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Influence of forage type in the diet of sheep on rumen microbiota and fermentation characteristics

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ABSTRACT

Four ruminally cannulated sheep were used in a cross-over design to assess the changes in rumen fluid microbial populations during the feeding cycle as affected by the type of forage (FOR) in the diet. The two experimental diets contained 70% of either alfalfa hay (AL) or grass hay (GR) as FOR and 30% of concentrate. Sheep were fed the diets twice daily and samples from rumen fluid were taken at 0, 4 and 8 h after the morning feeding. Ruminal pH, lactate concentrations and xylanase activity were not affected ($P > 0.05$) by FOR, but concentrations of NH_3–N and total volatile fatty acid (VFA), and carboxymethylcellullase (CMCase) and amylase activities were greater ($P < 0.05$) for AL compared with GR diet. Total protozoa numbers determined by microscopic counting were higher ($P < 0.05$) in AL-fed sheep than in sheep fed the GR diet; in contrast, concentrations of protozoal DNA determined by quantitative real-time PCR tended ($P = 0.06$) to be higher in GR-fed sheep, and no correlation ($P > 0.05$) between protozoal numbers and total protozoal DNA concentrations was detected. Sheep fed GR had higher ($P < 0.001$–0.05) relative abundance of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and fungi than did AL-fed sheep, but bacterial DNA concentrations and relative abundance of *Ruminococcus albus* and methanogenic archaea were unaffected ($P > 0.05$) by FOR. Postprandial changes of DNA concentrations of all determined microbial populations were similar for the two diets. Total bacterial and protozoal DNA concentrations decreased ($P < 0.05$) at 4 h post-feeding but recovered before-feeding values at 8 h post-feeding. No correlations ($P > 0.05$) were found between CMCase and xylanase activities and either the concentration of total bacterial DNA or the relative abundance of the three cellulolytic bacteria, but xylanase activity was positively correlated ($P < 0.05$) with both protozoa numbers and protozoal DNA concentration. Although the postprandial evolution of fermentation parameters and microbes were similar for both forages, sheep fed the lower quality forage showed higher abundance of some cellulolytic bacteria and fungi, which could be interpreted as an adaptation to digest a more fibrous and complex forage.

1. Introduction

High-forage diets are widely used in the feeding of small ruminants in many parts of the world, but the interactions between rumen microbes and forage fermentation are not yet fully understood. Knowledge of diet-based microbial population shifts and why they occur will enable to apply appropriate techniques to improve the efficiency of microbial fermentation (Edwards et al., 2008). Conventional culture-based methods of counting rumen microbes are quickly being replaced by molecular techniques which are suitable to characterize complex microbial communities. Although these techniques provide useful information on composition of microbial populations, they usually offer

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little or no information on the functional role of specific rumen microbes; therefore, the combination of molecular techniques with measurements of rumen function would help to understand the mechanisms involved in the complex rumen function, as well as to develop strategies for enhancing the ruminant productivity by selective manipulation of microbial populations (Morgavi et al., 2013).

The quantitative PCR (qPCR) assay has been used to assess changes in microbial populations when ruminants are switched from forage-based to high-concentrate diets, but information on the influence of the forage type (FOR) in the diet on rumen microbes is more limited and most of it refers to cattle (Chanthakhoun and Wanapat, 2010; Huws et al., 2010; Halmemies-Beauchet-Filleau et al., 2013; Kong et al., 2010). Although the use of high-forage diets for sheep feeding is a common practice in many parts of the world and has increased in previous years due to increased grain prices, information on the influence of FOR type on sheep rumen microbes is very scarce (Ouwerkerk et al., 2008; Martínez et al., 2010).Therefore, the objectives of this study were to determine how the quality of dietary FOR affected the postprandial changes of total bacteria, cellulolytic bacteria, protozoa, fungi and methanogenic archaea in the rumen of sheep, and to investigate the relationships between the determined rumen microbial communities and fermentation parameters. Alfalfa and grass hay were chosen as FOR because they are widely used in sheep practical feeding and differ in chemical composition and fermentation rate and extent in the rumen, thus promoting different microbial communities.

1.1. Animals, diets and experimental procedure

Four ruminally-cannulated Merino sheep (58.3 ± 3.27 kg body weight) were housed in individual pens with continuous access to fresh water and vitamin/mineral block. Animals were cared and handled in accordance with the Spanish Animal Care Regulations, and the experimental protocols were approved by the León University Institutional Animal Care and Use Committee. The two experimental diets contained (DM basis) 70% of either alfalfa hay (AL) or grass hay (GR) as forage source and 30% of concentrate. The alfalfa hay was a second-cut harvested at 30% flowering, and the grass hay was harvested at post-flowering stage and consisted primarily of perennial ryegrass (81%), red and white clover (11%) and other grasses (8%). Chemical composition of individual feeds and diets is shown in Table 1. Diets were offered to the animals twice daily (08:00 h and 20:00 h) at a daily rate of 52 g DM/kg body weight$^{0.75}$ to minimize feed selection. All animals consumed all the diets offered, with the exception of one sheep that occasionally left some refusals (< 100 g/day) with diet GR.

The experiment had a crossover design and each of the two 24-day periods consisted of 21 days of dietary adaptation and three days for sample collection. Samples of offered diets were collected daily, composited and dried at 55 °C in an oven for 48 h and ground (1-mm) before chemical analyses. On day 22 and 24 of each period, ruminal content samples (about 500 g) were taken through the cannula of each sheep immediately before the morning feeding (0 h), at 4 and at 8 h after feeding. Ruminal content was strained through two layers of cheesecloth, the pH of the fluid was immediately measured, and the following samples were taken. About 20 mL were placed in sterile containers and stored frozen at −80 °C until DNA extraction. Five mL of fluid were added to 5 mL of deproteinizing solution (García-Martínez et al., 2005) for volatile fatty acids (VFA) analysis, 2 mL were added to 2 mL 0.5 M HCl for NH_3–N determination, and 5 mL were frozen at −20 °C for total lactate analyses. 10 mL of fluid was immediately frozen at −80 °C for determination of enzymatic activities and 5 mL of fluid was added to 5 mL of formalin solution (120 mL formalin/L) and stored until protozoa counting. The remaining ruminal fluid was used to isolate pellets of prokaryotes and protozoa by differential centrifugation as described by Ramos et al. (2009) and Saro et al. (2012), respectively. Pellets were freeze-dried and used to obtain prokaryotic and protozoal DNA to be used as standard for the qPCR analysis. Within each experimental period, the two samples taken on the two sampling days for each sheep at each sampling time were composited before analysis.

1.2. DNA extraction and analysis of microbial communities by quantitative PCR (qPCR)

DNA was isolated in duplicate from centrifuged ruminal fluid (4 mL, 20,000 × *g*, 5 min, 4 °C) and pellets of prokaryotes

Table 1
Chemical composition of feed ingredients (g/kg dry matter unless stated otherwise) and of experimental diets containing 70% of alfalfa hay (AL) or grass hay (GR) as forage and 30% of concentrate (dry matter basis)

Item	Ingredients			Diets[b]	
	Alfalfa hay	Grass hay	Concentrate[a]	AL	GR
Dry matter (g/kg fresh matter)	928	925	923	927	924
Organic matter	913	933	914	913	927
Nitrogen	26.7	14.6	30.4	27.8	19.3
NDF	466	569	335	427	499
ADF	331	286	125	269	238
Ether extract	17.9	16.8	25.0	20.0	19.3
NSC[c]	262	256	364	293	288

[a] Based on barley, gluten feed, wheat middlings, soybean meal, palm kernel meal, wheat, corn and mineral/vitamin premix in the proportions of 217, 204, 197, 134, 115, 50, 50 and 33 g/kg, respectively (dry matter basis)
[b] Chemical composition of diets calculated from ingredient composition.
[c] Non-fiber carbohydrates; calculated as 100 – (crude protein + ash + NDF + ether extract).