Anim. Res. 52 (2003) 27–36

DOI: 10.1051/animres:2003007

Original article

Relationship between ruminal degradability and chemical composition of dehydrated lucerne

José Luis Repetto*, Javier González**, Cecilia Cajarville*, María Remedios Alvir, Carlos Alberto Rodríguez

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

(Received 22 April 2002; accepted 20 January 2003)

Abstract — The rumen degradation characteristics and effective degradability (ED) of dry matter (DM) and crude protein (CP) of 21 samples of dehydrated lucerne from 5 different processing plants were studied in three rumen fistulated wethers using the nylon bag technique. The animals were fed at an intake level of 40 g DM·kg^{-1} BW$^{0.75}$ with a mixed diet of lucerne (40% dehydrated and 60% hay) and concentrate (2:1 on DM). The mean values of the ED of DM and CP, calculated for rumen outflow rates determined in each animal (2.28%·h^{-1}, as average), presented a low variation (mean = 61.2%; CV = 5.31% for DM and mean = 73.5%; CV = 4.95% for CP). Degradation of DM was directly related to lucerne quality, with negative and positive correlations with the contents of fibre and CP, respectively. The best prediction of the ED of DM was derived from the contents of acid detergent fibre, which explained 73.3% of the total variation. The best prediction of the ED of CP (R^2 = 0.592) was related negatively to the proportion of insoluble nitrogen in neutral detergent fibre and positively to the CP concentration as the first and second predictive variables. However, the first variable allowed a good estimation of the ED of CP for the dehydrated lucerne samples presented in a long form (R^2 = 0.818). Degradation studies of DM and CP also indicated that most of the N available to animals was derived from rumen microbial synthesis.

dehydrated lucerne / rumen degradability / protein / chemical composition / sheep

Résumé — **Relations entre la dégradabilité ruminale et la composition chimique de la luzerne déshydratée.** Les caractéristiques de dégradation et la dégradabilité théorique (DT) dans le rumen de la matière sèche (MS) et des matières azotées totales (MAT) de 21 échantillons de luzerne déshydratée ont été mesurées par la technique des sachets de nylon sur trois moutons munis d'une canule du rumen. Les animaux ont été nourris avec une ration composée de luzerne (40 % déshydratée et 60 %

* Present address: Departamento de Nutrición Animal, Facultad de Veterinaria, Universidad de la República Oriental del Uruguay, 11600 Montevideo, Uruguay
** Correspondence and reprints
Tel.: (34)915493069; fax: (34)915499763; e-mail: jgonzalez@pan.etsia.upm.es

foin) et d'aliment concentré dans des proportions 2:1 (sur MS) à un niveau de 40 g MS·kg^{-1} P^{0,75}. Les valeurs de DT, calculées avec les taux de sortie des particules du rumen mesurés pour chaque animal (moyenne = 2,28 %·h^{-1}), ont été peu variables tant pour la MS (moyenne = 61,2 % ; CV = 5,31 %) que pour les MAT (moyenne = 73,5 % ; CV = 4,95 %). La DT de la MS a été directement liée à la qualité de la luzerne, avec des corrélations négatives et positives pour les teneurs en fibre et MAT, respectivement. La meilleure prédiction de la DT de la MS a été obtenue avec la teneur en lignocellulose (ADF), qui explique 73,3 % de la variation totale. Pour les MAT, la meilleure prédiction de la DT (R^2 = 0,592) a été négativement corrélée à la proportion d'azote insoluble dans la solution au détergent neutre et positivement à la teneur en MAT, respectivement première et seconde variables indépendantes. La première variable a permis une prédiction convenable de la DT des MAT pour la luzerne déshydratée présentée sous forme de brins longs (R^2 = 0.818). Les études de dégradation de la MS et des MAT ont aussi montré que la plus grande partie de l'azote disponible pour l'animal est fournie par la synthèse microbienne dans le rumen.

luzerne déshydratée / dégradabilité ruminale / protéine / composition chimique / ovins

1. INTRODUCTION

Reliable estimates of rumen degradability of feed protein are essential for the sensible application of new systems for assessing protein nutrition in ruminants. However, these systems use fixed values of crude protein (CP) degradability for each feed type and do not integrate the possible intratype variability existing for this trait. Consequently, studies focused at predicting degradability or determining the factors affecting this variability are of interest, specially for the most commonly used feeds. Lucerne is characterised by a high content of protein with an extensive rumen degradation, which may reduce its protein value as a consequence of important nitrogen losses by ammonia absorption from the rumen [3]. Therefore, the knowledge of the possible factors conditioning rumen degradability are useful to improve rationing and, consequently, the nitrogen utilisation of this feed. Previous results on the effective degradability (ED) of CP of dehydrated lucerne show a great variability. Mean values obtained by the NRC [11], Verité et al. [19], Kowalski et al. [9], Faria-Marmol et al. [4] and Repetto et al. [13] were respectively: 41, 60, 64, 71 and 77.5%. Rumen degradability of dehydrated lucerne should be affected by multiple factors related to the crop, the post-harvest forage management and the industrial process of dehydration. The purposes of this research were: (i) to obtain information on the variation of rumen degradability of dehydrated lucerne and (ii) to study the effect of chemical composition and CP solubility of dehydrated lucerne on rumen degradation.

2. MATERIALS AND METHODS

2.1. Experimental procedures

A total of 21 samples of dehydrated lucerne presented in a long form (17) or pelleted (4) were studied. These samples were obtained during the same harvest season from 5 plants (A, B, C, D and E) located in the Lleida province (north-east Spain). The plants differed in technology and management of the post-harvest and industrial processes, except for the harvest stage, which was always at the beginning of flowering (about 5% of the plants). Plant A processed the lucerne directly, whereas the other plants made a field wilting period of different duration (between 12 and 72 h). All plants employed rotational driers, with one pass (plants B, D and E) or three passes (plants A and C). The mean technical specifications of the dehydration process and the initial and final DM content of the

Table I. Mean characteristics of the lucerne dehydration process in different plants.

	Dehydration plants				
Items	A	B	C	D	E
Mean dehydration time (min)	9	4	3	3/4	6
Drier outflow temperature[1] (ºC)	116.6 (5.72)	105.6 (8.70)	85.8 (4.98)	116 (9.00)	82.7 (7.67)
Initial DM content of lucerne[1] (%)	24.3 (7.30)	61.2 (10.8)	52.8 (8.28)	57.2 (4.16)	62.7 (10.9)
Final DM content of lucerne[1] (%)	83.9 (3.34)	94.3 (2.08)	85.6 (3.01)	86.2 (5.07)	96.0 (1.89)

[1] Determined on a total of 48 lucerne samples.
Values in bracket are standard deviations.

vegetable material are exposed in Table I. These latter values were determined on a total of 48 samples employed to select the 17 non conditioned samples employed in this study. All granulated samples were obtained from plant E. All materials were ground to pass a 2 mm screen for degradability trials and 1 mm screen for chemical analysis and buffer CP solubility measures.

All forages were incubated by the in situ method into the rumen of three rumen cannulated wethers of the Manchega breed, which were fed with a 2/3 chopped (6–8 cm) forage (40% dehydrated lucerne and 60% lucerne hay) and 1/3 concentrate diet, distributed at an intake level of 40 g DM·kg$^{-0.75}$ in two equal-weight meals at 9:00 and 17:00 h. The NDF and CP contents of this ration were 435 and 161 g·kg^{-1} (on DM), respectively.

The samples of dehydrated lucerne were incubated in nylon blutex bags (46 μm pore size; reference: 120 T, Tissages Tissues Techniques, France) of 11 × 7 cm (internal dimensions), heat-sealed and filled with approximately 3 g (air dry basis) of feed samples. The bags were incubated in the rumen for each wether at times of 3, 6, 12, 24, 48 and 72 h. Two series of incubation were conducted for each feed in two successive periods, in order to have two bags per animal and incubation time. Within each series, the rings of 12 bags were sequentially incubated. Each ring encompassed a randomly associated pair of dehydrated lucerne samples. All bags of each ring were inserted at the morning feeding time. After incubation, the bags were removed, washed with tap water, and stored frozen. After being defrosted, the bags were washed 3 times for 5 min in a turbine washing machine, dried in an air forced oven for 48 h at 80 ºC and weighed for DM determination. The residues were homogenised and analysed for N. Three additional bags of each sample were reserved for the zero incubation, which involved the washing procedure without prior rumen incubation. The specifications of the bags, their incubation and the post-incubation treatment of the bags were in agreement with the method proposed by Michalet-Doreau et al. [10].

The degradation characteristics of DM and CP were described using the model of Ørskov and McDonald [12]. To determine the ED of all samples, between both incubation series of the studied sample, the rate constant describing the passage from the rumen of dietary particles (k_p) was determined for the dehydrated lucerne included in the diet, which had been chopped at 6–8 cm and marked with Ytterbium (Yb) by immersion [6], at 10 mg Yb·g^{-1} of feed. The

procedure of this determination and the resultant k_p values ($2.28 \pm 0.22\% \cdot h^{-1}$) has been previously published [13].

2.2. Analytical

The samples of dehydrated lucerne were analysed for dry matter (DM), organic matter (OM), crude protein (CP) [2], neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) [14]. Fibre fractions were calculated ash free. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were performed by Kjeldahl analysis on the NDF and ADF residues, respectively, and the contents are expressed as percentage of total nitrogen. The solubility of CP was determined in Mc Dougall buffer for 6 h according to the procedure described by Alvir et al. [1].

2.3. Statistical analysis

A least-square non-linear iterative process was used to fit the kinetics of DM and CP disappearance by the NLIN procedure of the Statistical Analysis System for Windows software, version 6.12 [18]. The same programme was employed to perform correlation analyses between ruminal degradation characteristics and chemical composition of the samples. Then, multivariate regression equations for ED were obtained by using the stepwise procedure.

3. RESULTS

The chemical composition of dehydrated lucerne samples (Tab. II) showed a moderate variability, except for the proportion of NDIN (CV = 26.3%), which varied from 12.3 to 32.9% of total N. The solubility of CP in buffer also showed an important variability (from 20.2 to 44.6%). This last value was related to the proportions of ADIN ($r = -0.577$; $P < 0.01$) and NDIN ($r = -0.550$; $P < 0.01$).

Ruminal degradation characteristics of DM and CP for dehydrated lucerne samples are shown in Table III. The disappearance data of DM and CP fitted well with the model used, and did not show evidence of lag time for any sample. Both in DM and especially in CP results, the fractional

Table II. Mean values ($g \cdot kg^{-1}$ DM) and variability of chemical composition and crude protein solubility of dehydrated lucerne samples.

Items	Mean	C.V. (%)	Median	Range
Ash	100	8.38	99.0	88.0–124
Crude protein (CP)	170	8.16	169	147–203
Neutral detergent fibre (NDF)	465	6.81	465	401–515
Acid detergent fibre (ADF)	338	9.02	341	277–380
Acid detergent lignin (ADL)	80.7	8.32	80.2	67.7–91.7
NDIN[1]	20.0	26.3	19.3	12.3–32.9
ADIN[1]	8.61	9.68	8.48	7.3–10.5
CP solubility[1]	33.1	16.4	33.7	20.2–44.6

[1] % on total N or CP.
C.V.: coefficient of variation; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen.

Table III. Mean values and variability of in situ degradation characteristics of dry matter and crude protein in dehydrated lucerne samples.

	Dry matter				Crude protein			
Item	Mean	C.V. (%)	Median	Range	Mean	C.V. (%)	Median	Range
a (%)	29.6	13.3	28.6	25.7–39.6	37.7	14.0	38.0	25.7–47.0
b (%)	40.8	5.66	40.8	36.5–45.4	47.4	11.2	47.1	37.6–58.6
u (%)	29.6	15.4	30.3	18.8–35.9	14.9	15.0	15.4	9.9–19.0
k_d (%·h^{-1})	8.42	15.9	8.30	6.0–10.3	8.06	35.7	7.85	3.4–14.3
ED (%)	61.2	5.31	60.1	56.8–69.2	73.5	4.95	73.6	66.5–78.0

a, *b*, and *u* represent soluble, non-soluble degradable, and undegradable fractions, respectively; k_d: fractional degradation rate of fraction *b*; ED: effective degradability; C.V.: coefficient of variation.

degradation rate (k_d) showed higher variations than the different feed fractions. The lowest relative dispersion was observed for the ruminal ED of both DM and CP, which represented mean values of 61.2 and 73.5%, respectively. The ED of DM was closely related with their *a* (r = 0.856; $P < 0.001$) and *u* (r = –0.920; $P < 0.001$) fractions, whereas for CP, the ED was related with both *a* and *b* fractions (r = 0.607 and –0.534, respectively; $P < 0.01$) and more closely with k_d (r = 0.704; $P < 0.001$).

Correlation coefficients between the degradation characteristics of DM and CP and chemical composition including hemicellulose and cellulose contents (estimated respectively as NDF-ADF and ADF-ADL) are presented in Tables IV and V, respectively.

For DM, the degradation characteristics were mainly correlated with the contents of fibre (ADF or NDF), but a high positive correlation was also observed with the lucerne CP concentration. The content of

Table IV. Correlation coefficients between chemical composition and in situ degradation characteristics of dry matter of dehydrated lucerne.

	a	*b*	*u*	k_d	ED
CP	0.448*	0.689***	–0.736***	–0.243	0.705***
NDF	–0.712***	–0.109	0.673**	0.122	–0.755***
ADF	–0.735***	–0.490*	0.884***	0.423	–0.856***
ADL	0.024	–0.701***	0.334	0.294	–0.195
HCEL	0.007	0.577**	–0.297	–0.455*	0.126
CEL	–0.766***	–0.348	0.839***	0.371	–0.842***
NDIN	0.169	0.657**	–0.478*	–0.505*	0.329
ADIN	–0.238	0.188	0.116	0.089	–0.121

HCEL: hemicellulose; CEL: cellulose; for other abbreviations see Tables II and III.
*, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table V. Correlation coefficients between chemical composition, solubility of crude protein and in situ degradation characteristics of crude protein of dehydrated lucerne.

	a	*b*	*u*	k_d	ED
CP	0.150	0.036	–0.439*	–0.390	0.050
NDF	0.028	–0.326	0.712***	0.149	–0.203
ADF	–0.037	–0.257	0.699***	0.504*	0.087
ADL	–0.322	0.240	0.187	0.151	–0.166
HCEL	0.100	–0.118	0.047	–0.536*	–0.450*
CEL	0.035	–0.320	0.681***	0.488*	0.128
NDIN	–0.387	0.463*	–0.192	–0.692***	–0.665**
ADIN	–0.409	0.282	0.293	–0.267	–0.525*
CP solubility	0.838***	–0.821***	–0.017	0.257	0.626**

HCEL: hemicellulose; CEL: cellulose; for other abbreviations see Tables II and III.

ADF showed the closest correlation with the ED of DM and the different fractions, except *b*, which was mainly correlated with ADL (r = –0.701; $P < 0.001$). However, the effects of ADF should be mainly due to the cellulose content, since this carbohydrate showed a similar correlation coefficient with the ED of DM than ADF. The degradation rate of DM was related to the NDIN proportion (r = –0.505; $P < 0.05$) and, at a lower level, to the hemicellulose content ($P = –0.455$; $P < 0.05$). The best prediction of ED of DM (DMED) was derived from the ADF content (Fig. 1). The range of application of this equation was only from 275 to 380 g of ADF·kg^{-1} DM.

The CP solubility was related to the *a* and *b* fractions of CP (r = 0.838 and r = –0.821, respectively; $P < 0.001$) and, as a consequence, to the ED of CP (r = 0.626; $P < 0.01$). However, the most close correlation for ED was observed with NDIN (r = –0.665; $P < 0.01$), which also displayed the highest correlation with k_d (r = –0.692; $P < 0.001$). This last trait was also affected ($P < 0.05$) by the content of both hemicellulose (r = –0.536) and cellulose (r = 0.488). Conversely, only this last type of carbohydrate

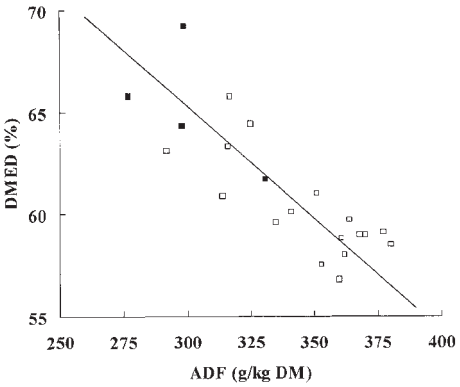

Figure 1. Relationship between the acid detergent fibre concentration (ADF) and the effective degradability of dry matter (DMED) of long (□) and granulated (■) dehydrated lucerne. The regression equation was: DMED (%) = 98.3 – 0.11 FAD (g·kg^{-1} DM); n = 21; RSD = 2.07; $R^2 = 0.733$; $P < 0.001$.

was related to the undegradable fraction (r = 0.681; $P < 0.001$). The best prediction of ED of CP (CPED) included NDIN and CP as the first and second predictive variables (Fig. 2), although its determination coefficient only reached a moderate level ($R^2 = 0.592$), mainly as a consequence of

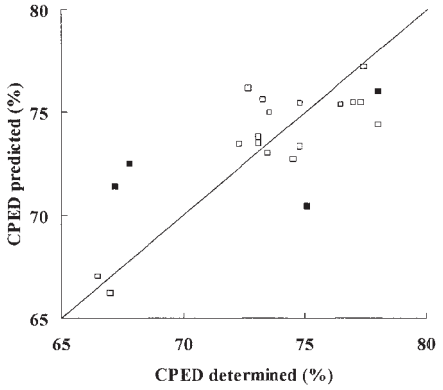

Figure 2. Relationship between determined and predicted values of effective degradability of crude protein (CPED) of long (□) and granulated (■) dehydrated lucerne samples. The predictive equation was: CPED (%) = 66.1 – 0.60 NDIN (% of N_t) + 0.114 CP (g·kg^{-1} DM); RSD = 2.46; $R^2 = 0.592$; $P < 0.001$.

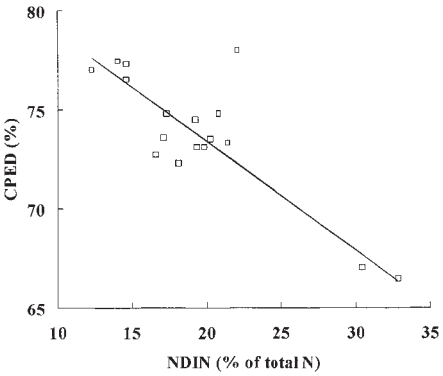

Figure 3. Relationship between the proportion of neutral detergent insoluble nitrogen (NDIN) and the effective degradability of crude protein (CPED) of long dehydrated lucerne. The regression equation was: CPED (%) = 84.3 – 0.55 NDIN (% of N_t); n = 17; RSD = 1.42; $R^2 = 0.818$; $P < 0.001$.

the important deviations observed for the granulated samples. When these samples were excluded from the regression, CPED was only dependent of NDIN (Fig. 3) and a higher accuracy was observed ($R^2 = 0.814$). The range of application of NDIN proportions in both equations was large: from 14 to 33% of total N.

4. DISCUSSION

The moderate variability observed in the study sample for most tested chemical parameters should be, in part, a consequence of the similar harvesting stage used for the different samples. Dehydration of lucerne leads to an increase in the proportions of ADIN and specially NDIN and to a decrease in CP solubility [13]. Therefore, the high variability observed for the proportion of NDIN (CV = 26.3%) or the values of CP solubility (CV = 16.4%) will be related with the intensity of heating effects derived from the forage management and industrial treatments employed for the different plants. On the contrary, ADIN proportions, which were slightly higher than the usual values of fresh lucerne [5, 7, 17] and its moderate variation (CV = 9.68%) showed a limited and relatively uniform effect of dehydration on this trait. The proportion of ADIN has been used to assess overheating [9]. Therefore, heat damage was not suspected in the present samples.

Considered globally, the correlation coefficients recorded between degradative characteristics of DM and the contents of CP or fibre related parameters (Tab. IV) showed that the ruminal availability of dehydrated lucerne is logically dependent on its quality. In this way, the CP content can be used as an index of its level of rumen fermentation, but a better evaluation can be derived from the ADF contents. On the contrary to what was expected, there were no relations between the lignin content and the undegradable fraction or the ED of DM. Jung and Allen [8] indicated that the relations between lignin and degradability or digestibility are consistent when forages with different maturity stages or from

different species are considered. On the contrary, these relations are usually not found for forages of the same species with a similar maturity stage.

Faría-Mármol et al. [4] indicated that the differences in protein value due to lucerne quality are mainly derived from its potential to synthesise microbial protein in the rumen, as a consequence of the high CP degradability of lucerne and of the moderate intestinal digestibility of the undegraded protein (about 50–55% for dried lucerne). The interest in using dehydrated lucerne of a high quality in high productive ruminants is not only derived from its higher level of rumen fermentation, but also from its lower effect on rumen fill, which leads to a higher intake and, therefore, to a higher total microbial protein synthesis.

The values of ED of CP were higher than those reported by the NRC [11], Vérité et al. [19] and Kowalski et al. [9] (41, 60, and 64%, respectively). These disagreements can be associated with factors related to sample characteristics and also to differences in methodology. Thus, most of the values cited above were calculated for k_p values higher than those used in our work. On the contrary, our values agreed with those of Faría-Mármol et al. [4], estimated with determined k_p values similar to those observed in our work. The outflow rate of particles from the rumen is conditioned mainly by the rumen fill, which is basically associated with fibrous particles. When good quality lucerne is the only forage of a diet consumed at a restricted level, as in this work or that of Faria-Marmol et al. [4], this fill will be low. Therefore, rumen pressure, rumination activity, rumen motility (contractile and propulsive movements) and consequently the evacuation through the reticulo-omasal orifice will also be low. As a consequence of the low k_p values, our results of ED are logically higher than those of other works or those recorded in tables of nutritive value, which have been calculated using a fixed and higher k_p value. On average, 73.5% of total CP in dehydrated lucerne was apparently degraded in the rumen. Nevertheless, when these values were corrected for microbial contamination using the equation proposed by Rodriguez et al. [15] (which was obtained using the same methodology and with a diet with the same intake level and forage to concentrate ratio), the results showed that 79.1% of the CP of these forages is diverted to microbial ruminal fermentation. The underestimation of ED, produced by the microbial contamination, varied from 5.7 to 8.4% and averaged 7.1%. The high values of corrected ED were associated with low proportions of undegradable CP. So, based on the prediction of Rodriguez et al. [16], 51.4% on average of fraction *u* was of microbial origin. Consequently, the corrected *u* values represented only 7.2% of total CP on average. These authors showed that the microbial CP in the undegradable CP fraction is related positively with the cellulose content and negatively with the N concentration of feed, which agrees with the correlation coefficients observed between fraction *u* and the chemical composition of the samples. The lack of a relation between ADIN and this fraction reinforced the previous indication on the lack of heat damage.

The fractional degradation rate of CP was negatively related with the values of NDIN and hemicellulose. This same behaviour was observed for DM degradation, since a close correlation was recorded between both k_d values ($r = 0.813$; $P < 0.001$). The degradation rate of insoluble CP in dehydrated lucerne is, therefore, associated in great part to the progression of degradation in the feed, which seems to be diminished by the protein-hemicellulose complexes.

Solubility in buffer of CP was not only positively related to the soluble CP (*a*), but also negatively to the potentially degradable CP (*b*), since there was a close correlation between both fractions ($r = 0.911$; $P < 0.001$) as a consequence of the moderate value and variation of the undegradable fraction.

Nevertheless, the correlation of this trait with the ED of CP was insufficient to be considered as a valuable index. Similarly, the accuracy of the prediction of the ED of CP based on NDIN and CP only reached an intermediate level, mainly as a consequence of the deviation of granulated lucerne samples, which could have been mixed with other raw materials (straws, urea, ...) in the industrial process. However, NDIN allowed an accurate prediction of CPED of dehydrated lucerne presented in the long form.

Using fresh and dried lucerne samples, Faría-Mármol et al. [4] observed a close and negative relationship between ED of CP and the fraction of CP digested in the intestine, which is a consequence of the change of the digestion site. Since the ED of DM and CP were not related to the same parameters, it can be stated that the reduction of ED of CP always enhances the feed protein value, as a consequence of a reduction of N losses [3] and an increase of the CP digested in the intestine [4].

Therefore, the chemical ideotype derived from the present results, for a high protein value in dehydrated lucerne combines low contents of fibre (especially lignocellulose) and high proportions of NDIN.

ACKNOWLEDGEMENTS

Financial support was provided by C.I.C.Y.T. Project AGF 93-0549-CO2.

REFERENCES

[1] Alvir M.R., González J., Caja G., Gálvez J.F., Relación entre la solubilidad y la degradación ruminal de la proteína en alimentos concentrados, Invest. Agr.: Prod. Sanid. Anim. 2 (1987) 43–52.

[2] AOAC, Official Methods of Analysis (15th ed.), Association of Official Analytical Chemists, Arlington, VA, 1990.

[3] Broderick G.A., Alfalfa silage or hay versus corn silage as the sole forage for lactating dairy cows, J. Dairy Sci. 68 (1985) 3262–3271.

[4] Faría-Mármol J., González J., Rodríguez C.A., Alvir M.R., Effect of diet forage to concentrate ratio on rumen degradability and postruminal availability of protein from fresh and dried lucerne, Anim. Sci. 74 (2002) 337–345.

[5] Goering H.K., A laboratory assessment on the frequency of overheating in commercial dehydrated alfalfa samples, J. Anim. Sci. 43 (1976) 869–872.

[6] González J., Rodríguez C.A., Andrés S.G., Alvir M.R., Rumen degradability and microbial contamination of fish meal and meat meal measured by the in situ technique, Anim. Feed Sci. Technol. 73 (1998) 71–84.

[7] González J., Faría-Mármol J., Rodríguez C.A., Alvir M.R., Effects of stage of harvest on the protein value of fresh lucerne for ruminants, Reprod. Nutr. Dev. 41 (2001) 381–392.

[8] Jung H.G., Alleng M.S., Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants, J. Anim. Sci. 73 (1995) 2774–2790.

[9] Kowalski Z.M., Pisulewski P.M.P., Peyraud J.L., Kaminski J., The effect of drier outflow temperature on rumen protein degradability and intestinal digestibility of rumen-undegraded protein of dehydrated grass and lucerne, Ann. Zootech. 44 (Suppl.) (1995) 88.

[10] Michalet-Doreau B., Vérité R., Chapoutot P., Méthodologie de mesure de la dégradabilité in sacco de l'azote des aliments dans le rumen, Bull. Tech. CRZV Theix 69 (1987) 5–7.

[11] NRC, Nutrients Requirements of Dairy Cattle (6th ed.), National Academy Press, Washington, DC, 1989.

[12] 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.

[13] Repetto J.L., González J., Cajarville C., Effect of dehydration on ruminal degradability of lucerne, Ann. Zootech. 49 (2000) 113–118.

[14] 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 Fibre in Food, Marcel Dekker, New York, 1981, pp. 123–158.

[15] Rodríguez C.A., González J., Alvir M.R., Cajarville C., Underestimation of in situ effective degradability of N due to microbial contamination, in: Lobley G.E., White A., MacRae C. (Eds.), Book of abstracts of the VIIIth International Symposium on Protein Metabolism and Nutrition, Aberdeen, United Kingdom, 1999, p. 67.

[16] Rodríguez C.A., González J., Alvir M.R., Repetto J.L., Microbial nitrogen contamination of in sacco ruminal incubated feeds, in: Lobley G.E., White A., MacRae C. (Eds.), Book of abstracts of the VIIIth International Symposium on Protein Metabolism and Nutrition, Aberdeen, United Kingdom, 1999, p. 68.

[17] Sanderson M.A., Weding W.F., Nitrogen in the detergent fibre fractions of temperate legumes and grasses, Grass Forage Sci. 44 (1989) 159–168.

[18] SAS, SAS/STAT User's Guide (version 6, 4th ed.), Statistical Analysis System Institute Inc., Cary, NC, 1990.

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