Ann. Zootech. 48 (1999) 447–456

Original article

Relationship between in situ rumen protein degradability and chemical composition of alfalfa hays

Maria Remedios Alvir*, Javier González, Carlos Alberto Rodríguez

Dpto. Producción Animal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, 28040 Madrid, Spain

(Received 2 April 1999; accepted 14 September 1999)

Abstract — The variation in protein degradation kinetics and effective degradability of ten alfalfa hays was studied to evaluate the prediction of these parameters from chemical composition of the hays. The alfalfa hays were selected on the basis of the stage of maturity to obtain a wide range of variation in their chemical composition. Rumen degradation of crude protein (CP) was measured by the nylon bag technique on four cannulated wethers fed one of the tested alfalfa hays in two equal meals at an intake level of 80 g dry matter·kg^{-1} LW$^{0.75}$. Four of the studied hays with different vegetative state were mordanted with chromium (Cr) and rumen outflow rates determined from faecal Cr concentrations after single dose. The neutral detergent insoluble nitrogen (NDIN) showed a wide variation among hays, from 6.2 to 29.8 % of total N. In six of the ten hays the existence of a lag time was verified with values ranging from 2.70 to 6.75 h. The length of this period was positively related to the NDIN content of hays ($R^2 = 0.57$; $P < 0.05$). The NDIN was also found to be the best predictor of effective degradability of protein ($R^2 = 0.77$; $P < 0.001$), which ranged from 61.1 to 74.7 %. The soluble and insoluble potentially degradable protein fractions represented from 35.9 to 48.1 % and from 38.7 to 55.2 % of the total CP, respectively. Values of the degradation rate were highly variable among hays, ranging from 0.061 to 0.151 h^{-1}. The non-degradable protein fraction also showed a wide variation from 3 to 20 %. The best single predictor of this last fraction was the acid detergent lignin content ($R^2 = 0.83$; $P < 0.001$), but the prediction was improved when considering as the second variable the cellulose expressed as a fraction of the cellular wall ($R^2 = 0.92$; $P < 0.001$). (© Elsevier / Inra)

alfalfa hay / protein degradability / rumen outflow rate / chemical composition / sheep

Résumé — Relations entre la dégradabilité in situ des matières azotées et la composition chimique des foins de luzerne. La variation des cinétiques de dégradation et de la dégradabilité théorique des matières azotées (MAT) de dix foins de luzerne, a été étudiée et mise en relation avec celle de la composition chimique. Les foins ont été choisis en fonction de leur stade de végétation pour obtenir une variation importante de leur composition chimique. La dégradation dans le rumen des MAT a été mesurée à l'aide de la technique des sachets de Nylon sur quatre moutons adultes, munis d'une canule du rumen, et nourris avec un des foins étudiés, distribué en deux repas égaux à un niveau

* Correspondence and reprints
Tel.: (34) 91 54 93 069; fax (34) 91 54 99 763; malvir@pan.etsia.upm.es

d'ingestion de 80 g MS·kg^{-1} P^{0,75}. Quatre des foins étudiés, avec des stades de végétation différents, ont été marqués par mordançage au Cr pour estimer leurs taux de sortie du rumen à partir des concentrations de Cr dans les fèces après l'administration d'une dose simple. L'azote insoluble dans la solution au détergent neutre (NDIN) a été très variable entre les foins : de 6,2 à 29,8 % de l'azote total. Pour six des dix foins on a constaté l'existence d'un temps de latence de 2,7 à 6,7 h. Sa durée est positivement liée avec la proportion de NDIN des foins ($R^2 = 0,57$; $p < 0,05$). La proportion de NDIN fournit aussi la meilleure prédiction de la dégradabilité théorique des MAT ($R^2 = 0,77$; $P < 0,001$), qui a varié de 61,1 à 74,7 %. Les fractions des MAT solubles et insolubles mais potentiellement dégradables représentent, respectivement, de 35,9 à 48,1 % et de 38,7 à 55,2 % des MAT. Les vitesses de dégradation ont été très variables entre les foins (de 0,061 à 0,151 h^{-1}). De même, la fraction des MAT non dégradables a été très variable, de 3 à 20 %. La meilleure prédiction de cette fraction avec une seule variable indépendante a été obtenue avec la teneur en lignine ($R^2 = 0,83$; $P < 0,001$). Cependant, l'emploi comme seconde variable du rapport cellulose : parois cellulaires permet une amélioration importante de la prédiction ($R^2 = 0,92$; $p < 0,001$). (© Elsevier / Inra)

foin de luzerne / dégradabilité de l'azote / taux de sortie du rumen / composition chimique / ovins

1. INTRODUCTION

Current systems of protein evaluation for ruminants [1, 20, 27, 37] require reliable information on rumen protein degradability of feedstuffs. However, in relation to the nutritive value of forages these systems show two basic limitations: first, existing data on protein degradability of forages are limited, and second, there is little known concerning the variability of rumen degradability values within a given forage type [27]. Many factors influence the crude protein (CP) degradability of forages, such as stage of maturity [6, 11, 12], forage species [13, 26] and preservation method [5, 38]. The effect of stage of maturity at harvest and the loss of leaves during haymaking may affect the distribution of different nitrogen fractions of the hay and therefore the accessibility of microbial enzymes to tissular proteins. Hoffman et al. [11] demonstrated that stage of maturity affects all protein fractions and degradation rate. Balde et al. [6] also indicated a shift in CP degradability with stage of maturity and suggested that the use of fixed values for forage CP degradability are inappropiate. Moreover, advancing forage maturity may affect protein degradability by affecting physical breakdown and removal from the rumen. On the other hand, like in most forages, nitrogenous components are basically digested in the rumen [32] and differences in protein degradability are the main source of variation of their protein value.

The most frequently used method to estimate rumen protein degradability is that proposed by Ørskov and McDonald [28], which consists of the integration of rumen degradation and transit kinetics. However, quantification of kinetic parameters requires laborious and time-consuming techniques and surgically prepared animals, which are major inconveniences for advisory analytical services. Prediction of the effective degradability of protein from chemical composition is possible, but attempts made on forages are limited [11, 13, 21].

The aim of this work was to study the variations of protein degradation kinetics, rumen particulate outflow rate and effective degradability of alfalfa hays to develop predictive equations from their chemical composition.

2. MATERIAL AND METHODS

2.1. Feeds

Ten alfalfa hays (H1 to H10) with different origins were used in this study. The hay samples were selected on the basis of their stage of veg-

etation and cut number to obtain a wide range of variation in their chemical composition. Most hay samples were representative of typical hays used by dairy farmers, except alfalfa harvested at the early vegetative stage. All the hay samples were field dried without subsequent thermic treatment. The cut number and estimated stage of maturity are shown in *table I*.

2.2. In sacco trials

The CP ruminal degradation of the hays was measured in four adult wethers (LW = 67 kg ± 14.3) equipped with rumen cannula. Animals were fed alfalfa hay (H3) throughout an adaptation period of 15 days and the experimental period of 30 days. The hay was distributed in two equal meals per day (9.00 and 17.00 hours) at a level of 80 g dry matter·kg^{-1} LW$^{0.75}$. This level corresponds to 95 % of the average voluntary intake, which was determined over the first 10 days of the adaptation period.

The CP ruminal degradation kinetics of the ten alfalfa hays were determined by the in situ technique, using nylon bags with 1 200 µm^2 pore surface (reference F100, Tripette & Renauld, France) and a size of 14 × 8 cm. The bags were filled with hay samples (milled through a 2-mm mesh) at a rate of 15 mg of dry matter (DM) per cm^2 of bag surface area. One bag of each hay was incubated in the rumen of each animal for periods of 1, 3, 6, 9, 15, 24 and 48 h. One incubation, including the bags of the ten hays, was conducted for each incubation period. The bags of each incubation were always inserted in the rumen at the time of the morning feeding. After being removed from the rumen, the bags were rinsed with tap water (twice for 5 min), dried at 60 °C for 48 h, weighed and analysed for Kjeldahl-N. Nitrogen disappearance was expressed as a proportion of the initial N in the sample. Microbial contamination of the bags was not estimated.

2.3. Rumen particulate outflow rate

Four of the alfalfa hay samples (H1, H5, H9 and H10) were selected to study the effect of alfalfa maturity on rumen outflow rate. Unchopped hays were mordanted with chromium (Cr) using the procedure described by Udén et al. [34]. A single dose of each labelled feed (30 g) was offered to each animal 30 min before the morning feeding and animals ingested the labelled feed within 30 min. From 12 h after this moment faecal grab samples (10 g) were collected from the rectum at intervals of 6 h during the first 5 days, 8 h on the 6th day and 12 h on the 7th day. Faeces were immediately frozen (–20 °C) until Cr analysis. These studies were carried out with only three animals because the fourth animal refused to eat systematically the labelled hay. A resting period of at least 3 days

Table I. Chemical composition of alfalfa hays (g.kg^{-1} dry matter).

Hays	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Cut number and stage of maturity[1]	1V	2V	2B	1BF	1BF	3BF	1F	2F	2F	4F
Ash	136.0	108.5	112.1	105.1	129.8	106.2	124.6	113.0	103.6	123.2
CP	282.3	242.4	201.8	189.5	181.1	176.7	182.3	197.9	169.7	191.8
NDF	218.6	281.2	366.0	414.3	333.7	323.9	376.8	383.2	402.8	397.1
ADF	196.6	232.9	273.8	285.1	286.6	271.0	293.0	330.1	350.6	330.7
ADL	34.7	47.7	53.2	61.6	60.5	60.0	66.3	64.6	74.8	63.5
HEM	22.0	48.3	92.2	129.2	47.1	52.9	83.8	53.1	52.2	66.4
CEL	161.9	185.2	220.6	223.5	226.1	211.0	226.7	265.5	275.8	267.2
NDIN[2]	6.2	8.8	22.8	29.8	11.5	13.5	13.0	18.8	18.0	14.5

[1] V: vegetative; B: bud; BF: beginning of flowing; F: flowing.
[2] Expressed as percent of total nitrogen, NDIN: neutral detergent insoluble nitrogen.
CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; HEM (hemicellulose): NDF – ADF; CEL (cellulose): ADF – ADL.

was introduced between two consecutive hay passage measurements.

2.4. Analytical methods

Dry matter, ash and CP were determined according to the methods of the AOAC [3]. Analysis for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were carried out according to the methods of Robertson and Van Soest [30]. The hemicellulose (HEM) was considered as the difference between NDF and ADF fractions. The cellulose (CEL) was measured as ADF – ADL. Insoluble N in neutral detergent solution (NDIN) was determined by Kjeldahl analysis on the NDF residues. Samples of faeces were dried at 80 °C for 24 h, ashed (500 °C, 3 h) and Cr concentrations determined by atomic absorption spectrometry [40].

2.5. Mathematical and statistical methods

The rumen fractional outflow rate of particles (k_p) was determined for each animal by fitting a non-linear regression to the decreasing phase of the faecal Cr excretion curve in accordance with the expression:

$$C = A(e^{-kp\,t})$$

where C represents the Cr concentration in faeces at a time t and A is a constant depending on the initial concentration of the marker in the rumen.

The evolution for each hay of the N disappearance from the bag (d) with incubation time (t) was fitted through non-linear regression for the group of four wethers, using all data points, to the model proposed by Ørskov and McDonald [28]:

$$d = a + b\,(1 - e^{-kd\,t}) \qquad (1)$$

In this model, a represents the soluble fraction and b the insoluble fraction potentially degradable by rumen microorganisms at a fractional rate k_d. The non-degradable fraction (u) was estimated as the difference $100 - (a + b)$.

When visual inspection of the degradation curves suggested the existence of a lag time (t_o), the modification of this model proposed by McDonald [19] was adopted:

$$d\,(t) = a \text{ if } t < t_o$$
$$d\,(t) = a_1 + b_1\,(1 - e^{-kd\,t}) \text{ if } t > t_o \qquad (2)$$

where a was determined as the average of the initial and constant disappearance values and the remainder of the data where used to establish the exponential equation. Estimations of t_o were obtained solving for t at the intersection of both equations and the insoluble degradable fraction (b) was estimated as: $b_1\,e^{-kd\,to}$.

The effective degradability (ED) of the hay samples adjusted to the models (1) and (2) was estimated in accordance with Ørskov and McDonald [28] and McDonald [19], respectively, as:

$$ED = a + (b\,k_d / (k_d + k_p))$$
$$ED = a + (b\,k_d\,e^{-kp\,to} / (k_d + k_p))$$

Degradation kinetics were fitted by an iterative least-squares procedure and best fit values were chosen using the Marquardt procedure of the SAS software (SAS Institute, Cary, NC, USA). Values of k_p were studied by analysis of variance examining the effect allocated to hay and animal. Possible effects of chemical composition of hays on their CP degradation characteristics were studied by correlation and uni- and multivariate (stepwise) regression analyses of data. All analyses were performed using the statistical program SAS for Windows version 6.08.

3. RESULTS AND DISCUSSION

Chemical composition of alfalfa hays ranked by their stage of maturity at harvest are shown in *table I*. The NDF, ADF and ADL contents tended to increase with maturity state at harvest and a reverse trend was observed for the CP content as shown by the negative correlations observed between CP and NDF ($r = -0.83$; $P < 0.01$), CP and ADF ($r = -0.82$; $P < 0.01$) and CP and ADL ($r = -0.93$; $P < 0.001$). The proportion of NDIN showed a wide variation among hays (6.2 to 29.8 %), which seemed to be associated with NDF content ($r = 0.77$; $P < 0.01$), but mainly with the hemicellulose content ($r = 0.85$; $P < 0.01$).

The mean values of CP disappearance from the bags at each incubation time and resulting values of degradation parameters are shown in *tables II* and *III*, respectively. In six of the tested hays the existence of a lag time was evident, with values ranging from 2.70 to 6.75 h. Nevertheless, real values of

Table II. Mean values of crude protein disappearance (%) at each incubation time (h).

Incubation	Hay									
time (h)	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
1	47.7	45.0	41.8	36.4	40.8	40.7	48.0	44.4	38.2	41.2
3	55.3	50.9	39.9	35.4	48.1	37.2	48.3	42.1	38.6	44.1
6	62.4	63.1	40.9	39.1	59.0	53.6	65.4	56.1	51.2	58.4
9	63.9	64.1	44.4	47.9	61.9	64.3	70.3	59.9	59.9	59.2
15	74.9	68.9	60.0	58.9	72.1	73.7	80.0	71.6	62.4	71.1
24	89.7	88.8	78.3	82.1	82.3	79.4	84.8	78.3	77.7	81.1
48	92.6	91.7	87.6	84.6	86.4	86.3	87.6	84.3	79.1	85.5

Table III. Degradation kinetics and effective degradability of crude protein of alfalfa hays.

Hay	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
lag	–	–	6.75	4.03	–	2.92	2.83	2.77	2.70	–
a	44.3	41.8	40.9	35.9	39.2	39.0	48.1	43.3	38.4	36.1
b	52.8	54.3	51.3	55.2	50.0	46.3	38.7	41.4	41.6	51.4
u	2.9	3.9	7.8	8.9	10.9	14.7	13.2	15.4	20.0	12.5
k_d	0.066	0.065	0.064	0.061	0.065	0.121	0.151	0.092	0.097	0.077
ED	74.7	72.8	62.0	61.1	67.7	67.9	73.7	67.0	62.8	67.6

lag: lag time (h); *a*: soluble fraction (%); *b*: insoluble potentially degradable fraction (%); *u*: non-degradable fraction (%); k_d: fractional degradation rate (h^{-1}); *ED*: effective degradability of protein (%) (calculated for a passage rate of $0.049 \cdot h^{-1}$).

lag time should be lower than these apparent values as a consequence of the effects of microbial contamination [26]. In an extensive study of 16 alfalfa hays, Von Keyserlingk et al. [38] also detected the existence of a lag time for CP degradation, with apparent values ranging from 0 to 3.15 h. However, in other experiments with alfalfa hays, the existence of a lag time for CP degradation has not been reported [26, 33]. The soluble protein fraction (*a*) represented from 35.9 to 48.1 % of total CP and the insoluble degradable protein fraction (*b*) from 38.7 to 55.2 %, and therefore the non-degradable protein fraction (*u*) was highly variable (3 to 20 %). The fractional degradation rate also varied considerably, ranging from 0.061 to 0.151 h^{-1}. The values obtained by Balde et al. [6] for the different fractions of four alfalfa samples (dried at 40 °C in an oven) with different maturities are within the range reported in the present study. However, the results obtained by Von Keyserlingk et al. [38] for the soluble fraction show a variation range from 48.3 to 75.4 %, the minimum value corresponding to the maximum value established in the present work (48.1 %). Consequently, the values indicated by the same authors for the potentially degradable fraction were lower (16.7 to 44.9 %) than those in the present work, although the average values obtained for the fractional degradation rate were similar in both experiments. The differences in both soluble and potentially degradable CP values may be a result of differences in measurement conditions and/or in growing conditions and practices on individual farms. Christensen and Fehr

[8] stated that alfalfa hay has very different nutrient profiles depending on the region where it is grown. The values obtained for the effective degradability of CP are also given in *table III*. Due to the lack of significant differences in the fractional outflow rate (as described later), an average value of 0.049 h^{-1} was used in the estimation of effective degradability. These values represent only apparent degradability as a consequence of the lack of correction for microbial contamination of incubated residues, which implies an underestimation of the values. Nevertheless, the error introduced for alfalfa hays should be moderate. Using ^{35}S or ^{15}N as microbial markers, Mathers and Aitchison [23], Bernard et al. [7] and Rodriguez [31] reported that microbial contamination underestimated *ED* of CP by 6.2, 8.8 and 4.8 %, respectively. Moreover, from the results of Kennedy et al. [16] it can be deduced that underestimation of *ED* may be lower than 4.8 %. A second reason for underestimation of the present results is derived from the calculation of *ED*, which considers the outflow rate of particles from the rumen and not the fractional rate of release of particles from the rumen non-escapable to escapable pool [4]. The assumption of total degradation of the soluble fraction (*a*), normally employed in nylon bag studies, represents an overestimation as a consequence of the loss of non-degraded small particles across the bag pores and also because the degradation rate of really soluble components presumably does not reach an infinite value. Nevertheless, for alfalfa hays this overestimation should be rare because an important part of soluble CP is non-protein nitrogen [35] and soluble protein seems to be relatively rapidly degraded [22]. In spite of its moderate value, this overestimation contributes to balance the above indicated underestimations. Thus, present values, which ranged in effective CP degradability from 61.1 to 74.7 %, are in agreement with the range of 62 to 78 % derived from different in vivo studies [15, 17, 24, 39]. The range of variation of *ED* values corrected for microbial contamination is lower than those indicated above. Thus, the application of the corrective equations proposed by Michalet-Doreau and Ould-Bah [25] and Rodriguez [31] give values from 67.6 to 80.1 % and from 66.2 to 78.7, respectively. The range of variation of both sets of corrected values [2] for oven-dried alfalfa (60 °C for 48 h), collected between the vegetative stage and the end of budding, resulted from 77.4 to 81.4 %.

The rumen fractional outflow rate (h^{-1}) did not differ ($P > 0.05$) among hays, averaging 0.050 (±0.002), 0.049 (±0.006), 0.051 (±0.005) and 0.044 (±0.006) for H1, H5, H9 and H10, respectively. Similar values have been reported for long or chopped alfalfa hays by Colucci et al. [9] using Cr, and Llamas-Lamas and Combs [18] using Yb, La and Cr as markers. On the other hand, lower values were obtained in cows by Ehle [10] with a rich concentrate mixed diet and by Susmel et al. [33] with a forage diet fed to a low plane of nutrition, using, respectively, chopped and unchopped hays labelled with Cr (0.023 and 0.037 h^{-1}, respectively). The absence of significant differences among outflow rates was also observed by Llamas-Lamas and Combs [18] for three alfalfa hays at early vegetative, late bud and full bloom states using La and Cr; however, with Yb a higher value was observed for the early vegetative state in comparison to late bud and full bloom hays. Also, Kawas et al. [14] using Yb as a marker did not obtain significant differences among three chopped alfalfa hays at pre-, early and mid-bloom maturity states, with NDF contents of 40.5, 42.0 and 52.5 %, respectively, although a significant decrease of this parameter was found for a hay with a high degree of maturity (full bloom, 59.5 % NDF). These authors reported that the organic matter intake decreased as the NDF and ADF contents of hays increased and this appeared to be related to a decrease in digestibility and in rumen outflow rates. The amount of feed consumed is probably the most important variable associated with retention time of

digesta in the rumen. In our experiment, the hays offered at constant intake level could explain the absence of significant differences among rumen outflow rates.

The correlation coefficients between chemical fractions related to nitrogen or fibre composition (CP, NDF, ADF, ADL, HEM, CEL and NDIN) and degradation parameters of CP are given in *table IV*. Prediction equations obtained from these chemical fractions and including also the CEL/NDF ratio are shown in *table V*. The duration of the lag period showed correlations with the hemicellulose content (r = 0.66; $P < 0.05$) and the NDIN proportion (r = 0.76; $P < 0.05$), which suggests a lower accessibility of microbial proteolytic enzymes to the proteinaceous substrate as the feed protein fraction bound to the cell wall increases. No correlations were observed between chemical composition and *a*, *b*, or k_d parameters, except between the fraction *b* and the ADL content (r = –0.63; $P < 0.05$), which shows that lignin reduces the extent of the potential degradation of the insoluble CP. On the contrary, the non-degradable protein fraction exhibited significant positive correlations with NDF (r = 0.69; $P < 0.05$), ADF (r = 0.88; $P < 0.001$), ADL (r = 0.91; $P < 0.001$) and CEL (r = 0.85; $P < 0.001$) and a negative correlation with CP (r = –0.83; $P < 0.001$). In multiple regression analysis (stepwise), the ADL content was

Table IV. Correlation coefficients between protein degradation parameters and chemical composition of hays.

	CP[1]	NDF[1]	ADF[1]	ADL[1]	HEM[1]	CEL[1]	NDIN[2]
lag	–0.39	0.49	0.23	0.27	0.66*	0.21	0.76**
a	0.36	–0.40	–0.34	–0.28	–0.29	–0.35	–0.46
b	0.50	–0.36	–0.57	–0.63*	0.14	–0.53	0.05
u	–0.83***	0.69*	0.88***	0.91***	0.05	0.85***	0.28
k_d	–0.44	0.22	0.30	0.48	–0.01	0.24	–0.15
ED	0.59	–0.71**	–0.56	–0.51	–0.60	–0.56	–0.88***

[1] Expressed on dry matter, CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; HEM (hemicellulose): NDF – ADF; CEL (cellulose): ADF – ADL.
[2] Expressed as percent of total nitrogen, NDIN: neutral detergent insoluble nitrogen.
lag: lag time (h); *a*: soluble fraction (%); *b*: insoluble potentially degradable fraction (%); *u*: non-degradable fraction (%); k_d: fractional degradation rate (h^{-1}); *ED*: effective degradability of protein (%).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table V. Regression equations between lag time (*lag*, h), crude protein (CP) non-degradable fraction (*u*, %) or CP effective degradability of protein (*ED*, %) and chemical composition of hays.

Parameter	Equation	R^2	RSD	*P*
lag	–1.61 + 0.243 NDIN	0.57	1.55	*
u	– 14.3 + 4.32 ADL	0.83	2.30	***
u	– 35.6 + 4.75 ADL + 0.288 CEL/NDF	0.92	1.70	***
ED	77.3 – 0.612 NDIN	0.77	2.46	***

NDIN: neutral detergent insoluble nitrogen (% of N); ADL: acid detergent lignin (%).
CEL/NDF: cellulose expressed over cellular wall (%).
* $P < 0.05$; *** $P < 0.001$.

the first variable considered ($R^2 = 0.83$; $P < 0.001$) followed by CEL expressed as a proportion of NDF ($R^2 = 0.92$; $P < 0.001$) (*table V*). Although ADL was the best single predictor, results suggest that the non-degradable N fraction is not only determined by the lignin content, but also by the carbohydrate composition of the cell wall. In forages an important part of their non-degradable fraction would be constituted by microorganisms, which are attached to the residue and are not washed out by the washing procedure [7, 26, 36]. Rodriguez [31], using ^{15}N as microbial marker and 16 samples of different feeds, reported that 43.7 % of the non-degradable protein fraction of an alfalfa hay was of microbial origin and that microbial contamination of this fraction of feedstuffs was mainly related positively to the cellulose content of samples, which is in agreement with the correlations obtained for the non-degradable protein fraction in our study.

Correlations between *ED* values and degradation kinetics parameters of hays showed that the lag time ($r = -0.64$; $P < 0.05$) and the proportion of soluble CP ($r = 0.69$; $P < 0.05$) are the main factors conditioning degradability. Effective degradability values were also negatively correlated to NDIN ($r = -0.88$; $P < 0.001$) and NDF content ($r = -0.71$; $P < 0.01$) (*table IV*). Nevertheless, in the stepwise procedure only NDIN was determined as the best independent variable, which enabled an acceptable prediction of CP degradability ($R^2 = 0.77$; $P < 0.001$) (*table V*). This relationship is supported in part by the positive effect of NDIN on lag time and therefore on the protein escape from the rumen by transit, which reduces protein degradation. Nevertheless, the relationship between *ED* and NDIN could also be based on other reasons. Repetto [29] observed that for a total of 21 samples of dehydrated alfalfa, none of which were presented with a lag time, NDIN was the best predictor of in situ CP degradability, mainly as a consequence of a negative correlation between NDIN and the fractional degradation rate. Janicki and Stallings [13] also observed a significant negative correlation ($r = -0.83$; $P < 0.001$) between NDIN and the in situ CP degradability of four lucerne and five cocksfoot hay samples. However, Hoffman et al. [11], working with legumes and grasses with different stages of maturity and oven-dried at 55 °C, observed that CP content was the best predictor of CP degradability ($r = 0.86$).

Results obtained from this study established that there are important differences in the degradation characteristics among alfalfa hays, which should have a practical relevance in the supply of degradable N to ruminal microorganisms and of rumen escape protein to the animal. These results confirm that a large proportion of the variation of CP degradability can be attributed to the NDIN variation. Therefore, the determination of this variable can be a simple and rapid method to characterize CP degradability of these hays.

REFERENCES

[1] AFRC, Agricultural and Food Research Council, Technical Committee on Responses to Nutrients. Report No. 9. Nutritive Requirements of Ruminant Animals: Protein, Nutr. Abstr. Rev. (*B*) 62 (1992) 787–835.

[2] Amrane R., Michalet-Doreau B., Effect of maturity stage or Italian rye grass and lucerne on ruminal nitrogen degradability, Ann. Zootech. 42 (1993) 31–37.

[3] AOAC, Official Methods of Analysis, 14th ed., Association of Official Analytical Chemists, Arlington, VA, 1984.

[4] ARC, The Nutrient Requirements of Ruminant Livestock, Suppl. 1, Commonwealth Agricultural Bureau, Slough, England, 1984.

[5] Aufrère J., Boulberhane D., Graviou D., Andrieu J.P., Demarquilly C., Characterisation of in situ degradation of lucerne proteins according to forage type (green forage, hay and silage) using gel electrophoresis, Anim. Feed Sci. Technol. 50 (1994) 75–85.

[6] Balde A.T., Vandersall J.H., Erdman R.A., Reeves III J.B., Glenn B.P., Effect of stage of maturity of alfalfa and orchardgrass on in situ dry matter and crude protein degradability and amino acid composition, Anim. Feed Sci. Technol. 44 (1993) 29–43.

[7] Bernard L., Marvalin O., Yang W., Poncet C., Colonisation bactérienne de differents types d'aliments incubés in sacco dans le rumen ; conséquences pour l'estimation de la dégradabilité de l'azote, Reprod. Nutr. Dev. 28 (1) (1988) 105–106.

[8] Christensen D.A., Fehr M.I., The dairy industry, in: Martin J., Hudson R.J., Young B.A. (Eds.), Animal Production in Canada, University of Alberta, Edmonton, Canada, 1993, pp. 75–91.

[9] Colucci P.E., MacLeod G.K., Grovum W.L., McMillan I., Barney D.J., Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes, J. Dairy Sci. 73 (1990) 2143–2156.

[10] Ehle F.R., Influence of feed particle density on particulate passage from rumen of Holstein cow, J. Dairy Sci. 67 (1984) 693–697.

[11] Hoffman P.C., Sievert S.J., Shaver R.D., Welch D.A., Combs D.K., In situ dry matter, protein, and fiber degradation of perennial forages, J. Dairy Sci. 76 (1993) 2632–2643.

[12] Hume I.D., Purser D.B., Ruminal and post-ruminal protein digestion in sheep fed on subterranean clover harvested at four stages of maturity, Aust. J. Agric. Res. 26 (1974) 199–208.

[13] Janicki F.J., Stallings C.C., Degradation of crude protein in forages determined by in vitro and in situ procedures, J. Dairy Sci. 71 (1988) 2440–2448.

[14] Kawas J.R., Jorgensen N.A., Lu C.D., Influence of alfalfa maturity on feed intake and site of nutrient digestion in sheep, J. Anim. Sci. 68 (1990) 4376–4386.

[15] Kennedy P.M., Christopherson R.J., Milligan L.P., Effects of cold exposure on feed protein degradations, microbial protein synthesis and transfer of plasma urea to the rumen of sheep, Br. J. Nutr. 47 (1982) 521–535.

[16] Kennedy P.M., Hazlewood G.P., Milligan L.P., A comparison of methods for the estimation of the proportion of microbial nitrogen in duodenal digesta, and of correction for microbial contamination in nylon bags incubated in the rumen of sheep, Br. J. Nutr. 52 (1984) 403–417.

[17] Lindsay J.R., Hogan J.P., Digestion of two legumes and rumen bacterial growth in defaunated sheep, Aust. J. Agric. Res. 23 (1972) 321–330.

[18] Llamas-Lamas G., Combs D.K., Effect of alfalfa maturity on fiber utilization by high producing dairy cows, J. Dairy Sci. 73 (1990) 1069–1080.

[19] McDonald I., A revised model for the estimation of protein degradability in the rumen, J. Agric. Sci. Camb. 96 (1981) 251–252.

[20] Madsen J., The basis for the proposed Nordic protein evaluation system for ruminants. The AAT-PPV system, Acta Agric. Scand. 25 (1985) 9–20.

[21] Madsen J., Hvelplund T., Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements, Acta Agric. Scand. 25 (Suppl.) (1985) 103–124.

[22] Makoni N.F., Shelford J.A., Fisher L.J., Initial rates of degradation of protein fractions from fresh, wilted and ensiled alfalfa. J. Dairy Sci. 77 (1994) 1598–1603.

[23] Mathers J.C., Aitchison E.M., Direct estimation of the extent of contamination of food residues by microbial matter after incubation within synthetic fibre bags in the rumen, J. Agric. Sci. Camb. 96 (1981) 691–693.

[24] Mathers J.C., Miller E.L., Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped lucerne and rolled barley, Br. J. Nutr. 45 (1981) 587–604.

[25] Michalet-Doreau B., Ould-Bah M.Y., Estimation of the extent of bacterial contamination in bag residues and its influence on in sacco measurements of forage nitrogen degradation in rumen, in: Proc. 16th Int. Grassl. Congress, Nice, France, 1989, pp. 909–910.

[26] Nocek J.E., Grant A.L., Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages, J. Anim. Sci. 64 (1987) 552–564.

[27] NRC, Nutrient Requirements of Dairy Cattle, 7th rev. ed., National Academy Press, Washington, D.C., 1989.

[28] Ørskov E.R., McDonald I., The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage, J. Agric. Sci. Camb. 92 (1979) 499–503.

[29] Repetto J.L., Degradabilidad ruminal de la alfalfa deshidratada, PhD thesis, Universitat de Lleida, Spain, 1997.

[30] Robertson J.B., Van Soest P.J., The detergent system of analysis and its application to human foods, in: James W.P.T., Theander O. (Eds.), The Analysis of Dietary Fiber in Foods, Marcel Dekker, New York, 1981, pp. 123–158.

[31] Rodriguez C.A., Estudio de la colonizacion microbiana de los alimentos en el rumen. Implicaciones sobre la estimacion de la degradabilidad ruminal de las materias nitrogenadas mediante tecnicas in situ, PhD thesis, Universidad Politecnica de Madrid, Spain, 1996.

[32] Sauvant D., Grenet E., Doreau M., Dégradation chimique des aliments dans le réticulo-rumen: cinétique et importance, in: Jarrige R., Ruckebusch Y., Demarquilly C., Farce M.H., Journet M. (éd.), Nutrition des ruminants domestiques. Ingestion et digestion, Inra, Paris, 1995, pp. 383–406.

[33] Susmel P., Stefanon B., Mills C.R., Spanghero M., Rumen degradability of organic matter, nitrogen and fibre fractions in forages, Anim. Prod. 51 (1990) 515–526.

[34] Udén P., Colucci P.E., Van Soest P.J., Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies, J. Sci. Food Agric. 31 (1980) 625–632.

[35] Van Soest P.J., Nutritional Ecology of the Ruminant, Cornell University Press, Cornell, New York, 1994.

[36] Varvikko T., Lindberg J.E., Estimation of microbial nitrogen in nylon bag residues by feed ^{15}N dilution, Br. J. Nutr. 54 (1985) 473–481.

[37] Vérité R., Michalet-Doreau B., Chapoutot P., Peyraud J.L., Poncet C., Révision du système des protéines digestibles dans l'intestin (PDI), Bull. Tech. C.R.Z.V. Theix Inra 70 (1987) 19–34.

[38] Von Keyserlingk M.A.G., Swift M.L., Puchala R., Shelford J.A., Degradability characteristics of dry matter and crude protein of forages in ruminants, Anim. Feed Sci. Technol. 57 (1996) 291–311.

[39] Walker D.J., Egan A.R., Nader C.J., Ulyatt M.J, Storer G.B., Rumen microbial protein synthesis and proportions of microbial and non-microbial nitrogen flowing to the intestines of sheep, Aust. J. Agric. Res. 2 (1975) 699–708.

[40] Williams C.H., David D.J., Iismaa O., The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry, J. Agric. Sci. Camb. 59 (1962) 381–385.