**Effect of feeding young oak (*Quercus pyrenaica*) leaves to cattle on
ruminal fermentation***

Effect of the administration of young oak (*Quercus pyrenaica*) leaves to
cattle on ruminal fermentation*

R.R. Doce, G. Hervás, A. Belenguer, P.G. Toral, F.J. Giráldez, P. Frutos¹

Estación Agrícola Experimental (CSIC), Finca Marzanas, 24346-Grulleros, León, Spain

* Presented in part at the VII International Symposium on the Nutrition of Herbivores (ISNH-7), 16-21 September 2007. Beijing, China.

* Presented in part at the VII International Symposium on the Nutrition of Herbivores (ISNH-7), 16-21 September 2007. Beijing, China.

¹ Corresponding author: p.frutos@eae.csic.es (Dr. P. Frutos)

Abstract

Two experiments were conducted to study effects of feeding young Pyrenean oak leaves (OLs) to beef cattle on *in vitro* ruminal fermentation. A total of twelve ruminally cannulated Brown Swiss bulls were divided into experimental groups that were fed different quantities of OLs via the ruminal cannula (on average 0, 2.5, 5.2 and almost 10 kg fresh matter/animal and day; the treatments designated control, L-OL, H-OL and VH-OL, respectively). Batch cultures of rumen microorganisms and the *in vitro* gas production technique were used to study ruminal fermentation of two substrates (grass hay and OL) incubated with rumen inocula derived from each bull. Results showed not only a dose-dependent negative effect of feeding tannin-containing OL on ruminal fermentation of conventional feeds, such as the grass hay, but also improved fermentation of tannin-containing feeds, such as the OLs, in bulls fed moderate quantities of OLs (i.e., L-OL and H-OL), which suggests adaptation of the rumen microbial population. Detrimental effects of the VH-OL treatment indicate that the high level of tannins in the rumen of bulls on the highest dose of OLs would have exceeded the capacity of the microorganisms to resist or detoxify them.

Keywords: Beef cattle; *In vitro* gas production; Pyrenean oak; Hydrolysable tannin

Abbreviations: A, asymptotic gas production; ADF, acid detergent fibre; AFR, average fermentation rate; c, fractional rate of gas production; CP, crude protein; DM, dry matter; DMD, DM disappearance; ED, extent of degradation; HT, hydrolysable tannins; LW, live weight; NDF, neutral detergent fibre; OL, oak leaves; OM, organic matter; TT, total tannins; VFA, volatile fatty acids.

1. Introduction

Oak trees, shrubs and saplings are found worldwide and, wherever cattle eat their young leaves (OLs), cases of intoxication can occur. In the hill areas of northern Spain, where the Pyrenean oak (*Quercus pyrenaica*) is found, intoxications of beef cattle occur

recurrently in the spring when scarcity of other feeds leads cattle to consume immature oak leaves. Young OL contain a high level of hydrolysable tannins (HTs; Salminen et al., 2004) which are considered to be responsible for the intoxication (Plumlee et al., 1998). Although clinical and pathological signs associated with this toxicosis have been described (Spier et al., 1987; Yeruham et al., 1998), very little is known about the effect of consumption of young oak leaves on ruminal fermentation.

High levels of tannins are known to exert negative effects, including a reduction of voluntary feed intake and nutrient digestibility as well as impairment of ruminal fermentation (McAllister et al., 1994; Frutos et al., 2004; Mueller-Harvey, 2006). However, it has also been shown that a gradual increase in the intake of secondary compound-containing plants may increase the ruminant's ability to tolerate or degrade them (Duncan et al., 1997). Hydrolysable tannins can undergo significant transformation in the rumen due to the presence of ester bonds that are cleaved by microbial enzymes, and there is sufficient evidence in the literature to support rumen microbial adaptation and degradation of those secondary compounds (McSweeney et al., 2003).

It was against this background that this study, part of a research project on *Quercus pyrenaica* intoxications in cattle, was conducted to investigate effects of feeding young Pyrenean oak leaves to beef cattle on *in vitro* ruminal fermentation and potential adaptation of rumen microorganisms to the presence of tannins in the diet.

2. Material and methods

2.1 Oak Leaves

Very young oak (*Quercus pyrenaica*) leaves were manually collected from saplings during the spring on a country estate in northwest Spain (Sahechores, León; latitude 42° 37' 49''N, longitude 5° 09' 25''W), at a mean altitude of about 900 m, and frozen within one hour at -30°C until used.

2.2 Animals and Diets

The experiments were in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes.

2.2.1. Experiment 1

Six young Brown Swiss bulls (about 1.4 years old, 534 ± 29.6 kg live weight (LW) at the beginning of the experiment), each fitted with a ruminal cannula of 10 cm internal diameter (Ankom Technology Corp., Macedon, NY, USA) were used. Bulls were individually penned and divided into three groups: control, L-OL and H-OL, balanced for LW. All bulls were fed a limited amount of grass hay (i.e., about 5 kg/animal and day; DM = 875 g/kg) for a 14 day adaptation period, after which the control bulls continued on this diet. Bulls on treatment L-OL were fed daily 14 g DM of grass hay plus 7 g DM of OLs/kg $LW^{0.75}$ (on average 1.7 kg of hay and 2.5 kg of OLs; $DM_{OL}=298$ g/kg) and those on treatment H-OL received 14 g DM of grass hay plus 14 g DM of OLs/kg $LW^{0.75}$ (on average 1.8 and 5.2 kg of hay and OLs, respectively). The oak leaves, defrosted and slightly chopped, were administered twice/day at approximately 08.30 and 20.00 h, through the rumen cannula to ensure that all bulls received the appropriate amount. Treatments lasted for 14 days and, on day 15, rumen fluid from each animal was collected for the *in vitro* ruminal fermentation study. Clean water and vitamin-mineral blocks were permanently available.

2.2.2. Experiment 2

Since none of the bulls in Experiment 1 showed any sign of toxicity, Experiment 2 was designed to consider that probably neither the feed scarcity simulated during the adaptation period (i.e., observed under practical conditions when cattle ingest OLs) nor the amount of OLs administered in the first experiment were sufficient to cause intoxication.

In this experiment, another six ruminally cannulated young bulls (same breed, similar age, 502 ± 25.5 kg LW at the beginning) were individually penned and divided into two groups of three, control and VH-OL, balanced for LW. They were fed a very limited amount of grass hay for 8 days (about 4 kg/animal for 2 days, 3 kg the next day, then 2 kg/day for 4 days, followed by one day of fasting). Thereafter, control bulls received 35 g DM of grass hay/kg LW^{0.75} (on average 3.6 kg per day) daily, while those on treatment VH-OL received a very high quantity of OLs (35 g DM of OL/kg LW^{0.75}, averaging almost 10 kg/day). The oak leaves were fed daily through the rumen cannula at three-hourly intervals from 8.30 to 20.30 h, in equal parts, for 3 days. Feeding then stopped as the bulls on VH-OL developed clinical signs of intoxication. On day 4, ruminal fluid from each animal was collected for the *in vitro* incubations. Clean water and vitamin-mineral blocks were permanently available.

2.3 In Vitro Ruminal Fermentation

2.3.1. Experiment 1

Rumen microbial fermentation was assessed *in vitro* using a modification of Theodorou et al.'s gas production technique (1994), as adapted by Mauricio et al. (1999).

Substrates incubated were grass hay and oak leaves (see Table 1 for chemical composition). Eighteen samples per substrate (500 mg DM; 3 treatments $\times$ 2 inocula/treatment $\times$ 3 flasks/inoculum) were incubated in sealed serum flasks at 39°C with 10 ml strained rumen fluid and 40 ml phosphate-bicarbonate buffer (McDougall, 1948). Ruminal inocula were obtained from each bull pre-feeding through the ruminal cannula,

from multiple sites, transferred to the laboratory in pre-warmed thermos flasks and then strained through a double layer of muslin and kept under CO₂ flushing.

Accumulated head-space gas pressures were measured at 2, 4, 6, 8, 10, 15, 19, 24, 30, 36, 48, 72, 96 and 120 h post-inoculation using a pressure transducer. Pressure values, corrected for the quantity of substrate organic matter (OM) incubated and gas released from the blanks (i.e., ruminal fluid plus buffer medium, without substrate, a total of 18 blank flasks), were used to estimate gas volumes by means of a predictive equation derived from earlier simultaneous pressure and volume measurements (Hervás et al., 2005).

Fermentation was stopped by swirling the flasks on ice. *In vitro* DM disappearance (DMD; g/kg) after 120 h incubation was estimated by filtering residues using pre-weighed sintered glass crucibles (100–160 µm; Pyrex, Stone, UK).

A further eighteen flasks per substrate (3 treatments × 2 inocula/treatment × 3 flasks/inoculum) were incubated for 24 hours and treated identically to those explained above. On termination of incubation, samples were immediately centrifuged (600 × *g* at 4°C for 15 min) to eliminate feed particles. Five ml of supernatant were acidified with 5 ml 0.2 N HCl for ammonia determination. A further 0.8 ml of the supernatant was added to 0.5 ml of a deproteinizing solution (0.02 metaphosphoric and 0.04 crotonic acids, in 0.5 N HCl) for determination of volatile fatty acids (VFA). All samples were stored at –30°C until analysis. The remaining contents of the centrifuge tube were returned to the flasks and the residues filtered through pre-weighed sintered glass crucibles, as explained above, to estimate DM disappearance after 24 h incubation (DMD₂₄).

2.3.2. Experiment 2

In the second experiment, the *in vitro* ruminal fermentation study was as described for Experiment 1, with incubation of 18 samples/substrate (2 treatments × 3 inocula/treatment × 3 flasks/inoculum) plus 18 blank flasks for the gas production kinetics and DMD and a further eighteen flasks for ammonia, VFA and DMD₂₄ determinations.

2.4. Chemical Analyses

Prior to incubations and analyses, freeze-dried samples of OLs and grass hay were ground to pass a 1 mm sieve. They were then analysed for DM (ISO 6496:1999), ash (ISO 5984:2002) and crude protein (CP, ISO 5983-2:2005). Neutral (NDF) and acid (ADF) detergent fibre were determined by methods described by Mertens (2002) and the AOAC (2006; official method 973.18), respectively, using an Ankom²⁰⁰⁰ Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). NDF was assayed with sodium sulphite but without alpha amylase and expressed with residual ash (the latter also for ADF).

Total tannin (TT) content was assayed in the OLs using the Folin-Ciocalteu method in combination with polyvinyl-polypyrrolidone, with tannic acid (Merck, Darmstadt, Germany) as the reference standard (Makkar, 2003). For determination of hydrolysable tannins (i.e., gallotannins and ellagitannins, as gallic and ellagic acid) the OLs were processed according to Makkar (2003) and analysed by ion-pair high-performance liquid chromatography (IP-RP-HPLC) as explained by del Moral et al. (2007).

Ammonia concentration was determined as described by Weatherburn (1967) and VFA by gas chromatography using crotonic acid as the internal standard (Ottenstein and Bartley, 1971).

2.5. Calculations and Statistical Analysis

Gas production (G) data were fitted to the exponential model:

$$G = A \times [1 - e^{-c \times (t - lag)}]$$

Where: *A* represents the asymptotic gas production, *c* is the fractional fermentation rate, *lag* is the initial delay in the onset of gas production and *t* is the gas reading time. The parameters *A*, *c* and *lag* were estimated by an iterative least squares procedure using the NLIN procedure of the Statistical Analysis System (SAS, 1999).

The extent of degradation in the rumen (ED) was estimated by assuming a rumen particulate outflow (k_p) of 0.042/h, according to the equation proposed by France *et al.* (2000):

$$ED = [c \times DMD / (c + k_p)] \times e^{-k_p \times lag}$$

The average fermentation rate (AFR; ml gas/h) was defined as the average gas production rate between the start of the incubation and the time at which the cumulative gas production was half of its asymptotic value, and was calculated as:

$$AFR = A \times c / [2 \times (\ln 2 + c \times lag)]$$

Data were analysed by one-way analysis of variance, using the General Linear Models (GLM) procedure of SAS (1999). The statistical model included the fixed effect of treatment (i.e., control vs. L-OL vs. H-OL in the first experiment, and control vs. VH-OL in the second) and the inoculum replicates nested within treatment. In the first experiment, treatment means were separated using the ‘pdiff’ option of the ‘lsmeans’ statement of the GLM procedure of SAS (1999). Significant differences were declared at $P < 0.05$ and tendencies accepted if $P < 0.10$.

3. Results

3.1. Experiment 1

As shown in Figure 1, feeding OLs to the bulls (treatments L-OL and H-OL) negatively affected *in vitro* gas production from grass hay. However, gas production increased when OL were incubated.

The fractional rate of gas production (c), the average fermentation rate (AFR) and the extent of degradation (ED) were lower ($P < 0.001$) in grass hay incubations with rumen inocula derived from L-OL and H-OL bulls, as was DM disappearance (Table 2) estimated at 24 h post-incubation ($P < 0.001$). Very little differences occurred between treatments L-OL and H-OL. In contrast, when OLs were incubated, all these *in vitro* ruminal

fermentation parameters (i.e., c , lag , AFR, ED and DMD₂₄) were higher in bulls on treatments L-OL and H-OL *versus* the controls ($P<0.001$), the effect being, in general, stronger in treatment H-OL.

Ammonia concentration and net production of isobutyrate + isovalerate + valerate + caproate (referred to as ‘others’ in Table 3) decreased in grass hay incubations with rumen inoculum from bulls fed OLs ($P<0.05$). The negative effect of the treatments with tannin-containing oak leaves on ammonia concentration also occurred in the OL incubations ($P<0.001$), where, however, total net VFA production was higher ($P<0.001$). The three major VFA (acetate, propionate and butyrate) increased ($P<0.05$), but not proportionally, such that molar proportions of butyrate were higher ($P<0.001$) and those of propionate lower ($P<0.01$), compared with the control incubations (data not shown).

3.2. Experiment 2

In vitro gas production from grass hay was much higher in incubations with inoculum from the control animals versus those fed the highest amount of oak leaves (VH-OL); with greater differences than in Experiment 1. In contrast, no substantial differences were observed for OL incubations due to inoculum source (see Figure 2).

In the grass hay cultures, both cumulative gas production (A) and rate (c) were decreased by the consumption of young oak leaves ($P<0.001$), whereas lag time increased ($P<0.05$). This was reflected in lower values of AFR and ED ($P<0.001$). The DMD₂₄ was also lower in VH-OL bulls ($P<0.001$). However, when OL were incubated with rumen inoculum from those animals, although there was also a tendency ($P<0.10$) to higher values of A and AFR in treatment VH-OL, only the lag time was increased in comparison with control bulls ($P<0.01$).

Ammonia concentration was always reduced (Table 3) in incubations with rumen fluid from bulls consuming tannin-containing leaves ($P<0.001$). For grass hay cultures, total VFA showed a tendency ($P<0.10$) to a lower net production when VH-OL rumen

fluid was used, which was accounted for by a reduction of acetate and ‘others’ ($P<0.05$). Propionate, in contrast, was lower in control incubations. When the substrate incubated was OL, cultures with VH-OL rumen inoculum decreased net production of acetate and ‘others’ ($P<0.05$), whereas butyrate increased ($P<0.001$).

4. Discussion

Young oak leaves have a high content of tannins, basically HTs, which may be toxic to grazing ruminants (Makkar et al., 1988; Salminen et al., 2004). On the contrary, mature leaves of several oak species are a major component of livestock diets in less favoured areas of many countries (Garg et al., 1992; Makkar and Becker, 1998). In Northern Spain, mature oak leaves are not fed to livestock, but cattle may browse very young leaves in spring, especially if other feeds are scarce.

The results of our study showed that the effect of young Pyrenean oak leaves fed to beef cattle on *in vitro* ruminal fermentation depended on the dose administered to the animals and on the substrate incubated.

As batch cultures of rumen microorganisms and the *in vitro* gas production technique are closed systems, they are especially reliable for detection of ruminal fermentation inhibiting compounds (Khazaal et al., 1994). Tannins have been widely reported to decrease both the rate and the extent of gas production (e.g., Getachew et al., 2000; Frutos et al., 2004), with this suppressive effect probably being the result of a reduction in the attachment of microbes to feed particles (McAllister et al., 1994) and an inhibition of microorganisms growth and microbial enzymes activity (McSweeney et al., 2003; Smith et al., 2005). In our study, however, the negative effect of tannin supply only occurred when the grass hay (conventional feed model) was incubated. Conversely, opposite results were observed for the ruminal fermentation of oak leaves (see Table 2 and Figure 1), with significantly higher values for the rate and extent of degradation of OLs when they were

incubated with rumen inoculum derived from animals fed the lowest and the intermediate doses of oak leaves (L-OL and H-OL, Experiment 1) and with no significant differences (with the exception of the lag time) when incubated with rumen fluid from bulls fed the highest amount of oak leaves (VH-OL, Experiment 2). These results confirm that the effect of tannins on ruminal fermentation activity is dose-dependent (Aerts et al., 1999; Hervás et al., 2003), although very little differences occurred between the L-OL and H-OL treatments. Furthermore, they suggest that the presence of tannins (mainly HTs) in the rumen of the bulls on treatments L-OL and H-OL may have elicited changes in their predominant microbial communities and/or favoured development of microbial mechanisms of adaptation, whereas the highest level (VH-OL) might have exceeded the ability of the microbes to resist tannins, which is suggested by the clinical intoxication suffered by the bulls on this treatment. The very high level of tannins that caused toxicity, and the resulting short period of OL feeding, probably overwhelmed the adaptation mechanisms of the ruminal microorganisms.

Notwithstanding, the microbiota of the gastrointestinal tract of ruminants have an extraordinary ability to adapt to a vast array of nutrients and secondary compounds present in the diet (McSweeney et al., 2003). Several ruminal microorganisms able to resist relatively high concentrations of tannins, as well as various strategies to overcome the inhibitory effects of tannins have been described (Smith et al., 2005). In this regard, for example, Krause et al. (2005) observed that *Streptococcus gallolyticus*, a bacteria found ubiquitously in the rumen, increased an extracellular polysaccharide coat gene expression in response to either hydrolysable or condensed tannins. Bae et al. (1993) reported that the net effect of exposure of *Fibrobacter succinogenes*, a predominant cellulolytic rumen bacterial species, to condensed tannins (0 to 300 µg/mL) was a transient increase in the activity of cell-associated endoglucanases that compensated the decline in the activity of

extracellular endoglucanases. As the concentrations of the tannins approached 400 µg/ml, all endoglucanases were inhibited and cellulose degradation ceased.

Rumen bacterial species that enzymatically cleave structural bonds in HT molecules, and utilize the degradation products for their own growth, such as *Selenomonas ruminantium*, have also been described (Brooker et al., 2000). In our case, the composition of the rumen bacterial population, determined by fluorescence *in situ* hybridization and real-time PCR (Polymerase Chain Reaction), has been observed to be affected by feeding young OL (Belenguer et al., 2008). Nevertheless, even though the population of tannin-tolerant and/or tannin-degrading microorganisms may increase in ruminants consuming tannins, these changes do not appear to prevent reduction in the fermentation of many feeds caused by these secondary compounds (McSweeney et al., 2003), which would explain the negative effect observed on grass hay fermentation.

Effects of administration of tannin-rich oak leaves were also reflected in commonly studied indicators of utilization of N and energy (i.e., ammonia and VFA). Ammonia concentration was always reduced when the substrates, either grass hay or OLs, were incubated with rumen fluid from bulls fed OL, even from those receiving the lowest dose (L-OL). Many authors have indicated that the principal effects of tannins in ruminal fermentation include a reduction in proteolysis of dietary protein and subsequently lower concentrations of ammonia in rumen fluid (Frutos et al., 2004; Mueller-Harvey, 2006). All ammonia concentrations were higher than the 100 mg/l reported by Van Soest (1994) as optimal for efficiency of amino acid synthesis and microbial growth. Although that value might depend on a number of factors, such as the amount of available fermentable energy (Huntington and Archibeque, 1999), ammonia concentrations were probably adequate for optimal rumen fermentation in all cases. Consistent with the lower concentrations of ammonia, the reduced net production of the 'other' VFA (originating mostly from deamination of some amino acids) in most incubations with inocula from bulls fed OL,

could also reflect effects of tannins on proteolysis. However, that these ‘other’ VFA were not reduced in the first experiment in OL cultures suggests that a better ruminal utilization of nitrogenous compounds in adapted animals (i.e., L-OL and H-OL) should not be ruled out as another explanation for the lower concentrations of ammonia.

While most studies agree on the inhibitory effect of tannins on ruminal proteolysis, effects reported on VFA production are not consistent, with different authors reporting different results (e.g., Getachew et al., 2000; Hervás et al., 2003), probably owing to the amount and type of tannins and/or the animal species involved. In this study, net VFA production was hardly affected by treatments L-OL and H-OL in grass hay cultures, but increased when OL were incubated, which supports improved ruminal fermentation of tannin-rich feeds in bulls consuming tannins. In addition, HTs have been reported to undergo degradation by microbial enzymes in the rumen to form gallic and ellagic acid. Gallic acid can then be decarboxylated to pyrogallol and converted to resorcinol and phloroglucinol before cleavage of the phloroglucinol ring to acetate and butyrate (Murdiani et al., 1992; McSweeney et al., 2003). However, a very high supply of OLs (treatment VH-OL) produced a detrimental effect on net VFA production in grass hay cultures, but did not improve *in vitro* fermentation of OLs, confirming that the level of HTs in the rumen of the bulls on this treatment exceeded the ability of the microbial populations to resist or detoxify them. Interestingly, divergences in the fermentation pathways were also observed, reflected in the different molar proportions of VFA, which might be due to stimulation of specific microbial groups to the detriment of others, or by a shift in microbial degradation pathways (Russel and Wallace, 1997). The clearest example of this possibility was the molar proportion of butyrate, which was higher in all incubations with rumen fluid from bulls fed high or very high quantities of oak leaves (i.e., H-OL and VH-OL) and even treatment L-OL stimulated its accumulation in OL cultures.

5. Conclusions

Results show a dose-dependent negative effect of tannin-containing oak leaves on *in vitro* ruminal fermentation of conventional feeds (e.g., grass hay), while also suggesting adaptation of the rumen microbiota in bulls consuming this feed, which may be of benefit to cattle fed tannin-rich forages. Further research is necessary to determine the role of rumen microbial populations to better utilize tannin-containing diets and prevent intoxications in cattle unadapted to consumption of HTs that may sporadically consume young oak leaves.

Acknowledgements

R.R. Doce, P.G. Toral and A. Belenguer gratefully acknowledge receipt of two predoctoral grants and a research contract, respectively, from the Ministry of Education and Science (MEC, Spain) and the Spanish National Research Council (CSIC). This work was supported by the MEC (project AGL2004-06076-C02-01). The authors wish to thank Drs. M.J. Arín and M.T. Díez (University of León, Spain) for the analysis of hydrolysable tannins.

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.
- AOAC, 2006. Official Methods of Analysis of the Association of Official Agricultural Chemists. 18th Edn. (1st. revision). AOAC International: Gaithersburg, MD, USA.
- Bae, H.D., McAllister, T.A., Yanke, J., Cheng, K.-J., Muir, A.D., 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. *Appl. Environ. Microbiol.* 59, 2132-2138.
- Belenguer, A., Hervás, G., De Paz, P., Ezquerro, C. Frutos, P., 2008. Changes in rumen bacterial communities from cattle receiving immature oak (*Quercus pyrenaica*) leaves

- determined by fluorescence in situ hybridization and real-time PCR. In Abstracts of: 6th INRA-RRI Symposium. Gut Microbiome: Functionality, interaction with the host and impact on the environment. INRA, Clermont-Ferrand, France, p.27.
- Brooker, J.D., Donovan, O., Skene, I., Sellick, G., 2000. Mechanisms of tannin resistance and detoxification in the rumen. In: J.D. Brooker (Ed), Tannins in Livestock and Human Nutrition. Proceedings of an International Workshop, Adelaide, Australia. ACIAR Proceedings No. 92, Australian Centre for International Agricultural Research, Adelaide, Australia, pp.117-122.
- del Moral, P.G., Arín, M.J., Resines, J.A., Díez, M.T., 2007. Determination of ellagic acid in oak leaves and in sheep ruminal fluid by ion-pair RP-HPLC. J. Chromatogr. B. 855, 276-279
- Duncan, A.J., Frutos, P., Young, S.A., 1997. Rates of oxalic acid degradation in the rumen of sheep and goats in response to different levels of oxalic acid administration. Anim. Sci. 65, 451-455.
- France, J., Dijkstra, J., Dhanoa, M.S., López, S., 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.
- Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R., 2004. An *in vitro* study on the ability of polyethylene glycol to inhibit the effect of quebracho tannins and tannic acid on rumen fermentation in sheep, goats, cows and deer. Aust. J. Agr. Res. 55, 1125-1132.
- Garg, S.K., Makkar, H.P.S., Nagal, K.B., Sharma, S.K., Wadhwa, D.R., Singh, B., 1992. Oak (*Quercus incana*) leaf poisoning in cattle. Vet. Human Toxicol. 34, 161-164.

- Getachew, G., Makkar, H.P.S., Becker, K., 2000. Effect of polyethylene glycol on *in vitro* degradability and microbial protein synthesis from tannin-rich browse and herbaceous legumes. Br. J. Nutr. 84, 73-83.
- Hervás, G., Frutos, P., Giráldez, F.J., Mora, M.J., Fernández, B., Mantecón, A.R., 2005. Effect of preservation on fermentative activity of rumen fluid inoculum for *in vitro* gas production techniques. Anim. Feed Sci. Technol. 123-124, 107-118.
- 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.
- Huntington, G.B., Archibeque, S.L., 1999. Practical aspects of urea and ammonia metabolism in ruminants. Proc. Am. Soc. Anim. Sci., online (<http://www.asas.org/jas/symposia/proceedings/0939.pdf>).
- Khazaal, K., Boza, J., Orskov, E.R., 1994. Assessment of phenolics-related antinutritive effects in Mediterranean browse: a comparison between the use of the *in vitro* gas production technique with or without insoluble polyvinylpolypyrrolidone or nylon bag. Anim. Feed Sci. Technol. 49, 133-149.
- Krause, D.O., Smith W.J.M., Brooker J.D., McSweeney, C.S., 2005. Tolerance mechanisms of streptococci to hydrolysable and condensed tannins. Anim. Feed Sci. Technol. 121, 59-75.
- Makkar, H.P.S., 2003. Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Makkar, H.P.S., Becker, K., 1998. Do tannins in leaves of trees and shrubs from African and Himalayan regions differ in level and activity? Agr. Syst. 40, 59-68.
- Makkar, H.P.S., Dawra, R.K., Singh, B., 1988. Changes in tannin content, polymerisation and protein precipitation capacity in oak (*Quercys incana*) leaves with maturity. J. Sci. Food Agric. 44, 301-307.

- 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.
- McDougall, E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* 43, 99-109.
- McSweeney, C.S., Makkar, H.P.S., Reed, J.D., 2003. Modification of rumen fermentation to reduce adverse effects of phytochemicals. *Proceedings of the VI International Symposium on the Nutrition of Herbivores*, Univ. Aut. Yucatán, Mérida, Mexico, pp. 239-268.
- Mertens, D. R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* 85, 1217-1240.
- Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.* 86, 2010-2037.
- Murdiati, T.B., McSweeney, C.S., Lowry, J.B., 1992. Metabolism in sheep of gallic acid, tannic acid and hydrolysable tannin from *Terminalia oblongata*. *Aust. J. Agr. Res.* 43, 1307-1319.
- Ottenstein, D.M., Bartley, D.A., 1971. Improved gas chromatography separation of free acids C2-C5 in dilute solution. *Anal. Chem.* 43, 952-955.
- Pell, A.N., Woolston, T.K., Nelson, K.E., Schofield, P., 2000. Tannins: Biological activity and bacterial tolerance. In: J.D. Brooker (Ed), *Tannins in Livestock and Human Nutrition. Proceedings of an International Workshop*, Adelaide, Australia. ACIAR Proceedings No. 92, Australian Centre for International Agricultural Research, Adelaide, Australia, pp.111-117.

- Plumlee, K.H., Johnson, B., Galey, F.D., 1998. Comparison of disease in calves dosed orally with oak or commercial tannic acid. *J. Vet. Diagn. Invest.* 10, 263-267.
- Russel, J.B., Wallace, R.J., 1997. Energy-yielding and energy-consuming reactions. In: P.N. Hobson, C.S. Stewart (Eds), *Rumen Microbial Ecosystem*. Chapman and Hall, London, UK, pp.246-282.
- Salminen, J.P., Roslin, T., Karonen, M., Sinkkonen, J., Pihlaja, K., Pulkkinen, P., 2004. Seasonal variation in the content of hydrolyzable tannins, flavonoid glycosides, and proanthocyanidins in oak leaves. *J. Chem. Ecol.* 30, 1693-1711.
- SAS, 1999. SAS OnlineDoc[®], Version 8, SAS Institute Inc., Cary, NC, USA.
- Smith, A.H., Zoetendal, E., Mackie, R.I., 2005. Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microb. Ecol.* 50, 197-205.
- Spier, S.J., Smith, B.P., Seawright, A.A., Norman, B.B., Ostrowski, S.R., Oliver, M.N., 1987. Oak toxicosis in cattle in northern California: clinical and pathologic findings. *J. Am. Vet. Med. Ass.* 191, 958-964.
- Theodorou, M.K., Williams, 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.
- Van Soest, P.J., 1994. *Nutritional Ecology of the Ruminant* (2nd Edition), Cornell University Press, Ithaca, NY, USA.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583-3597.
- Weatherburn, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* 39, 971-974.

Yeruham, I., Avidar, Y., Perl, S., Yakobson, B., Shlosberg, A., Hanji, V., Bogin, E., 1998.

Probable toxicosis in cattle in Israel caused by the oak *Quercus calliprinos*. Vet.

Human Toxicol. 40, 336-340.

Table 1

Chemical composition of the substrates incubated (g/kg DM).

	CP	NDF	ADF	TT*	Ellagic acid	Gallic acid
Grass hay	158 (0.3)	482 (4.9)	299 (5.8)	nd	nd	nd
Oak leaves	194 (4.3)	284 (5.9)	181 (2.5)	230 (3.8)	18.8 (0.97)	11.0 (1.13)

* TT: Total tannins (expressed as tannic acid equivalents). nd = not determined.
Standard error of the means in brackets (n=2)

Table 2

In vitro gas production parameters (*A*, mL/g OM; *c*, /h and *lag*, h), average fermentation rate (AFR, ml/h), extent of degradation (ED, g/kg), and DM disappearance after 24 h incubation (DMD₂₄, g/kg) from grass hay and oak leaves incubated with rumen inocula from bulls receiving no (control), low (L-OL), high (H-OL) or very high (VH-OL) quantities of oak leaves

	Grass hay						Oak leaves					
	<i>A</i>	<i>c</i>	<i>lag</i>	AFR*	ED	DMD ₂₄	<i>A</i>	<i>c</i>	<i>lag</i>	AFR	ED	DMD ₂₄
Experiment 1												
Control	305.9	0.069 ^a	2.54 ^{ab}	12.13 ^a	382.4 ^a	536.4 ^a	221.9	0.013 ^c	0.07 ^b	2.02 ^c	146.7 ^c	431.2 ^b
L-OL	306.4	0.043 ^b	2.67 ^a	8.17 ^b	292.7 ^b	424.4 ^b	227.2	0.022 ^b	1.39 ^a	3.37 ^b	201.1 ^b	516.2 ^a
H-OL	302.1	0.044 ^b	2.35 ^b	8.27 ^b	279.0 ^b	451.2 ^b	231.4	0.026 ^a	2.10 ^a	4.00 ^a	229.0 ^a	511.5 ^a
sed	2.33	0.0010	0.105	0.151	7.88	18.32	4.31	0.0015	0.389	0.179	9.75	18.28
<i>P</i>	0.2605	<0.0001	0.0325	<0.0001	<0.0001	0.0001	0.1270	<0.0001	0.0007	<0.0001	<0.0001	0.0008
Experiment 2												
Control	306.0 ^a	0.057 ^a	1.04 ^b	11.56 ^a	371.5 ^a	557.2 ^a	157.9	0.025	0.09 ^b	2.82	222.6	462.9
VH-OL	207.3 ^b	0.038 ^b	1.75 ^a	5.17 ^b	272.5 ^b	399.6 ^b	163.3	0.028	1.36 ^a	2.97	233.4	451.4
sed	1.96	0.0013	0.301	0.123	6.53	10.50	2.80	0.0021	0.397	0.080	7.70	9.30
<i>P</i>	<0.0001	<0.0001	0.0353	<0.0001	<0.0001	<0.0001	0.0751	0.1846	0.0077	0.0842	0.1860	0.2363

* Average gas production rate between the start of the incubation and the time at which the cumulative gas production was half of its asymptotic value.

P: level of significance.

For each experiment, means in a column with different letters differs significantly ($P < 0.05$).

Table 3

Ammonia (NH₄) concentration and net VFA (mmol/L) production from grass hay and oak leaves incubated for 24 h with rumen inocula from bulls receiving no (control), low (L-OL), high (H-OL) or very high (VH-OL) quantities of oak leaves

	Grass hay						Oak leaves					
	NH ₄	Total VFA	Acet	Prop	But	Others*	NH ₄	Total VFA	Acet	Prop	But	Others*
Experiment 1												
Control	333.8 ^a	38.43	24.91	8.75	3.03	1.74 ^a	213.3 ^a	15.67 ^b	11.39 ^b	2.99 ^b	1.08 ^c	0.21
L-OL	186.7 ^b	34.95	21.51	9.83	2.77	0.84 ^b	138.5 ^b	24.42 ^a	18.23 ^a	3.65 ^{ab}	2.28 ^b	0.26
H-OL	176.9 ^c	35.25	22.91	8.25	3.45	0.63 ^b	143.7 ^b	27.24 ^a	19.93 ^a	4.25 ^a	2.88 ^a	0.18
sed	3.62	3.119	2.051	0.833	0.234	0.288	2.94	2.137	1.576	0.407	0.215	0.126
<i>P</i>	<0.0001	0.5417	0.4155	0.3714	0.0947	0.0103	<0.0001	0.0004	0.0004	0.0301	<0.0001	0.8107
Experiment 2												
Control	274.5 ^a	36.82	24.14 ^a	8.42 ^b	2.71	1.55 ^a	177.4 ^a	20.29	14.48 ^a	3.91	1.53 ^b	0.36 ^a
VH-OL	178.8 ^b	32.60	20.38 ^b	9.55 ^a	2.51	0.16 ^b	142.0 ^b	19.07	12.79 ^b	4.21	2.06 ^a	0.02 ^b
sed	3.14	1.980	1.327	0.478	0.146	0.150	2.62	0.952	0.752	0.217	0.070	0.039
<i>P</i>	<0.0001	0.0547	0.0149	0.0350	0.2043	<0.0001	<0.0001	0.2256	0.0436	0.1946	<0.0001	0.0017

* Calculated as the sum of isobutyrate, isovalerate, valerate and caproate. VFA = volatile fatty acids, Acet=acetate, Prop=propionate, But=butyrate.

P: level of significance.

For each experiment, means in a column with different letters differs significantly ($P<0.05$).

FIGURES CAPTION

Figure 1.

Experiment 1. Cumulative gas production profiles (ml/g OM) from grass hay and oak leaves incubated with rumen inocula from bulls fed no (control), low (L-OL) or high (H-OL) quantities of oak leaves (sed for the interaction incubation time x treatment = 2.28 for grass hay and 5.19 for oak leaves).

Figure 2.

Experiment 2. Cumulative gas production profiles (ml/g OM) from grass hay and oak leaves incubated with rumen inocula from bulls fed no (control) or a very high (VH-OL) quantity of oak leaves (sed for the interaction incubation time x treatment = 3.32 for grass hay and 1.19 for oak leaves).

FIGURE 1

FIGURE 2