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Original article

Degradation in the rumen of proteins from fresh lucerne forage in various stages of growth and conserved as silage or hay

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Abstract — The extent to which rumen soluble nitrogen can contribute to the intestinal flow is unknown. Therefore a study was carried out to simultaneously assess the dynamics of protein disappearance from dacron bags placed in the rumen and the amount of various N products (total nitrogen (tN), ammonia nitrogen (NH3-N), non-ammonia nitrogen (NAN). Measurements were carried out on 4 sheep fed various lucerne forages. These forages included fresh lucerne cut at the vegetative or bud stage, fresh lucerne cut at the 6-week second growth stage and at stemmy regrowth stage. In addition two silages made from lucerne at the bud stage, with or without formic acid were also given. The hay was dried on the ground in good weather. The effective degradability of nitrogen (DegN) estimated from the nylon bag procedure was lower ($p < 0.05$) with the latter vegetation stage (0.80 for vegetative stage vs. 0.76 for bud stage). This value was 0.81 for the regrowth stage. The DegN of the silages was higher ($p < 0.05$) without additive (0.84) than with formic acid (0.80) and the DegN of the hay was markedly lower (0.66, $p < 0.05$) than with the original fresh forage. Whatever the forage studied, tN and NAN rumen fluid contents were high at 1 h or 2 h after feeding (from 0.458 $mg \cdot g^{-1}$ for hay to 0.813 $mg \cdot g^{-1}$ for fresh forage at the vegetative stage) and diminished rapidly up to 7 h after feeding except for the silages, for which the minimum content was observed 4 h after feeding. A part of the solubilised nitrogen remained as proteins at 1 h and 2 h after feeding for fresh lucerne at various stages of harvesting (from 0.187 $mg \cdot g^{-1}$ to 0.221 $mg \cdot g^{-1}$ at 1 h) while no protein could be seen in the rumen fluid after feeding of sheep fed silage (with or without preservative) or hay. The part of NAN escaping rumen degradation and transiting with the rumen fluid represented 7 to 11% of the nitrogen disappearing from the nylon bags placed in the rumen. There was little difference linked to the vegetation stage of the forage or its mode of conservation in particular. This content remained high for hay while its effective degradability (0.66) was much lower than for other forages (from 0.76 to 0.84).

lucerne / green forage / silages / hay / vegetation stage / protein degradation / rumen fluid composition

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Résumé — Dégradation dans le rumen des protéines d'un fourrage vert de luzerne récolté à différents stades de végétation et conservé sous forme d'ensilage ou de foin. L'importance du flux d'azote solubilisé dans le rumen pouvant contribuer au flux d'azote intestinal est mal connu. C'est pourquoi une étude a été conduite pour estimer simultanément en cinétique la disparition des matières azotées des sachets de nylon déposés dans le rumen et la teneur en différentes fractions azotées du jus de rumen (azote total (tN), azote ammoniacal (NH3-N), azote non ammoniacal (NAN)). Les mesures ont été effectuées sur 4 moutons alimentés avec différents fourrages de luzerne : luzerne récoltée en vert au 1er cycle, stades végétatif et bourgeonnement, et luzerne récoltée à 6 semaines de repousse à tiges au 2e cycle, 2 ensilages (conservés avec et sans acide formique) et un foin réalisé à partir du fourrage vert au stade bourgeonnement séché au sol par beau temps. La dégradabilité effective de l'azote estimée à partir de la méthode des sachets de nylon (DegN) diminue significativement ($p < 0,05$) avec le stade de végétation (0,80 pour le stade végétatif contre 0,76 pour le stade bourgeonnement) ; elle est de 0,81 pour les repousses. En comparaison avec le fourrage vert récolté au stade bourgeonnement, la DegN des ensilages est significativement plus élevée ($p < 0,05$; 0,84 pour l'ensilage sans acide formique et 0,80 pour celui avec acide formique) et la DegN du foin est fortement diminuée (0,66 ; $p < 0,05$). Quel que soit le fourrage étudié, les teneurs en tN et NAN dans le jus de rumen sont élevées au temps 1 h ou 2 h après le repas (de 0,458 $mg \cdot g^{-1}$ pour le foin à 0,813 $mg \cdot g^{-1}$ pour le fourrage vert au stade végétatif) et diminuent rapidement jusqu'à 7 h après le repas sauf pour les ensilages pour lesquels on observe une teneur minimum au temps 4 h. Une partie des matières azotées solubilisées reste sous forme de protéines à 1 h et 2 h après le repas pour le fourrage vert de luzerne aux différents stades de récolte (de 0,187 $mg \cdot g^{-1}$ à 0,221 $mg \cdot g^{-1}$ à 1 h) alors que l'on n'observe pas de protéines après le repas dans le jus de rumen des moutons ayant consommé de l'ensilage (avec ou sans conservateur) ou du foin. La part de l'azote non ammoniacal qui échappe à la dégradation dans le rumen et transite avec le liquide ruminal représente de 7 à 11 % de l'azote disparaissant des sachets de nylon déposés dans le rumen. Elle est peu différente selon l'âge du fourrage ou son mode de conservation en particulier cette teneur reste élevée pour le foin alors que sa dégradabilité effective (0,66) est beaucoup plus faible que celle des autres fourrages (de 0,76 à 0,84).

luzerne / fourrage vert / ensilage / foin / stade de végétation / dégradation des protéines / composition du jus de rumen

1. INTRODUCTION

The protein value of feeds for ruminants is based on an estimation of the quantity of dietary and microbial protein absorbed in the small intestine. Dietary nitrogen that escapes degradation in the rumen is therefore an important factor in determining the protein value.

In France, the in situ method [31] is used to predict the quantity of protein from dietary sources escaping degradation in the rumen. Because of a lack of data on forages, the feeding systems suggested by INRA [22] use fixed nitrogen degradability (DegN) for each of the most representative forage types, respectively fresh forages, hay, and silages with and without preservatives.

The in situ method assumes that the solubilised nitrogen is completely degraded into ammonia inside the rumen. Studies carried out on concentrates [7] have shown that part of the solubilised nitrogen is indeed transformed into ammonia after a meal but that a part could also remain as protein or non-ammonia nitrogen (NAN) and leave the rumen with the liquid phase.

We studied the degradation in the rumen of nitrogen from fresh lucerne forage, cut at various growth stages, (vegetative, bud stage and at the second growth cycle). For bud stage, forage was harvested as silage with or without preservatives and as hay. The objectives of the study were, firstly, to investigate the effect of the growth stage and of the various modes of conservation on the in situ degradation (nylon bag

procedure) of nitrogen in the rumen and, secondly, to establish whether a part of the solubilised nitrogen would remain long enough in the rumen as proteins, peptides and amino-acids, to escape ruminal degradation by transiting with the liquids.

2. MATERIALS AND METHODS

2.1. Forages

The study was carried out with fresh lucerne cut at two stages in the first growth cycle (60 cm high vegetative stage and at the end of the bud/flowering stage) and in the second growth cycle (stemmy regrowth) at 6-week regrowth after the end of the bud stage.

Two silages and one hay were made from the fresh lucerne cut at the bud stage. The fresh forage was ensiled with 19% dry matter in 4 m^3 experimental silos. One silage was without additives and the other with formic acid (800 $g \cdot kg^{-1}$) 5 $liters \cdot t^{-1}$. The hay was dried on the ground in good weather.

2.2. Animals and experimental design

The study was carried out on 4 Texel sheep weighing about 60 kg and fitted with a ruminal cannula. During the experimental periods, the animals were housed in individual pens and allowed free access to water and the salt block.

The animals were given the fresh forage or conserved forage in a chopped form. They were fed two meals per day ad libitum (10% refusal) at 09.00 h and 17.00 h.

Two series of measurements were conducted: the first during the spring /summer with the fresh forages at various growth stages (vegetative stage: May 1997, bud stage: June 1997 and stemmy regrowth: July 1997); the second during autumn with the conserved forages (silage without additive, silage with formic acid, then hay).

Each measurement period included a 2 week adaptation phase and 2 weeks for measurements: one week was used to measure the kinetics sampling of the ruminal fluid and ruminal content measurements and the second week was used to measure the in situ degradation kinetics. Forages placed in the bags were the same as in the diet. They were made at the end of the week of kinetic sampling and rapidly frozen to avoid modifications of forages (see Sect. 2.3).

In parallel, the digestibility of organic matter (OMD) was measured on another group of 6 intact sheep kept in metabolism cages. The measurements were carried on fresh forages and not on frozen forages because freezing at –20 °C modifies the characteristics of forages and their degradability [20, 26].

2.3. In situ degradation

Nitrogen degradability was measured using the nylon bag procedure, as described by Michalet-Doreau et al. [31]. Dacron bags (Ankom) (pore size 30–60 μm) with an internal surface of 5 × 11 cm and closed by two stitches were used. Forage samples weighed into bags were prepared according to Dulphy et al. [17]. Fresh forages and silages were cut into particle sizes of 4–5 mm length. Hay was ground to a mesh size of 4 mm. A quantity equivalent to 2.5 g dry matter (DM) was weighted into bags followed by incubation in the rumen of the four fistulated sheep fed the same forage. Incubation periods were 2, 4, 8, 16, 24 and 48 h. Two replications per sheep were used for 2, 4 and 8 h whereas three replications were used for 16, 24 and 48 h. A standard hay was incubated (8 h) daily in duplicate in the rumen of each of the animals used, in order to detect any changes in the level of degradation during the experiment. After the removal of bags from the rumen, they were kept at –20 °C until analysis. Prior to analysis, the bags were defrosted and then rinsed with cold water until the rinse water ran clear. The bags were then beaten for 7 minutes in a

"stomacher" [29], followed by further washing and finally dried at 60 °C for 48 h. Michalet-Doreau and Ould-Bah [30] showed that beating the residues in the bags in a stomacher can significantly reduce microbial contamination of the undegraded fraction of the sample. Nitrogen solubility without incubation in the rumen (T 0 h) was determined by soaking the bags containing the samples in warm water (40 °C) for 1 h 30 min, followed by drying as before.

2.4. Ruminal fluid sampling

Rumen fluid was taken from the same sheep on two consecutive days, before the morning meal (T 0 h), and 1, 2, 4 and 7 hours after feeding. 150 mL of rumen fluid were taken and muslin-filtered then centrifuged for 5 min at 120 *g* to remove dietary particles and protozoa. The supernatant was centrifuged at +4 °C and 27 000 *g* for 20 min in order to remove dietary particles and bacteria. Proteins were then precipitated by adding sulfosalicylic acid (400 $g \cdot L^{-1}$) and separated after centrifuging (20 000 *g* for 10 min).

2.5. Rumen content and digesta kinetics

Total reticulo-rumen contents were determined by manually emptying the rumen before the morning meal (08.30 h) and after the evening meal (19.00 h). Rumen emptying were carried out with an interval of at least 60 h to ensure normal digestion [1].

After emptying, rumen contents were weighed, homogenised and sampled for DM determination and then reintroduced in the rumen. The total procedure did not exceed 30 min [10].

A 200 mL dose of a Cr-EDTA solution [11] was injected into the rumen 2 h before morning forage distribution. Samples of rumen fluid (50 mL) were taken from 09.00 h on, over a 36 h period and stored at –20 °C until Cr concentration analysis. The fractional turnover rate of rumen fluid was calculated by adjustment of the Cr-dilution curve [39].

The daily fractional turnover rate (kl) (h^{-1}) of a given digesta fraction was calculated by dividing its daily intake by the mean rumen content of the fraction [21].

2.6. Chemical analyses

The total nitrogen (tN) content forages, bag residues and soluble nitrogen content of the rumen fluids (before and after precipitation with sulfosalicylic acid) were determined using the Kjeldahl method [4]. The protein contents in the rumen fluid were obtained as the difference between total nitrogen in the rumen fluid before and after precipitation. The NH3-N values were determined on the acid supernatant (after precipitation with sulfosalicylic acid) by the Conway method [13].

The fibre contents (NDF, ADF, ADL) were determined for the forages as proposed by Goering and Van Soest [19].

The fermentative characteristics of the silages were analysed (pH, NH3-N, soluble N, volatile fatty acids and lactic acid) [16].

2.7. Calculations and statistical analysis

The in situ dry matter and N disappearances of lucerne forages were fitted to the model of Ørskov and McDonald [32] using a non-linear regression procedure [36]:

$$\% \text{ N degraded} = a + b\,(1 - \exp^{-ct}).$$

The effective degradability of nitrogen DegN was calculated as:

$$DegN = a + (bc) / (c + kp)$$

assuming $kp = 0.06\ h^{-1}$ [27].

The same model was used to calculate the effective degradability of dry matter (DegDM).

The various degradation parameters for the dracon bags were analysed using

Table I. Chemical composition of lucerne forages.

	Fresh forage 1st growth vegetative stage	Fresh forage 1st growth bud stage	Fresh forage 2nd growth stemmy regrowth	Silage without additive	Silage with formic acid	Hay
CP ($g \cdot kg^{-1}$ DM)*	201.5	172.8	184.3	179.9	179.7	174.8
NDF ($g \cdot kg^{-1}$ DM)	408	489	459	431	416	528
ADF ($g \cdot kg^{-1}$ DM)	235	312	314	313	294	357
ADL ($g \cdot kg^{-1}$ DM)	65	79	71	70	64	82
OMD (%)	65.7	60.0	63.8	63.5	61.7	61.0

Dry matter (DM); Crude Protein (CP); neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL); organic matter digestibility (OMD).
* Oven dry matter corrected for volatile losses for silages [16].

variance analysis (SAS GLM procedure) [36] according to the following model:

$$Y = M + Ai + Tj + Eij$$

M: overall mean;
Ai: animal effect (df = 3);
Tj: stage of vegetation (vegetative, bud or regrowth; df = 2) or conservation effect (fresh at bud stage, silage with or without preservative, hay; df = 3);
Eij: residual error (df = 6 or df = 9), respectively.

Since no significant difference appeared between the two measurement days (Duncan test) [36] for the parameters measured in the rumen fluid (tN, NH3-N, NAN) the mean values of the two measurement days were analysed following the same model.

3. RESULTS

3.1. Chemical composition, digestibility and conservation quality of the forages

The fibre content of green lucerne forage (NDF and ADF) increased according to the maturity of the plant while the nitrogen content and OMD decreased (Tab. I). For the 2nd growth (stemmy regrowth), the values for nitrogen and fibre contents and the OMD were between those for the 1st growth vegetative and bud stages.

The NDF content of fresh forage (bud stage) was higher than for silages but the digestibility of the silages was slightly higher than that of the corresponding fresh forage. It is with hay cut on the same day as the silages that the expected OMD is the lowest, in accordance with the highest fibre content (Tab. I).

Lucerne silage without a preservative was of inferior quality to the one preserved with formic acid: the pH and acetic acid content, the NH3-N/tN and soluble N/tN percentages were higher (Tab. II).

3.2. In situ degradation

Fresh forages were compared, on the one hand, according to their vegetation stage at the 1st growth stage and at the 2nd growth stage (Tab. III), and on the other hand, for the same vegetation stage according to their mode of conservation (silage or hay) (Tab. IV).

The degradability of the dry matter of the fresh forage was significantly higher at the 1st growth vegetative stage ($p < 0.05$) than at the other stages of harvesting. DegN for fresh forage at the vegetative stage was significantly higher than at the bud stage

Table II. Fermentation characteristics of the silages studied (determined on the fresh silages).

	Silage without additive	Silage with formic acid
pH	4.48	4.03
NH3-N (% Nt)	12.86	7.62
Sol N (% Nt)	65.52	54.90
Acids ($g \cdot kg^{-1}$ DM)		
Lactic	97.36	45.60
Acetic	42.35	29.6
Propionic	1.11	0.3
Butyric	0	0
Alcohols ($g \cdot kg^{-1}$ DM)		
Methanol	3.52	2.50
Ethanol	4.98	3.03
Propanol	0.99	0.26
Butanol	0.04	0

($p < 0.05$) but no different from the 2nd growth stage results ($p > 0.05$). For nitrogen, modelling parameters (a, b, c) were significantly different between the vegetative stage and the 2nd growth stage on the one hand, and the 1st growth vegetative stage and bud stage on the other.

DegDM for fresh forage and the 2 silages were identical and significantly higher than for hay, whose b fraction was much higher ($p < 0.05$) and furthermore degraded at a low degradation rate.

DegN for the 2 silages were higher than that of fresh forage (from 4 to 8 points) while for hay it was 10 points lower than that of fresh forage. DegN for silage without an additive was significantly higher ($p < 0.05$) than for silage with formic acid, with a degradation rate c of the b fraction which was twice as high.

As for DegDM, DegN of hay was significantly lower (10 to 17 points) than for the 3 other forages since its b fraction was much higher and the degradation rate c, 2 to 4 times lower. On the contrary, the sum (a + b) was identical for the 3 preserved forages (0.86), and slightly higher than for the original fresh forage (0.83).

Table III. In situ degradation parameters for lucerne forages according to the stage of growth.

	Fresh forage 1st growth vegetative stage	Fresh forage 1st growth bud stage	Fresh forage 2nd growth stemmy regrowth	RSD
DM				
a	0.313^{a}	0.278^{b}	0.310^{a}	0.0043
b	0.459^{a}	0.428^{b}	0.409^{c}	0.0073
c	0.162^{a}	0.131^{b}	0.278^{b}	0.0126
DegDM	0.645^{a}	0.568^{c}	0.588^{b}	0.0053
N				
a	0.396^{c}	0.410^{b}	0.460^{a}	0.0029
b	0.476^{a}	0.421^{b}	0.418^{b}	0.0065
c	0.341^{a}	0.304^{b}	0.307^{b}	0.0188
DegN	0.800^{a}	0.761^{b}	0.810^{a}	0.0068

DM: dry matter; N: nitrogen; a: rapidly degraded fraction (%); b: slowly degraded fraction (%); c: rate of degradation (h^{-1}), Deg: degradability (%) a + (bc/(c + k)); RSD: residual standard deviation; differents superscripts in a same line correspond to a significant difference ($p < 0.05$).

Table IV. In situ degradation parameters for lucerne forages according to the conservation method.

	Fresh Forage 1st growth bud stage	Silage without additive	Silage with formic acid	Hay	RSD
DM					
a	0.278[c]	0.331[a]	0.301[c]	0.189[d]	0.0126
b	0.428[b]	0.370[c]	0.408[b]	0.533[a]	0.0159
c	0.131[b]	0.120[a]	0.114[a]	0.066[b]	0.0176
DegDM	0.568[a]	0.578[a]	0.568[a]	0.453[b]	0.0152
N					
a	0.410[b]	0.618[a]	0.601[a]	0.345[c]	0.0190
b	0.421[b]	0.251[c]	0.261[c]	0.517[a]	0.0152
c	0.304[b]	0.427[a]	0.214[c]	0.105[d]	0.0325
DegN	0.761[c]	0.838[a]	0.804[b]	0.664[d]	0.0171

DM: dry matter; N: nitrogen; a: rapidly degraded fraction (%); b: slowly degraded fraction (%); c: rate of degradation (h^{-1}), Deg: degradability (%) a + (bc/(c + k)); RSD: residual standard deviation; different superscripts in a same line correspond to a significant difference ($p < 0.05$).

3.3. Rumen fluid composition (total N (tN), non-ammonia N (NAN), ammonia N (NH3-N), protein nitrogen (protein-N))

As for the in situ degradation study, forages were compared according to the stage of growth for fresh forage (Fig. 1) and according to the conservation mode for the bud stage (Fig. 2).

The variation coefficient of tN content in rumen fluid varied from 3 to 20% respectively for the stage of growth and 6 to 27% for harvesting silage with or without preservative and hay. For NH3-N contents, the variation coefficient varied from 2 to 29% and 4 to 27%.

Rumen fluid content measurements for the various nitrogenous forms (tN, NH3-N, protein N) at T 0 h (before the meal) and 7 h after feeding were very similar whatever the type of forage.

For all fresh forages, whatever their harvesting stage, the tN content in rumen fluid was maximum 1 h or 2 h after feeding (0.633 $mg \cdot g^{-1}$ to 0.813 $mg \cdot g^{-1}$) and decreased up to 7 h after feeding (0.270 $mg \cdot g^{-1}$ to 0.325 $mg \cdot g^{-1}$). Among fresh forages the tN content was the highest ($p < 0.05$) 1 h and 2 h after feeding for the vegetative stage, but 4 h after feeding the contents did not vary significantly any more (Fig. 1).

NH3-N contents were at their maximum 2 h and 4 h after feeding (0.350 $mg \cdot g^{-1}$ on average for fresh forage at the vegetative stage) but there was not significant difference between forages although they were always slightly higher for fresh forages in the vegetative stage ($p > 0.05$) except 2 h ($p < 0.05$) after feeding. The NAN content was the difference between tN and NH3-N. The NAN content followed the same evolution over time as tN. It represented a large part of tN since it was between 50% and 60% 1 h and 2 h after feeding and between 34% and 46% 4 h and 7 h after feeding.

For all fresh forages, the NH3-N content in rumen fluid was maximum 2 h after the meal (0.321 $mg \cdot g^{-1}$ to 0.349 $mg \cdot g^{-1}$) then decreased up to 7 h after feeding (0.135 $mg \cdot g^{-1}$ to 0.183 $mg \cdot g^{-1}$).

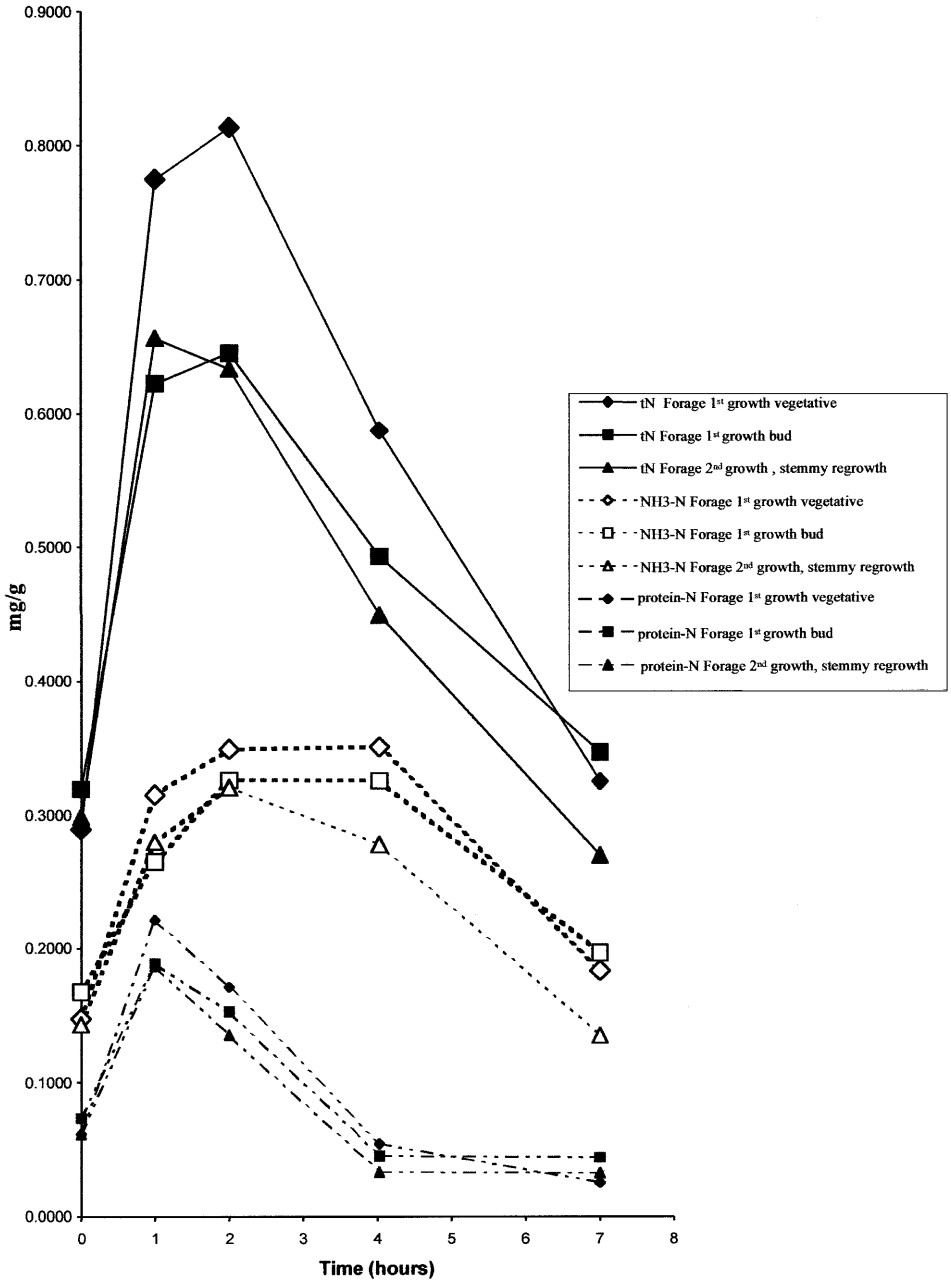

Figure 1. Evolution of the concentration ($mg \cdot g^{-1}$) of tN, NH3-N, Protein-N in the rumen fluid for lucerne fresh forages at different vegetation stages, before the morning meal (T 0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

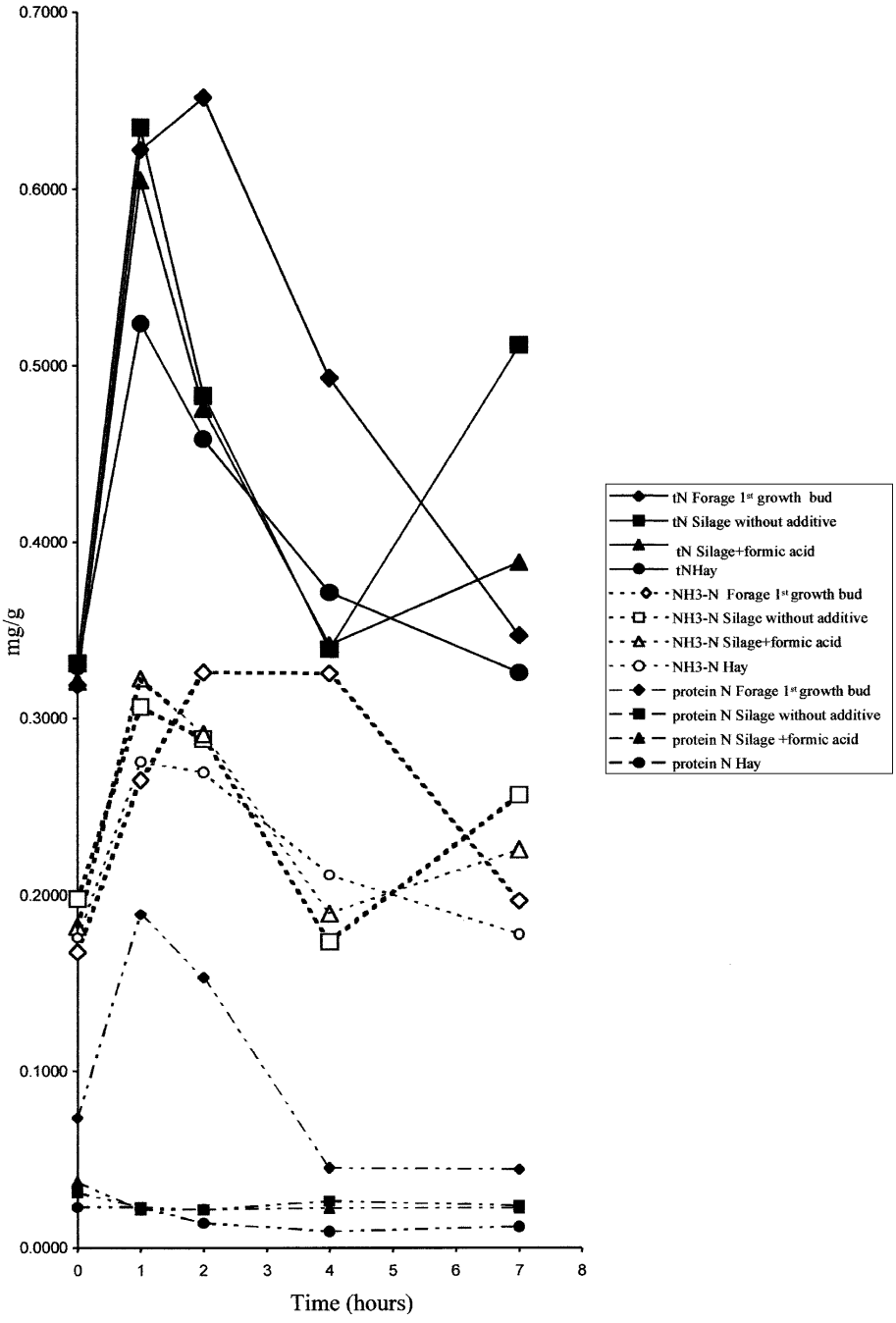

Figure 2. Evolution of the concentration ($mg \cdot g^{-1}$) of tN, NH3-N, Protein-N contents in the rumen fluid for lucerne fresh forage, silage without additive, silage+formic acid and hay before the morning meal (T 0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

1 h and 2 h after feeding, protein could be found in the rumen fluid (Fig. 1) for the three fresh forages cut at the various stages (from 0.187 $mg \cdot g^{-1}$ to 0.221 $mg \cdot g^{-1}$ 1 h after feeding). These proteins represented 20 to 30% tN 1 h and 2 h after feeding but none was found from 4 h after feeding onward.

The evolution of the concentration of the various nitrogenous forms (tN, NH3-N, NAN) were similar for the two silages. On the contrary to what was observed for fresh forages cut at the same stage, (Fig. 2), the rumen fluid showed a high content of these 3 parameters 1 h after feeding, a minimum amount 4 h after feeding and an increased content again at 7 h after feeding, which was higher than that at T 0 h (Fig. 2).

Although tN contents were lower than for fresh forages at all sampling times, the evolution of the tN content for hay was comparable with that of fresh forages (0.524 $mg \cdot g^{-1}$ at T 1 h and 0.326 $mg \cdot g^{-1}$ at T 7 h). NH3-N content was maximum 1 h and 2 h after feeding (about 0.270 $mg \cdot g^{-1}$) and decreased up to 7 h after feeding.

Proteins were not observed in the rumen fluid of sheep being fed hay or silages, but the NAN content remained high for all kinetic times since it was between 40% and 50% tN.

4. DISCUSSION

Concentration of crude protein (CP) in lucerne decreases with plant maturity because the leaf proportion decreases while the fibre content increases. Silages had lower NDF contents than corresponding fresh forages. Such differences have been previously observed by Dulphy and Demarquilly (unpublished results): hemicellulose is partly hydrolysed in the silo. Fermentative characteristics of lucerne silages with and without preservative were very comparable with the results obtained by Demarquilly and Jarrige [14] and to those found in the INRA tables [22].

The DegN for fresh forages and silages were close to those found by other authors (0.80 in Demarquilly and Jarrige's review [15], Amrane and Michalet-Doreau [2]) and to what we obtained previously [6]. The decrease observed for DegN with age agreed with the results of the bibliography [9, 24]. The a + b fraction decreased slightly during the growth stage, as observed by Antoniewicz et al. [3]. In accordance with other results [6, 23, 25], the immediately soluble fraction a of the silages was higher than for fresh forages while the fraction b was lower. The fraction a did not differ for the silages while the soluble N/tN was lower for the silage treated with formic acid. So, the DegN difference between silages was only due to the difference between the degradation rate which was twice as low for the silage treated with formic acid ($p < 0.05$).

In rumen fluid, the maximum value of tN was reached 2 h after feeding for the fresh forages and decreased up to 7 h while for the silages, there was a maximum value at 1 h, then a decrease at 4 h followed by an increase at 7 h. These results can be explained by a difference in the feeding behaviour of the sheep which eat several small meals if they are fed silage while they eat fewer larger meals when fed fresh forage or hay, as observed by Baumont et al. (unpublished results) on a group of sheep in parallel with the same forages as those studied here and previously in a comparison between hay and silage made from the same orchard grass forage [12].

The average NH3-N content in the rumen tended to decrease with the age of the plant which was consistent with the results of Kawas et al. [24]. It remained higher for fresh forages than for silages (except at 2 h), which was in agreement with the results obtained by Mc Donald and Edwards [28] and Flores et al. [18]. NH3-N content was nevertheless lower for hay as observed by Mc Donald and Edwards [28] and Vagnoni and Broderick [37]. However, a part of the solubilised nitrogen remained as protein-N

1 h and 2 h in the rumen after feeding of fresh lucerne, as we had observed on some single feeds [7, 8].

The same degradation profile of nitrogen fractions observed in rumen fluid for fresh lucerne cut at various stages, was in good accord with the modelling parameter (a, b, c) value.

On the contrary to sheep fed fresh forage, no true protein content in the rumen fluid of sheep fed silage was observed, since the proteins, especially ribulose 1–5 diphosphate carboxylase, had already been degraded in the silo; these results confirmed the ones we observed previously with lucerne [5]. As might be expected, for hay that degrades less and more slowly, nitrogen degrades progressively to NH3-N, peptides-N plus the amino-acid-N stage without any intermediate protein accumulation.

The NAN, which is the difference between tN and NH3-N, may have been surestimated. NAN in the rumen fluid can include other N forms as nucleic acids [33] that we find in low quantity [35]. We can also find other nitrogen forms (saliva N, endogenous N, microbial nitrogen).

We quantified the part of the NAN in the rumen fluid, which escapes degradation in the rumen and arrives in the small intestine in order to estimate the nutritional consequences (Tab. V). From our results of NAN content at different kinetic times, the NAN flow (Tab. V) was calculated as the area under the curve (extended to 12 h) multiplied by two, to take into account the evening meal. It was expressed as daily dry matter intake ($CP \cdot kg^{-1}$ $DM \cdot d^{-1}$). In the calculation of NAN flow, the mean volume of the liquid phase of the rumen and the fractional passage of the liquid phase were taken into account. The volume of the liquid phase of the rumen was calculated from the average of the measures carried out by emptying before the morning meal and after the evening meal for each of the forages (see Sect. 2.5). The fractional passage of the liquid phase (kl) was determined from the measurements carried out with the chromium-EDTA. DegN measured by dracon bags were calculated by taking into account either a 6% per hour for a fractional rate of the particles from the rumen for all forages [22]. The ratio between the quantity of NAN (calculation Tab. V) of the rumen fluid able to escape degradation in the rumen and the nitrogen degraded in the rumen enabled us to assess an underestimation of 7 to 11% of the nitrogen degraded in the rumen. This also amounted to modulating the 1.11 factor used for estimating the proportion of our estimates of dietary protein reaching the intestine, using the dracon bag procedure [38]. This factor calculated with our estimates of NAN flow varied from 1.20 to 1.47 (Tab. V). The ratio between the quantity of the NAN in the rumen fluid liable to escape degradation in the rumen and was 6.8 to 9.5% of the nitrogen degraded in the rumen.

The quantity of NAN able to leave the rumen varied very little in relation to the age of the forage or its mode of conservation. This content remained particularly high for the hay while its DegN (0.664) was much lower than for other forages (0.761 to 0.838). These results agree with those of Prange et al. [34] who carried out in vivo measurements and found an equivalent NAN flow at the level of the duodenum when the animals were fed silage lucerne hay.

5. CONCLUSION

The DegN of nitrogen in fresh lucerne decreased only slightly with the age of the forage (from 0.800 to 0.761). It was slightly higher for silages (0.838 and 0.804), but much lower for hay cut at the same stage (DegN = 0.664).

In rumen fluid, part of the solubilised nitrogen remained as proteins 1 h and 2 h after the meal for fresh forages at different stages of harvesting while no proteins were

Table V. Ratio between the amount of non-ammonia nitrogen (NAN) liable to escape degradation in the rumen and degraded protein for the different lucerne forages.

	Fresh forage 1st growth vegetative	Fresh forage 1st growth bud	Fresh forage 2nd growth stemmy regrowth	Silage without additive	Silage with formic acid	Hay
Crude Protein (CP) ($g \cdot kg^{-1}$ DM)	201.5	172.8	184.3	179.9	179.7	174.8
DM intake (DMI) ($kg \cdot d^{-1}$)	2.200	2.210	2.310	1.980	1.950	1.640
Effective degradability of nitrogen (DegN) (kp = 0.06 h^{-1})	0.800	0.761	0.810	0.838	0.804	0.664
Mean volume of liquid phase (L)	8.8	10.9	8.2	9.8	9.4	11.6
Daily digesta fractional turnover rates (h^{-1}) (kl)	0.094	0.091	0.103	0.090	0.091	0.069
NAN content ([NAN]) (g)[a]	5.460	4.640	4.560	4.900	4.000	3.960
NAN flow ($CP \cdot kg^{-1}$ DMI$\cdot d^{-1}$) expressed in g[b]	12.79	13.07	10.45	13.64	10.95	12.00
CP × DegN[c]	161.2	131.5	149.3	150.8	144.5	116.1
NAN/(CP × DegN) (%)	7.93	9.94	7.00	9.05	7.58	10.34
CP × (1-DegN)[d]	40.29	41.29	35.01	29.15	35.22	58.74
coeff = ((CP × (1-DegN) + NANflow))/(CP × (1-DegN))	1.32	1.32	1.30	1.47	1.31	1.20

[a] NAN content was calculated from our results of kinetics of rumen juice at 0 h, 1 h, 2 h,4 h, 7 h after the meal and extended to 12 h, then to 24 h.
[b] NAN flow = ([NAN]/DMI) × kl × 6.25 × mean volume of liquid phase.
[c] Degraded protein in the rumen.
[d] Undegraded protein in the rumen.

observed after a meal in the rumen fluid of sheep who had received silage (with or without preservative) or hay.

The part of NAN of the rumen fluid escaping degradation in the rumen was 7 to 11% of the nitrogen that disappeared from the dracon bags placed in the rumen.

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