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in

Ruiz R. (ed.), López-Francos A. (ed.), López Marco L. (ed.).
Innovation for sustainability in sheep and goats

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 123

2019
pages 167-171

Effect of levels of intake on rumen fermentation, digestibility, methane emissions and behaviour

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Abstract. The objective of this experiment was to examine the effect of differing levels of intake on rumen fermentation profile, nutrient digestibility, methane emissions, and feeding behaviour in sheep. Six Aberdale cross Texel ewes (90.2 ± 1.89 kg BW) fitted with rumen fistula were used in a duplicated 3×3 Latin Square with three 21-d experimental periods. Treatments comprised dried grass nuts fed to meet one (1M), 1.5 times (1.5M), or two times (2M) maintenance energy requirements (NRC, 2007). The average daily gain was greater in 2M than 1M ewes. The 1M ewes had a lower VFA concentration than 1.5M and 2M, and a greater acetic-to-propionic ratio than 1.5M. The ammonia concentration was greater in 1.5M than in 2M ewes. Dry and organic matter digestibility per unit of metabolic weight were greater in 1M than 1.5M and 2M ewes. Emissions of methane per unit of DM intake were greater in 1M than 1.5M and 2M ewes. No differences were found in protozoa count, feeding behaviour or haematology. Results show that increased levels of intake have a measureable impact on diet fermentation and digestibility, reducing the acetate-to-propionate ratio, ammonia production, digestibility rate, and methane emissions. These changes could be attributed to the different nutrient fermentation pattern and the higher flow rate from the rumen to the lower digestive tract.

Keywords. Rumen fermentation – Nutrient digestibility – Methane emissions – Feeding behaviour.

Impact de différents niveaux d'ingérés sur la fermentation du rumen, la digestibilité, les émissions de méthane et le comportement

Résumé. *L'objectif de cette étude était d'examiner l'impact de différents niveaux d'ingérés sur le profil de fermentation du rumen, la digestibilité, la production de méthane et les comportements alimentaires chez les moutons. Six brebis croisées Texel-Aberdale (90,2 ± 1,89 kg poids moyen) canulées ont été utilisées dans un carré latin double 3 × 3 de trois périodes expérimentales de 21 jours. Les traitements consistaient en différentes quantités de granulés d'herbe afin de répondre à une fois (1M), 1 fois et demi (1,5M), ou deux fois (2M) aux besoins énergétiques (NRC, 2007). Le gain de poids moyen journalier était plus important chez les brebis 2 M que les 1M. Les brebis 1M avaient une concentration en AGV inférieure aux 1,5 M et 2 M, et un rapport acétique-propionique plus élevé que 1,5 M. La concentration d'ammoniaque était supérieure chez les brebis 1,5M que chez les brebis 2M. La digestibilité des matières sèches et organiques par unité de poids était plus élevée chez les brebis 1M que 1,5M et 2M. La production de méthane par unité de matières sèches ingérées était plus importante chez les brebis 1M. Aucune différence n'a été trouvée sur le nombre de protozoaires, le comportement alimentaire ou les paramètres hématologiques. Ces résultats montrent qu'une augmentation de l'ingéré a un impact quantifiable sur la fermentation et la digestibilité en réduisant le ratio acétate-propionate, la production d'ammoniaque, le taux de digestibilité et la production de méthane. Ces changements pourraient être attribués aux différentes voies de fermentation utilisées et à un transit plus élevé du rumen vers le tractus digestif inférieur.*

Mots-clés. *Fermentation du rumen – Digestibilité – Émissions de methane – Comportement alimentaire.*

I – Introduction

Improving our understanding of tree-animal-soil interactions in upland systems in Wales is a vital component for future sustainable intensification of ruminant livestock systems (MULTI-LAND, 2015). In order to develop a systems-level understanding of management on phenotypic characteristics of grazing animals it is essential to identify and implement new methodologies for accurately estimating for-

age intake by free-ranging ruminants. Existing methodologies, however, are labour intensive and are often associated with large measurement error and low repeatability (Burns *et al*., 1994).

In recent years, alternative methods have been developed that can potentially be used to estimate intake in grazing animals with sufficient accuracy and precision. These include specific biomarkers excreted in urine or in plasma compounds (e.g. nitrogen, sugars, potassium, or vitamins; Fukuwatari and Shibata, 2012), near infrared reflectance spectroscopy of faecal constituents (e.g. fibre fractions; Dixon and Coates, 2015), spot short-term quantification of carbon dioxide and methane (Huhtanen *et al*., 2015), and automatic classification of feeding behaviour using 3-axis accelerometers (Marais *et al*., 2014).

The objectives of this experiment were to evaluate combinations of existing methodologies to estimate intake, and to assess the effects of increasing levels of intake on the rumen microbiome in sheep. Our hypothesis were that intake and digestibility could be estimated individually in grazing animals using a combination of automated methods and biomarkers, and that changes in the level of intake without changes in diet composition have a measureable impact on rumen microbial diversity and composition.

II – Material and methods

1. Animals, housing and experimental design

All animal procedures were carried out according to the Home Office scientific Procedures (Act 1986). Seven Aberdale cross Texel ewes (48 ± 0.1 months of age, and 90.2 ± 1.89 kg BW) fitted with rumen fistula (as described in an earlier experiment; ref.: 101091) were used during 14 weeks from April to July 2016.

Six of the 7 ewes were assigned to one of two groups of 3 animals according to live weight. The remaining ewe was kept as a spare animal in an individual pen with food to cover maintenance requirements. All sheep were moved to the individual pens and fed the experimental diet for 14 d. Thereafter, sheep were used in a replicated 3x3 Latin Square with three 21 d experimental periods. Each period consisted of a 14 d adaptation and 7 d sampling interval. To implement the experimental design, animals in the second group started the experiment 7 d later.

At the beginning of the experiment individual energy requirements were calculated to maintain BW according to the energy evaluation system described by NRC (2007). After the acclimation period, where all sheep were fed at maintenance, animals were allocated to Latin squares according to BW and randomly assigned to experimental treatments. Treatments comprised dried grass nuts fed to meet one (1M), 1.5 times (1.5M), or two times (2M) maintenance energy requirements (NRC, 2007). Allocation of experimental treatments is shown in Table 1.

Table 1. Distribution and permutes of treatments over the experiment

	Period 1	Period 2	Period 3
Animal 1	1M	1.5M	2M
Animal 2	1M	2M	1.5M
Animal 3	1.5M	1M	2M
Animal 4	2M	1M	1.5M
Animal 5	2M	1.5M	1M
Animal 6	1.5M	2M	1M

Treatments comprised dried grass nuts fed to meet one (1M), 1.5 times (1.5M), or two times (2M) maintenance energy requirements (NRC, 2007).

2. Sample collection and analyses

At the beginning of the adaptation period, animals were weighed in two consecutive days to calculate the maintenance requirements. During each of the three experimental periods, sheep were weighed at d 1, 7, 14, and 17 to calculate average daily gain and feed efficiency, and to ensure there were no abrupt changes in BW and that maintenance requirements are being fulfilled.

Feed offered and refusals were recorded daily during the length of the experiment to measure feed intake. Samples of the feed offered were collected weekly for determination of DM by oven drying at 55 ºC for 48 h. A subsample was composited for every experimental period and stored at -20 ºC for later analysis of composition of NDF, ADF, and CP.

A total of 15 mL of blood were taken from jugular venipuncture immediately before feeding and 4 h later during the third day at the digestibility crate (d 17 of each period). One out of the three tubes contained anticoagulant (K3-EDTA), and the sample was kept at 4 ºC until the haematology analysis with the Mythic 18 Vet Haemotology Analyser. The second and third tube contained Lithium Heparin and a clot activator, respectively, and the samples were immediately centrifuged at 2,000 x g for 15 min.

Approximately 100 mL of ruminal content were collected from the ruminal cannula immediately before feeding, and 2 and 4 h after feeding during the third day at the digestibility crate (d 17 of each period). Rumen fluid was filtrated trough 250 µm pore size nylon mesh, and with 50 mL of rumen fluid pH was determined using a pH meter and several sub-samples were taken: 1) 4 mL were transferred into a previously labelled 15 mL falcon tube containing 1 ml 20% V/V orphophosphoric acid with 4 mM 4 ethyl butyric acid for the subsequent analysis of VFA; 2) 1 mL was transferred to a 2 mL eppendorf tube containing 0.25 ml of 25% W/V trichloroacetic acid (TCA) for the analysis of ammonia; and 3) 0.5 mL were transferred to a 1.5 mL eppendorf tube containing 0.5 mL of saline formaldehyde 9.25% in NaCl 0.9% solution and dyed by adding a drop of methylene blue for protozoa count.

The first three days of the sampling week (d 15 to 18) sheep, while sheep were housed individually in metabolic crates, total amount of faeces and urine produced were recorded daily. Urine samples were collected into a container with approximately 100 ml of 10% sulphuric acid in order to maintain the pH below 3 (acidification of the urine prevents bacterial destruction of purine derivatives). A 20% of the faeces and a 10% of the urine were stored in plastic bags and processed accordingly to determine nutrient digestibility, N and energy balances, and purine derivative excretion.

From day 18 to 21 of each experimental period, ewes were housed in methane chambers where methane emissions were quantified. A MGA 3000 series multi gas analyser was used and calibrated and auto zeroing daily using oxygen free nitrogen. Chamber emissions were corrected for background concentrations of methane and for mean gas airflow.

The second day of the sampling week (d 16), from 0 to 6 h after feeding, different measurements were collected to determine individual feeding behaviour: 1) A digital voice recorder (GH609 Digital Voice Recorder) attached to the left side of a head harness captured the bite and chewing sounds of the animal while eating, that were later uploaded into a computer to be processed with an acoustic analysis software; 2) A 3-axis accelerometer (HOBO pendant G data logger) was attached to the right side of a head harness to capture the position and movement of the head while eating, that were later uploaded into a computer to be processed with its specific software to determine magnitude and frequency of the movements; and 3) A video camera recorded the front of the animals to be visualized later and be used as the gold standard for the former behavioural measurements.

3. Statistical analyses

Data were analysed using the MIXED procedure of SAS (University Edition 2.3, SAS Institute, Inc., Cary, NC). Rumen pH, VFA profile, ammonia production, and protozoa count was analysed as repeated measures over the hours after feeding nested within period, considering ewe as the subject with the first-order Autoregressive covariance structure. The model included the square of the Latin Square arrangement, the period, the animal nested within square, the treatment, the hour after feeding, and the interaction treatment x hour as fixed effects. The nutrient digestibility, methane production and average daily gain were calculated once per period, and therefore the model only included the square of the Latin Square arrangement, the period, the animal nested within square, and the treatment as fixed effects, with no repeated measures. For all the statistical analyses, significance was declared at $P < 0.05$ and trends at $0.05 \leq P < 0.10$, using the Tukey multiple comparison test to separate means.

II – Results

The average daily gain was greater (P = 0.01) in 2M than 1M ewes (0.49 vs -0.09 ± 0.079 kg BW/d; Table 2). The 1M ewes had a lower (P < 0.01) VFA concentration than 1.5M and 2M (69.9 vs 101.8 and 111.4 ± 6.99 mM), and a greater (P = 0.04) acetic-to-propionic ratio than 1.5M (2.40 vs 1.97 ± 0.108). The ammonia concentration was greater (P = 0.01) in 1.5M than in 2M ewes (14.14 vs

Table 2. Rumen fermentation profile, nutrients digestibility, methane emissions and growth performance

	Treatment[1]				P-Value[2]		
	1X	1.5X	2X	SEM	Treatment	Hour	Hour*Treatment
Rumen pH	6.25	5.97	6.09	0.089	0.09	<0.01	0.60
Total VFA production, mM	69.9^{b}	101.8^{a}	111.4^{a}	6.99	<0.01	<0.01	0.83
Acetic,%	54.8	53.0	54.8	1.06	0.34	<0.01	0.24
Propionic,%	23.8^{b}	27.5^{a}	25.6^{ab}	0.97	0.04	<0.01	<0.01
Butyric,%	14.5	13.7	14.3	0.68	0.59	0.04	0.55
Valeric,%	1.6	1.5	1.7	0.10	0.28	<0.01	0.63
Branched chain VFA,%	5.2	4.0	3.6	0.43	0.06	<0.01	0.28
Acetic:Propionic ratio	2.4^{a}	2.0^{b}	2.2^{ab}	0.10	0.04	<0.01	0.01
Ammonia, mM	12.2^{ab}	14.1^{a}	8.9^{b}	1.02	0.01	<0.01	0.04
Protozoa count, □ 10^5	12.7	21.7	25.9	3.40	0.10	0.04	0.98
Digestibility,%/$BW^{0.75}$							
DM	2.61^{a}	2.47^{b}	2.43^{b}	0.029	0.01	–	–
OM	2.68^{a}	2.55^{b}	2.50^{b}	0.027	0.01	–	–
NDF	2.34	2.20	2.14	0.075	0.20	–	–
ADF	2.27	2.21	2.15	0.081	0.55	–	–
CP	2.53	2.32	2.32	0.066	0.09	–	–
Retained CP,%	1.01	1.03	1.29	0.132	0.26	–	–
Retention rate,%	1.46	1.58	1.98	0.195	0.18	–	–
Methane emissions, g/d	20.1^{b}	25.0^{ab}	30.7^{a}	1.97	0.03	–	–
Kg^{-1} metabolic weight	0.74^{b}	0.88^{ab}	1.10^{a}	0.077	0.04	–	–
Kg^{-1} DMI	18.3^{a}	15.1^{b}	13.5^{b}	0.77	0.01	–	–
ADG, kg BW/d	-0.09^{b}	0.17^{ab}	0.49^{a}	0.079	0.01	–	–

[1] Treatments comprised dried grass nuts fed to meet one (1M), 1.5 times (1.5M), or two times (2M) maintenance energy requirements (NRC, 2007).

[2] Fixed effects were treatment, hour after feeding, and the interaction hour within treatment.

[a, b] Means with different superscripts in the same row are different (P < 0.05).

8.89 ± 1.024 mM). Dry and organic matter digestibility per unit of metabolic weight were greater (P < 0.05) in 1M than 1.5M and 2M ewes (2.61 vs 2.47 and 2.43 ± 0.029%/BW^0.75; 2.68 vs 2.55 and 2.50 ± 0.027%/BW^0.75, respectively). Emissions of methane per unit of DM intake were greater (P < 0.05) in 1M than 1.5M and 2M ewes (18.33 vs 15.08 and 13.53 ± 0.772 g/d x kg DMI). No differences (P > 0.05) were found in protozoa count, feeding behaviour or haematology.

III – Conclusions

Changes in the level of intake, without changing the diet composition, have a measureable impact on rumen fermentation profile, as seen by the greater total VFA production and the lower acetic-to-propionic ratio, DM and OM digestibility, and methane emissions by unit of DMI of x1M ewes compared to x1.5M and x2M. These changes could be attributed to the greater amount of food fermented in the rumen and the higher flow rate from the rumen to the lower digestive tract.

Acknowledgments

The authors acknowledge the financial support provided by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment.

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