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Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats

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ABSTRACT

Rumen cannulation is the reference method for collection of representative samples of rumen digesta. However, it is not always viable, which obliges to depend on less invasive techniques, such as stomach tubing. The aim of this work was to study if the differences in fermentation parameters and rumen microbial populations observed between species (sheep and goats), diets (forage and forage plus concentrate) and sampling times (pre- and post-feeding) are consistent when collecting the samples through stomach tube or rumen cannula, in an attempt to validate the use of the former as an alternative to the latter. Four sheep and four goats, fitted with ruminal cannula, were fed either forage (F diet; alfalfa hay) or forage plus concentrate (1:1; FC diet), in two 15-day periods. At the end of each period (days 14 and 15), samples of rumen digesta were taken by stomach tube and rumen cannula, before and 4 h after morning feeding, for determination of ruminal fermentation parameters (pH, and lactate, ammonia and total VFA concentrations). The three main rumen microbial groups (bacteria, protozoa and methanogenic archaea) and two fibrolytic bacteria (*Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) were quantified by real-time PCR and, additionally, PCR-DGGE analysis of the bacterial community on the rumen digesta samples collected post-feeding was carried out. Overall, sampling through ruminal cannula and stomach tube gave similar results regarding fermentation parameters when comparing species, diets and sampling times. Despite samples for microbiology assays contained liquid plus solid fractions when collected through rumen cannula and mostly liquid when collected through stomach tube, both techniques showed certain consistency in the effects of treatments on the rumen microbiota (*e.g.*, both revealed no differences between

Abbreviations: ADF, acid detergent fibre; CP, crude protein; D, diet; DGGE, denaturing gradient gel electrophoresis; DM, dry matter; F, forage; FC, forage plus concentrate; FM, fresh matter; G, goat; LW, live weight; MEI, metabolizable energy intake; N, nitrogen; NDF, neutral detergent fibre; OM, organic matter; PCA, principal components analysis; PCR, polymerase chain reaction; qPCR, real-time quantitative PCR; S, sheep; Sp, species; VFA, volatile fatty acids; T, sampling time.

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species in total bacteria, archaea and *R. flavefaciens* concentrations, and higher protozoa numbers in goats than in sheep). However, there was also some discrepancy regarding microorganism concentrations, particularly concerning sampling times (*e.g.*, differences between pre- and post-feeding samplings were only observed in rumen cannula samples for total bacteria and methanogenic archaea, and in stomach tube samples for *R. flavefaciens* concentrations). Therefore, this study supports that non-invasive stomach tubing is a feasible alternative to surgical rumen cannulation in sheep and goats to examine ruminal fermentation. Nonetheless, caution should be taken when using this technique to assess the structure and composition of the rumen microbial community.

1. Introduction

Rumen cannulation is considered the reference method for collection of representative samples of rumen digesta and is therefore widely used in ruminant nutrition research (Komarek, 1981; Kristensen et al., 2010). However, rumen cannulation is not feasible in lactating ewes or goats, because of potential adverse effects on animal performance, which obliges to depend on less invasive alternatives, such as oral stomach probing.

Rumen cannulation and stomach tubing have been mainly used to assess ruminal fermentation (Geishauser and Gitzel, 1996; Duffield et al., 2004) and, more recently, to analyse the structure of the rumen microbial community (Hook et al., 2009; Lodge-Ivey et al., 2009; Terré et al., 2013). In the few studies in which the two techniques were used together, comparisons of fermentation profile and microbiota resulted in either significant differences (*e.g.*, Geishauser and Gitzel, 1996; Duffield et al., 2004) or similar results (*e.g.*, Lodge-Ivey et al., 2009; Shen et al., 2012; Terré et al., 2013) and the reasons for this discrepancy are probably related to the probing procedure to avoid saliva contamination, the type of sample obtained and the rumen sampling site.

While negligible amounts of solid material can be collected with stomach probe, rumen cannula allows collection of both solid and liquid fractions of the rumen digesta. This may be relevant when the treatments to be studied are not expected to have the same effect on microbial populations attached to solids or inhabiting the liquid phase (Martínez et al., 2010).

Regarding the rumen sampling site, Shen et al. (2012) obtained significant variations in ruminal fermentation parameters (pH, VFA, ammonia N and ion concentrations) when sampling at different locations through ruminal cannula. Differences between samples collected via cannula or stomach tube were also observed and attributed to the sampling site when the probe was not inserted to a depth enough to reach the central sac. Otherwise, no significant differences were detected between methods (Shen et al., 2012). Unfortunately, probe insertion in an accurate location of the rumen is very complicated in small ruminants.

To our knowledge, reports analysing methods of rumen sampling are very scant in sheep and practically non-existent in goats. Therefore, this experiment was conducted with ruminally cannulated sheep and goats to validate the use of the stomach probing as an alternative to rumen cannulation in small ruminants. The main aim of this work was to assess the ability of both approaches to detect differences between treatments (*i.e.*, species, diets or sampling times) in ruminal fermentation and microbial community, rather than a direct comparison of methods.

2. Materials and methods

2.1. Animals, diets and experimental design

Four Segureña sheep (S; mean live weight 56.4 ± 2.66 kg) and four Murciano-Granadina goats (G; 37.8 ± 1.65 kg), fitted with a ruminal cannula (35 mm internal diameter), were individually penned and fed alfalfa hay for 2 weeks. After that adaptation, animals were fed two different diets in two consecutive 15-day periods (for each period, two animals/species and diet): forage (F diet; alfalfa hay) or forage plus concentrate (1:1; FC diet). Concentrate (Pacsa Sanders, Seville, Spain) was provided as pellets. Chemical composition of the diets (g/kg DM) and dry matter intake (g/kg) and metabolizable energy intake (MEI; MJ/d) is shown in Table 1. Experimental diets were offered in two meals (60% at 9:00 h and 40% at 18:00 h) at estimated energy requirements for maintenance for sheep (Aguilera et al., 1986) and goats (Prieto et al., 1990). Clean water and mineral supplement were always available.

All experimental procedures were approved and completed in accordance with the Spanish Royal Decree 53/2013 for the protection of animals used for experimental purposes.

2.2. Measurements and sampling procedures

On days 14 and 15 of each period, samples of rumen digesta were obtained, via stomach tube and rumen cannula, from each animal.

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