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Effects of stage of harvest on the protein value of fresh lucerne for ruminants

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Abstract — The ruminal degradation of dry matter (DM) and crude protein (CP) and the intestinal availability of CP of four fresh lucerne (*Medicago sativa* L.) samples, corresponding to a 3rd growing cycle and harvested at 2-week intervals, were determined. Rumen degradability, measured by the nylon bag technique, and rumen outflow rates were determined on three rumen-cannulated wethers. Intestinal digestibility was determined by the mobile bag technique on three duodenal fistulated wethers. Both groups of animals were fed a 2:1 lucerne hay to concentrate diet at an intake level of 40 g DM·kg^{-1} BW$^{0.75}$. The effective degradability (*ED*) of DM decreased with maturity in linear and quadratic form, as a consequence of a decrease in the soluble fraction and a similar increase in the undegradable materials. The resultant values were 0.795, 0.661, 0.600, and 0.576 for harvests at 2, 4, 6, and 8 weeks. The *ED* of CP showed the same trend. However, the variations (values of 0.896, 0.832, 0.791, and 0.817, respectively), were moderate and mainly due to the reduction of the proportion of soluble CP. The intestinal digestibility of CP of all samples showed a downward trend with the increase in the ruminal incubation time, as modelled according to a logistic function. The undegraded CP digested in the gut (D_i) and therefore the effective intestinal digestibility (*EID*) were derived from this function according to the rumen outflow of undegraded CP. The effects of maturity on the mean values of D_i, expressed as a proportion of the original CP content, were the opposite of those recorded for the *ED* of CP. These values were 0.067, 0.102, 0.115, and 0.089 for samples harvested at 2, 4, 6, and 8 weeks, respectively. Nevertheless, when D_i was expressed as g CP·kg^{-1} DM, these values (18.0, 17.4, 17.1, and 14.3, respectively) decreased in linear form. The same trend was observed for *EID* values, which represent 0.641, 0.609, 0.549, and 0.488, respectively. The change of the digestion site produced by the reduction of *ED* of CP was also associated with an increase in the undigested CP (values of 0.037, 0.066, 0.094, and 0.094, at the four harvesting times).

lucerne / stage of maturity / rumen degradability / intestinal digestibility / protein

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Résumé — Effets du stade de maturité sur la valeur protéique du fourrage vert de luzerne. La dégradation dans le rumen de la matière sèche (MS) et des matières azotées totales (MAT), ainsi que l'utilisation dans l'intestin des MAT de 4 fourrages de luzerne (*Medicago sativa* L.) recoltés en vert au 3e cycle à 2 semaines d'intervalle, ont été determinées. La dégradabilité effective (*DE*), mesurée en sachets de nylon, et le taux de sortie de particules du rumen ont été determinés sur 3 moutons fistulés dans le rumen, tandis que la digestibilité dans l'intestin a été mesurée, à l'aide des sachets mobiles, sur 3 moutons munis d'une canule du duodénum. Les deux groupes d'animaux ont été nourris avec une ration mixte de foin de luzerne et concentré (2:1 sur MS) distribuée à 40 g MS·kg^{-1} P^{0,75}. La *DE* de la MS a diminué de façon linéaire et quadratique avec l'âge du fourrage, par suite d'une diminution de la fraction soluble et d'une augmentation similaire de la fraction non dégradable. Les valeurs de *DE* ont été : 0,795, 0,661, 0,600 et 0,576 pour la fauche à 2, 4, 6 et 8 semaines. Une évolution similaire, mais plus modérée, a été observée pour la *DE* des MAT (valeurs de 0,896 ; 0,832 ; 0,791 et 0,817, respectivement). Ceci est principalement la conséquence de la réduction des proportions des MAT solubles. L'augmentation du temps de séjour dans le rumen a entraîné pour tous les échantillons une diminution de la digestibilité dans l'intestin, qui a pu être convenablement décrite par une fonction logistique. La proportion des MAT de l'aliment digérées dans l'intestin (D_i) et la digestibilité intestinale effective (*DIE*) résultante ont été déduites de cette fonction et du flux de sortie du rumen des MAT non dégradées. L'évolution en fonction de l'âge, des valeurs de D_i– exprimées comme proportion des MAT – a montré des effets inverses à ceux observés pour la *DE* des MAT. Ainsi, ces valeurs ont été : 0,067, 0,102, 0,115 et 0,089 pour les fourrages recoltés à 2, 4, 6 et 8 semaines, respectivement. Par contre, si D_i est exprimé en g MAT·kg^{-1} MS, les teneurs respectives (18,0, 17,4, 17,1 et 14,3) ont montré une diminution linéaire. Une évolution similaire a été observée pour les valeurs de *DIE*: 0,641, 0,609, 0,549 et 0,488, respectivement. Le déplacement du lieu de digestion, associé avec la réduction de la *DE* des MAT, donne lieu aussi à une augmentation des MAT non digérées. Ainsi, ces valeurs représentent 0,037, 0,066, 0,094 et 0,094, respectivement.

luzerne / stade de maturité / dégradabilité ruminale / digestibilité intestinale / protéine

1. INTRODUCTION

The sensible application of the new systems for assessing protein nutrition in ruminants needs suitable information about the rumen effective degradability and intestinal digestibility of the undegraded protein of feeds, as well as of the synthesis of microbial proteins promoted in the rumen. However, the variability existing for some of these parameters within a given feed type is not usually considered in these systems. Lucerne (*Medicago sativa* L.) is a major forage for feeding productive ruminants in temperate countries. Nevertheless, its extensive rumen degradation may reduce its protein value as a consequence of important nitrogen losses associated with the ammonia absorption from the rumen [9]. On the contrary, the stage of maturity is a main factor related to the protein value of forages through its influence on microbial synthesis and the digestion site (rumen vs. small intestine) of the feed nitrogenous compounds.

The mobile nylon bag technique, based on the incubation of preincubated-rumen samples in bags inserted in the intestines through a duodenal cannula, is a simple and adaptable method that estimates the intestinal digestibility of rumen undegraded protein in feedstuffs. In vivo essays cannot carry out these systematic estimations, since it is an indirect, complex, laborious and time-consuming process. On the contrary, the mobile bag technique can be used in systematic studies with a large number of feeds. This technique can be used even if the intestinal digestibility values change with rumen preincubation time as occurs in many types of feeds, because the effective digestibility can be estimated based on solutions by integration of the functions which

describe this behaviour and the outflow from the rumen of undegraded protein [15]. The simplicity of this technique can be enhanced, in addition, by suppressing the incubation in the abomasum (or the pepsin–HCl preincubation), since different works have shown that the lack of this step does not affect the values of intestinal protein digestion [7, 30, 34]. Also, the recovery of bags directly from the faeces instead of the ileum does not represent an important source of error [18, 34].

The objective of this research was to evaluate the effect of maturity on the ruminal degradation and the intestinal digestibility of fresh lucerne.

2. MATERIALS AND METHODS

2.1. Lucerne samples

Four samples of fresh lucerne corresponding to a 3rd growing cycle were harvested in a field located near Madrid (Spain) at intervals of two weeks from the previous cut (June 2 of 1998). The lucerne crop was in its fourth growing season and was managed under irrigated on-farming conditions. The samples of 2, 4, 6 or 8 weeks were collected at vegetative, bud, full flowering, and early pod setting stages, respectively. However, the sample harvested at 8 weeks partially included vegetative biomass from basal bud regrowth. These forage samples were frozen (–20 °C), freeze-dried and grounded and passed through a 2-mm screen for nylon bag assays and passed through a 1-mm screen for chemical composition analyses (Tab. I).

2.2. Animals and feeding

Two groups of three wethers fitted with rumen cannulas (group 1) or "T" duodenal simple cannulas (group 2) were used. The animals were housed in metabolism cages and fed with a mixed diet consisting of lucerne hay and concentrate in a 2 to 1 ratio (on a DM basis). The hay and the concentrate contained (per kg DM) 188 and 172 g of crude protein (CP), 488 and 188 g of neutral detergent fibre (NDF), 356 and 49 g of acid detergent fibre (ADF), and 81 and 11 g of acid detergent lignin (ADL), respectively. This diet was offered at an intake level of 40 g DM·kg^{-1} BW$^{0.75}$ in two equal meals (8:00 and 16:00 h) from 15 days before and throughout the experimental period.

2.3. Rumen degradability

Bags of 11 × 7 cm (inner dimensions) were made by heat-sealing (Preci-Pack P30N, Dover Pack S.A., Barcelona, Spain)

Table I. Chemical composition (g·kg^{-1} DM) of fresh lucerne samples.

	Harvesting treatment (weeks)			
Items	2	4	6	8
Organic matter	880	895	907	913
Crude protein	270	170	149	160
Neutral detergent fibre	294	411	471	488
Acid detergent fibre	242	329	369	379
Acid detergent lignin	60.5	71.7	78.6	72.1
NDIN[1]	14.1	16.2	11.2	11.7
ADIN[1]	2.1	4.6	6.5	7.3

[1] Percentage of total nitrogen.

from nylon cloth with a pore size of 46 µm (Saatilon, SAATI Serigrafia Iberica S.A., Almazora, Castellón, Spain). The bags were filled with approximately 3 g (freeze-dried) of feed samples and incubated in the rumen of each animal of group 1 for intervals of 2, 4, 8, 15, 24 and 48 h, with an additional period of 72 h for the sample collected at 8 weeks. Two series of incubation with duplicate bags were performed for each feed. At each series of incubation, all bags were placed simultaneously in the rumen just before the sheep were offered their first meal in the morning. After being collected from the rumen, the bags were washed with tap water and stored at –20 °C. Later, the bags were thawed and washed three times for 5 minutes in a turbine washing machine. The same procedure was applied to two series of two bags of each feed to obtain the zero hours value. For each sheep and incubation time, one bag of each incubation series was oven dried for 48 h at 80 °C and analysed for DM and nitrogen. The other bag was stored at –20 °C, freeze-dried and destined to intestinal digestibility studies.

The disappearance of DM and CP from the nylon bags at each incubation time was calculated from their respective amounts remaining after incubation in the rumen. The pattern of DM or CP disappearance (p) with the incubation time (t) was described for each animal using the model proposed by Ørskov and McDonald [21]:

$$p = a + \mathrm{b}\,(1 - \mathrm{e}^{-k_d t}). \qquad (1)$$

In this model, the constants a and b represent, respectively, the soluble fraction and the non-soluble degradable component, which disappears at a constant fractional rate k_d per unit of time. The fraction of undegradable CP (r) was estimated as $1 - (a + b)$. Effective degradability (ED) values were estimated, in accordance with the same authors, using the passage rate through the rumen (k_p) of the lucerne hay included in the diet, which had been marked by immersion with 10 mg Yb·g^{-1} of feed as described by González et al. [14]. To determine k_p values, a pulse dose (40 g) of labelled lucerne hay was fed to each animal immediately before the first daily meal. A total of 22 samples of faeces were obtained from the rectum of each animal, the first sample before supplying the marker and the remainder between 12 and 144 h afterwards. These samples were dried, milled and analysed for Yb. The pattern of Yb concentrations in the faeces over time was described for each animal by fitting to the model of Dhanoa et al. [11] and rate constants derived from the decreasing phase of concentrations were used as k_p values for all samples.

2.4. Intestinal digestibility

For each tested lucerne sample, the freeze-dried residues were pooled for each incubation time and the resulting samples were analysed for DM and nitrogen. Six sub-samples of 0.2 g were taken from each pooled sample and placed in heat-sealed nylon bags of the above indicated material having an approximately round shape (diameter 3 cm). Two bags from each rumen incubation time of each feed and three additional empty bags (used as blanks) were inserted using a spindle through the duodenal cannula into the small intestine of each animal of group 2. Six bags per sheep per day were inserted at random, at a rate of one bag every 15 min. Once recovered from the faeces, the bags were washed with tap water and deep-frozen. After thawing, they were mechanically washed, as indicated for the bags incubated in the rumen, and destined intact to nitrogen analysis. Blanks containing a known weight of nylon were used to correct for nitrogen content. The intestinal disappearance (ID) of undegraded CP was calculated as the amount of CP lost from the bag divided by the amount of CP in the bag before the intestinal passage. Then, the evolutions of ID values with the rumen preincubation time were fitted for each animal to a logistic function (see later) to determine the effective intestinal digestibility by the method proposed by González et al. [15].

2.5. Chemical analyses

Samples of tested lucerne were analysed for DM, ash, and CP (Kjeldahl N × 6.25) by AOAC methods [3] and for NDF [31], ADF, and ADL [27]. Insoluble nitrogen in neutral detergent (NDIN) and in acid detergent (ADIN) solutions was determined by Kjeldahl analysis of the NDF and ADF residues, respectively. Rumen incubation residues and intestinal mobile nylon bags were also analysed for nitrogen by the Kjeldahl method. Samples of faeces collected for transit studies were analysed for ytterbium by atomic absorption spectrometry as described by González et al. [14].

2.6. Statistical methods

The different kinetics associated with the employed models were fitted using a non-linear regression programme. Statistical analyses of rumen degradation parameters were made by variance analysis with animals and samples as factors in the model. Then, linear and quadratic effects of maturity were established by orthogonal polynomials. The results of intestinal digestibility for each lucerne sample were analysed considering animals and rumen pre-incubation times as factors in the model, before modelling these data. Then, maturity effects were studied on the effective parameters of intestinal digestion as indicated above for rumen studies. All the statistical analyses were performed using the Statistical Analysis System for Windows software, version 6.12 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of lucerne samples

Maturity leads to a decrease in the CP concentration and to an increase in the contents of OM and fibre fractions, but the rate of variation of all these parameters decreased as maturity progressed (Tab. I). Nevertheless, in the last harvest time the concentration of CP increased and that of ADL decreased, probably due to the presence in the sample of vegetative material corresponding to the regrowth of basal buds. The observed evolutions agree with previous results [6, 12, 16] and should be related to the main morphologic and chemical changes associated with maturity such as the reduction of the cell content to cell wall and leaf to steam ratios. The evolution of the ADIN proportions was similar to those of fibre. On the contrary, those of NDIN did not show a clear trend with maturity.

3.2. Ruminal degradability

The parameters of the degradation kinetics and the effective degradabilities of DM and CP in the rumen are shown in Table II. Mean values and standard error of k_p values used to establish the *ED* estimates were 0.0226 ± 0.0021 (h^{-1}). These values agree with those proposed by the AFRC [1] as a function of the intake level and they are very close to those obtained in similar conditions for dehydrated lucerne by Repetto et al. [26].

As maturity advanced, opposite linear and quadratic variations for the soluble and undegradable DM fractions were observed, in agreement with the reduction of cell contents and the increase of cell walls and lignin. A slight variation of the potentially degradable materials was observed with a quadratic effect. A tendency ($P = 0.055$) to an opposite quadratic evolution was also observed for the fractional degradation rate of this fraction. Therefore, the linear and quadratic decrease recorded for the *ED* of DM (from 0.795 to 0.576) was only the consequence of the variations induced by maturity on the *a* and *r* fractions. The decrease observed for the *ED* of DM with maturity agrees with other reported results [6, 12, 16] and showed a reduction of the fermentation level and therefore of the rumen microbial synthesis promoted by fresh lucerne.

Table II. Mean values of rumen degradation parameters and effective degradability of dry matter and crude protein of fresh lucerne samples (values are expressed as a proportion of the original feed).

	Harvesting treatment (weeks)					Effects	
Item	2	4	6	8	*M.S.E.*	*L*	*Q*
Dry matter							
a	0.506	0.370	0.314	0.302	0.0033	< 0.001	< 0.001
b	0.355	0.369	0.365	0.335	0.0082	0.135	0.039
r	0.139	0.262	0.321	0.363	0.0059	< 0.001	0.005
k_d (per h)	0.104	0.084	0.085	0.103	0.0079	0.933	0.055
ED	0.795	0.661	0.600	0.576	0.0048	< 0.001	< 0.001
Crude protein							
a	0.595	0.474	0.471	0.515	0.0052	< 0.001	< 0.001
b	0.342	0.411	0.374	0.348	0.0149	0.781	0.019
r	0.064	0.114	0.156	0.137	0.0152	0.009	0.063
k_d (per h)	0.179	0.149	0.140	0.168	0.0299	0.769	0.372
ED	0.896	0.832	0.791	0.817	0.0096	< 0.001	0.003

a, *b*, and *r* represent soluble, non-soluble degradable, and undegradable fractions, respectively, k_d: fractional degradation rate of fraction *b*.
ED: effective degradability. *M.S.E.*: mean standard error. *L* and *Q*: significance of linear and quadratic effects, respectively.

Effects of maturity on *a* and *r* fractions of CP showed a similar trend as for DM. However, the extreme values for both parameters were obtained in this case for the harvest at 6 weeks, suggesting an offsetting effect of the lucerne regrowth observed at 8 weeks. In addition, the increase of the undegradable fraction was lower than for DM, specially considering that an important part of this fraction is composed by adherent microorganisms. Therefore, based on the predictive equation obtained in similar conditions by Rodríguez et al. [29], this microbial proportion increases with maturity and represents 37.6, 53.4, 61.6, and 61.6% for 2, 4, 6, and 8 weeks, respectively. Therefore, the respective corrected fractions of undegradable CP from the lucerne samples were 0.040, 0.053, 0.060 and 0.053. The evolution of the potentially degradable fraction (*b*) showed, as for DM, a quadratic effect. Nevertheless, the variation between samples for these values or for their contribution to *ED* values was greater than for DM. Maturity had a linear and quadratic effect on the *ED* of CP. However, this variation was moderate (from 0.896 to 0.791), and even more so when the values were corrected by microbial colonisation using the equation of Rodríguez et al. [28]: 0.923, 0.886, 0.854 and 0.880 for 2, 4, 6, and 8 weeks at harvest, respectively. The mean value of apparent *ED* of CP compares closely with reported in situ [4, 5, 6, 12, 26] or in vivo [13] values. In addition, the effect of maturity is in agreement with that of previous results [2, 6, 12, 16].

The results of this experiment showed that plant maturity leads to an important decrease of rumen use of the whole forage by the reduction of the cell contents to cell walls ratio, but it only has a little effect on the rumen degradation of CP. The results also showed that the contents of insoluble bypass protein in fresh lucerne is small and they support the observation of Elizalde et al. [12] that the rumen escape protein of fresh lucerne is not affected by maturity. So, considering the CP concentration of the samples and their *ED*, values of the rumen escape protein of 28, 28.5, 31, and 29 g CP·kg^{-1} DM were estimated for samples harvested at 2, 4, 6 and 8 weeks.

3.3. Intestinal digestibility of undegraded crude protein

The effect of ruminal pre-incubation time on *ID* of undegraded CP is shown in Table III. As previously indicated, the 72-h rumen incubation was only considered necessary for the 8-week sample. A decrease in the *ID* values with the increase of rumen incubation time was observed for all samples. This reduction should be a consequence of the progressive enrichment in indigestible nitrogenous compounds of feed particles produced by the extent of rumen degradation [15]. In this last study, the use of an exponential equation to fit these changes has been proposed in order to obtain the effective proportion of feed protein digested in the intestine, which is carried out considering both this function and that which describes the outflow of undegraded protein from the rumen. Nevertheless, for samples harvested at 2 and 4 weeks, the results recorded up to 2 h were similar. In these conditions, the use of an exponential model would lead to an overestimation of the initial intestinal digestibility and therefore of the effective proportion of feed protein digested in the intestines. For this reason, the evolution of these values for all samples was fitted to a logistic equation:

$$ID = 1 - \frac{n\,(n+h)}{n + h\mathrm{e}^{-k_i t}} \cdot \qquad (2)$$

In this model, the $1 - n$ value represents the intestinal digestibility of the total insoluble feed protein, whereas the asymptotic value $1 - (n + h)$ corresponds to the intestinal digestibility of the protein fraction that was apparently undegradable in the rumen. The constant k_i is a rate of decrease of intestinal digestibility derived from the sample enrichment in undigestible compounds with

the extent of rumen degradative actions. The use of this model showed a better fitting for all samples than an exponential equation, although the more important differences between both models were obtained for the samples harvested at 2 and 4 weeks. Mean values of these parameters (n, h, and k_i) for the different samples are also shown in Table III.

The utilisation of a function to describe the evolution of the *ID* of undegraded residues allows the estimation of the effective intestinal digestibility from this function and from the post-ruminal flow of undegraded CP. González et al. [15] have established this last function considering that undegraded nitrogen is defined by $u = r + b\,e^{-k_d t}$ and that the rumen outflow of any feed constituent is defined by $f = 1 - e^{-k_p t}$. Thus, the corrected outflow rate from the rumen undegraded nitrogen is $u(df/dt)$. Therefore, the corrected rate of digested protein in the intestine can by obtained as $IDu(df/dt)$, and their cumulative proportion up to time t ($D_{i(t)}$) can be derived from:

$$D_{i(t)} = \int_0^t IDu \frac{df}{dt}\,dt = \int_0^t \left(1 - \frac{n\,(n+h)}{n + re^{-k_i t}}\right) \times (r + be^{-k_d t})\,k_p e^{-k_p t}\,dt. \quad (3)$$

As the time from feeding increases, the fraction of CP flowing into the intestines tends to zero, so that the proportion of CP from the feed digested in the intestines tends to:

$$D_i = r + \frac{bk_p}{k_d + k_p} - \int_0^\infty \frac{n\,(n+h)}{n + he^{-k_i t}} (r + be^{-k_d t}) \times k_p e^{-k_p t}\,dt. \quad (4)$$

The primitive function of the last term of this equation has not been found. Nevertheless, it can be easily calculated by numerical approximation using a mathematical software such as Derive 2 (Soft Warehouse Inc., Honolulu, USA).

From the resultant value of equation (4), the effective intestinal digestibility of undegraded nitrogen (*EID*) can be obtained as:

$$EID = \frac{D_i}{(1 - ED)}. \quad (5)$$

The important variations of *ID* associated with the rumen incubation time (Tab. III) show that the estimation of this parameter based on a single rumen incubation time can lead to errors. The use of a function to describe the evolution of the *ID* of CP in rumen undegraded residues together with estimations of the ruminal flow of undegraded CP allows for the estimation of the protein effectively digested (D_i) and solves the problems of accuracy derived from the use of a fixed but arbitrary time of ruminal pre-incubation.

Mean values of the initial digestibility ($1 - n$) and the final digestibility ($1 - (n + h)$) showed a linear decrease in all samples with maturity (Tab. IV). The rate of change between initial and final values (k_i) also showed a linear downward trend with maturity. Nevertheless, this reduction was mainly observed between the sample harvested at 2 weeks and the remainder samples as indicated by the quadratic tendency ($P = 0.06$) observed. The values of D_i varied in linear and quadratic form, showing its maximum for the 6 weeks harvest. This variation was opposite that observed for the *ED* of CP and evidenced a change of the digestion site from the rumen to the intestines with the reduction of ruminal degradability. The same effect was also observed on different forages [32] or when the reduction of the *ED* of CP was produced by the thermic treatment of different concentrates [15, 22, 24, 25].

When D_i values were expressed as concentration (g CP·kg^{-1} DM), a linear decrease was observed with maturity (Tab. IV). Nevertheless, this reduction was small and mainly associated with the last harvest treatment. This low variation shows that differences due to lucerne quality would be mainly derived from the synthesis of microbial protein. A superior forage quality is not only associated with higher levels of rumen

Table III. Effect of rumen incubation time on intestinal digestibility (*ID*) of undegraded crude protein and resulting fitting parameters for different fresh lucerne samples.

	Harvesting treatment (weeks)			
RPT (h)	2	4	6	8
0	0.876	0.784	0.760	0.720
2	0.851	0.790	0.707	0.547
4	0.812	0.715	0.725	0.633
8	0.692	0.729	0.616	0.634
15	0.519	0.517	0.572	0.471
24	0.533	0.548	0.424	0.419
48	0.531	0.498	0.464	0.396
72	–	–	–	0.384
M.S.E.	0.0174	0.0248	0.0500	0.0395
Significance	< 0.001	< 0.001	0.003	< 0.001
Fitting parameters[1]				
n	0.090	0.193	0.235	0.291
h	0.402	0.343	0.327	0.347
k_i	0.276	0.124	0.128	0.101

RPT: rumen pre-incubation time.
M.S.E.: mean standard error.
[1] Parameters obtained by fitting the equation $ID = 1 - \frac{n\,(n+h)}{n + h e^{-k_i t}}$; where, $1-n$: intestinal digestibility of total insoluble feed CP, $1-(n+h)$: intestinal digestibility of rumen undegradable CP; k_i: rate of decrease of the intestinal digestibility.

fermentation and therefore of microbial synthesis, but also with a higher intake and, consequently, with an additional increase of the microbial protein synthesised. The low values obtained in all samples for the total protein digested in the intestine also show that the main part of the supply of amino acids to the animal from this forage is derived from microbial protein.

Maturity was associated with a linear reduction of the *EID* (Tab. IV), at a mean rate of 2.6 percentage points per week. This effect shows that intestinal digestibility of fresh lucerne cannot be considered as a constant value. Present *EID* values were lower than those estimated in an indirect form by INRA, who propose intestinal digestibility values of 0.75 for fresh forages [33]. Values of intestinal digestibility higher than those obtained with the mobile bag technique could be expected if a fraction of the feed soluble CP escapes rumen degradation. Aufrère et al. [5] observed an accumulation of protein in the rumen liquid fluid of sheep fed fresh lucerne ad libitum, which represent some evidence of rumen escape of soluble proteins. However, this accumulation was small, short and associated with a larger meal. Therefore, the contribution of the soluble proteins to the *EID* value should be low. On the contrary, the mathematical calculations from present results of intestinal digestibility for total protein (undegraded protein plus microbial protein) are higher than in vivo measurements. So, assuming that the effective degradability of OM should be similar to that of DM and based on the specifications of the PDI system [33], the estimated mean value for the intestinal digestibility of total protein was 0.730, which is higher than the value (0.587) recorded by

Table IV. The effects of maturity on intestinal digestion of crude protein of fresh lucerne (values are expressed as proportions of the original feed CP).

	Harvesting treatment (weeks)					Effects	
Item	2	4	6	8	*M.S.E.*	*L*	*Q*
Initial digestibility ($1 - n$)	0.910	0.807	0.765	0.709	0.0260	0.002	0.409
Final digestibility ($1-(n + h)$)	0.508	0.464	0.439	0.362	0.0168	< 0.001	0.362
k_i (h^{-1})	0.276	0.124	0.128	0.101	0.0271	0.005	0.060
Intestinal digested CP (D_i)							
– as proportion of original CP	0.067	0.102	0.115	0.089	0.0044	0.006	< 0.001
– as $g \cdot kg^{-1}$ DM	18.0	17.4	17.1	14.3	0.67	0.010	0.152
Effective digestibility (*EID*)	0.641	0.609	0.549	0.488	0.0224	0.002	0.543
Whole tract undigested CP	0.037	0.066	0.094	0.094	0.0044	< 0.001	0.016

Elizalde et al. [13] for fresh lucerne or the mean value (0.631) recorded for legumes [8, 10, 13, 17, 19, 20].

The effect of maturity on the lucerne undigested CP content after ruminal and intestinal incubations is also shown in Table IV. These values increased in linear and quadratic form, reaching a maximum for the harvest treatments of 6 and 8 weeks. These values displayed in a similar ranking to that of ADIN, except for the sample at 8 weeks. ADIN is usually considered as indigestible [23], though some rumen degradation has been shown for fresh lucerne by Aufrere et al. [4]. In addition, the content of undigested CP should also be related with the proportions of rumen undegradable CP. So, the similar contents of undigested CP observed for samples at 6 and 8 weeks should be explained by an offsetting effect between ADIN proportions and the content of rumen undegradable CP.

4. CONCLUSION

The *ED* of fresh lucerne markedly decreased with maturity for DM and only slightly for CP. Considering both effects and considering the high *ED* of CP, it can be stated that the differences associated with lucerne quality are mainly related to the microbial protein synthesis promoted in the rumen. Although the reductions of protein degradability produced by maturity shifted the site of protein digestion from the rumen to the intestine, the progression of maturity also implied an increase of the undigested CP and, therefore, a reduction of its *EID*. Nevertheless, the most important changes associated with the protein value took place up to 4 weeks and, consequently, for the usual harvesting stages, the negative effect of maturity on the total supply of intestinal digestible proteins seems to be small.

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