Ann Zootech (1998) 47, 185–196

Original article

Addition of urea to lucernes before industrial dehydration: Effect on nutritional value and amino acid digestion

Nathalie Ballet, Laurence Bernard, Jean-Michel Besle*, Camille Demarquilly, Claude Poncet

Station de recherches sur la nutrition des herbivores, Inra, centre de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France

(Received 22 September 1997; accepted 6 February 1998)

Abstract – *Medicago sativa* L. var. Resis was harvested at two successive stages during its first growth cycle and at the end of the third cycle: lucernes 1, 2 and 3. These three samples were submitted to an industrial dehydration treatment with or without prior addition of urea (30 g kg^{-1} DM). The effect of adding urea was studied: i) on nitrogen content, cell wall composition and in vivo organic matter (OMd) and cell wall (CWd) digestibility measured in sheep using the three dehydrated lucernes; and ii) on ruminal degradation and intestinal digestibility of nitrogen (N) and amino acids (AA) of dehydrated lucerne 1. Urea treatment increased significantly ($P < 0.05$) the nitrogen content of the three lucernes (+ 4 to 13 g kg^{-1} DM). Nitrogen gain comes mainly from remaining urea-N. Urea treatment decreased the NDF content of lucerne 3 only (–37 g kg^{-1} DM). This treatment improved the OMd (2.9 to 3.7 points) of the three lucernes and the CWd of lucernes 2 (+3.1 points) and 3 (11.7 points). It tended to increase the effective ruminal degradability of nitrogen of lucerne 1 (+ 2.1 points taking into account the microbial contamination). This increase was essentially due to that of nitrogen being rapidly solubilized in the rumen, some of which coming from the nitrogen supplied by the treatment. Correlatively, the urea treatment increased the 16-h ruminal degradation of the AA (+ 5.2 points for total AA), but no effect on intestinal digestibility was observed. However, this result requires confirmation, since a probable excessive heating suffered by untreated lucerne 1 may have overprotected the AA from ruminal degradation, which could explain the difference from treated lucerne 1. In that case, the urea treatment would not affect the PDIA content of lucerne 1. The corrected intestinal digestibility value of total AA (0.86) was appreciably higher than that (0.70) used in the PDI system. (© Elsevier/Inra)

urea treatment / dehydrated lucerne / cell walls / digestibility / amino acids

* Correspondence and reprints
Tel.: (33) 04 73 62 40 51; fax: (33) 04 73 62 42 73; e-mail: bernard@clermont.inra.fr

Résumé – Effet de l'addition d'urée avant le processus industriel de déshydratation sur la valeur alimentaire et la digestion des acides aminés des lucernes. *Medicago sativa* L. var. Résis a été récoltée à deux stades de maturité différents au cours du premier cycle de croissance et à un stade tardif au troisième cycle, soit par ordre croissant de maturité les luzernes 1, 2 et 3. Ces trois échantillons de plante entière ont subi une déshydratation industrielle à haute température précédée ou non d'une addition d'urée (30 g kg^{-1} MS). L'effet de l'addition d'urée avant la déshydratation industrielle a été étudié i) sur la teneur en azote, la composition des parois et la digestibilité sur moutons de la matière organique et des parois des trois luzernes déshydratées et ii/ sur la dégradation ruminale de l'azote (N) et des acides aminés (AA) et sur la digestibilité dans l'intestin grêle de l'N et des AA non dégradés dans le rumen de la luzerne déshydratée 1. Le traitement à l'urée a entraîné une augmentation significative ($p < 0,05$) de la teneur en azote des trois luzernes déshydratées (4 à 13 g kg^{-1} MS). Le gain d'azote provenait surtout de l'azote restant sous forme uréique. Le traitement à l'urée n'a diminué la teneur en NDF que pour la luzerne 3 (– 37 g kg^{-1} MS). L'addition d'urée avant la déshydratation a entraîné une amélioration de la digestibilité de la MO (2,9 à 3,7 points) mais n'a augmenté celle des parois que pour les luzernes 2 (+ 3,1 points) et 3 (+ 11,7 points). De plus, le traitement à l'urée tend à augmenter la dégradabilité théorique de l'azote dans le rumen de la luzerne 1 (+ 2,1 points après déduction de la contamination microbienne). Cette augmentation est due essentiellement à celle de l'azote rapidement solubilisé dans le rumen dont une partie provient de l'azote apporté par le traitement. Le traitement à l'urée a augmenté la dégradation ruminale à 16 heures des AA (+ 5,2 points pour les AA totaux) sans toutefois affecter leur digestibilité intestinale. Cependant, ce résultat provenant de l'analyse d'un seul échantillon a besoin d'être confirmé car le chauffage vraisemblablement excessif subi par la luzerne 1 non traitée peut avoir surprotégé les acides aminés de la dégradation ruminale, ce qui expliquerait la différence avec la luzerne 1 traitée. Le traitement à l'urée n'affecterait donc pas la teneur en PDIA (protéines vraies digestibles dans l'intestin d'origine alimentaire) de la luzerne 1. La valeur corrigée de digestibilité dans l'intestin des AA totaux (0,86) est nettement supérieure à celle (0,70) utilisée dans le système PDI. (© Elsevier/Inra)

traitement à l'urée / luzerne déshydratée / parois / digestibilité / acides aminés

1. INTRODUCTION

With its high crude protein (CP) content, dehydrated lucerne could play an important part in feedstuffs of high-producing ruminants. The dehydration process increases the protein value by protecting part of the plant proteins from degradation in the rumen [27, 32] without decreasing their intestinal digestibility [18] and does not modify the energy value. Heat treatment, however, impairs the nutritive value of forages if the temperature or duration of the treatment is too high [18]. However, the incorporation of dehydrated lucerne into the diet of dairy cows and steers is relatively limited because of its low energy value, at least compared with maize silage, which often forms the basic diet of these animals in France [26].

A preliminary study (C. Demarquilly, unpublished results) showed that adding urea at 30 g per kg DM to lucerne just before dehydration improved CP content by 4 to 6 points and in vivo dry matter digestibility by 3.5 points. The positive effect of urea treatment (as an ammonia source) on nutritive value has been clearly demonstrated in grasses [3, 10]. Alkalis are reputed to protect dietary proteins from solubilization and degradation in the rumen [30]. Treatment with sodium hydroxide (30 g kg^{-1} DM) effectively protects proteins of soya and rapeseed from degradation in the rumen without impairing their intestinal digestibility [21, 22]. Similarly,

P. Chapoutot and D. Sauvant (unpublished results) observed that adding urea to lucerne before dehydration had a protective effect on the degradation of proteins. However, little work has been published on this subject and the first results on urea treatment of lucerne require confirmation. Accordingly, we examined the effect of urea treatment of lucerne on nitrogen content, cell wall composition and digestibility of lucerne harvested at three growth stages. In addition, ruminal degradation and intestinal digestibility of nitrogen and amino acids of lucerne were studied.

2. MATERIALS AND METHODS

2.1. Forages and urea treatment

A lucerne (*Medicago sativa* L. var. Resis) was harvested at bud stage (lucerne 1) and flowering stage (lucerne 2) during its first growth cycle and at the end of third cycle (lucerne 3). Immediately after harvest, the three whole plant samples were dried by high temperature industrial drier (Van den Brock, inlet temperature 600 °C, outlet temperature 110 °C) with or without prior addition of urea (30 g kg^{-1} DM) then milled (3 mm screen) and pelleted.

The dehydrated lucerne pellets were used as they were for animal digestibility measurements, and milled (4 mm screen) for in situ disappearance measurements and chemical analyses.

2.2. Experimental studies

2.2.1. Experiment 1

The effect of urea treatment on nitrogen content, cell wall composition and in vivo digestibility of dehydrated lucerne was studied at three growth stages.

2.2.2. Experiment 2

The effect of urea treatment on nitrogen and amino acid degradation in the rumen and on the digestibility in the small intestine of nitrogen and amino acids not degraded in the rumen was studied only on dehydrated lucerne 1. Degradabilities and digestibilities were estimated by the nylon bag method.

2.3. Digestibility measurements

2.3.1. In vivo digestibility

Two groups (one for lucernes 1 and 2, one for lucerne 3) of six castrated male Texel sheep weighing about 60 kg were used for the measurement of in vivo digestibility of treated (T) and untreated (UT) dehydrated lucerne pellets. Sheep maintained individually in metabolism cages were fed at maintenance level, i.e., 45 g DM per kg $P^{0.75}$. UT and T lucernes for each growth stage were given successively to one and same group of sheep. For each pair of dehydrated lucernes (T and UT), the experimental procedure was composed of three successive phases of measurement: i) chopped dehydrated lucerne alone; ii) 60% untreated lucerne pellets and 40% chopped dehydrated lucerne; and iii) 60% treated lucerne pellets and 40% chopped dehydrated lucerne. The chopped dehydrated lucerne was added to allow proper functioning of the rumen and maintain minimal rumination. Each measurement phase consisted of a 15-day period of adaptation to the diet followed by a measurement period lasting 6 days with total collection of feces. The dry matter (DM) digestibility of the experimental lucernes (UT or T) was calculated by difference, assuming no associative digestibility. Earlier measurements of the digestibility of concentrate feed showed that 40/60 forage-concentrate diets offered at maintenance level exhibited only very slight, if any, digestive interaction [9].

2.3.2. Degradation in the rumen and digestibility in the intestine

Nitrogen (N) and amino acid (AA) degradation kinetics in the rumen and intestinal digestibilities of nitrogen and amino acids were measured for lucerne 1 using the incubated nylon bag method for the rumen, and the mobile bag method for the intestine.

These measurements were made on three castrated male Texel sheep weighing about 60 kg, equipped with ruminal and duodenal cannulas. These sheep were fed with a diet of 70% lucerne hay and 30% barley at 1.2 kg DM/day.

2.3.3. Preparation and treatment of rumen incubated bags

Nylon bags (Ankom, USA, 5 × 10 cm, pores 50 μm ± 10) containing 3 g of milled sample were incubated in the rumen of fistulated sheep for 0, 2, 4, 8, 16, 24 and 48 h. Each kinetic run was repeated once on each sheep so that each kinetic point corresponded to six measurements (three sheep twice). The control and treated sample bags were introduced simultaneously in the rumen of each sheep. After removal from the rumen, the bags were rapidly washed in water and frozen. After thawing, the bags were thoroughly washed in a washing machine (5–6 cycles of 5 min) until rinse water was clear, and freeze-dried for 48 h. Nitrogen content of the bag residues was determined after pooling the two bags corresponding to the same incubation time on the same sheep. Individual amino acid contents in the residues of bags incubated for 16 h (average residence time of particles in the rumen; [33]) were determined on a pooled sample from the three sheep (six bags).

To calculate the nitrogen degradability in the rumen, the degradation kinetics were fitted to the exponential model described by Orskov and Mc Donald [24], i.e.:

$$D(t) = a + b\,(1\text{-}e^{-ct})$$

where D(t) is the percentage of nitrogen lost from the bag after an incubation time of t hours, a (%) is the immediately degradable fraction, b (%) is the slowly degradable fraction, and c (h^{-1}) is the degradation rate constant for fraction b.

The effective ruminal nitrogen degradability is calculated using the following formula:

$$D = a + bc / (c + k)$$

where k (h^{-1}) is the rumen particle outflow rate. This rate was fixed at 0.06 h^{-1} corresponding to an average residence time in the rumen of 16.5 h [33]. The correction of D to take into account microbial contamination of feed particles incubated in the rumen was done according to Michalet-Doreau and Ould-Bah [20], using the equation:

$$\Delta D = 6.4 - 0.353CP + 0.170NDF \pm 1.9,$$

with CP and NDF in % DM

2.3.4. Preparation and treatment of mobile bags

Intestinal digestibility of nitrogen and amino acids was estimated on pooled 16-h rumen fermented residues. 150 mg of 'rumen' samples were placed in a hexagonal microbag (15 mm width, pores 50 μm ± 10) and immersed in a pepsine-0.01 M HCl solution for 2 h at 39 °C to simulate digestion in the abomasum. Four microbags, two containing the control and two the treated sample, were then introduced daily during 4 days into the small intestine of three sheep through the duodenal cannula. They were recovered in the feces but the bags with intestinal residence time greater than 30 h were discarded.

After recovery in the feces, the microbags were washed in a 0.9% sodium chloride solution until rinse water was clear, and freeze-dried for 48 h.

Nitrogen and individual amino acid contents in residues of microbags were determined on an average sample after pooling the eight bags of individual sheep.

2.3.5. Estimation of microbial contamination

The contamination of the 16-h rumen incubated residues by rumen bacteria leads to an underestimation of feed nitrogen and amino acid degradability. To estimate N and AA from microbial origin in the bag residues, an ADF residue [13] of lucerne hay which was presumably indigestible, was introduced in both the rumen and the small intestine. However, this ADF nitrogen proved to be partly digestible, and the microbial contamination was very low. Therefore, we evaluated the microbial contamination from literature data. According to Bernard et al. [2] and Ould-Bah et al. [25], the microbial contamination of dehydrated lucernes incubated for 16-h in nylon bags in the rumen represented on average 10% of the residual DM. In addition, from the results of 21 research groups compiled by Clark et al. [8], the mean N content of bacterial DM appeared to be 7.7% and the proportion of amino acid nitrogen in total nitrogen was 0.66. The true degradation in the rumen could therefore be calculated using:

$$\frac{I - (R - M)}{I}$$

where I is the amount of nitrogen or amino acids in the feed before rumen incubation, R the

amount of nitrogen or amino acids in the 16-h rumen residue, and M the amount of nitrogen or amino acids in bacteria attached on the rumen residue.

Individual amino acid contents of the particle attached bacteria were also estimated from data in the review of Clark et al. [8].

Hvelplund [14] showed that the microbial contamination of the residues from microbags recovered in the feces could be ignored probably because the slight microbial contamination in the large intestine compensates for the weak digestion of feed nitrogen there.

The intestinal digestibility was calculated using:

$$\frac{Rc - F}{Rc}$$

where Rc is the amount of nitrogen or amino acids in the feed sample preincubated in the rumen for 16 h after deduction of the microbial contamination, and F the amount of nitrogen or amino acids in the bags recovered in feces.

2.4. Chemical analyses

The cell wall (neutral detergent fiber, NDF), lignocellulose (acid detergent fiber, ADF) (method of Goering and Van Soest [13]), crude fiber (CF) (method of Weende) and nitrogen (totalN, method of Kjeldahl) contents were determined in treated and untreated lucernes and in the corresponding feces.

Additionally, the cell wall residues (CWRs) of treated and untreated lucernes were prepared by the method of Jarrige [16], which consists in washing with water at 40 °C following by two reflux extractions in a Soxhlet apparatus with ethanol and then ethanol-toluene (1/2, v/v). The carbohydrate and lignin contents were determined by the sequential method of Jarrige [16]. After hydrolysis, hemicelluloses and cellulose were estimated by colorimetry as reducing sugars with xylose and glucose as standards respectively [4]. Uronic acids were hydrolyzed according to Englyst et al. [12] and determined by the colorimetric method of Blumenkrantz and Asboe-Hansen [5].

Urea was analyzed according to Sahnoune et al. [28]. The degree of ureolysis corresponds to the percentage of urea hydrolyzed, e.g., (ureaN added - ureaN recovered) × 100/ureaN added.

Individual amino acid contents in initial feeds and in rumen and intestine samples were determined after hydrolysis of the sample with 6 M HCl for 24 h at 120 °C. Hydrolysates were evaporated and taken up in lithium citrate buffer (pH 2.2). To determine methionine, the hydrolysis was preceded by an oxidation step to protect it. The amino acids were then separated by liquid phase chromatography (Beckman 6 300) on a cation exchange column with an eluant lithium citrate buffer of increasing pH.

2.5. Statistical analyses

Data were submitted to analysis of variance [29]. The Duncan test was used to identify the significant differences between means. The nitrogen degradation kinetics were fitted by the method of Marquardt [29].

3. RESULTS

3.1. Experiment 1: Chemical composition and digestibility

The treatment with urea caused a significant increase ($P < 0.05$) in the nitrogen content of the dehydrated lucerne at all three growth stages (*table I*). This increase ranged from 3.7 to 13.3 g kg^{-1} DM and from 14 to 47% of the initial N content. The industrial treatment was characterized by a relatively low degree of ureolysis (about 35%) except for lucerne 2 for which the degree of ureolysis was 70%. Interestingly, the greater the ureolysis is, the lower the nitrogen gain; this therefore comes mainly from nitrogen remaining in urea form.

The urea treatment had no effect on the NDF content and cell wall composition of lucernes 1 and 2, but caused a reduction of the NDF content (–37 g kg^{-1} DM) of lucerne 3 (*table I*). These results agree with those obtained by the method of Jarrige and the soluble fraction of treated lucerne 3 had the same composition as untreated lucerne 3.

The urea treatment improved the digestibilities of DM and organic matter measured in the sheep (*table II*). This increase ranged from 3.8 to 4.6 points for DM (on average + 7%) and from 2.9 to 3.7 points for organic matter (on average + 6%).

The improvement of the DM digestibility is partly due to an improvement in cell wall digestibility for lucernes 2 and 3 which increased 3.1 and 11.7 points respectively, but not for lucerne 1. The increase in the digestibility of the cell walls is partly due to the increase of the ADF fraction digestibility (4.7 to 11.3 points). Likewise, CF digestibility was increased 2.2 to 8.4 points. However, an abnormal decrease in the ADF and CF digestibilities was observed for lucerne 1.

The urea treatment improved CP digestibility in lucerne at all three growth stages (+ 10.1 to 13.2 points) and decreased the non-digestible CP content (NDCP) (*table II*).

3.2. Experiment 2: Degradation in the rumen and intestinal digestibility of nitrogen and amino acids

After urea treatment of lucerne 1, the amount of immediately degradable nitrogen (a) increased from 14 ± 0.9 g kg^{-1} DM to 20.2 ± 0.8 g kg^{-1} DM, representing nearly the half of total nitrogen (*figure 1*). In parallel, the amount of slowly degraded nitrogen (b) remained constant at 17 ± 1.3 g kg^{-1} DM. The non-degradable nitrogen fraction accounted for 2.6 ± 0.8 g kg^{-1} DM before treatment and 2.7 ± 0.4 g kg^{-1} DM after treatment. This fraction (100-a-b) represented 6.7% of total nitrogen against 7.7% before treatment. The degradation rate (c) tended to decrease from 8.2 ± 2.4% h^{-1} to 7.7 ± 2.6% h^{-1}. Lastly, the urea treatment increased the effective degradability (D) of nitrogen in the rumen of lucerne 1, which rose from 70.9 ± 3.4% to 74.4 ± 3.0%. This increase was essentially due to the increase in the immediately degradable fraction. Taking into account

Table I. Chemical composition (g kg^{-1} DM) of dehydrated lucernes with and without added urea (30 g kg^{-1} DM) before dehydration.

Sample	Lucerne 1		Lucerne 2		Lucerne 3		RSD
	– urea	+ urea	– urea	+ urea	– urea	+ urea	
Total N	33.6[b]	40.2[a]	26.9[b]	30.6[a]	28.3[b]	41.6[a]	0.1
Ureolysis (%)		42.0		70.3		33.0	
NDF	440	440	493	495	520	483	N.D.
ADF	294	273	294	304	331	308	N.D.
Crude fiber	232	231	263	271	301	270	N.D.
CWR	540[a]	520[a]	618[a]	629[a]	659[a]	579[b]	3
Hemicellulose	48[a]	51[a]	68[a]	71[a]	94[a]	84[b]	2
Cellulose	154[a]	157[a]	183[a]	187[a]	214[a]	192[b]	4
Lignin	74[a]	66[a]	86[a]	89[a]	89[a]	71[b]	3
Uronic acids	63[a]	65[a]	63[a]	59[a]	N.D.	N.D.	2

[ab] Values with different letter superscripts are significantly different ($P < 0.05$) for a given lucerne pair. Total N, total nitrogen; NDF, neutral detergent fiber; ADF, acid detergent fiber. RSD, residual standard deviation. N.D., not determined.

Table II. Effect of adding 30 g of urea per kg DM before dehydration on in vivo digestibility of DM, OM, CP, cell walls and different constituents of the cell wall of dehydrated lucernes.

Sample	Lucerne 1		Lucerne 2		Lucerne 3		RSD
	– urea	+ urea	– urea	+ urea	– urea	+ urea	
DMd	62.9[b]	67.5[a]	53.0[b]	56.9[a]	55.0[b]	58.8[a]	2.31
OMd	65.0	68.7	57.8	60.7	54.9	58.3	–
CPd	67.1	78.7	63.1	73.2	67.0	80.2	–
NDFd	58.4	59.0	47.5	50.6	40.6	52.3	–
ADFd	55.8	51.5	34.9	39.6	34.6	45.9	–
CFd	52.6	48.4	38.3	40.5	30.5	38.9	–
NDCP (g kg^{-1} DM)	69	53	62	51	57	51	–

[ab] Values with different letter superscripts are significantly different ($P < 0.05$) for a given lucerne pair. DMd, dry matter digestibility; OMd, organic matter digestibility; CPd, crude protein digestibility; NDFd, neutral detergent fiber digestibility; ADFd, acid detergent fiber digestibility; CFd, crude fiber digestibility; NDCP non-digestible crude protein. RSD, residual standard deviation.

the microbial contamination, the effective degradability of the nitrogen rose to 77.4 ± 3.4% for untreated lucerne 1 and to 79.5 ± 3.0% for treated lucerne 1. Thus the urea treatment tended to slightly increase the corrected effective degradability of nitrogen. However, it can be calculated that the amount of total N effectively escaping degradation in the rumen increased from 7.6 to 8.2 g kg^{-1} DM (with correction for microbial contamination).

The proportion of amino acid nitrogen in total N was 0.72 for untreated lucerne 1 and 0.60 for treated lucerne 1 (*table III*). The urea treatment had no effect on the amino acid profile of the dehydrated lucerne. It increased the 16-h ruminal degradation (corrected for microbial contamination) of nitrogen (+ 4.2 points), total amino acids (+ 5.2 points) and individual amino acids (on average + 4.2 points), but had no significant effect on their intestinal digestibility. Microbial contamination caused an underestimation of the 16-h ruminal degradation of nitrogen (–6.1 points), total amino acids (–6.4 pts) and individual amino acids (on average –6.2 pts), and an overestimation of intestinal digestibility of nitrogen (+ 10.5 pts). In contrast, microbial contamination had little effect on the intestinal digestibility of amino acids.

The corrected 16-h degradation of total amino acids in the rumen was lower than that of nitrogen in both untreated lucerne 1 (respectively 72.9% and 83.7%) and treated lucerne 1 (respectively 78.1% and 87.9%); the opposite was observed for intestinal digestibility (respectively 85.8% and 63.0% for untreated lucerne 1 and 83.9% and 64.7% for treated lucerne 1). The highest 16-h ruminal degradation and intestinal digestibility values were observed for aspartic acid and arginine, the lowest for methionine and serine respectively, for both untreated lucerne 1 and treated lucerne 1.

4. DISCUSSION

Urea treatment applied on an industrial scale moderately improved the digestibility of dehydrated lucernes. The limited effect of urea, and alkali treatments in general, on the digestibility of lucernes has also been observed by Waiss et al.

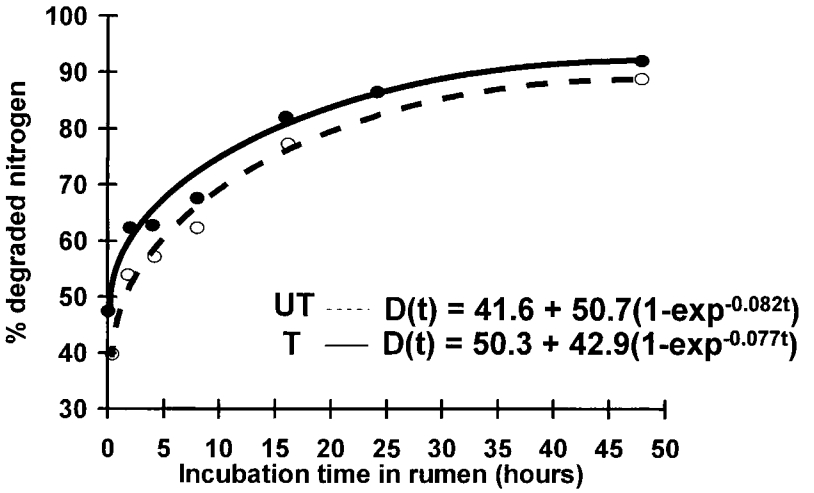

Figure 1. Nitrogen degradation kinetic in the rumen of treated (T) (●) and untreated (UT) (○) lucerne 1with 30 g urea per kg DM.

[34], Nishino et al. [23] and Ballet et al. [1]. Chesson [6, 7] and Ballet et al. [1] reported that alkalis have little effect on the degradation of the cell walls of dicotyledons, as their hemicellulose-lignin complexes contain mostly alkali-resistant bonds.

The non-digestible CP content (69 g kg^{-1} MS) of untreated lucerne 1 was abnormally high; the average value is 54.2 g kg^{-1} DM with a range from 45 to 65 g kg^{-1} DM (average of 41 dehydrated lucernes) (C. Demarquilly, unpublished results). This high non-digestible CP content of untreated lucerne 1 may be due to excessive heating during the industrial dehydration. Hence the improvement in the in vivo digestibility observed with treated lucerne 1 could result from a reduction in the digestibility of untreated lucerne 1 rather than from the urea treatment. Overheating is known to be detrimental to the digestibility of lucernes [18]. The in vivo DM digestibility of untreated lucerne 1 may therefore be higher than 62.9% which is a low figure for lucerne harvested at bud stage of the first cycle. It is thus impossible to tell how much of the observed improvement is due to urea treatment. The urea treatment had no positive effect on in vivo cell wall digestibility of lucerne 1 which initially had the greatest digestibility. This result confirms that of Ballet et al. [1] who found that improvement of cell wall digestibility obtained by urea treatment is inversely proportional to the initial digestibility. Allowing for the degree of ureolysis, the positive effect of the urea hydrolyzed to ammonia during dehydration (equivalent to 5–10 g NH_3 per kg DM) on digestibility is close to that obtained by Ballet et al. [1] using an ammonia treatment (15 g kg^{-1} DM) (on average + 3.5 points) applied at 80 °C on a laboratory scale. The improvement of digestibility obtained by addition of urea before industrial dehydration was apparently amplified by the dehydration temperature (110 °C). This improvement in digestibility seems to result mainly from an increase of the accessibility of cell walls to enzymatic hydrolysis, since it was observed in the absence of any change in cell wall content.

Table III. Contents, 16-h ruminal degradations and intestinal digestibilities of nitrogen and amino acids of treated and untreated lucerne 1 (nylon bag method).

	Ntotal	AAtot	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ileu	Leu	Tyr	Phe	Lys	His	Arg
Nitrogen and amino acid contents in feed (% DM)																		
UT	3.36	15.1	2.71	0.90	0.79	1.78	0.86	0.82	0.97	0.82	0.32	0.66	1.33	0.62	0.84	0.75	0.31	0.64
T	4.04	15.1	2.74	0.90	0.84	1.76	0.90	0.81	0.99	0.78	0.35	0.62	1.31	0.60	0.81	0.75	0.31	0.66
16-h ruminal degradation (%)																		
UT	77.1	66.5	79.5	63.5	72.3	68.7	69.0	64.1	65.4	65.0	57.6	65.4	63.3	67.0	64.6	64.1	64.4	64.9
UT_c	83.7	72.9	83.1	69.9	71.5	74.7	71.9	70.9	72.4	70.6	65.2	73.3	69.1	74.3	70.0	73.7	70.8	71.6
T	82.3	71.8	81.0	67.2	70.4	72.1	74.4	67.0	70.3	68.8	62.7	68.9	68.3	70.9	67.0	65.5	70.1	70.6
T_c	87.9	78.1	85.7	73.3	75.8	79.0	77.3	73.8	77.6	76.3	69.6	77.2	73.4	79.9	72.4	76.5	75.9	76.9
Intestinal digestibility (%)																		
UT	74.2	88.5	87.8	88.1	85.4	90.0	86.9	86.0	86.9	88.6	85.4	89.1	90.4	89.9	89.3	88.5	88.1	93.6
T	74.8	87.5	86.3	86.4	82.7	89.6	86.4	88.5	85.7	86.7	85.8	87.8	89.5	89.3	89.2	88.2	86.5	92.4
RSD	*3.1*	*2.0*	*2.5*	*1.0*	*2.6*	*0.2*	*0.3*	*1.5*	*1.9*	*3.0*	*2.0*	*1.9*	*0.7*	*0.9*	*0.9*	*0.3*	*1.3*	*2.7*
UT_c	63.0	85.8	85.0	85.4	82.4	87.7	85.3	83.3	83.8	86.7	82.1	86.2	88.4	87.0	87.5	84.2	85.5	91.2
T_c	64.7	83.9	82.6	84.2	79.9	86.1	84.7	85.0	81.8	81.6	82.4	83.8	87.6	85.2	87.3	83.3	83.4	90.4
RSD	*4.7*	*2.0*	*2.2*	*0.9*	*0.8*	*2.0*	*0.6*	*2.9*	*3.8*	*1.5*	*2.6*	*3.2*	*1.5*	*1.4*	*2.1*	*2.1*	*1.2*	*2.8*

The coefficient of variation was lower than 0.5% for the amino acid and nitrogen analysis. UT, untreated lucerne 1; T, treated lucerne 1 (30 g urea per kg of DM); c, corrected for microbial contamination; RSD, residual standard deviation.

The effective nitrogen degradability value in the rumen for untreated lucerne 1 (0.71) was appreciably higher than that (0.60) used in the PDI system [15]. It is, however, close to that (0.70) obtained by J.L. Peyraud (personal communication) for six samples of Resis lucerne with average crude protein contents of 22% DM. The observed difference thus comes from the fact that the dehydrated lucerne used here had a higher crude protein value (21%) than that (18%) of the dehydrated lucernes studied to determine the effective degradability of the dehydrated lucernes listed in the Inra tables [15].

The amino acid profile of the dehydrated lucerne presented here is similar to that given by Skórko-Sajko et al. [31] for a dehydrated lucerne with a nitrogen content of 2.8% DM. This profile was not influenced by urea treatment as observed by Mason et al. [17] for ammonia-treated hay. This means that the urea treatment did not alter the proteins of the lucerne.

Mir et al. [21, 22] and Waltz and Loerch [35] showed that alkali treatments could reduce ruminal degradation of proteins without affecting their intestinal digestibility. However, the ruminal nitrogen degradability of lucerne 1 was increased by urea treatment. This increase was due essentially to that of nitrogen being rapidly solubilized in the rumen, some of which comes from the nitrogen supplied by the treatment. The same observation was made by Michalet-Doreau and Guedes [19], and by Dryden and Kempton [11] on straws and hays treated with ammonia. The intestinal nitrogen digestibility was not affected by the urea treatment [30]. The absence of any protective effect of urea on the degradation of proteins in the rumen is confirmed by the study of the digestion of amino acids. The urea treatment studied did not decrease the degradation of the amino acids in the rumen, in fact an opposite effect was observed. The amount of amino acids available for absorption in the small intestine thus decreases after urea treatment, with no effect on intestinal digestibility. Consequently, urea treatment has a negative effect on PDIA (protein truly digestible in the intestine of feed origin) content.

However, this result is derived from the analysis of a single sample and requires confirmation. The probably excessive heating suffered by untreated lucerne 1 may have overprotected the amino acids from ruminal degradation, which could explain the difference from treated lucerne 1. In that case, the urea treatment would not affect the PDIA content of lucerne 1.

The intestinal digestibility of total amino acids of untreated dehydrated lucerne 1 was appreciably higher than that of nitrogen, as was also observed by Skórko-Sajko et al. [31] on dehydrated lucerne pellets (74.8% and 84.4% respectively for intestinal digestibility uncorrected for microbial contamination of nitrogen and total amino acids) This result may be explained by a high proportion of non-amino acid nitrogen in the non-digestible fraction. The corrected digestibility of the total amino acids in untreated lucerne 1 was about 0.86, comparable to that of microbial amino acids [33]. Therefore, the microbial contamination has little or no effect on the true digestibility of the lucerne AA. The corrected intestinal digestibility value of total AA (0.86) was appreciably higher than that (0.70) used in the PDI system [15].

5. CONCLUSION

Urea treatment applied on an industrial scale enhances the energy value of dehydrated late lucernes, but seems not to improve the nitrogen value of lucernes, at least in regard to PDIA content.

ACKNOWLEDGMENTS

The authors thank M.T. Beaufort, M.P. Maillot, J.P. Chaise, and E. Delval for their technical assistance, and the Syndicat National des Déshydrateurs de France (SNDF) for their financial support.

REFERENCES

[1] Ballet N., Besle J.M., Demarquilly C., Effect of ammonia and urea treatments on digestibility and nitrogen content of dehydrated lucerne, Anim. Feed. Sci. Technol. 67 (1997) 69–82.

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

[3] Ben Salem H., Nefzaoui A., Rokbani N., Upgrading of sorghum stover with anhydrous ammonia and urea treatments, Anim. Feed. Sci. Technol. 48 (1994) 15–26.

[4] Besle J.M., Lassalas B., Thivend P., Digestion des glucides cytoplasmiques de la féverole par le veau préruminant, Reprod. Nutr. Dev. 21 (1981) 629–649.

[5] Blumenkrantz N., Asboe-Hansen G., New method for quantitative determination of uronic acids, Anal. Biochem. 54 (1973) 484–489.

[6] Chesson A., Lignin-polysaccharide complexes of the plant cell wall and their effect on microbial degradation in the rumen, Anim. Feed. Sci. Technol. 21 (1988) 219–228.

[7] Chesson A., Mechanistic models of forage cell wall degradation, in: Jung H.G., Buxton D.R., Hartfield R.D., Ralph J. (Eds.), Forage cell wall structure and digestibility, Madison, Wisconsin, USA, 1993, pp. 347–376.

[8] Clark J.H., Klusmeyer T.H., Cameron M.R., Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows, J. Dairy Sci. 75 (1991) 2304–2333.

[9] Demarquilly C., Chenost M., Giger S., Pertes fécales et digestibilité des aliments et des rations, in : Jarrige R., Ruckebush Y., Demarquilly C., Farce M.H., Journet M. (Eds.), Nutrition des ruminants domestiques : ingestion et digestion, Inra éditions, Paris, France, 1995, pp. 601–647.

[10] Dias-Da-Silva A.A., Mascarenhas-Ferreira A., Guedes C.V.M., Effects of moisture level, treatment time and soya bean addition on the nutritive value of urea-treated maize stover, Anim. Feed. Sci. Technol. 19 (1988) 67–77.

[11] Dryden G.M.L., Kempton T.J., Digestion of organic matter and nitrogen in ammoniated barley straw, Anim. Feed. Sci. Technol. 10 (1983/84) 65–75.

[12] Englyst H., Wiggins H.S., Cummings J.H., Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituents sugars as alditol acetate, Analyst 107 (1982) 107–318.

[13] Goering H.K., Van Soest P.J., Forage fiber analysis (apparatus, reagents, procedures and some applications), Agric Handbk, no 379, Agricultural Research Service, USDA, 1970.

[14] Hvelplund T., Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by in sacco procedure, Acta Agric. Scand. 25 (1985) 132–144.

[15] Inra tables, in: Jarrige R. (Ed.), Alimentation des bovins, ovins et caprins, Inra éditions, Paris, France, 1988.

[16] Jarrige R., Analyse des constituants glucidiques des plantes fourragères : 1. Fractionnement des constituants de la membrane par des hydrolyses acides, Ann. Biol. Anim. Biochim. Biophys. 1 (1961) 163–212.

[17] Mason V.C., Keene A.S., Cooper E.M., Hoadley C.J., Hartley R.D., Oven and stack ammoniation of grass hays. II. Voluntary intake, digestibility and faecal nitrogen excretion in sheep in relation to hay composition, Anim. Feed. Sci. Technol. 24 (1989) 313–326.

[18] McKinnon J.J., Olubobokum J.A., Mustafa A., Cohen R.D.H., Christensen D.A., Influence of dry treatment of canola meal on site and extent of nutrient disappearance in ruminants, Anim. Feed. Sci. Technol. 56 (1995) 243–252.

[19] Michalet-Doreau B., Guedes C.V.M. Influence du traitement des fourrages à l'ammoniac sur leur dégradation azotée dans le rumen, Ann. Zootech. 38 (1989) 259–268.

[20] 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: XVI International Grassland Congress, Nice, France, 1989, pp. 909–910.

[21] Mir Z., MacLeod G.K., Buchanan-Smith J.G., Grieve D.G., Grovum W.L., Effect of feeding soybean meal protected with sodium hydroxide, fresh blood, or fish hydrolysate to growing calves and lactating dairy cows, Can. J. Anim. Sci. 64 (1984) 845–852.

[22] Mir Z., MacLeod G.K., Buchanan-Smith J.G., Grieve D.G., Grovum W.L., Methods for protecting soybean and canola proteins from degradation in the rumen, Can. J. Anim. Sci. 64 (1984) 853–865.

[23] Nishino N., Ohshima M., Uchida S., A note on the fermentation characteristics and rumen degradation of low moisture alfalfa silage treated with sodium hydroxide or ammonia, Anim. Feed. Sci. Technol. 47 (1994) 297–304.

[24] Orskov E.R., Mc Donald I., The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage, J. Agric. Sci. Cambridge 92 (1979) 499–503.

[25] Ould-Bah M.Y., Michalet-Doreau B., Jamot J., Colonisation bactérienne des résidus alimentaires des sachets incubés dans le rumen ; utilisation du " stomacher " pour la réduire et conséquences sur la mesure de la dégradabilité de l'azote, Reprod. Nutr. Dev. 28 (1988) 107–108.

[26] Peyraud J.L., Delaby L., Marquis B., Intérêt de l'introduction de luzerne déshydratée en substitution de l'ensilage de maïs dans les rations des vaches laitières, Ann. Zootech. 43 (1994) 91–104.

[27] Price S.G., Satter L.D., Jorgensen N.A., Dehydrated alfalfa in dairy cow diets, J. Dairy Sci. 71 (1988) 727–736.

[28] Sahnoune S., Besle J.M., Chenost M., Jouany J.P., Combes D., Treatment of straw with urea. 1. Ureolysis in a low water medium, Anim. Feed. Sci. Technol. 34 (1991) 75–93.

[29] SAS Institute Inc., SAS user's guide: statistics, SAS Institute, Cary, NC, USA, 1989.

[30] Schwab C.G., Protected proteins and amino acids for ruminants, in: Wallace R.J., Chesson A. (Eds.), Biotechnology in animal feeds and animal feeding, Weinhein, RFA, 1995, pp. 115–141.

[31] Skórko-Sajko H., Hvelplund T., Weisbjerg M.R., Rumen degradation and intestinal digestibility of amino acids in different roughages estimated by nylon bag techniques, J. Anim. Feed. Sci. 3 (1994) 1–10.

[32] Urbaniak M., Przybecki T., Effect of dehydrated alfalfa on ruminal characteristics and amino acids flow through lambs duodenum, Anim. Feed. Sci. Technol. 54 (1995) 121–131.

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

[34] Waiss A.C. Jr., Guggolz J., Kohler G.O., Walker H.G. Jr., Garret W.N., Improving digestibility of straws for ruminant feed by aqueous ammonia, J. Anim. Sci. 35 (1972) 109–112.

[35] Waltz D.M., Loerch S.C., Effect of acid and alkali treatment of soybean meal on nitrogen utilization by ruminants, J. Anim. Sci. 63 (1986) 879–887.