Changes in ruminal fermentation in sheep fed either alfalfa hay or grass hay after changing to a high-concentrate diet

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in

Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009
pages 285-290

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S. Ramos, M.L. Tejido, M.E. Martínez, M.J. Ranilla and M.D. Carro

Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León,
Campus de Vegazana s/n, 24071 León (Spain)

Abstract. Four rumen-fistulated Merino sheep were used in a crossover design experiment with two 44-day periods each to evaluate the changes in ruminal parameters including enzymatic activities that occur after an abrupt change from a 70:30 forage:concentrate diet (HF) to a 30:70 forage:concentrate diet (HC) when forage was either alfalfa hay (AL) or grass hay (GR). Sheep were fed the diets at a daily rate of 55 g/kg body weight$^{0.75}$. During the first 15 days of each experimental period sheep were fed HF diets, and from day 16 thereafter they received HCAL and HCGR. On days 0, 2, 5, 12, 20 and 28 after diet change, ruminal samples from each sheep were collected 3 h after feeding. There were no differences ($P > 0.05$) between diets (HCAL and HCGR) in any of the measured variables, with the exception of NH_3-N and total short chain fatty acid (SCFA) concentrations which were greater ($P < 0.001$ and 0.028) for HCAL than for HCGR. For both diets, ruminal pH decreased significantly 2 days after the change to HC diets, and values continued being lower ($P < 0.001$) 28 days after the diet change. Total SCFA concentrations increased ($P < 0.05$) by 19 and 24% for HCAL and HCGR, respectively, on day 2, but values on day 28 did not differ ($P > 0.05$) from those on day 0. No effect ($P > 0.05$) of diet change was observed on endoglucanase, exoglucanase and xylanase activities in ruminal fluid on day 2, but activities of all these enzymes increased ($P < 0.05$) by day 28. Amylase activity increased ($P < 0.05$) on day 2 for diet HCAL and remained greater ($P < 0.05$) for the rest of the trial. For diet HCGR, amylase activity increased ($P < 0.05$) on day 12 and remained greater through to the end of the trial.

Keywords. High-concentrate diets – Ruminal fermentation – Enzymatic activity – Sheep.

Variation de la fermentation ruminale chez des ovins alimentés avec du foin de luzerne ou du foin d'herbe puis un régime riche en concentré

Résumé. *Quatre moutons de race Mérinos, munis de canules ruminales, ont été utilisés pour évaluer les variations des paramètres de fermentation ruminale et les activités enzymatiques qui se produisent après le changement brusque d'une ration 70:30 fourrage:concentré (HF) à une ration 30:70 fourrage:concentré (HC). Le foin de luzerne (AL) ou le foin d'herbe (GR) étaient utilisés comme fourrage, selon un dispositif en cross-over, avec deux périodes de 44 jours chacune. Les ovins ont reçu 55 g/kg^{0,75} par jour de chacune des rations. Pendant les 15 premiers jours de chaque période expérimentale, les animaux ont reçu les rations HF, et à partir du jour 16, ils ont reçu HCAL et HCGR. Des échantillons du jus de rumen de chaque mouton ont été prélevés pendant les jours 0, 2, 5, 12, 20 et 28 après le changement de régime, 3 h après la prise d'aliment. Il n'y a eu aucune différence ($P > 0,05$) entre les rations (HCAL et HCGR) pour les différentes variables mesurées, sauf pour l'azote ammoniacal et les concentrations d'acides gras à courtes chaînes (SCFA), qui étaient plus élevés ($P < 0,001$ et $P < 0,028$) pour HCAL que pour HCGR. Pour les deux régimes, le pH ruminal a nettement diminué pendant les 2 premiers jours après le changement au régime HC, et les valeurs sont restées plus faibles ($P < 0,001$) 28 jours après. Au deuxième jour, les concentrations en acides gras volatils totaux ont augmenté ($P < 0,05$) de 19 et 24% pour HCAL et HCGR, respectivement, mais après 28 jours elles ont été similaires ($P > 0,05$) à celles du jour 0. Aucun effet ($P > 0,05$) du changement de régime n'a été observé sur les activités d'endoglucanase, d'exoglucanase et de xylanase du jus de rumen le 2ème jour, mais les activités de toutes ces enzymes ont été plus élevées ($P < 0,05$) au 28ème jour. L'activité d'amylase a augmenté ($P < 0,05$) le 2ème jour avec le régime HCAL et le 12ème jour avec la ration HCGR et elle est restée plus élevée jusqu'à la fin de l'essai.*

Mots-clés. *Régime riche en concentré – Fermentation ruminale – Activité enzymatique – Mouton.*

I – Introduction

Feeding shift from high-forage (HF) to high-concentrate (HC) diets produce marked changes in the ruminal environment, which usually result in alterations of microbial population and fermentation. These changes could be affected by the forage source in the diet (Mertens, 1997), but most of the studies investigating this issue have been conducted in cows. The objective of the present study was to investigate the long-term changes in ruminal variables and enzymatic activities that occur in the rumen of sheep after an abrupt shift from a 70:30 forage:concentrate diet to a 30:70 forage:concentrate diet. Forage component of the diet was either alfalfa hay or grass hay.

II – Materials and methods

Four rumen-fistulated Merino sheep [58.5 ± 3.16 kg live weight (BW)] were used in a cross-over design with two consecutive 44-day experimental periods. Sheep were housed in individual pens and had continuous access to fresh water and vitamin/mineral block. During the first 15 days of each experimental period, sheep were fed a mixed diet composed of 70% forage and 30% concentrate (fresh matter basis). Two sheep received alfalfa hay as forage (HFAL) and the other two received grass hay (HFGR). The concentrate was based on barley, gluten feed, wheat middlings, soybean meal, palm kernel meal, wheat, corn and mineral/vitamin premix at proportions of 21.5, 20.4, 20.0, 13.5, 11.5, 5.0, 5.0 and 3.1%, respectively (fresh matter basis). The diet was offered to the animals twice daily (8:00 and 20:00 h) at a rate of 55 g fresh matter/kg $BW^{0.75}$ per day to minimize feed selection. Individual feed intakes were recorded daily through the experiment. After ruminal sampling on day 16, the proportion of ingredients in the diet was changed to 70% concentrate and 30% forage (HCAL and HCGR for alfalfa hay and grass hay, respectively) and was maintained for the remainder of each experimental period. Chemical composition of diets is given in Table 1.

Table 1. Dry matter content (g/kg) and chemical composition (g/kg DM) of experimental diets

Diets†	Dry matter	Organic matter	Crude protein	Neutral-detergent fibre	Acid-detergent fibre
HFAL	927	913	160	429	270
HCAL	925	913	173	381	189
HFGR	925	927	124	502	239
HCGR	924	919	157	412	176

†HFAL: 70:30 alfalfa hay:concentrate; HCAL: 30:70 alfalfa hay:concentrate; HFGR: 70:30 grass hay:concentrate; HCGR: 30:70 grass hay:concentrate.

On days 0, 2, 5, 12, 20 and 28 after diet change, samples of ruminal content were taken through the cannula of each sheep 3 h after the morning feeding. Ruminal content was homogenised and strained through two layers of cheesecloth. The pH of the fluid was immediately measured and the following samples were taken: 5 ml were added to 5 ml of deproteinising solution [metaphosphoric acid (100 g/l) and crotonic acid (0.6 g/l)] for short chain fatty acids (SCFA) analyses, 2 ml were added to 2 ml 0.5 M HCl for NH_3-N and total lactate determination, and 5 ml were frozen at –80°C immediately for assay of enzymatic activities. Procedures for analysis of dry matter (DM), crude protein, ash, neutral detergent fibre, acid detergent fibre, SCFA, total lactate and NH_3-N have been described previously (Giraldo *et al.*, 2007). For determination of enzymatic activities in ruminal fluid samples, cells were lysed using a Mini-Beadbeater™ (BioSpec Products Inc., Bartlesville, OK, USA) to release intracellular enzymes. Samples were centrifuged (10,000 × *g*, 10 min, 4°C) and the supernatant was used for analyses. Endoglucanase, exoglucanase, xylanase and amylase

activities were determined as described by Giraldo *et al.* (2007) and using carboxymethylcellulose, Avicel PH-101, oat spelt xylan and soluble starch, respectively, as substrates.

Data were analyzed according to a repeated measures model using the MIXED procedure of the Statistical Analysis Systems statistical package version 8.02 (SAS Institute, Cary, NC, USA). The effect of diet (HCAL and HCGR) was considered fixed, and sheep and period effects were considered random. Data for each variable were analysed using compound symmetry, unstructured and auto regressive covariance structures, and the one that produced the minimum Akaike Information Criterion was chosen. Mean effects were declared significant at $P < 0.05$, and $P < 0.10$ values were considered as trends and discussed. When a significant ($P < 0.05$) effect of time was detected, differences between means were assessed by LSD test. Correlations between fermentation variables were determined by Pearson correlation.

III – Results and discussion

The effects of feeding HCAL and HCGR diets on ruminal variables and enzymatic activity are shown in Tables 2 and 3, respectively. Sheep fed HCGR tended ($P = 0.067$) to exhibit higher ruminal pH than those fed HCAL. For both diets ruminal pH decreased significantly ($P = 0.002$ and <0.001 for HCAL and HCGR, respectively) 2 days after the abrupt change to high-concentrate diets, and values continued being lower after 28 days ($P = 0.005$ and <0.001, respectively). A change from HF to HC diets has been shown to decrease ruminal pH in sheep (Mackie *et al.*, 1978; Brossard *et al*., 2003) and cattle (Goad *et al*., 1998; Bevans *et al*., 2005).

SCFA concentrations followed a pattern inverse to that observed for pH. In agreement with the literature (Mackie and Gilchrist, 1979; Brossard *et al.*, 2003), the concentrations of total SCFA increased ($P < 0.05$) by 19 and 24% for HCAL and HCGR, respectively, 2 days after the diet change. However, SCFA concentrations measured on day 28 did not differ ($P > 0.05$) from those determined on day 0 before the change of diet. The increase in SCFA is usually due to presence of readily fermentable carbohydrates, despite the concomitant decrease in ruminal pH which should facilitate a higher post-prandial absorption rate of SCFA (Mackie and Gilchrist, 1979). Sheep fed HCAL showed greater ($P = 0.028$) total SCFA concentrations than those fed HCGR all through the experimental period.

For both diets, molar proportion of acetate decreased ($P < 0.001$) by 8.0% on day 2, and remained lower over the rest of the trial. The decrease in the molar proportion of acetate was similar in both groups, and there were no differences due to the type of forage in the diet ($P = 0.424$). The change of diet tended ($P = 0.079$) to increase the molar proportion of propionate, but values on day 28 (17.0 and 19.7% for HCAL and HCGR, respectively) did not differ ($P > 0.05$) from those determined on day 0 (17.0 and 17.2%). Acetate:propionate ratio (4.03 and 4.04 for HCAL and HCGR on day 0) decreased significantly ($P < 0.05$) 2 days after the diet change (3.15 and 3.25), but whereas values recovered to 3.62 on day 28 in HCAL-fed sheep, acetate:propionate ratio remained low (3.15) in sheep fed the HCGR diet. Molar proportions of butyrate increased ($P < 0.001$) after feeding both HC diets, and values on day 28 were 45.2 and 37.3% greater than those on day 0 for HCAL and HCGR, respectively. Similar fermentation shifts towards propionate and butyrate at the expense of acetate have been reported during adaptation to HC diets in sheep (Mackie *et al.*, 1978; Brossard *et al.*, 2003) and cattle (Eadie *et al*., 1970; Goad *et al*., 1998). These changes in fermentation pattern may reflect concomitant changes in ruminal bacterial populations. A change from HF to HC diets has been shown to reduce the numbers of fibrolytic bacteria and to increase the amylolytic population (Goad *et al*., 1998; Tajima *et al.*, 2001). In addition, ruminal pH values lower than 6.0 are known to depress cellulolytic activity (Stewart, 1977).

For both diets, ruminal NH_3-N concentrations increased ($P < 0.05$) on day 5 and remained greater than those on day 0 for the rest of the trial. HCAL-sheep showed greater ($P < 0.001$) NH_3-N concentrations than HCGR-fed animals, probably due to the greater crude protein content of HCAL diet compared to HCGR diet (Table 1).

Table 2. Mean values of ruminal pH, concentration of total short chain fatty acids (SCFA; mmol/l), molar proportions (mol/100 mol) of acetate, propionate and butyrate, acetate/propionate ratio (Ac/Pr; mol:mol) and concentrations of NH_3-N (mg/l) and lactate (mg/l) in sheep after an abrupt change (after sampling on day 0) in diet from 70:30 hay:concentrate to 30:70 hay:concentrate (n = 4)

Variable and diet†	Sampling day							Significance of effects		
	0	2	5	12	20	28	SED††	Diet	Day	Diet × Day
pH										
HCAL	6.07^{b}	5.69^{a}	5.63^{a}	5.85^{ab}	5.86^{ab}	5.72^{a}	0.116	0.067	<0.001	0.193
HCGR	6.23^{c}	5.62^{a}	5.97^{b}	5.96^{b}	5.89^{b}	5.69^{a}				
Total SCFA										
HCAL	134^{a}	160^{b}	159^{b}	151^{ab}	139^{ab}	143^{ab}	8.2	0.028	0.018	0.870
HCGR	123^{a}	152^{b}	139^{ab}	138^{ab}	137^{ab}	139^{ab}				
Acetate										
HCAL	67.7^{b}	62.3^{a}	61.9^{a}	62.6^{a}	61.5^{a}	60.6^{a}	2.31	0.424	<0.001	0.612
HCGR	68.7^{c}	63.2^{b}	57.5^{a}	61.8^{ab}	61.2^{ab}	59.5^{ab}				
Propionate										
HCAL	17.0	20.7	18.1	17.9	15.9	17.0	1.63	0.111	0.079	0.549
HCGR	17.2	20.2	17.8	19.5	18.8	19.7				
Butyrate										
HCAL	11.5^{a}	12.8^{ab}	14.2^{b}	14.3^{b}	16.6^{c}	16.7^{c}	1.04	0.291	<0.001	0.393
HCGR	11.0^{a}	13.6^{b}	15.0^{b}	13.5^{b}	15.0^{b}	15.1^{b}				
Ac/Pr										
HCAL	4.03^{b}	3.15^{a}	3.52^{ab}	3.49^{ab}	3.88^{b}	3.62^{ab}	0.355	0.285	0.044	0.811
HCGR	4.04^{b}	3.25^{a}	3.44^{ab}	3.42^{ab}	3.44^{ab}	3.15^{a}				
NH_3-N										
HCAL	234^{a}	298^{ab}	339^{b}	316^{b}	368^{b}	313^{b}	37.6	<0.001	<0.001	0.178
HCGR	80.4^{a}	145^{ab}	207^{b}	241^{bc}	292^{c}	274^{bc}				
Lactate										
HCAL	45.5	168	151	108	63.6	198	77.9	0.699	0.124	0.323
HCGR	26.5	59.2	102	65.9	210	196				

†Diets differ in forage source; HCAL: alfalfa hay; HCGR: grass hay. Rumen content was sampled 3 h after the morning feeding.
††Standard error of the difference.
^{a,b,c}Within a variable and diet, mean values with unlike superscripts differ (P < 0.05).

Table 3. Mean values of enzymatic activities in ruminal fluid of sheep after an abrupt change (following sampling on day 0) in diet from 70:30 hay:concentrate to 30:70 hay:concentrate (n = 4)

Variable and diet†	Sampling day						SED††	Significance of effects		
	0	2	5	12	20	28		Diet	Day	Diet × Day
Endoglucanase										
HCAL	629^{a}	634^{a}	857^{b}	1025^{b}	1048^{b}	945^{b}	107.8	0.201	<0.001	0.308
HCGR	697^{a}	671^{a}	786^{a}	831^{a}	1071^{b}	737^{a}				
Exoglucanase										
HCAL	20.9^{a}	39.8^{ab}	38.0^{ab}	40.8^{b}	44.7^{b}	44.7^{b}	9.45	0.583	0.004	0.827
HCGR	20.5^{a}	33.1^{ab}	43.8^{b}	49.9^{b}	42.5^{b}	51.9^{b}				
Xylanase										
HCAL	6212^{a}	6029^{a}	8786^{ab}	8846^{ab}	12567^{b}	11332^{b}	2013.1	0.986	<0.001	0.959
HCGR	6186^{a}	5512^{a}	8560^{ab}	10500^{b}	12632^{b}	10297^{b}				
Amylase										
HCAL	1119^{a}	4575^{b}	4750^{b}	5046^{b}	3692^{b}	5712^{b}	1214.6	0.833	<0.001	0.447
HCGR	1425^{a}	3431^{ab}	3432^{ab}	4494^{bc}	5643^{c}	5837^{c}				

†Diets differ in forage source; HCAL: alfalfa hay; HCGR: grass hay.
††Standard error of the difference.
^{a,b,c}Within a variable and diet, mean values with unlike superscripts differ (P < 0.05).

Ruminal lactate concentrations were not affected neither by the forage to concentrate ratio ($P = 0.124$) nor by the type of forage in the diet ($P = 0.699$). The interaction diet × sampling day was not significant ($P = 323$). Readly fermentable carbohydrates fermented by the amylolytic bacteria represent the main source of lactic acid, which is the growth substrate of the lactate-utilizing bacteria (Mackie and Gilchrist, 1979). During cases of subacute acidosis, lactic acid may not accumulate and the concentrations seldom exceed 900 mg/l (Mackie *et al.*, 1978; Goad *et al.*, 1998). Values of lactate concentrations in this study were in the range of those reported by others (Mackie *et al*., 1978; Brossard *et al*., 2003) in sheep in latent acidosis fed HC diets. Brossard *et al.* (2003) tried to explain the butyric rather than lactic fermentative pattern during latent acidosis in sheep by changes in the microbial community. The acetate and butyrate pathways are pH dependent and the production of butyrate from lactate is maximal at low pH. In addition, butyrate synthesis from acetate uses hydrogen and would provide protection against increased ruminal acidity (Brossard *et al.*, 2003).

In general, it is accepted that increased SCFA concentration, rather than lactate accumulation, is responsible for the decreased ruminal pH in sheep during adaptation to concentrate feeding (Mackie *et al.*, 1978). In the present experiment, there were negative relationships between pH and both SCFA ($r = 0.706$; $P < 0.001$; $n = 48$) and lactate concentrations ($r = 0.587$; $P < 0.001$; $n = 48$).

Diet had no effect ($P > 0.05$) on endoglucanase, exoglucanase and xylanase activities in ruminal fluid on day 2, but all enzymatic activities were increased ($P < 0.05$) by day 12 and remained higher thereafter in sheep fed both experimental diets. Amylase activity increased ($P < 0.05$) 2 days after diet change in HCAL-fed sheep and on day 12. In HCGR-fed sheep and values remained greater ($P < 0.05$) until the end of the trial. No differences between diets ($P = 0.201$ to $P = 0.986$) were observed in any enzymatic activities.

IV – Conclusions

An abrupt change from HF to HC diets produced marked changes in the ruminal fermentation profile of sheep. The results indicate that the nature of the forage fed before diet's change did not affect most of the ruminal variables measured.

Acknowledgements

The authors acknowledge the financial support received from the CICYT of Spain (Project AGL2004-04755-C02-01).

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