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Ruminal degradation evaluation of different feedstuffs by using single-flow continuous culture fermenters

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Abstract. Two experiments were carried out to study the ruminal degradation profiles of three different quality experimental diets: alfalfa hay (AH), olive leaves (OL) and a 60:40 mixture of AH and concentrate (AHCO). The concentrate (CO) consisted of barley grain and two-stage olive cake (2:1). In the first experiment, the samples were incubated in the rumen of 3 Granadina dry goats and 3 Segureña wethers fed the corresponding experimental diets (AH, OL or AHCO). Samples were incubated in nylon bags by following the methodology described by Madsen and Hvelplund (1994). In the second experiment, samples were incubated in small nylon bags into 4 continuous culture fermenters inoculated with rumen liquor from goats and 4 inoculated with rumen liquor from wethers. Fermenters were fed the same diets as ruminal inoculum donor animals. Data from goats and wethers were pooled and those from fermenters inoculated with rumen liquor from goats and wethers were pooled as well and the profiles of dry matter (DM) and crude protein (CP) degradation were determined using the Ørskov and McDonald (1979) exponential model. Additionally correlations equations between data obtained *in situ* and those derived from fermenters were obtained in order to check the potential of continuous fermenters for predicting rumen degradation activity. Significant correlations were obtained for the soluble fraction of DM ($P < 0.05$, RSD = 7.52, $r^2 = 0.715$), the potential degradability of DM ($P < 0.01$, RSD = 7.68, $r^2 = 0.612$) and, the DM effective degradability ($P < 0.01$, RSD = 6.58, $r^2 = 0.872$). Statistically significant equations were also obtained between data obtained *in situ* and those derived from fermenters for crude protein degradability (CPD) concerning the soluble fraction ($P < 0.01$, RSD = 8.02, $r^2 = 0.888$), potentially degradable fraction ($P < 0.01$, RSD = 6.92, $r^2 = 0.867$), rate of degradation ($P < 0.05$, RSD = 0.025, $r^2 = 0.688$), potential degradability ($P < 0.01$, RSD = 10.24, $r^2 = 0.933$) and, effective degradability ($P < 0.01$, RSD = 6.88, $r^2 = 0.946$). Overall, no statically significant ($P > 0.05$) differences were found between animal species in experiment 1 and 2. From these results it may be concluded that *in situ* ruminal degradation can be estimated accurately by using continuous fermenters.

Keywords. Rumen degradability – Sheep – Goats – Fermenters.

Évaluation de la dégradation ruminale de différents aliments en utilisant les fermenteurs à flux continu

Résumé. *Deux expériences ont été réalisées pour étudier les profils de la dégradation ruminale de trois régimes expérimentaux : foin de luzerne (AH), feuilles d'olivier (OL) et un mélange de AH et de concentré (CO) dont la proportion était de 60:40 (AHCO). Le concentré est constitué d'orge et de grignons d'olive (2:1). Dans la première expérience, les échantillons ont été incubés dans le rumen de 3 chèvres de race Granadina et 3 moutons de race Segureña alimentés avec les régimes expérimentaux correspondants (AH, OL ou AHCO). Des échantillons ont été incubés dans des sachets de nylon selon la méthodologie décrite par Madsen et Hvelplund (1994). Dans la deuxième expérience, les échantillons ont été incubés dans de petits sachets en nylon dans 4 fermenteurs à flux continu inoculés avec le jus de rumen de chèvres et 4 inoculés avec le jus de rumen de moutons. Les fermenteurs recevaient les mêmes régimes que les animaux donneurs d'inoculum ruminal. Les données des chèvres et des moutons ont été mises en commun et celles des fermenteurs inoculés avec le jus de rumen de chèvres et de moutons ont été aussi mises en commun et les profils de dégradation de la matière sèche (DM) et de la protéine brute (CP) ont été déterminés en utilisant le modèle exponentiel de Ørskov et McDonald (1979). En outre, les équations de régression entre les données obtenues in situ et celles dérivées des fermenteurs ont été analysées afin d'examiner le potentiel des fermenteurs à flux continu pour prédire la dégradabilité dans le rumen. Des corrélations significatives ont été obtenues pour la fraction soluble de la MS ($P < 0,05$, RSD = 7,52, $r^2 = 0,715$), la dégradabilité potentielle de la MS ($P < 0,01$, RSD = 7,68, $r^2 = 0,612$) et la*

dégradabilité effective de la MS (P < 0,01, RSD = 6,58, r^2 = 0,872). Des équations statistiquement significatives ont été également obtenues entre les données obtenues in situ *et celles issues des fermenteurs pour la dégradabilité des protéines brutes concernant la fraction soluble (P < 0,01, RSD = 8,02, r^2 = 0,888), la fraction potentiellement dégradable (P < 0,01, RSD = 6,92, r^2 = 0,867), le rythme de dégradation (P < 0,05, RSD = 0,025, r^2 = 0,688), la dégradabilité potentielle (P < 0,01, RSD = 10,24, r^2 = 0,933) et la dégradabilité effective (P < 0,01, RSD = 6,88, r^2 = 0,946). En général, aucune différence significative (P > 0,05) n'a été obtenue entre les espèces animales dans les expériences 1 et 2. On peut conclure que la dégradabilité ruminale* in situ *peut être estimée en utilisant les fermenteurs à flux continu.*

Mots-clés. *Dégradabilité ruminale – Mouton – Chèvre – Fermenteurs.*

I – Introduction

Rumen degradability is one of the key factors that determine the potential utilization of a feedstuff by ruminants and it is needed when formulating diets. The nylon bag technique is nowadays widely accepted as a valid method for measuring this parameter (Madsen and Hvelplund, 1994). However, it implies the use of ruminal cannulated animals. The use of *in vitro* techniques that simulate rumen activity to study the rumen fermentation promoted by different feedstuffs has been largely accepted as a less time consuming and valid approach as it uses less cannulated animals than *in vivo*. The continuous culture has been shown to provide the best estimations. However, the possibility of measuring the *in sacco* degradability in fermenters has not been addressed yet and it could add an extra value to the *in vitro* approach. These experiments were designed to asses the suitability of the *in sacco* technique measured in fermenters to predict the values obtained in animals by incubating three different quality diets. The effect of the animal species (sheep *vs* goats) was also studied.

II – Materials and methods

Two consecutive experiments were conducted in adult dry non-pregnant Granadina goats and Segureña wethers. In both experiments three ruminally cannulated goats (43 ± 2.1 kg LW) and three ruminally cannulated wethers (69 ± 4.3 kg LW) were fed three experimental diets (Table 1): alfalfa hay plus a commercial mineral-vitamin supplement (diet AH), alfalfa hay and a concentrate formulated with barley, two-stage dried olive cake (TSDOC) and a mineral vitamin mixture (diet AHCO), and olive leaves (diet OL). The first two diets were fed at maintenance level and OL *ad libitum* with a 20-d adaptation period to each diet. Mineral requirements of the animals were guaranteed (ARC, 1980). The average intakes of OL (g, DM ± SD) were 545.4 ± 39.5 and 50 ± 44.5, respectively in goats and wethers.

Table 1. Chemical composition of the experimental diet's ingredients (g/100 g DM)

	AH	AHCO	OL
DM, g/100 g FM	90.0	90.3	93.7
OM	88.5	94	88.6
NDF	42.0	37.6	40.1
ADF	27.6	15.6	26.9
ADL	5.93	6.04	15.4
CP	17.9	11.7	7.66
EE	1.76	2.63	8.03
EB, MJ/kg DM	17.7	18.4	21.1

1. *In situ* experiment

Samples of the experimental diets were mill ground through a 2-mm screen and aliquots of approximately 2 g were placed in nylon bags (7 × 10 cm and 46 µ pore, Sefar Maissa SA, Barcelona, Spain). Bags were incubated during 0, 4, 8, 16, 24, 48 and 72 h in the rumen of cannulated animals fed one of the experimental diets, containing the ingredient to be tested. Two bags per animal, ingredient and incubation time were used. After incubation, bags were washed in a washing machine during 20 min, then stomached (residual material was subjected to vigorous mechanical pummelling between two metal plates, IUL Instruments GmbH) for 5 min (Michalet-Doreau and Ould Bah, 1992) to remove the bacterial population from bag residues, finally, dried at 60℃. Aliquots of the residual dry matter were us ed for N analyses. The degradation profiles were calculated by the non-linear model described by Ørskov and McDonald (1979). The effective degradability (ED) in the rumen was calculated, using the Nonlinear Regression (NLIN) procedure of SAS, as ED = a + [(b × c) / (c + k)], where *a* is the water soluble fraction, *b* the potentially degradable (insoluble) fraction, *c* the rate of degradation of *b* and, *k* the passage rate of the digesta out of the rumen. The passage rate was assumed to be 0.031 and 0.025 h^{-1} in goats and wethers, respectively (García *et al.*, 1995).

2. Fermenters experiment

A continuous culture system with eight fermenters was used. Fermenters were inoculated with rumen liquor collected from the same animal species and received the same diets as described above. The rumen content for each animal species was strained through two layers of cheesecloth and liquid used as inoculum (700 ml per fermenter). Four fermenters were inoculated with pooled rumen liquor from wethers and other four with pooled rumen liquor from goats, within 30 minutes after rumen content collection. Each fermenter vessel received 60 g of dry matter per day of the corresponding experimental diet, previously ground through a 2 mm screen, in two equal meals at 09:00 and 16:00 hours. Artificial saliva (McDougall, 1948) was continuously infused into each fermenter at a dilution rate of 40 ml/h. The anaerobic conditions in the vessels were maintained by continuous infusion of CO_2.

A. Degradation profile

Samples of the ingredients of the experimental diets were mill ground through a 2-mm screen and aliquots of approximately 1.4 g were placed in nylon bags (4 × 5 cm; 46 µm of pore size). The *in vitro* degradation parameters were determined as described *in situ*. The artificial saliva flow was considered as passage rate (k) value to determine the effective degradability *in vitro*.

Correlations equations considering data obtained from the three diets and two animal species between values obtained *in situ* and those derived from fermenters were obtained by using Statgraphics software package (1989).

III – Results and discussion

In general, degradation profiles of both alfalfa hay and concentrate were similar in goats and wethers (Table 2). These results corroborate those found in previous studies (Isac *et al.*, 1994; Molina Alcaide *et al.*, 2000), concerning the lack of significant differences between goats and sheep in DM and crude protein (CP) degradation rates and effective degradability of medium-good quality feedstuffs. Values obtained in fermenters inoculated with rumen liquor from wethers showed the same trend. However, samples of OL incubated in the rumen of goats showed higher b and ED values for DM and CP than in wethers. These results corroborate those found in previous studies (García *et al.*, 1995; Martín García *et al.*, 2003) indicating that goats have a better capacity for degrading poor quality feedstuffs. The interspecies differences found when OL was incubated were not observed in fermenters, which may be related to the differences in the passage rate of digesta that obviously did not exist *in vitro*.

Table 2. Dry matter (DMD) and crude protein (CPD) rumen degradation profiles of the experimental diets obtained either in fermenters or *in situ*

	AH				AHCO				OL			
	Fermenters		*In situ*		Fermenters		*In situ*		Fermenters		*In situ*	
	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
DMD†												
a	48.2	45.5	34.1	32.8	53.7	54.2	53.4	56.6	31.7	31.8	29.2	28.8
b	27.3	32.5	37.7	37.9	12.4	12.8	24.5	30.1	2.62	3.83	24.2[a]	45.2[b]
c	0.02	0.031	0.074	0.100	0.032	0.042	0.058	0.047	0.047	0.026	0.001[a]	0.014[b]
a + *b*	75.5	78.1	71.8	70.7	66.1	67.1	77.9	86.7	34.3	35.6	53.2[a]	73.4[b]
ED	56.8	59.2	61.2	61.8	60.2	61.3	69	74.9	33	32.9	30.5[a]	44.9[b]
CPD												
a	61.2	58.6	40.1	38.8	64.3	65.2	59.5	62.7	30.7	30.2	14.0	12.8
b	29.5	31.6	48.6	51	16.2	13.8	32.5	33.3	3.88	4.15	5.01[a]	23.4[b]
c	0.034	0.044	0.114	0.127	0.052	0.05	0.102	0.078	0.034	0.063	0.106	0.016
a + *b*	90.7	90.2	88.7	89.8	80.5	79.0	92.0	96.0	34.6	34.4	19.1	28.4
ED	74.2	74.7	82.7	84.4	74.7	73.7	84.0	86.7	32	32.2	18.0	22.4

†*a*: water soluble fraction; *b*: potentially degradable (insoluble) fraction; *a* + *b*: potential degradability; *c*: rate of degradation.
[a,b]Values in columns, between sheep and goats, are different $P < 0.05$.

Significant correlations were obtained for the soluble fraction (*a*) of DM ($P < 0.05$, RSD = 7.52, r^2 = 0.715), the potential degradability (*a* + *b*) of DM ($P < 0.01$, RSD = 7.68, r^2 = 0.612) and the DM effective degradability (ED) ($P < 0.01$, RSD = 6.58, r^2 = 0.872). Statistically significant equations were also obtained between data obtained *in situ* and those derived from fermenters for crude protein degradability (CPD) concerning *a* ($P < 0.01$, RSD = 8.02, r^2 = 0.888), *b* ($P < 0.01$, RSD = 6.92, r^2 = 0.867), *c* ($P < 0.05$, RSD = 0.025, r^2 = 0.688), *a* + *b* ($P < 0.01$, RSD = 10.24, r^2 = 0.933) and, ED ($P < 0.01$, RSD = 6.88, r^2 = 0.946). Overall, the fermenters approach overestimates *a* fraction and underestimates *b* fraction compared with values obtained *in situ*, resulting in slightly lower ED values for AH and AHCO diets. In the case of OL diet, the discrepancy was higher between the fermenters and the *in situ* values.

IV – Conclusion

It may be concluded that *in situ* ruminal degradation can be estimated by using continuous fermenters, although the accuracy of this prediction seems to decrease when low quality feeds are used.

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