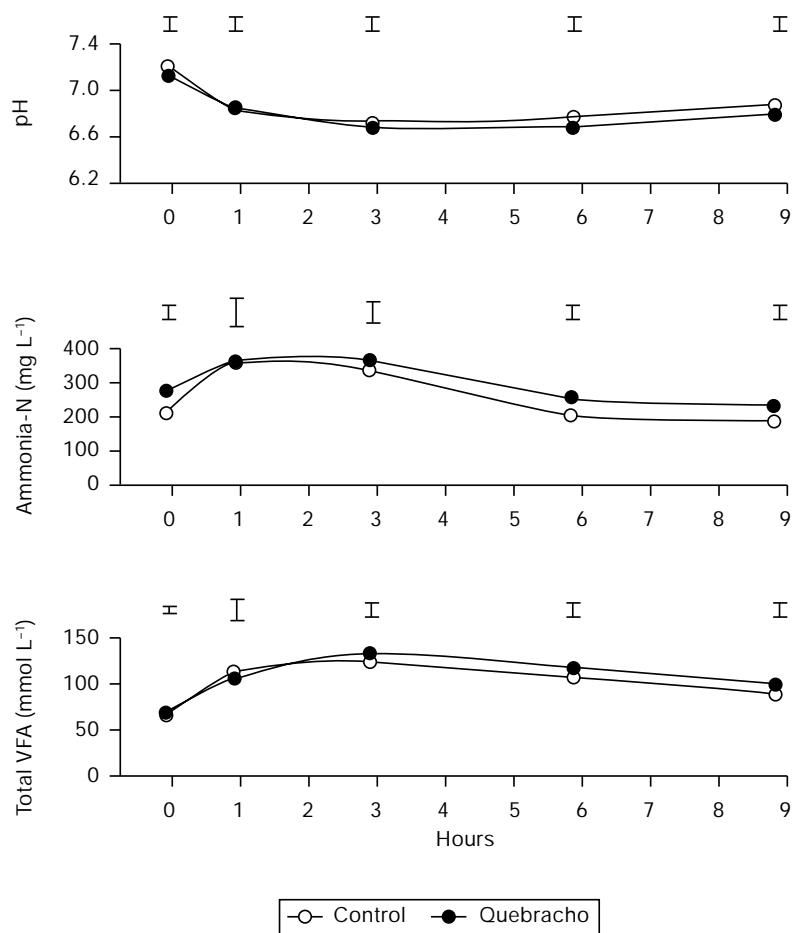

Figure 1. Effect of quebracho administration on the evolution of ruminal pH and concentrations of ammonia-N and total VFA at 0, 1, 3, 6 and 9 h after administration of the morning feed.

Table 3. Effect of quebracho administration on the *in vivo* digestibility (g g^{-1}) of alfalfa hay DM, N, NDF and ADF, in weeks 5 and 10 of the experiment

	DM			N			NDF			ADF		
	Week 5	Week 10	sed	Week 5	Week 10	sed	Week 5	Week 10	sed	Week 5	Week 10	sed
C	0.662	0.678	0.0082	0.780	0.794	0.0105	0.484	0.533	0.0230	0.487	0.542	0.0266
Q	0.618	0.635	0.0082	0.747	0.753	0.0105	0.440	0.487	0.0230	0.459	0.499	0.0266
Signif. level (P)												
Treat.		***			**			*			NS	
Week		**			NS			**			**	
Treat. × Week		NS			NS			NS			NS	

sed: standard error of difference. C: control treatment. Q: quebracho treatment. Treat.: treatment. NS: $P > 0.10$. *: $P < 0.05$. **: $P < 0.01$. ***: $P < 0.001$.

In vivo digestibility

Ruminal administration of quebracho extract (see Table 3) reduced the digestibility of the DM ($P < 0.001$), of N ($P < 0.01$) and of the NDF ($P < 0.05$) of alfalfa hay. In the case of ADF digestibility, the reduction (0.515 *vs.* 0.479 g g^{-1}, $P = 0.1059$) did not reach the required level of significance. In most cases, a significant variation was observed with week, with slightly higher digestibility values being obtained in week 10 of the experiment.

Discussion

The results of *in situ* incubations of alfalfa hay confirm the known fact that the presence of CT in ruminant diets can reduce ruminal degradation of feeds (Mueller-Harvey and McAllan, 1992; Aerts *et al.*, 1999; Barry and McNabb, 1999). This effect seems to be mainly due to the lower rate of degradation and to the reduced amount of substrate available for microorganisms, since tannins interfere with microbial attachment (and consequently inhibit digestion by rumen bacteria) and the complexes formed between tannins and other molecules makes them inaccessible to ruminal microorganisms (Mueller-Harvey and McAllan, 1992; McAllister *et al.*, 1994; Aharoni *et al.*, 1998; Makkar, 2001).

In this work, administration of quebracho extract was found to significantly reduce the fractional rate of degradation of the alfalfa hay N. It also reduced the potentially degradable fraction (*b*) of the DM, which could be attributed to the inaccessibility of ruminal microorganisms to the feed, as a consequence of the treatment with CT.

It is noteworthy that, contrarily to what has been mentioned by other authors (Aharoni *et al.*, 1998; Frutos *et al.*, 2000), no negative effect of CT on the immediately degradable fraction (*a*) occurred in this experiment. However, it should be taken into account that reduction in the *a* fraction has been observed when the feed themselves contain tannins which, in relation to the above mentioned substrate deprival effect, probably cannot have the same effect as the presence of tannins in the rumen. Moreover, it could be suspected that complexes with the condensed tannins administered in the rumen were not formed immediately, which would also explain the lack of effect on the *a* fraction. Nevertheless, this point cannot be confirmed given the methodology followed here.

Although one of the most common effects of CT is the reduction of rumen protein degradation (given the great affinity between these phenolic compounds and proteins; McLeod, 1974), in our work, the slight decline observed in the group of animals treated with quebracho (Q) was not significant ($P = 0.1422$). Nevertheless, this result should be interpreted with care since this absence of variation could possibly be because the 24 h incubations in nylon bags were too long to clearly observe any effect on protein degradation.

In general, the administration of quebracho extract for a relatively long period of time would be expected to cause the development of different defense mechanisms by the ruminal microorganisms in response to these secondary compounds (O'Donovan and Brooker, 2001). This response may be very variable depending on the rumen microbiota, since different ruminal bacterial strains present different susceptibility to CT (Nelson, 1996). This would explain why, after an initial negative effect on DMD and NDFD, values

of rumen disappearances were similar in both experimental treatments. However, this point was not clear for the case of protein (ND). On the one hand, as mentioned above, 24 h could be too long a time to observe effects on protein degradation but, on the other hand, according to the results of ruminal degradation kinetics (see Table 1), the rate of protein degradation was still lower in the Q group even in week 10 of the experiment.

Changes in alfalfa hay rumen degradation could be reflected in certain parameters of ruminal fermentation such as pH and ammonia-N and VFA concentrations. However, in general, these changes were not observed in our experiment. Ruminal pH was maintained at all times within a «normal» range in both treatments (6.6–7.4), suggesting that the administration of the quebracho solution (pH ≈ 5.5) was rapidly compensated in the rumen. Ammonia-N and total VFA concentrations were similar to those obtained by other authors using comparable diets. Similar variations were observed among sampling days in both treatments. Several authors have observed that CT intake usually causes a reduced ammonia-N concentration resulting from the decline in ruminal protein degradation. However, not only were differences not found in most cases, but also the mean concentration of ammonia-N, at 6 and 9 h post-feeding, was significantly higher in animals treated with quebracho (Q). This was probably largely due to a higher initial ammonia-N concentration in animals in this experimental treatment ($P < 0.05$), in spite of these having received the same treatment for the 15 pre-experimental days. Nevertheless, in other experiments performed on sheep fed a diet containing quebracho extract (Salem, 2002), increased ammonia-N concentration with no variation in VFA was also observed, in accordance with our results. Several authors (for example, Aerts *et al.*, 1999; Barry and McNabb, 1999) have reported that CT concentrations lower than 50 g per kg DM do not affect most ruminal fermentation parameters.

One of the best known effects of CT on diet digestibility is the reduction of apparent protein digestibility. However, although many studies have reported a rise in faecal N excretion in response to increasing dietary tannin concentration (Barry *et al.*, 1986; Mehansho *et al.*, 1987; Komolong *et al.*, 2001; McSweeney *et al.*, 2001), the increased faecal N in the presence of these compounds would be more likely to correspond to an increase of faecal metabolic N (comprised of cells from the intestinal mucosa, digestive enzymes, mucus, salivary proteins, etc.) (Mitjavila *et al.*, 1977; Dawson *et al.*, 1999), than to a real reduction in the amount of protein absorbed, giving rise, therefore, to clear underestimations of the true digestibility of dietary protein (Waghorn, 1996). Nonetheless, the possibility that the tannins could prevent absorption of amino acids from the intestine (McNeill *et al.*, 1998; Dawson *et al.*, 1999) or that the tannin-protein complexes had not been completely dissociated after crossing the rumen (McSweeney *et al.*, 2001) cannot be ruled out.

Regarding the digestibility of structural carbohydrates, Barry *et al.* (1986) reported that the CT of *Lotus corniculatus* affected neither cellulose nor hemicellulose digestibility. In contrast, Waghorn and Shelton (1995) observed that the digestibility of hemicellulose was reduced in a group of male sheep fed *ad libitum* a mixture of ryegrass and *Lotus pedunculatus*. This disparity could be due to the different tannin contents of *L. corniculatus* and *L. pedunculatus* (30-45 *vs.* 70-100 g CT kg^{-1} DM). Salawu *et al.* (1997) supplemented sheep with 50 g quebracho per kg of DM and observed, like in our experiment (in which the dose corresponded to *ca.* 37.5 g quebracho extract kg^{-1} DM), not only a reduced digestibility of the NDF but also of the ADF. The greater effect of the quebracho extract on NDF digestibility than on ADF digestibility could be related to the greater susceptibility of hemicellulolytic enzymes to tannins (Waghorn, 1996).

Acknowledgements

References

AERTS R.J., BARRY T.N., McNABB W.C., 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agric 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.

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

BARRY T.N., MANLEY T.R., DUNCAN S.J., 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influence by dietary reactive tannin concentration. Br J Nutr 55, 123-137.

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

CARRO M.D., LÓPEZ S., VALDÉS C., GONZÁLEZ J.S., 1999. Effect of nitrogen from casein and urea on the in vitro degradation of cell walls from six forages. J Anim Physiol Anim Nutr 81, 212-222.

DAWSON J.M., BUTTERY P.J., JENKINS D., WOOD C.D., GILL M., 1999. Effects of dietary quebracho tannin on nutrient utilisation and tissue metabolism in sheep and rats. J Sci Food Agric 79, 1423-1430.

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., HERVÁS G., GIRÁLDEZ F.J., FERNÁNDEZ M., MANTECÓN A.R., 2000. Digestive utilization of quebracho-treated soy bean meal in sheep. J Agric Sci, Camb 134, 101-108.

GOERING M.K., VAN SOEST P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook, No. 379. Agricultural Research Service, USDA. Washington.

HERVÁS G., FRUTOS P., GIRÁLDEZ F.J., MANTECÓN A.R., ÁLVAREZ DEL PINO M.C., 2003. Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. Anim Feed Sci Technol 109, 65-78.

KOMOLONG M.K., BARBER D.G., McNEILL D.M., 2001. Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucerne hay (*Medicago sativa*) with increasing levels of quebracho tannins. Anim Feed Sci Technol 92, 59-72.

MAKKAR H.P.S., 2001. Chemical, protein precipitation and bioassays for tannins, effect and fate of tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. 9th Seminar of the FAO-CIHEAM sub-network, Nutrition and feeding strategies of sheep and goats under harsh climates, 8-10 November 2001. Hammamet, Tunisia. 60 pp.

McALLISTER T.A., BAE H.D., YANKE L.J., CHENG K.-J., MUIR A., 1994. Effect of condensed tannins from birdsfoot trefoil on the endoglucanase activity and the digestion of cellulose filter paper by ruminal fungi. Can J Microbiol 40, 298-305.

McLEOD M.N., 1974. Plant tannins - Their role in forage quality. Nutr Abst Rev 44, 803-812.

McNEILL D.M., OSBORNE N., KOMOLONG M.K., NANKERVIS D., 1998. Condensed tannins in the genus *Leucaena* and their nutritional significance for ruminants. Proceedings of a Workshop on Leucaena adaptation, quality and farming systems (Shelton H.M., Gutteridge R.C., Mullen B., Bray R.A., eds.) ACIAR, Canberra, Australia, pp. 205-214.

McSWEENEY C.S., PALMER B., McNEILL D.M., KRAUSE D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim Feed Sci Technol 91, 83-93.

MEHANSHO H., BUTLER L.G., CARLSON D.M., 1987. Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms. Annual Rev Nutr 7, 423-440.

MILLER S.M., BROOKER J.D., BLACKALL L.L., 1995. A feral goat rumen fluid inoculum improves nitrogen retention in sheep consuming a mulga (*Acacia aneura*) diet. Aust J Agric Res 46, 1545-1553.

MITJAVILA S., LACOMBE C., CARRERA G., DERACHE R., 1977. Tannic acid and oxidised tannic acid on the functional state of rat intestinal epithelium. J Nutr 107, 2113-2121.

MUELLER-HARVEY I., McALLAN A.B., 1992. Tannins. Their biochemistry and nutritional properties. In: Advances in plant cell biochemistry and biotechnology (Morrison I.M., ed.). JAI Press Ltd., London, Vol. 1., pp. 151-217.

NELSON K.E., 1996. Tannins and ruminal bacteria. Ph.D. Thesis. Cornell University, Ithaca, NY.

O'DONOVAN L., BROOKER J.D., 2001. Effect of hydrolysable and condensed tannins on growth, morphology and metabolism of *Streptococcus gallolyticus* (*S. caprinus*) and *Streptococcus bovis*. Microbiol 147, 1025-1033.

SALAWU M.B., ACAMOVIC T., STEWART C.S., HOVELL F.D.DeB., 1997. Quebracho tannins with or without Browse Plus (a commercial preparation of polyethylene glycol) in sheep diets: effect on digestibility of nutrients *in vivo* and degradation of grass hay *in sacco* and *in vitro*. Anim Feed Sci Technol 69, 67-78.

SALEM A.Z.M., 2002. Producción y composición de saliva por la glándula parótida, comportamiento alimentario, fermentación ruminal, digestibilidad y parámetros plasmáticos en el ganado ovino y caprino: evolución de la respuesta a la presencia de taninos condensados de quebracho en la dieta. Doctoral thesis. Universidad de León, Spain.

SAS, 1989. SAS/STAT®. User's Guide Int. (V. 6, 4th ed.). SAS Institute Inc. Cary, NC, USA.

SCHWAB C.G., 1995. Protected proteins and amino acids for ruminants. In: Biotechnology in animal feeds and animal feeding (Wallace R.J., Chesson A., eds.) V.C.H. Press, Weinhein, Germany, pp. 115-141.

WAGHORN G., 1996. Condensed tannins and nutrient absorption from the small intestine. Proc of the Canadian Society of Animal Science Annual Meeting (Rode L.M., ed.) Lethbridge, Canada, pp. 175-194.

WAGHORN G.C., SHELTON I.D., 1995. Effect of condensed tannins in *Lotus pedunculatus* value of ryegrass (*Lolium perenne*) fed to sheep. J Agric Sci 125, 291-297.

WAGHORN G.C., SHELTON I.D., McNABB W.C., McCUTCHEON S.N., 1994. Effects of condensed tannins in *Lotus pedunculatus* its nutritive value for sheep. 2. Nitrogenous aspects. J Agric Sci 123, 109-119.

WEATHERBURN M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 39, 971-974.