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The effect of the diet fed to donor sheep on *in vitro* methane production and ruminal fermentation of diets of variable composition

M.E. Martínez[a,b], M.J. Ranilla[a,b], M.L. Tejido[a,b], C. Saro[a,b], M.D. Carro[a,b,*]

[a] *Departamento de Producción Animal, Universidad de León, 24007 León, Spain*
[b] *Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n. 24346 Grulleros, León, Spain*

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ABSTRACT

Six rumen-fistulated sheep were fed 4 diets in a partially replicated 4 × 4 Latin square design to study the effects of two forage:concentrate (F:C) ratios and two types of forage (FOR) in the diet on *in vitro* methane production and microbial fermentation of the same 4 diets. The diets consisted on either 70:30 (HF) or 30:70 (HC) F:C, and two FOR (alfalfa hay and grass hay), and were designated as HFA, HFG, HCA and HCG. In each period, ruminal fluid from each sheep was used to inoculate batch cultures containing 400 mg DM of each diet. Cultures were incubated at 39 °C for 24 h and the main ruminal fermentation parameters were determined. There were no F:C × FOR interactions ($P>0.05$) for any measured variable. Methane production after 8 h was not affected by F:C ratio in the diet of donors, but after 24 h of incubation the amount of methane was 1.2 times greater ($P<0.05$) with inoculum from HC-fed sheep compared with HF inoculum. Total volatile fatty acid (VFA) production was about 10% greater for HC compared with HF inoculum for both HF and HC substrates. Changing the F:C ratio in the diet did not affect propionate production for any substrate, but production of butyrate was augmented ($P<0.01$) as F:C ratio in the diet increased. Methane:VFA ratio and apparent dry matter digestibility were not affected by F:C ratio in the diet of donors. For all substrates, diets including alfalfa hay promoted greater ($P<0.05$) production of methane and total and individual VFA, as well as greater ($P<0.01$) acetate:propionate ratios, NH_3-N concentrations and apparent dry matter digestibility compared with grass hay diets. Whereas FOR in the diet of donor animals did not affect methane:VFA ratio when HF substrates were incubated, for HC substrates methane:VFA ratio was greater ($P<0.05$) with inoculum from sheep fed alfalfa hay diets. There were clear differences in methane production among inocula from different sheep, which persisted across diets and substrates. The results indicate that methane production *in vitro* is affected by both F:C ratio and type of forage in the diet of donors, and these variables should be taken into account when conducting *in vitro* experiments.

1. Introduction

In the last years there has been an increased use of batch cultures of ruminal microorganisms (BCRM) to study ruminal fermentation. The BCRM have been used for nutritive evaluation and for investigating the effects of additives on ruminal

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; BCRM, batch cultures of ruminal microorganisms; DM, dry matter; HF, high-forage; HC, high-concentrate; VFA, volatile fatty acids.

* Corresponding author at: Departamento de Producción Animal, Universidad de León, 24071 León, Spain. Tel.: +34 987 291240; fax: +34 987 291288.
E-mail address: mdcart@unileon.es (M.D. Carro).

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Table 1
Chemical composition (g/kg dry matter) and estimated energy content (MJ/kg dry matter) of the experimental diets.

Item	Diet[a]			
	HFA	HFG	HCA	HCG
Dry matter	927	925	925	924
Organic matter	913	927	913	919
Nitrogen	26.9	19.4	28.3	25.6
aNDF	426	499	374	401
ADF	269	238	187	174
ME[b]	8.87	8.37	10.3	10.0

[a] HFA: 700:300 alfalfa hay:concentrate; HFG: 700:300 grass hay:concentrate; HCA: 300:700 alfalfa hay:concentrate; HCG: 300:700 grass hay:concentrate. DM basis.
[b] Estimated according to NRC (1985).

fermentation (López et al., 1998; Carro et al., 2005), among other purposes. Although BCRM are a rapid and precise method that only needs a small amount of substrate, they still require an inoculum to create the fermentative environment, and the microbial populations in the ruminal fluid can affect the *in vitro* fermentation of the incubated substrate (Mould et al., 2005). Several studies have indicated that the diet of donor animals influences gas production and substrate degradability (Nagadi et al., 2000; Tejido et al., 2002). We are not aware, however, of studies that have investigated the effect of the diet of donors on *in vitro* methane production.

Methane is one of the major end products of anaerobic fermentation of feeds in the rumen. Reducing methane production is an important goal of ruminant nutritionists as it represents a significant loss of energy for the host animal and contributes to global warming (Moss et al., 2000). Many attempts have been made to depress rumen methanogenesis through the use of feed additives or diet modifications, and the number of studies using BCRM has noticeably increased in the last years as *in vivo* determination of methane emission is laborious, time-consuming, and requires complex and expensive equipment. Methane production *in vivo* has been reported to be close to that measured *in vitro* in some studies (Getachew et al., 2005; Bhatta et al., 2008), but the relationship between both values has been shown to be poor in another study (Moss and Givens, 1997). Methane production is affected by many factors, such as the level of intake, type and composition of the diet and ruminal pH. The composition of the diet seems to be one of the most important factors, and fewer methanogens have been detected in the rumen of concentrate-fed animals than in the rumen of forage-fed ones (Demeyer and Fievez, 2000). The objective of this study was to examine the effect of varying the forage to concentrate ratio (F:C) and the quality of forage (FOR) in the diet of donors on *in vitro* methane production and fermentation of diets of variable composition in BCRM.

2. Materials and methods

2.1. Diets

Four total mixed diets were formulated according to a 2 × 2 factorial arrangement of treatments. The diets had F:C (dry matter (DM) basis; g/g) ratios of 700:300 (HF) or 300:700 (HC) with either alfalfa hay or grass hay as FOR, and were designated as HFA, HFG, HCA, and HCG, respectively. Chemical composition of diets is shown in Table 1. The alfalfa hay was a second-cut harvested at 30% flowering and contained 913 g of DM/kg fresh matter and 26.7 g of nitrogen (N), 466 g of neutral detergent fibre (aNDF) and 331 g of acid detergent fibre (ADF) per kg DM. The grass hay consisted primarily of perennial ryegrass (0.81), red and white clover (0.11), and other grasses (0.08), was harvested at post-flowering stage, and contained 933 g of DM/kg fresh matter, 14.6 g of N, 569 g of aNDF and 286 g ADF per kg DM. The concentrate contained 914 g of DM/kg fresh matter, 30.4 g of N, 335 g of aNDF and 125 g of ADF per kg DM.

2.2. Animals and inocula

Six rumen-fistulated Merino sheep (59.0 ± 4.5 kg body weight) were used in a partially replicated 4 × 4 Latin square with 4 dietary treatments and 4 15-day periods. Sheep 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. Sheep were housed in individual pens and had continuous access to fresh water and vitamin/mineral block over the experimental period. Diets were offered to the animals twice daily (8.00 and 20.00 h) at a daily rate of 56 g DM/kg body weight$^{0.75}$ to minimize feed selection. This level of intake was estimated to meet 1.2, 1.1, 1.4 and 1.3 times the energy maintenance requirements of the experimental sheep for HFA, HFG, HCA and HCG diets, respectively (NRC, 1985).

On day 15 of each period, about 500 g of ruminal contents were taken through the cannula of each sheep immediately before the morning feeding and strained through 4 layers of cheesecloth into an Erlenmeyer flask with an O_2-free headspace. The pH of the fluid was immediately measured, 5 mL of fluid were added to 5 mL of deproteinizing solution (100 g of metaphosphoric acid and 0.6 g of crotonic acid per litre) for volatile fatty acid (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, and 5 mL were immedi-