

Effect of immature oak (*Quercus pyrenaica*) leaves intake on ruminal fermentation and adaptation of rumen microorganisms in cattle*

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ABSTRACT

Two experiments were conducted to study the effect of the consumption of different amounts of immature Pyrenean oak leaves (OL) by beef cattle on *in vitro* ruminal fermentation and potential adaptation of rumen microorganisms. A total of twelve ruminally cannulated young Brown Swiss bulls were divided in experimental groups that received different amounts of OL *via* the ruminal cannula (on average 0, 2.5, 5.2 and almost 10 kg fresh matter per animal and day). The gas production technique was used to study *in vitro* fermentation of two substrates (grass hay and OL) incubated with rumen inocula derived from each bull. Results suggest not only a dose-dependent negative effect of tannins consumption on ruminal fermentation of common feeds (e.g., grass hay), but also an adaptation of rumen microbial populations from animals receiving moderate amounts of OL. The high level of tannins in the rumen of bulls that received the highest amount of OL would have exceeded the ability of microorganisms to tolerate or detoxify them.

KEY WORDS: beef cattle, *in vitro* gas production, Pyrenean oak, tannin

INTRODUCTION

Oak trees, shrubs and saplings can be found worldwide, and wherever cattle eat their immature leaves, cases of intoxication can occur. In the hill areas of northern Spain, intoxications of beef cattle occur recurrently in the spring, when the animals graze in Pyrenean oak (*Quercus pyrenaica*) areas and consume immature oak leaves. These leaves

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contain a high level of hydrolysable tannins (HTs) which are thought to be responsible for the intoxication (Plumlee et al., 1998). Although the clinical and pathological signs associated with this toxicosis have been described, very little is known about the effect of oak leaves intake on ruminal fermentation and about the potential adaptation of rumen microbial populations to the inclusion of tannins in the diet.

High levels of tannins are known to exert antinutritional effects and impair ruminal fermentation (Makkar, 2003). However, it has been indicated that a gradual increase in the intake of secondary compound-containing plants may increase the ability of the ruminant to tolerate or degrade them (Duncan et al., 1997). In addition, the literature contains sufficient evidence supporting ruminal adaptation and degradation of hydrolysable tannins (McSweeney et al., 2001).

This work, which forms part of a research project on intoxications with *Quercus pyrenaica*, was conducted to study the effect of the consumption of different amounts of immature Pyrenean oak leaves by beef cattle on *in vitro* ruminal fermentation and potential adaptation of rumen microorganisms.

MATERIAL AND METHODS

Immature oak (*Quercus pyrenaica*) leaves (OL; DM - 297 g/kg) were collected from saplings during the spring and frozen at -30°C until the experiments. Aliquots to be used as the substrate for *in vitro* incubations were freeze-dried and chemically analysed. Their total tannin content (determined using the Folin-Ciocalteu method; Makkar et al., 1993) was 230 ± 3.8 g of tannic acid equivalents/kg DM.

Two experiments were carried out:

Experiment 1

Six young Brown Swiss bulls (about 1.4 years old, 534 ± 29.6 kg BW at the beginning of the experiment), each equipped with a ruminal cannula, were divided into three groups of two animals: control, 2.5-OL and 5-OL. All animals were fed a limited amount of grass hay (on average 5 kg per animal and day; DM-875 g/kg) for a 14-day adaptation period. Then, the bulls used as control continued receiving the same amount of grass hay. Animals on treatment 2.5-OL received daily 14 g DM of grass hay plus 7 g DM of OL/kg $BW^{0.75}$ (on average 1.7 kg of hay and 2.5 kg of OL) and those on treatment 5-OL received 14 g DM of grass hay plus 14 g DM of OL/kg $BW^{0.75}$ (on average 1.8 and 5.2 kg of hay and OL, respectively). The oak leaves, defrosted and slightly chopped, were administered twice per day (at 08.30 and 20.00 h approx.) through the rumen cannula to ensure that all animals received the established amount. Treatments lasted for 14 days and, on day 15th, rumen fluid from each animal was collected for the *in vitro* study.

In vitro gas production was studied using a modification of the technique described by Theodorou et al. (1994), as explained by Frutos et al. (2004). The substrates incubated were the two feeds used in the *in vivo* trial: grass hay (CP-158, NDF-452, ADF-279, g/kg DM) and OL (CP-194, NDF-323, ADF-172, g/kg DM).

Eighteen samples per substrate (500 mg DM) (3 treatments \times 2 inocula (replicates)/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 (Goering and Van Soest, 1970). The ruminal inocula were obtained from each animal through the ruminal cannula, 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 four times during the incubation period (10, 24, 48 and 120 h post-incubation), using a pressure transducer. Pressure values, corrected for the quantity of substrate OM incubated and gas released from the blanks (i.e. ruminal fluid plus buffer medium, without substrate, 18 blank flasks in total), were used to generate gas volume estimates using a predictive equation derived from earlier simultaneous pressure and volume measurements.

Experiment 2

Since none of the animals in the Experiment 1 showed any sign of toxicity, this Experiment 2 was planned considering that probably neither the feed scarcity observed under practical conditions when animals ingest OL (simulated during the adaptation period) nor the amount of OL administered in the first trial were sufficient to elicit intoxication.

In this experiment, another six ruminally cannulated young bulls (same breed, similar age, 502 \pm 25.5 kg BW at the beginning of the trial) were divided into two groups of three animals: control and 10-OL. The animals were fed a very limited amount of grass hay for 8 days (on average 4 kg/animal for 2 days, 3 kg for next day, 2 kg for next 4 days and then one day of fast). Afterwards, 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 10-OL received 35 g DM of OL/kg LW^{0.75} (on average almost 10 kg per day). As in the Experiment 1, the oak leaves were administered through the rumen cannula, twice daily, but only for 3 days because the animals developed clinical signs of intoxication. On day 4th, ruminal fluid from each animal was collected for the *in vitro* incubations.

In vitro gas production study was conducted as explained above for Experiment 1, with eighteen samples per substrate [2 treatments \times 3 inocula (replicates)/treatment \times 3 flasks/inoculum] plus 18 blank flasks being incubated.

Statistical analyses

The data of both experiments were analysed by one-way analysis of variance, with treatments as the only source of variation, using the general lineal model (GLM) procedure of the SAS (1999).

RESULTS AND DISCUSSION

Immature oak leaves have a high content of tannins, basically HT, which may be toxic to grazing ruminants. On the contrary, mature leaves of several species of oak are an important component of livestock diets in less favoured areas of many countries.

The results of this study showed that the effect of the immature Pyrenean oak leaves administered to beef cattle on *in vitro* ruminal fermentation was dependent both on the dose administered and on the substrate incubated. These results are consistent with other parameters of rumen fermentation (VFA and ammonia concentrations, pH, DM disappearance, etc.) estimated *in vivo* and *in situ* and published elsewhere (e.g., Frutos et al., 2007).

As shown in Figure 1, gas production from grass hay was always greater when using ruminal fluid from control animals ($P < 0.001$) and no significant differences were observed between treatments 2.5-OL and 5-OL. In the second experiment (Figure 2), gas production from grass hay was much greater in incubations with inocula from the control animals than from those that had received the highest amount of oak leaves (10-OL; $P < 0.001$); differences being stronger than in Experiment 1.

The fact that the *in vitro* gas production technique is a closed system rendered it specially reliable for the detection of ruminal fermentation inhibitory compounds. The negative effect of tannins consumption on ruminal fermentation (reflected in a reduction of the gas production) has previously been reported as dose-dependent (Hervás et al., 2003) and to be due to an inhibition of microorganisms growth and microbial enzymes activity (McSweeney et al., 2001).

According to these results, the presence of tannins (mainly HT) in the rumen of animals on treatments 2.5-OL and 5-OL would have elicited changes in their microbial populations or favoured the development of microbial mechanisms of adaptation, whereas the highest level (10-OL) would have exceeded their ability to tolerate or detoxify tannins, in agreement with the intoxication signs observed in animals on this treatment. Several ruminal microorganisms have been identified that can tolerate relatively high concentrations of tannins, and even degrade HTs, both in a dose-dependent fashion. In this respect, for example, Bae et al. (1993) observed that the net effect of the exposure of *Fibrobacter succinogenes* S85,

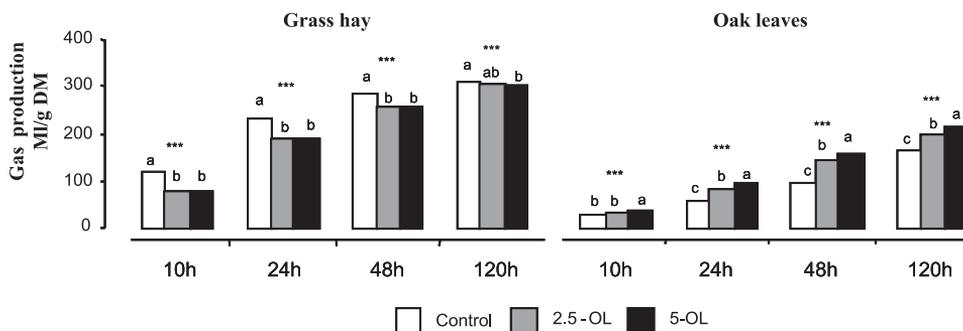


Figure 1. *In vitro* gas production from cultures of grass hay and oak leaves with rumen inocula from young bulls on treatments control, 2.5-OL and 5-OL (Experiment 1)

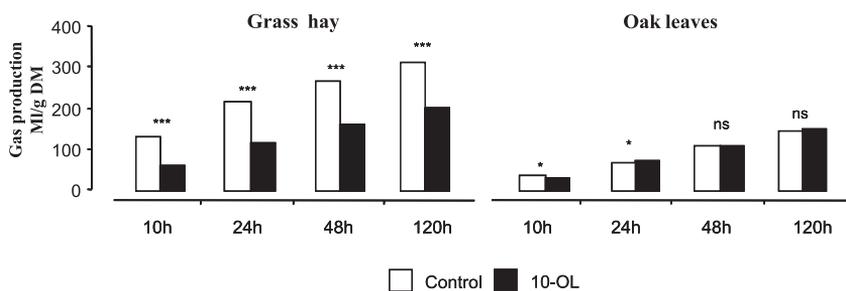


Figure 2. *In vitro* gas production from cultures of grass hay and oak leaves with rumen inocula from young bulls on treatments control and 10-OL (Experiment 2)

a predominant cellulolytic rumen bacterial species, to condensed tannins (0 to 300 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$, all endoglucanases were inhibited and cellulose degradation ceased.

The results of this study show a negative effect of oak tannins on *in vitro* ruminal fermentation of common feeds (e.g., grass hay), and suggest a potential adaptation of rumen microbiota in animals consuming OL, both in a dose-dependent manner.

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