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Original article

Study of modes of preparation of fresh and conserved forage samples for measurement of their dry matter and nitrogen degradations in the rumen

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Abstract — Six trials were conducted to study the conditions of preparation and treatment of moist forage samples for the in situ measurement of their ruminal dry matter and nitrogen degradations. The following preparations were compared in five trials: Trial I on fresh forages: lacerated fresh before bagging and freezing in liquid nitrogen and then stored at –20 °C, forage dried at 60 °C and ground to 0.8 mm, forage dried at 80 °C and ground to 0.8 mm, forage dried at 60 °C and ground to 4 mm. Trial II on lacerated fresh forages: put immediately in bags then in rumen, frozen in liquid nitrogen and used immediately, frozen in liquid nitrogen and stored at –20 °C, freeze dried then stored at –20 °C. Trial III on silages: lacerated silage, frozen in liquid nitrogen and then stored at –20 °C, silage dried at 80 °C and ground to 0.8 mm. Trial IV on hays: undried ground to 12 or 4 mm, dried at 80 °C and ground to 0.8 mm. Trial V: after ruminal incubation, bags beaten or not with a 'stomacher' after washing and before oven-drying. In trial VI, particle losses through the bag pores were measured. The main objective of all these trials was to evaluate a mode of sample preparation of moist materials (fresh and silage) in two steps: processing in a 'universal mill' to particles in about 5 mm length, bagging and rapid freezing of the bags in liquid nitrogen. There was no difference between fresh forage placed immediately in the rumen, fresh forage frozen in nitrogen and placed immediately in the rumen, and the same stored and then used several months later. Drying, even at 60 °C, lowered effective nitrogen degradability against moist forage; drying at 80 °C lowered it by 10 points ($P < 0.01$) (1 point = 1 % on a scale from 0 to 100). Freeze-drying had a weak negative effect (–3.1 points; $P < 0.05$). The nitrogen degradability of hays increased with decreasing particle size (+7.7 points, $P < 0.01$, from 12 to 0.8 mm mesh size). Beating with a 'stomacher' is useful for reducing microbial contamination of bag residues (increasing nitrogen degradability by +4.3 points, $P < 0.05$, for a poorly digestible forage, but only +1 point [not significant] for a digestible one). Finally, losses of particles through bag pores were low, 1.3 % of used dry matter. The mode of preparation tested is suit-

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able for the study of nitrogen degradation of moist forages in the rumen. It is therefore recommended that such moist forages be used directly or after freezing in liquid nitrogen without either oven-drying or freeze-drying. (© Elsevier / Inra)

rumen / in situ / protein degradation / forages / sample processing

Résumé — Études méthodologiques sur la préparation des échantillons de fourrage humide destinés à la mesure in situ de la dégradation ruminale de leurs matières azotées. Une série de 6 essais a été effectuée pour étudier les conditions de préparation et de traitement des échantillons de fourrage humide destinés à être placés dans le rumen pour mesurer la dégradation en sachets de nylon de leur matière sèche et de leurs matières azotées. Les traitements suivants ont été comparés : Essai I sur fourrages verts : fourrage frais, lacéré, mis en sachets plongés dans l'azote liquide et conservés à –20 °C ; fourrage séché à 60 °C, broyé avec une grille de 0,8 mm et mis en sachets ; fourrage séché à 80 °C, broyé avec une grille de 0,8 mm puis mis en sachets ; fourrage séché à 60 °C, broyé avec une grille de 4 mm. Essai II sur fourrages verts : fourrage frais, lacéré, mis en sachets déposés immédiatement dans le rumen ; fourrage frais, lacéré, mis en sachets plongés dans l'azote liquide et placés immédiatement dans le rumen ; la même préparation, avec conservation des sachets à –20 °C ; fourrage lacéré puis lyophilisé. Essai III sur ensilages : ensilage frais, lacéré, mis en sachets plongés dans l'azote liquide et conservés à –20 °C ; ensilage séché à 80 °C, broyé avec une grille de 0,8 mm. Essai IV sur foins : foin non séché, broyé avec une grille de 12 mm ou de 4 mm ; foin séché à 80 °C, broyé avec une grille de 0,8 mm. Essai V : sachets battus ou non au stomacher après leur lavage et avant leur passage à l'étuve. Enfin, dans un Essai VI les pertes en particules à travers les mailles des sachets ont été mesurées. Le principal objectif de tous ces essais était de tester l'intérêt d'une préparation des échantillons humides (verts et ensilages) en deux temps : lacération, avec un appareil multi-broie-tout, en particules d'environ 5 mm, mise en sachets et congélation rapide des sachets pleins dans de l'azote liquide. Il n'y a pas de différence entre le fourrage frais mis directement dans le rumen, le fourrage frais plongé dans l'azote liquide et mis dans le rumen immédiatement ou plusieurs mois plus tard. Le séchage, même à 60 °C, fait diminuer la dégradabilité de l'azote par rapport au fourrage de départ. La diminution est de 10 points pour le séchage à 80 °C. La lyophilisation a un léger effet négatif (–3,1). La dégradabilité de l'azote des foins augmente quand le diamètre de la grille du broyeur diminue (+7,7 points en passant de la grille de 12 mm à celle de 0,8 mm). Par ailleurs le battage au stomacher est utile pour diminuer la contamination microbienne des résidus de sachets (soit un effet de +4,3 points pour un fourrage peu digestible et pauvre en azote, mais seulement +1 point pour un fourrage digestible et riche en azote). Les pertes de particules à travers les mailles des sachets ont été très faibles, égales à 1,3 % de la MS mise en sachets. Finalement le mode de préparation testé semble adéquat pour l'étude de la dégradation de l'azote des fourrages humides dans le rumen. Il est donc proposé d'utiliser directement ou après congélation dans l'azote liquide les fourrages humides sans les sécher, que ce soit dans une étuve ou dans un lyophilisateur. (© Elsevier / Inra)

rumen / dégradation de l'azote / fourrages / préparation des échantillons

1. INTRODUCTION

The in situ measurement of food degradation in the rumen is widely used to predict the nutritional value (via effective degradability of dry matter and nitrogen) and ingestibility of forages (as reviewed by Michalet-Doreau and Ould-Bah [15] and Huntington and Givens [5]). The degradation kinetics depend on numerous factors, making it necessary to standardize the method (size and porosity of bags, fineness of grinding, procedure for washing the bags, animal feeding, etc.) as proposed by Michalet-Doreau et al. [16] for dry feeds. However, standardization with moist forages still presents problems, particularly that of sample preparation.

It has long been known that oven-drying tends to lower nitrogen degradability,

increasingly so as the oven temperature is raised [1, 3, 12, 20]. Some authors recommend a maximum temperature of 60 °C when samples are dried for grinding [20]. Huntington and Givens [7] recommend 65 °C as a maximum because of modifications of chemical composition by drying. However, even at 60 °C, drying reduces nitrogen degradability, although this reduction may be compensated for by fine grinding [20]. Kabuga and Darko [9] found no difference in nitrogen degradation after drying at 45 °C and grinding to 2 mm, or freezing after chopping four tropical grasses to 2–5 mm. Kamoun and Thewis [11] claim freeze-drying is the best preparation method, the closest to using raw forage, but the freeze-dried samples should not be ground [22].

The influence of particle size on effective nitrogen degradability of forages has not been widely studied. Comparing three grinding sizes (mesh sizes of 0.8, 3 and 6 mm), Michalet-Doreau and Cerneau [14] observed a reduction of the degradability of nitrogen with increasing mesh size for a cocksfoot hay, but no change for a lucerne hay. Aumont et al. [2] reported reduced nitrogen degradability in one grass and two tropical legumes when the mesh size is increased from 0.5 to 1 and then to 2 mm.

Ideally, 'chewed' moist forage should be placed in the bags [7], but this is very difficult to do in practice. We considered that a close approximation to this ideal situation might be achieved by chopping and lacerating moist forage simultaneously in a 'universal mill' to obtain particles with a length of 4–5 mm, but with a small diameter (1–2 mm). This mode of preparation was compared with others described in the literature, using different drying methods (oven- or freeze-drying), different oven-drying temperatures, and different particle sizes of the samples. The choice of modes of preparation was limited by both the availability of literature data and our own resources. However, in three cases, the mode of forage preparation used for the measurement of dry matter digestion of forages [4], namely drying at 80 °C followed by grinding to 0.8 mm mesh size, was compared with others.

After residence in the rumen, the bacterial contamination of the forage residues was evaluated for deduction using the method proposed by Ould-Bah et al. [21], involving beating the bags in a 'stomacher', with correction for the microbial dry matter (4 % of residue for a ruminal residence time > 24 h) not detached by the beating [19]. The 'stomacher' treatment is applied after freezing the bags at –15 °C, as the freezing-thawing sequence already detaches a large proportion of the bacteria [10]. An additional test was therefore conducted to measure the specific effect of the 'stomacher'.

Finally, the main objective of this work was to evaluate modes of sample preparation of moist materials (fresh and silage) in two steps: processing in a 'universal mill' to simulate chewing, bagging and rapid freezing of the bags in liquid nitrogen.

2. MATERIALS AND METHODS

Five trials were conducted to compare different modes of forage sample preparation.

2.1. Trial I: fresh forages 1996

In this first trial, two fresh forages were used: a hybrid rye-grass (*Lolium perenne* x *Lolium multiflorum*) and a perennial rye-grass in their first cycle at the vegetative stage. Four preparations were studied:

Mode A: fresh forage frozen in liquid nitrogen and stored at –20 °C.

Mode B: forage dried at 60 °C and ground to a mesh size of 0.8 mm.

Mode C: forage dried at 80 °C and ground to a mesh size of 0.8 mm.

Mode D: forage dried at 60 °C and ground to a mesh size of 4 mm.

Fresh forages were cut in the field at 8.00 hours. For mode A, the fresh forage was lacerated twice

in a 'universal mill' (grinder-chopper Law MS 3-1 with knives) to reduce the particle size to 4–5 mm in length and 1–2 mm in diameter (simulation of chewing). It was then immediately placed in bags (2.5 g of dry matter per bag, i.e. 12–16 g of fresh forage). The filled bags were frozen at about 11.00 hours in liquid nitrogen and stored at –20 °C for 4–8 months until used. For modes B, C and D, the fresh forage was dried for 48 h, ground, and placed in bags. For each forage, the bags corresponding to the four modes of forage preparation were placed in the rumen at the same times.

2.2. Trial II: fresh forages 1997

In this second trial, two forages were used: a lucerne and a natural grassland in their first cycle at the vegetative stage. Four preparations were also compared:

Mode E: fresh forage.

Mode F: fresh forage frozen in liquid nitrogen.

Mode A: as mode A in trial I.

Mode G: freeze-dried forage.

For mode E, the forage was processed in the 'universal mill' as in A, placed fresh in bags, and the bags placed immediately in the rumen. For mode F, the bags were prepared as for mode E, but frozen in liquid nitrogen before being placed immediately in the rumen. For mode G, the fresh forage, prepared as in A, E and F, was placed in bags that were then frozen in liquid nitrogen, stored for 2 h in a deep freezer (–20 °C) and, finally, freeze-dried for 70 h. The samples were then stored in this form for 4–8 months. Because the bags could not be studied synchronously on the cows, six other bags containing a control forage (lucerne hay) were therefore systematically introduced along with those containing experimental forages and withdrawn after 8 h., so that, if necessary, results could be corrected between series to make them comparable.

2.3. Trial III: silages 1996

The forages used in this trial were an ensiled hybrid rye-grass without preservative and an ensiled perennial ray-grass with formic acid, at first cycle, with 10 % of the ears at emergence. Two modes of preparation were compared:

Mode H: raw silage prepared with the mode A of the fresh forage.

Mode I: silage dried at 80 °C and ground as in mode C. Mode I is used classically to measure dry matter degradation. The bags for modes H and I were studied in the same series.

2.4. Trial IV: hays 1996

Two forages were studied: a hybrid rye-grass and a perennial ray-grass, at first cycle, with 10 % of the ears at emergence. Three preparations were studied:

Mode J: undried hay ground to a mesh size of 12 mm.

Mode K: undried hay ground to a mesh size of 4 mm.

Mode L: hay dried at 80 °C and ground as in mode C.

For modes J and K, the forage was not processed in the 'universal mill', but ground directly in a hammer mill fitted with the required mesh. Mode L was comparable to modes C and I. The bags corresponding to these three modes were studied in the same series.

2.5. Trial V: effect of stomacher 1997

From two forages, a natural grassland hay, undried and ground to a mesh size of 4 mm (preparation equivalent to mode K), and a lucerne silage (preparation identical to mode A), two series of 72 bags were prepared for ruminal incubation during 4, 8, 24, 48 and 72 h. After incubation in the rumen, the bags were, as usual, quickly washed and frozen at –20 °C. After thawing, all the bags were washed and half of them were beaten in the stomacher and washed again. Finally, all the bags were dried at 60 °C for 72 h.

The forages in trials I, II and III were different, but trials are in progress to study the effect of vegetation stage and cycle of growth of fresh forages and the effect of ensilage and hay-making.

2.6. Trial VI

In trial VI, a study of particle losses through the bag pores was made with two fresh forages processed as proposed (particles of 4–5 mm in length). For that purpose, bags containing processed forage were washed as explained in later and particles were recuperated on filter.

2.7. Degradability measurement

In situ nitrogen degradation was measured following the method of Michalet-Doreau et al. [16], except for the grinding size and the drying temperature, which were subject to study. Incubation times were 0, 2, 4, 8, 16, 24, 48 and 72 h. The bags were always placed at 08.00 hours, before the morning meal (except for the '16.00 hour incubated' bags, placed before the evening meal at 16.00 hours) in the rumen of three different cows in two successive periods. In all, six bags per incubation time and per treatment were studied. On withdrawal from the rumen, all the bags were washed promptly in cold water and frozen. After thawing, the bags incubated in cows or not ('0.00 hour incubated'), were washed in a washing machine (four 3-min runs), then beaten in a stomacher for 7 min as recommended by Ould-Bah et al. [21], and washed again (two 5-min runs). The dry matter contents of the silages were corrected to make up for volatile components lost during the oven-drying [5]. The nitrogen contents of the residues were corrected to deduct the adhering microbial nitrogen [19].

2.8. Chemical analysis

The following values were determined on forages placed in the bags:

- dry matter (48 h, 80 °C)
- residue after ashing at 550 °C (ashes)
- crude fibre by the method of Weende
- nitrogen by the Kjeldahl method
- volatile fatty acids and alcohols [8] and lactic acid [17] for silages to correct their dry matter [5]

On the residues from bags pooled for each residence time ($n = 6$), the dry matter weight was measured. The nitrogen content after drying at 60 °C for 72 h and very fine grinding was then determined.

2.9. Data processing and statistical analysis

Two types of results were considered: raw data, i.e. degradation of dry matter and nitrogen, and fitted data, i.e. parameters of the degradation curves fitted to an exponential model according to Ørskov and McDonald [18]:

- a: initial intercept = immediately soluble, rapidly degradable fraction
- b: potentialy degradable fraction
- c: rate of degradation of b

The curves were fitted with the nlin procedure of the Statistical Analysis System (SAS) [23].

For raw results concerning dry matter, the statistical analyses, performed with the SAS software, comprised analysis of variance to compare the treatments applied. Total degrees of freedom (d.f.) were, respectively, 335, 335, 167, 251 and 71, for trials I to V. The 'animal' effect (2 d.f.), 'forage' effect (1 d.f.), 'time' effect (6 d.f.) and 'treatment' effect were evaluated and the standard deviations for the degradations were calculated for each residence time and each treatment (six numbers per point).

The same was done for results concerning nitrogen, but with a lower total d.f. (respectively, 63, 55, 31, 47, and 23), without 'animal' or 'period' effects.

Taking into account the parameters a, b and c, an effective degradability in the rumen (DMDeg for dry matter, NDeg for nitrogen) was then calculated for a rate of passage of 0.06 per h [16].

For fitted results, the total d.f. values were low (7 for the first two trials), so the effect of treatments was only evaluated for these first two trials.

3. RESULTS

The chemical characteristics of the forages used are given in *table I*. The forages were different in each trial. Hence, the results of each trial will not be compared.

3.1. Dry matter degradability (DMDeg)

The results are given in *table II*. Each value corresponds to the average of two forages. On average, the initial intercept at 0 h (fraction a) and the real initial degradation (after washing, without residence in the rumen) were very close (+1.45 points for the real; 1 point = 1 %, on a scale from 0 to 100). The greatest discrepancies here were for the fresh forage dried at 80 °C and ground to 0.8 mm (+3.2), the hay ground to

Table I. Characteristics of used forages.

Trial	DM content g/1 000	g·kg^{-1} DM			%
		Ashes	CF	CP	OMD in vivo
Trial I: fresh forages					
Hybrid RG - vegetative	112	140	259	171	78.6
Perennial RG - vegetative	135	141	250	194	80.3
Trial II: fresh forages					
Lucerne - vegetative	171	162	236	222	65.7
Natural grassland - vegetative	197	105	302	174	69.2
Trial III: silages					
Hybrid RG	157	95	309	128	73.9
Perennial RG	194	80	307	119	70.5
Trial IV: hays					
Hybrid RG	869	97	382	70	56.3
Perennial RG	875	88	343	67	59.3
Trial V:					
Natural grassland hay	877	61	386	86	50.2
Lucerne silage	206	102	298	168	63.3

RG: rye grass; DM: dry matter; CF: crude fibre; CP: crude protein; OMD: organic matter digestibility.

4 mm (+2.8), and the hay ground to 0.8 mm (+2.9), i.e. the samples most liable to lose small particles during washing of the bags. This results from a short lag time of ruminal attack. The average initial degradation rate was 2.2 points/h.

The average effective DMDeg was 58.8 (range, 40–72), corresponding to the degradation observed after 12.5 h, but which varied between trials: 10.9 h in trial I, in which the DMDeg was high; 13.8 h in trial IV, in which DMDeg was low. In addition, the sum of parameters a + b of the fitted curves was barely greater than the degradation observed at 72 h (+1.8).

The drying of the samples, even accompanied by grinding (trials I and III), lowered DMDeg (–3.5 for 60 °C/4 mm and 80 °C/0.8 mm; $P < 0.05$). The DMDeg was closest to that of fresh frozen forage (–1.7 points; not significant) after drying at 60 °C and grinding to 0.8 mm. This effect of oven-drying is explained, for fresh forages, by a significant decrease of parameter c, whereas for the silages, parameter a was decreased.

In comparison to fresh samples directly introduced in the rumen, fast freezing in liquid nitrogen and freeze-drying did not modify DMDeg in the samples (trial II). Retarding introduction in the rumen had a positive though very slight effect (1.9 points; $P < 0.05$).

Although the drying of hay probably had little effect on dry matter degradability, grinding (trial IV) had a marked effect on DMDeg (+6.8 between 12 and 0.8 mm; $P < 0.01$) through a marked lowering of parameter a.

The effect of the stomacher after washing off the bags (trial V) was small (+0.8 point; not significant).

Table II. Degradation of dry matter (%).

Trial	Treatments	Hours							Effect of treatments*	Standard deviation by treatment	Effective DMDeg	a	b	c	a + b
		0	4	8	16	24	48	72							
I –	Fresh frozen stored[A]	39.9	53.5	69.5	81.3	88.3	93.2	93.7	a	1.98[a]	72.2[a]	38.7[a]	55.4[b]	0.0918[a]	94.1
	Dried 60 °C, 0.8 mm[B]	42.1	51.4	63.5	78.8	88.4	92.9	93.8	b	1.71[a]	70.5[ab]	40.4[a]	54.7[b]	0.0730[b]	95.1
	Dried 80 °C, 0.8 mm[C]	39.8	47.9	60.6	79.0	88.4	93.4	94.4	bc	1.91[a]	68.8[b]	36.6[b]	59.4[a]	0.0718[b]	96.0
	Dried 60 °C, 4.0 mm[D]	38 6	48.6	61.5	77.2	86.4	92.5	93.6	c	1.70[a]	68.4[b]	36.7[b]	58.0[a]	00729[b]	94.7
II –	Fresh[E]	36.2	46.2	57.7	–	73.0	77.5	80.3	a	1.71[a]	60.6[b]	35.1[a]	44.7[a]	00862[a]	79.8
	Fresh frozen[F]	35.5	46.7	57.1	–	73.3	77.7	79.4	a	1.74[a]	60.6[b]	34.5[a]	44.9[a]	0.0901[a]	79.4
	Fresh frozen stored[A]	37.4	48.7	58.1	68.3	75.5	82.0	84.1	b	1.51[a]	62.5[a]	36.9[a]	47.8[a]	0.0778[b]	84.7
	Freeze-dried[G]	36.6	45.7	56.0	68.1	75.0	81.3	84.0	b	1.48[a]	61.4[ab]	35.6[a]	48.8[a]	0.0741[b]	84.4
III –	Fresh silage[H]	42.0	51.1	57.4	70.2	80.7	89.6	91.8	a	2.19[a]	65.7	41.4	52.5	0.0520	93.9
	Dried silage 80 °C, 0.8 mm[I]	36.7	44.7	52.6	68.5	79.3	88.9	91.0	b	1.98[b]	62.3	35.1	58.3	0.0525	93.4
IV –	Hay 12 mm[J]	18.7	23.3	28.8	44.2	55.7	67.9	72.5	a	1.92[a]	40.1	16.2	61.3	0.0384	77.5
	Hay 4 mm[K]	23.3	25.9	32.7	46.4	58.1	69.2	74.1	b	2.06[a]	43.0	20.5	58.4	0.0375	78.9
	Hay dried 80 °C, 0.8 mm[L]	27.5	30.2	37.6	51.4	61.1	72.6	76.3	c	2.03[a]	46.9	24.6	55.9	0.0405	80.5
V –	Without stomacher	24.6	33.0	39.9	–	57.7	67.1	70.9	a		46.1	24.0	49.1	0.0588	73.1
	With stomacher	24.6	34.1	40.7	–	58.2	68.3	72.1	a		46.9	24.5	49.8	0.0582	74.3
Standard deviation by incubation time		1.35	1.62	2.43	3.48	2.00	1.20	1.22							

* Treatment with a same letter are not statistically different ($P < 0.05$).
In the first seven columns are the degradations measured in nylon bags (raw data) with signification of the effect of treatments; in the last five columns are fitted data (a, b, c from an exponential model). Effective dry matter degradability (DMDeg) is calculated by the formula: eff deg: $a + [(b \times c) / (c + 0.06)]$.

Finally, putting fresh forage in the bags instead of dried ground forage had no significant effect on the variability of the results (*table II*). This variability for the fresh silage was, however, significantly wider than that of the same forages that were dried, but the standard deviations were close. Overall, the standard deviations were low (average of 1.8), except for the 16-h incubation time, probably because the bags were then placed in the rumen at a time when the range of microbial activities between days and between cows is widest.

3.2. Nitrogen degradability (NDeg)

The results are given in *table III* and *figures 1* to *4*. On average, the fitted and real initial degradations were very close: +0.6 point for the real value, which corresponds to a lag time close to zero, the slope of the degradation curve being 2.7 points·h^{-1} at the start.

The average effective degradability (NDeg) was 75.6 (range, 50–87), corresponding to the real degradation after 10.7 h (8 h for the early fresh forage, 12 h for the coarsely chopped hays).

Lastly, the sum of parameters a + b (92.5) was equal to the real degradation at 72 h (92.2), which was already 91.1 at 48 h.

Drying the samples (trials I and III, *figures 1* and *3*) always lowered effective NDeg, the grinding not making up for the observed diminution. NDeg was closest to that of frozen fresh forage (–2.3; not significant) after drying at 60 °C and grinding to 0.8 mm.

Fast freezing in liquid nitrogen did not modify NDeg (trial II; *figure 2*). Retarding introduction of the bags also had no effect. In contrast, freeze-drying tended to lower NDeg (–3.1; $P < 0.05$), essentially by lowering the rate of degradation c of fraction b.

The effect of grinding, studied in trial IV on hay (*figure 4*), was appreciable: an increase in NDeg of 7.7 points ($P < 0.01$) when the mesh size ranged from 12 to 0.8 mm, essentially due to the increase in fraction a. Conversely, for high NDeg forages (trial I), there was barely any difference between mesh sizes 4 and 0.8 mm after drying at 60 °C (+0.8 point).

Lastly, the effect of the stomacher was appreciable for the hay, which had a poor nitrogen level: an increase of 4.3 points ($P < 0.05$), but weak for the silage, in which the nitrogen level was higher: + 1 point (not significant).

3.3. Losses of particles out of the bags

The weight of particles found on the filters was, respectively, 1.33 and 1.23 % of the dry matter introduced in the bags.

4. DISCUSSION

It is important to know the effect of modifications of the NDeg value in the calculation of the nitrogen value. First, the a value has a marked effect: a is about 63 % of effective NDeg. In addition, a, b, and c are interrelated; for example, if a increases, b decreases. The value of k has been set at 0.06 (value for dairy cows), but if NDeg has to be calculated for sheep, a value of 0.045 is better and effective Deg will be higher. However, our objective here was to compare different modes of preparation of forage samples. To do so, some PIA values (dietary protein undegraded in the rumen) were calculated and are given in *table IV*. PIA values were greatly modified by drying at 80 °C (+19 g·kg^{-1} dry matter), which gives an important error: the animals receiving fresh forages and not dried forages. Any modification of sample preparation has some effect: +4 g with drying at 60 °C, +5 g with freeze-drying, +3 g between particles of 4 and 0.8 mm, +4 g if the stomacher was used. Thus, the effect of mode of preparation of the sample has to be taken into account.

Measurement of the degradation kinetics of fresh forages should ideally be made

Table III. Degradation of nitrogen (%).

Trial	Treatments	Hours								Effect of treatments*	Effective NDeg	a	b	c	a + b
		0	2	4	8	16	24	48	72						
I –	Fresh frozen stored[A]	45.5	60.4	67.9	86.7	95.0	96.3	97.8	97.5	a	84.0[a]	44.9[ab]	53.0[ab]	0.1686[a]	97.9
	Dried 60 °C, 0.8 mm[B]	50.8	63.1	66.2	78.3	91.5	95.4	97.0	97.2	a	81.7[a]	51.2[a]	46.5[b]	0.1148[b]	97.7
	Dried 80 °C, 0.8 mm[C]	40.9	45.1	49.5	65.6	87.6	93.7	96.6	97.0	b	72.8[b]	36.6[b]	62.3[b]	0.0866[b]	98.9
	Dried 60 °C, 4.0 mm[D]	47.2	60.2	63.4	78.5	91.9	95.3	97.0	97.3	a	80.9[ab]	47.2[ab]	50.5[ab]	0.1201[b]	97.7
II –	Fresh[E]	53.7	67.1	71.7	80.6	–	90.3	91.5	92.7	a	81.6[a]	54.8[a]	37.0[b]	0.1622[a]	91.8
	Fresh frozen[F]	53.7	68.2	72.1	80.7	–	90.5	92.0	93.3	a	82.0[a]	55.2[a]	37.0[ab]	0.1624[a]	92.2
	Fresh frozen stored[A]	56.6	66.6	71.1	80.6	–	91.1	93.1	94.0	a	81.9[a]	57.0[a]	36.4[b]	0.1333[ab]	93.4
	Freeze-dried[G]	53.2	61.5	65.3	76.1	–	90.0	92.6	93.8	b	78.5[b]	53.3[a]	40.2[a]	0.1016[b]	93.5
III –	Fresh silage[H]	72.3	77.1	81.5	87.6	93.9	95.3	96.1	96.7	a	88.7	72.0	24.6	0.1305	96.6
	Dried silage 80 °C, 0.8 mm[I]	59.0	62.7	66.8	73.7	88.9	93.3	94.2	95.2	b	80.1	57.0	39.5	0.0847	96.5
IV –	Hay 12 mm[J]	22.3	32.0	37.7	45.9	64.4	73.3	80.6	83.0	b	55.4	22.7	60.8	0.0695	83.5
	Hay 4 mm[K]	33.6	40.2	40.4	49.0	70.0	78.6	82.8	85.4	a	60.2	31.5	55.3	0.0649	86.8
	Hay dried 80 °C, 0.8 mm[L]	39.5	43.1	41.0	51.9	74.6	81.3	86.5	86.2	a	63.1	35.0	54.3	0.0680	89.3
V –	Hay without stomacher	25.4	–	36.1	43.3	–	65.7	70.9	75.1	a	50.6	25.0	50.1	0.0626	75.1
	Hay with stomacher	25.4	–	41.1	48.1	–	69.6	77.0	80.9	b	54.9	26.3	53.9	0.0680	80.2
	Silage without stomacher	70.7	–	82.6	86.1	–	91.0	91.3	92.2	a	86.5	70.9	20.6	0.1878	91.5
	Silage with stomacher	70.7	–	83.1	86.9	–	92.3	93.2	93.8	a	87.5	70.9	22.2	0.1763	93.1

* Treatment with a same letter are not statistically different ($P < 0.05$).
In the first seven columns are the degradations measured in nylon bags (raw data) with signification of the effecty of treatments; in the last five columns are fitted data (a, b, c from an exponential model). Effective degradability is calculated by the formula: eff deg: $a + [(b \times c) / (c + 0.06)]$. NDeg: nitrogen degradability.

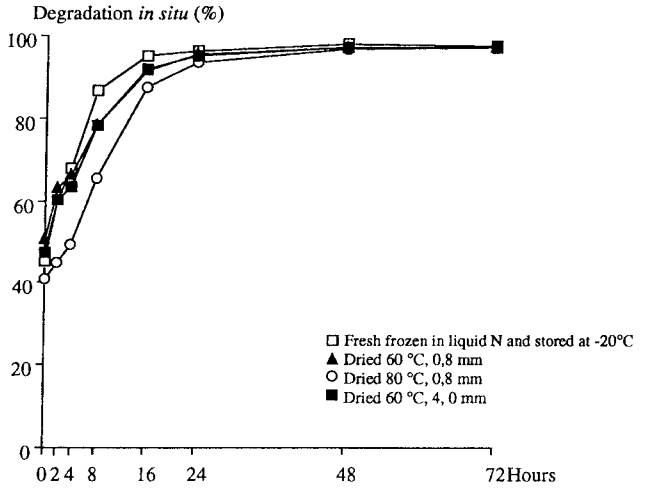

Figure 1. Degradation in situ of nitrogen (N) in trial I.

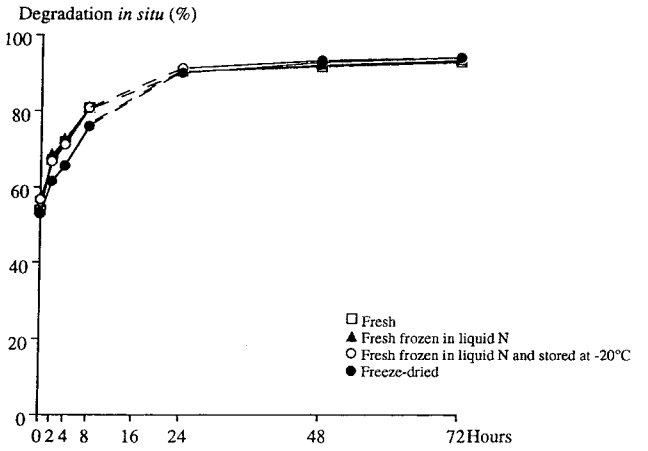

Figure 2. Degradation in situ of nitrogen (N) in trial II.

on fresh forage, but this is difficult because the measurements would have to be concomitant with sampling. It is therefore useful to have a mode of sample preparation that allows measurements to be deferred so they can be made together on animals in controlled conditions. However, such sample preparation must not appreciably modify the degradation kinetics. For silage, concomitance of sampling and measurement may be less of a problem, but it is still difficult.

If only the DMDeg of fresh forages is wanted, various modes of preparation are possible: forage frozen in liquid nitrogen and stored at –20 °C or forage dried at 60 °C and ground to 0.8 mm. For silage, only freezing is recommended, because oven-drying, even at low temperatures, causes losses of volatile compounds. Beating with a stomacher is superfluous for both fresh forage and silage.

However, the picture is different if NDeg is also needed. Our results confirm the effect of oven-drying on degradation kinetics and in situ nitrogen-effective degradability. Although the discrepancies are not always significant, the highest NDeg values were

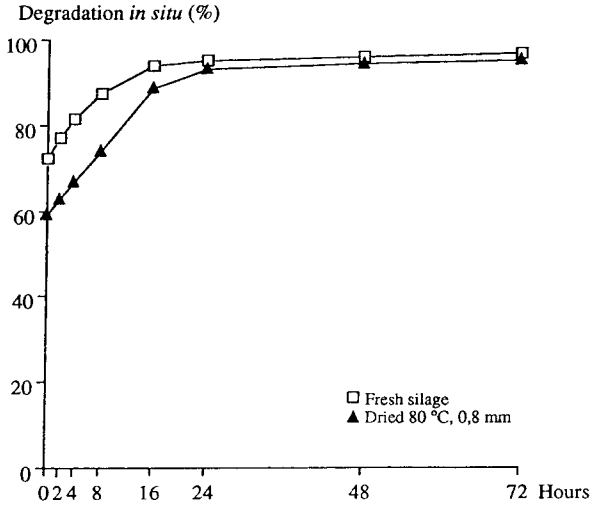

Figure 3. Degradation in situ of nitrogen (N) in trial III.

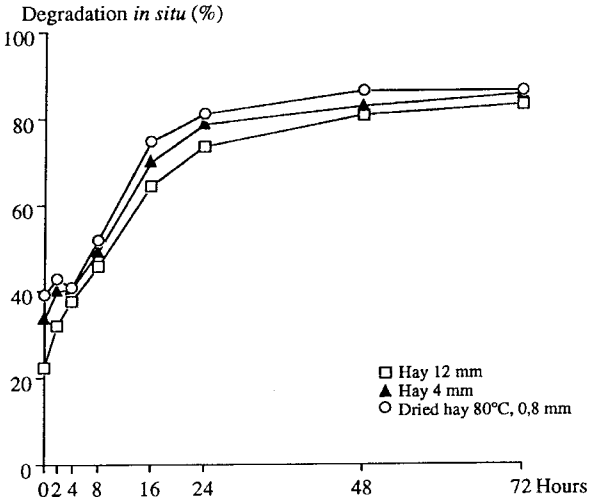

Figure 4. Degradation in situ of nitrogen (N) in trial IV.

observed with fresh forage. Even drying at 60 °C had an adverse negative effect, although it was partly compensated for by fine grinding, as indicated by Ould-Bah and Michalet-Doreau [20]. Drying, and particularly at 80 °C, appreciably slowed nitrogen degradation up to 24 h (*figure 1*).

Freeze-drying of forages processed in the 'universal mill' slightly lowered NDeg. In the literature, after grinding to the same size, the nitrogen degradability tended to be higher after freeze-drying than after oven-drying [12, 20, 24]. Comparisons between freeze-dried and fresh forages were made by Kamoun and Thewis [11] and gave the same results as in our trial.

Classically, the reduction of particle size of samples put in the bags increased nitrogen degradability. The fineness of grinding probably increases the attack surface area of the particles, but also favours fine particle loss through the bag pores. For this reason,

Table IV. Some results of PIA obtained with effective nitrogen degradability (NDeg) obtained in different situations.

	Crude protein g·kg^{-1} MS	Effective Ndeg × 100	PIA g·kg^{-1} MS
Fresh	150	83	28.3
Dried 60 °C, 0.8 mm	150	80.7	32.1
Dried 80 °C, 0.8 mm	150	71.8	46.9
Fresh	150	83	28.3
Freeze-dried	150	80	33.3
Hay 60 °C, 4 mm	90	60	40.0
Hay 60 °C, 0.8 mm	90	63	37.0
Hay with stomacher	90	50.6	49.3
Hay without stomacher	50	54.9	45.0

PIA: "proteines alimentaire entrant dans l'intestin", i.e. dietary proteins undegraded in the rumen entering in the small intestine. PIA = 1.11 × CP × (1 – NDeg), where CP = crude protein.

Michalet-Doreau and Ould-Bah [15] recommended using a mesh size no smaller than 3 mm. In our trials, a verification made elsewhere with two fresh forages gave particle losses through the bag pores equivalent to 1.3 % of the dry matter introduced, which is very low. This last value can be compared to those of Ould-Bah [19]: 6 to 9 % for samples ground using a mesh size of 1.5 mm.

The drying temperature and the fineness of grinding therefore have opposite effects, and therefore, by simultaneously varying these two parameters, representative fresh forage can probably be simulated at any given stage. However, these parameters vary with the type of forage and its growth stage, and thus are probably not easy to handle.

The rapid freezing of fresh forage in liquid nitrogen had no effect on the ruminal degradation kinetics compared with unfrozen fresh forage. This is probably because in liquid nitrogen, cells are not destroyed. Results in the literature concerning freezing are conflicting. For MacRae [13], extractable nitrogen is considerably reduced, but after classical freezing, due to a breakdown of the vacuolar membrane. For this author this leads to mixing of vacuolar and cytoplasmic contents, with resulting precipitation of protein at the lower vacuolar pH (precipitation probably aided by grinding before analysis). In addition, the samples are not introduced in the rumen. For Hristov [6], freezing leads to an increase of nitrogen degradability in the rumen, probably because of bursting of the cells of forages, allowing a more rapid release of cell contents. These problems are probably not important for silages (cells already destroyed), but the use of liquid nitrogen guarantees a representative sample without loss of volatile components. This is, therefore, an excellent method for studying fresh forages, particularly as several months storage at –20 °C did not modify the results. It remains to be determined how closely processing in the 'universal mill' models the effects of ingestive mastication. Even so, this mode of preparation offers the advantage of affording a thorough homogenization of the forage, allowing measurements as accurate as those obtained with dry ground samples, but probably without loss of particles from the bags; grinding is known to lead to loss of particles from the bags in the rumen

The method used to measure in situ dry matter degradation rate [4] is, therefore, not advocated for effective nitrogen degradability evaluation. With this mode of preparation, degradability of fresh forages is underestimated, and that of hays overestimated.

Moreover, our results confirm that processing of in situ degradation residues with a 'stomacher' is useful with forage containing poorly and slowly degradable nitrogen (high CWC), but has a weak effect when the nitrogen is rapidly degradable (low CWC).

The preliminary effect of freezing after rumen residence, then thawing is probably important for detaching bacteria [10], but this is integrated in the propositions of Michalet-Doreau et al. [16] (freezing, thawing, beating in a stomacher, and correction for estimated undetached bacteria) described in the thesis of Ould-Bah [19].

5. CONCLUSION

To study the in situ degradation of nitrogen in moist forages, these should be used directly without drying. Mechanical processing can be used to simulate chewing and for homogenisation of the sample. Freezing in liquid nitrogen and storage at –20 °C give excellent results. Processing of residues with a 'stomacher' before drying is advisable. The method proposed by Michalet-Doreau et al. [16] for dry feeds should therefore be modified as regards the preparation of fresh forage samples.

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