Ann Zootech (1994) 43, 125-134

Original article

Comparison of *in situ* degradation of cell-wall constituents, nitrogen and nitrogen linked to cell walls for fresh lucerne and 2 lucerne silages

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(Received15 March 1993; accepted 20 October 1993)

Summary — *In situ* degradation was studied on fresh lucerne and 2 silages prepared from fresh forage, one without added preservative, and the other with formic acid. Dry matter degradability is comparable for the fresh forage (68.1%) and silage with formic acid (66.1%) and lower for silage without added preservative (58.3%). The differences of nitrogen degradability between the 3 forages are small, 84.6%, 85.1% and 89.3% for fresh forage, silage without additive and silage with formic acid, respectively. The nitrogen linked to cell-wall residues represents a low proportion and does not have a great effect on degradability. However, on average the nitrogen of NDF residue (NDFN) and the ADF residue (ADFN) were degraded in the same manner whatever the forage. Some of the nitrogen linked to ADF appeared to be made up of a glycoprotein (extensin) which was in part degraded in the rumen.

rumen / nitrogen / cell wall / degradability

Résumé — Comparaison entre la dégradation *in situ* des constituants pariétaux, de l'azote, et de l'azote lié aux constituants pariétaux d'un fourrage vert de luzerne et de 2 ensilages. *Nous avons étudié la dégradation en sachets de nylon d'un fourrage vert de luzerne et de 2 ensilages préparés à partir de ce fourrage vert, l'un sans conservateur et l'autre avec addition d'acide formique. La dégradabilité de la matière sèche est comparable pour le fourrage vert (68,1%) et l'ensilage avec acide formique (66,1%) et plus faible pour l'ensilage sans conservateur (58,3%). La dégradabilité* in situ *de l'azote est peu diférente (respectivement de 84,6%, 85,1%, 89,3% pour le fourrage vert, l'ensilage sans conservateur, l'ensilage conservé avec de l'acide formique). L'azote lié aux constituants pariétaux représente une proportion faible et ne peut donc avoir une incidence importante sur la dégradabilité des fourrages. L'azote lié aux résidus NDF se dégrade dans le rumen de façon à peu près équivalente pour les 3 fourrages. On observe également une dégradation de l'azote lié aux résidus ADF, dont une partie serait constituée d'une protéine, l'extensine partiellement dégradable dans le rumen.*

rumen / azote / constituants pariétaux / dégradabilité

INTRODUCTION

The amount of protein that reaches the small intestine of ruminants depends on the protein content of the feed and on its degradability in the rumen. Chemical transformations during silage making, in particular proteolysis, lead to changes in the nitrogen compounds. The purpose of this study was to determine the effect of silage making on nitrogen degradation and the cell-wall constituents in the rumen. *In situ* degradations of 2 lucerne silages, one without preservative and the other with added formic acid were compared with that of the corresponding fresh forage.

MATERIALS AND METHODS

Forages

The study was made on fresh lucerne cut at the bud stage of the first cycle and 2 direct cut silages prepared from this fresh forage: one without preservative, and the other containing formic acid (800 g kg^{-1}) at 5 l t^{-1}, at 17.6% dry matter. The lucerne was ensiled in small experimental silos (4 m^3). The chemical compositions of the silages are given in table I and the fermentation characteristics in table II.

In situ degradation

Theoretical nitrogen degradability was measured using the nylon bag technique standardised by Michalet-Doreau *et al* (1987). The bags (pore size 46 μ) and interior dimensions of 6 x 14 cm are closed by 2 stitches. They contain about 3 g freeze-dried samples ground into particles of 0.8 mm. The bags were incubated for 2, 4, 8, 16, 24 and 48 h in the rumens of 4 sheep fed the same forage as that in the nylon bag. After removal from the rumen they were kept at −15°C until analysis.

The zero point was obtained by immersing the bags in distilled water at 40°C for 1.5 h.

After defrosting, the bags were rinsed in cold water until it was clear. They were then beaten in a stomacher (mechanical procedure) for 7 min, an apparatus used by Merry and McAllan (1983) to separate bacteria from rumen contents. Michalet-Doreau and Ould-Bah (1989) showed that beating in a stomacher considerably reduces microbial contamination. The bags were dried at 60°C for 48 h. Nitrogen content was then determined by the Kjeldhal method.

Chemical analysis

The solubility (S) of nitrogen in the fresh forage was measured in a phosphate buffer at pH 6.9, according to the method of Durand in Vérité and Demarquilly (1978). The solubility values given in table I are expressed as the ratios of the

Table I. Chemical composition of the forages studied*.

	Fresh lucerne	*Silage without formic acid*	*Silage with formic acid*
CP	184.4	173.8	189.4
S	46.1	69.9	62.3
NDF	407	471	427
ADF	296	368	326
NDFN (%Nt)	10.9	11.1	9.4
ADFN (%Nt)	6.3	7.3	5.9

* Chemical composition: crude protein (CP g kg^{-1}); solubility (S); cell-wall content (g kg^{-1}): neutral detergent fibre (NDF); acid detergent fibre (ADF); N linked to NDF (NDFN), N linked to ADF (ADFN).

quantity of nitrogen solubilised in the buffer solution and the initial nitrogen content of the feed.

The cell-wall contents (NDF and ADF) were determined in forages and bag residues according to the technique of Van Soest (1963) and Van Soest and Wine (1967). The total nitrogen contents of the NDF and ADF residues were determined by the Kjeldhal method.

The digestibility of organic matter was estimated by the pepsin-cellulase method (Aufrère and Michalet-Doreau, 1983).

Calculations and statistical analysis

Nitrogen disappearance in the rumen was adjusted to an exponential model (Orskov and McDonald, 1979):

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

This model supposes 3 fractions in the forage: one rapidly degradable fraction (a), one with slower degradation (b) at a rate reducing exponentially (exp–ct) and one non-degradable fraction (100 – a – b). Parameter values a, b and c of this model were obtained by fitting the data using a nonlinear regression procedure, based on Marquardt's method, performed by the NLIN procedure of SAS (SAS Institute, 1985). By fixing particle turnover at 0.06/h, in the French PDI system (Vérité *et al*, 1987), forage degradability was calculated using the equation of Orskov and McDonald (1979) :

$$\text{Deg} = a + (bc)/(c + 0.06)$$

The same model was used to calculate the degradability of dry matter of NDF and ADF and nitrogen in the detergent fibre fractions (NDFN and ADFN). The conservation effect (fresh forage or silages) on N degradation was tested by an analysis of variance using the SAS GLM procedure (SAS Institute, 1985) with 2 main effects: conservation and animal. Conservation effect differences were separated by the Duncan's multiple range test when the effect was significant.

Table II. Organic matter digestibility and fermentative characteristics of the silages studied*.

	Silage without additive	*Silage with formic acid*
OMD	57.9	60.5
Dcell (%MS)	57.0	60.6
pH	5.8	4.3
N-NH_3 (%Nt)	23.7	11.3
Nsol (%Nt)	69.9	62.3
Acids (g kg^{-1} MS)		
Lactic	4.0	81.0
Acetic	39.9	34.4
Propionic	4.4	1.4
Butyric	76.9	2.2
Valeric	2.9	0.2
Caproic	9.9	0.3

* Organic matter digestibility (OMD), pepsin-cellulose digestibility (Dcell).

RESULTS

Forage chemical composition

The results in table I indicate that the lucerne silage with no preservative was of poor quality. Acidification was insufficient (pH 5.83) and lactic fermentation was largely dominated by butyric fermentation. The large amount of protein degradation was evident from the high NH_3-N and soluble N contents.

Formic acid improved preservation quality. Acetic, propionic, and butyric acid contents were lower than those in the silage without preservative, but nevertheless this second silage was only of moderate quality. NH_3-N, soluble N and volatile fatty acid contents remained excessive.

The results presented in table II indicate that the total nitrogen content of the silage without preservative (2.78%) was lower than that of the other 2 forages (2.93% and 3.03%) following loss of NH_3 during or after freeze-drying since the difference did

not exist when the fresh forage was sampled (2.96%). In contrast, the proportion of nitrogen in NDF and ADF was slightly higher. The cell-wall contents (NDF, ADF, ADL) of the silages, especially the one without preservative were higher than for the fresh forage. The differences in digestibility obtained by the pepsin-cellulase method between the 2 silages agreed with those measured on sheep (OMD and Dcell of 57.9% and 57.0% respectively for silage without preservative and 60.5% and 60.6% for that containing formic acid).

Degradation in nylon bags

Dry matter

Residual dry matter after 48 h was 20.5 ± 1.8, 26.1% ± 2.4, and 28.0% ± 2.1, for the fresh forage, silage with preservative and silage without preservative, respectively (fig 1). Generally, most of the dry matter which disappears does so during the first 24 h in the rumen. The degradability of dry matter (adjusted by the equation of Orskov and McDonald, 1979) was comparable for fresh forage (68.1%) and silage with preservative (66.1%) but lower for silage without preservative (58.6%) (table III). Fraction a was found in greater quantity in silage with preservative (+3 points compared with fresh forage and +7 points compared with the silage without preservative). Fraction b was of lesser importance but its rate of degradation was higher.

Nitrogen

The degradation of nitrogen was rapid during the first 8 h in the rumen but then decreased. After 48 h, the residual nitrogen content was very similar for the fresh forage and silage with preservative (6% ± 0.5 and 8% ± 0.7, respectively) and was slightly higher (11% ± 0.9) for the silage without preservative (fig 2). The differences of nitrogen degradability (Deg N) between the 3 forages were thus small but result from very different fractions a and b and rates of fraction b degradation (table III). The fraction a of silages with and without preservative was distinctly higher than that of the

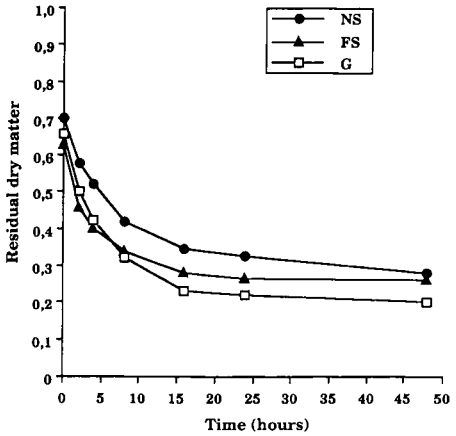

Fig 1. Variation of mean dry matter content of bag residues with time: 0, 2, 4, 8, 16, 24, and 48 h, for green forage (□), silage without preservative (●), and silage with formic acid (▲).

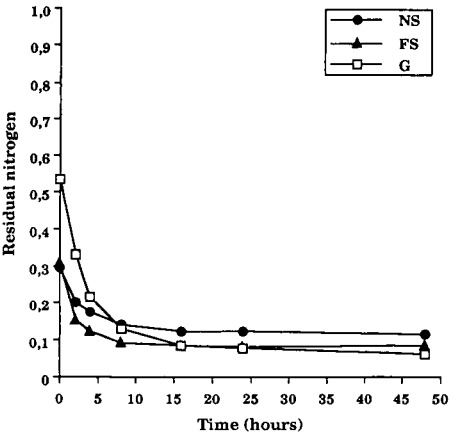

Fig 2. Variaton of mean nitrogen content of bag residues with time: 0, 2, 4, 8, 16, 24 and 48 h, for green forage (□), silage without preservative (●), and silage with formic acid (▲).

Table III. *In situ* degradation parameters for forages studied.

	Fresh lucerne	*Silage without formic acid*	*Silage with formic acid*
DM			
a	34.7[b]	30.5[c]	37.9[a]
b	44.2[a]	38.8[b]	35.1[c]
100 – (a+b)	21.1[b]	30.7[a]	26.9[ab]
c	0.191[ab]	0.162[b]	0.271[a]
Deg	68.1[a]	58.3[b]	66.1[a]
N			
a	46.4[c]	70.5[a]	69.4[b]
b	46.2[a]	17.3[c]	22.1[b]
100 – (a+b)	7.43[b]	12.2[a]	8.45[b]
c	0.291[b]	0.330[b]	0.537[a]
Deg	84.6[b]	85.1[b]	89.3[a]

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; different subscripts in a same column correspond to a significant difference ($P < 0.05$).

fresh forage (69.4% and 70.5% compared with 46.4%) and fraction b was smaller (22.1% and 17.3% compared with 46.4%) but was more rapidly degraded (0.537, 0.330 and 0.291 h).

NDF and ADF residues

The percentages of NDF and ADF degradation in the rumen were almost identical for all forages. There appeared very little difference between the fresh forage and the silages (fig 3).

The degradability of NDF (modelled by the equation of Orskov and McDonald, 1979) was comparable with that of ADF; it was also the same for silage with preservative (36.5% for NDF, 36.5% for ADF) and the fresh forage (37.7% for NDF, 35.2% for ADF) and smaller for silage without preservative. Fraction a was zero for fresh forage and the silage without preservative, and was very low for the silage with preservative which proved that loss of particles through the nylon bag pores was almost inexistent. Fraction b was high (56% for fresh forage, 50% for silage without preservative, and 49% for silage with formic acid) but was slowly degraded; rate c was the same for the degradation of NDF and ADF and for the 3 forages; it was relatively low (12–15% h^{-1}).

Nitrogen in the NDF and ADF

The nitrogen content of NDF and ADF of forages compared with total nitrogen were 10.9 and 6.3% for fresh forage, 9.4 and 5.9% for silage with preservative, 11.1 and 7.3% for silage without preservative. On average the nitrogen of the NDF residue and the ADF residue were degraded in the same manner whatever the forage. After 48 h incubation in the rumen the percentage degradation was slightly higher for the fresh forage (64 and 56%) than the silag-

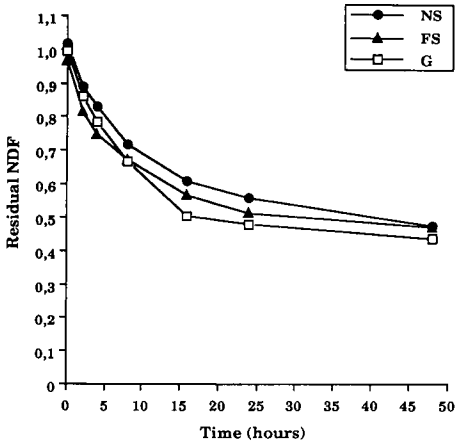

Fig 3. Variation of mean NDF content of bag residues after 0, 2, 4, 8, 16, 24 and 48 h, for green forage (□), silage without preservative (●), and silage with formic acid (▲).

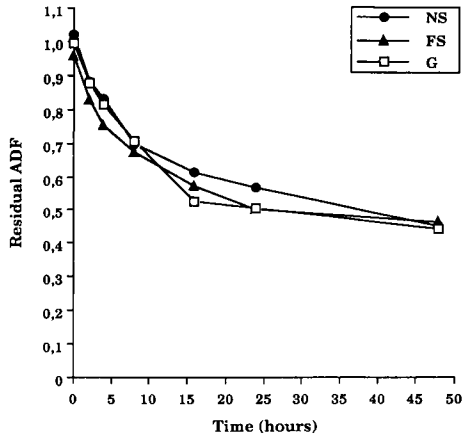

Fig 4. Variation of mean ADF contents of bag residues after 0, 2, 4, 8, 16, 24 and 48 h, for green forage (□), silage without preservative (●), and silage with formic acid (▲).

es, but also higher for the forage with preservative (53 and 47%) than the forage without preservative (48 and 51%) (fig 4). This gave a difference in the Deg of NDFN (40.9% for the fresh forage compared with 39% for the silage containing formic acid and 22% for the silage without preservative) and the ADFN (37.4% for fresh forage, 28% for silage with preservative and 15% for that without preservative).

DISCUSSION

During silage making, protein degradation and deamination led to an increase in soluble and non-protein nitrogen (Nocek and Grant, 1987; McDonald *et al,* 1991).

The high content of cell-wall constituents in silages, compared with that of fresh forages has also been observed by other authors (Demarquilly, 1973). This was due to loss of soluble compounds in neutral detergent, that was, in cytoplasmic constituents in liquids and gases during conservation, which led to a passive, relative increase in the concentration in cell-wall constituents (McDonald *et al,* 1991). This increase was amplified by the fact that, in this study, freeze-dried silages were used. This method of drying gave the least modification of silage composition especially nitrogen solubility. However, freeze-drying did cause a partial loss of volatile constituents: volatile fatty acids (VFA) and ammonia, giving a further passive increase in cell-wall constituents, in particular in the case of silage without preservative, rich in VFA and NH_3.

The dry matter degradation of silage with preservative was slightly lower than that of the original fresh forage. The slight difference (1.8 points of the Deg dry matter) could probably be explained by loss of volatile dry matter during freeze-drying. This dry matter was completely digestible. This almost identical degradation was normal since it was known that there was almost no reduction in digestibility of the dry matter or the organic matter between fresh

forage and well-conserved silage (Michalet-Doreau and Demarquilly, 1981). This was confirmed by the fact that the degradations and the Deg of NDF and ADF, for which the losses of volatile dry matter was not involved, were almost identical between the fresh forage and silage with preservative (respectively 37.5% and 36.4% for the Deg NDF, and 35.2% and 36.3% for the Deg ADF). The degradability or digestibility of cell-wall constituents determine that of the dry matter since the true digestibility or degradability of cell constituents (which can be considered as 100 – NDF) are very close to 1 (Jarrige, 1980; Aufrère *et al*, 1982).

In contrast, the degradation of dry matter in silage without preservative, which was of poor quality, was considerably lower than that of fresh forage: 9.5 points for the Deg of dry matter. This difference was increased by loss of volatile dry matter during freeze-drying, which was quite considerable for the silage, but such losses do not allow for the whole difference since there were also differences between the cell-wall degradation of the fresh forage and this silage (respectively 37.6% and 31.1% for Deg NDF, and 35.2% and 32.1% for Deg ADF). Moreover, the digestibility of the organic matter of this silage measured *in vivo* was lower than that of the silage with preservative of only 2.6 (57.9 *versus* 60.5%).

The *in situ* degradability of nitrogen (Deg N) of fresh forage was 84.7%. It was generally a little higher than most data in the literature, except for the results of Le Goffe (1991) on freeze-dried fresh forages. This present value was, nevertheless, in agreement with the mean degradation measured *in vivo*, which might be estimated at 80% from the review of Demarquilly and Jarrige (1982) on fresh forages.

The mean, Deg N of silages was 85.2% for the silage without preservative, very similar to that of fresh forage, but was higher for silage containing formic acid (89.3%). This difference between the 2 silages is surprising, but it might be explained in part by the loss of NH_3 during freeze-drying, for the silage without preservative. In addition, since the samples were decontaminated with a stomacher, the proportion of residual microbial nitrogen was proportional to the quantity of residual dry matter; it was thus slightly higher for the silage without preservative. Janicki and Stallings (1988) obtained comparable Deg values for lucerne silages (79.5% and 84.3%). The degradability of silage without preservative was probably underestimated since, as has already been noted, part of the ammonia was lost during conservation and this fraction would have disappeared very quickly in the rumen. Rooke *et al* (1983) found no differences in degradability between silages with and without preservative.

Thus, there was little difference between the Deg N of fresh forages and silages, as was also observed by Tamminga (1982) and Lopez *et al* (1991). These results did not agree with those of Ould Bah (1989) who found Deg N of lucerne silages lower than that of the fresh forage. This reduction must certainly result from the fact that these forages were dried at 80°C.

In addition, the present results agree with those of Janicki and Stallings (1988) in that the immediately soluble fraction (a) is much higher for silages than for the fresh forage, with a corresponding reduction in fraction b. The sum a + b remains the same, as underlined by Lopez *et al* (1991), although it is difficult to give a biological significance to these parameters.

For the fresh forages, the soluble nitrogen in the NDS solution was largely constituted of cytoplasmic and chloroplastic proteins which were rapidly degraded in the rumen. The most important protein was rubisco (ribulose 1–5 diphosphate carboxylase) which was rapidly solubilized in the

rumen (Mangan, 1982), as confirmed by results of electrophoretic profiles obtained on bag residues for the fresh forage (Aufrère *et al,* submitted) but this protein was also rapidly degraded in the silo to non-protein nitrogen.

Finally, the differences in protein degradability between fresh forage and silages could result mainly from the protein linked to cell walls, which was only slightly and slowly degraded. This protein fraction, which was not dissolved in neutral detergent, was, according to Sanderson and Wedim (1990), made up of the nitrogen linked to the ADF fraction, the nitrogen of extensin, a glycoprotein rich in hydroxyproline which is found only in primary walls, and also the nitrogen from proteins denatured by heat (Maillard reactions).

The nitrogen of cell walls also represents small a part of the total nitrogen (less than 11.0% for that in the NDF and less than 7% for that in the ADF) of the forages studied to have any important effect on the degradability of total proteins. This is especially true because its degradability was not very different between the 3 forages since it was largely related to the degradability of the cell-walls (the nitrogen content in the residues varied little according to deviation in the rumen), also showing little difference between forages.

The disappearance of nitrogen of the NDF residue in the nylon bags with time agreed with the results of Van Soest (1982) and Sanderson and Wedim (1989). The nitrogen of the NDF residue was partly degradable in the rumen, whereas the nitrogen in ADF residue was constituted largely by nitrogen linked to lignin and proteins denatured by heat and was not degradable (Van Soest, 1982). In contrast, in the present results, it may be noted that the ADF-linked nitrogen was partly degraded, as was observed also by Sanderson and Wedim (1990). Similarly, Mason (1969) and Goering *et al* (1973) observed a 40–63% degradation of ADF nitrogen over the whole gut.

Measurements of hydroxyproline (an amino acid that constitutes 50% of extensin) carried out on ADF residues (Aufrère *et al,* 1994) showed that the content of this amino acid in nylon bags was reduced with time for the 3 forages studied. This could partly explain the degradation of ADF protein with time.

CONCLUSION

The degradability of protein in lucerne forages measured with nylon bags does not differ greatly between the fresh forages and silages with and without preservative, because the protein degraded in the silo is the same as that degraded rapidly in the rumen. Protein linked to NDF residues, which represents a low proportion (about 10% of total N), is degraded in the rumen in a similar manner for the 3 forages. Protein linked to ADF residues is also partly degraded in the rumen and appears to be made up of a glycoprotein (extensin).

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