Original article

Effect of maturity stage of Italian rye grass and lucerne on ruminal nitrogen degradability

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Summary — Ruminal N degradability of Italian rye grass and lucerne was studied using the nylon bag technique: 5 samples of rye grass and 5 of lucerne were harvested during the 1st cycle of growth at different stages of maturity, between the leafy stage and the end of heading for rye grass and between the vegetative stage and the end of budding for lucerne. For grass, degradability values were high at the beginning of growth, with a mean of 72.2% in the period from leafy stage to heading, but they decreased rapidly after this stage to 64.6% (of 1.1%/d). For lucerne, the decrease in N degradability was approximately linear between the vegetative stage (79.0%) and the end of budding (70.0%), a decrease of 0.3%/d. After correction to take into account microbial contamination of bag residues, the reduction of N degradability with stage of maturity appears smaller, ie 5% for rye grass and 4% for lucerne.

in situ **degradability / nitrogen / fresh forage / maturity stage**

Résumé — **Influence du stade de végétation sur la dégradabilité** *in situ* **de l'azote dans le rumen d'un ray-grass Italien et d'une luzerne.** *La dégradabilité de l'azote dans le rumen de 2 fourrages était étudiée en utilisant la technique des sachets de nylon : un ray-grass Italien et une luzerne en étaient récoltés respectivement à 5 stades de maturité différents, du stade feuillu au stade fin de l'épiaison pour le ray-grass et du stade végétatif à la fin du bourgeonnement pour la luzerne. La dégradabilité de l'azote du ray-grass était élevée en début de végétation, soit en moyenne 72,2% entre le stade feuillu et l'épiaison, puis elle diminuait rapidement après l'épiaison à 64,6% (soit 1,1% / j). La dégradabilité de l'azote de la luzerne diminuait de façon presque linéaire entre les stades végétatif (79,0%) et fin du bourgeonnement (70,0%), soit une diminution de 0,3% / j. Après correction pour prendre en compte la contamination microbienne des résidus de sachets, la diminution de la dégradabilité de l'azote avec l'âge du fourrage est plus faible, soit 5% pour le ray-grass et 4% pour la luzerne.*

dégradabilité **in situ / azote / fourrage vert / stade de végétation /**

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INTRODUCTION

Most methods used to measure the nitrogen (N) values in ruminant feeds are based on estimation of the quantity of amino acids that can be absorbed by the small intestine. These amino acids have 2 possible origins: dietary or microbial. The feed N which escapes rumen degradation is one of the major elements in the determination of feed N value. *In vivo* measurement of dietary N degradation in the rumen is laborious and alternative methods microbiological, chemical and enzymatic have been sought (Lindberg, 1985; Nocek, 1988). Among these different methods, the nylon bag technique is the most commonly used (Madsen and Hvelplund, 1985; Nocek, 1988). This technique allows the description of the kinetics of dietary N degradation in the rumen (Orskov and McDonald, 1979). The extent of dietary N degradation in the rumen is determined by the rate of degradation of feed N and the rumen outflow rate of undigested N (Orskov and Mc Donald, 1979).

A considerable amount of data is available on N degradability of concentrates (Madsen and Hvelplund, 1985; Demarquilly *et al*, 1989; Tamminga *et al*, 1990). Recently, studies on fresh forages have increased, especially concerning variations of N degradability according to stage of maturity, but the results are incomplete (Van Eys and Reid, 1987), or it is difficult to compare them. Some trials do not take into account the microbial contamination of residues (Le Goffe, 1988; Van Vuuren *et al*, 1990). Omitting microbial contamination results in an overestimation of the decreasing effect of maturation on ruminal protein degradability. In other studies, only 2 stages of maturity are studied (Michalet-Doreau, 1990; Vik-Mo, 1989), and it is not possible to describe the changes in N degradability with stage of maturity.

Therefore, in this study, the variations in *in situ* N degradability of 2 forages have been examined during the first growth cycle, the results being corrected or not, taking into account the microbial contamination.

MATERIALS AND METHODS

Forages

The forages were grown at the Forage Research Station at Oued Smar in Algeria. The climate is subhumid with mild winters and hot summers. Average rainfall is 672 mm per yr. Rye grass received nitrogen fertilization, 60 kg N ha^{-1}, before first cutting. The samples of the same forage originated from the same plot.

Five samples of Italian rye glass (*Lolium multiflorum*) and 5 samples of lucerne (*Medicago sativa*) were cut during the first growth cycle at different stages of maturity between March 7 – May 2, 1990. The first sample corresponded to the leafy vegetative stage for rye grass and lucerne respectively, and the last to the end of heading and budding. Moving height was ≈ 6 cm; then samples were dried at 60 °C for 48 h and ground through a 0.8 mm screen.

In situ *measurements*

In situ measurements of rumen degradation was carried out using 3 dry Holstein cows fitted with a rumen cannula. They received 7 kg DM grass hay and concentrate (70/30) per animal per day, divided over 2 equal meals given at 8 and 17 h. The chemical composition of the concentrate has been described by Cerneau and Michalet-Doreau (1991). After 3 wk adaptation of animals to the diet, nylon bags (internal dimensions: 6 x 11 cm, pore size 46 μm), containing 3 g of forages, were all placed in the rumen at the same time, before the first meal of the day, and the removed after 2, 4, 7, 17, 24 or 48 h of incubation. After incubation, the bags were washed in cold water for 6 min (3 successive baths of 2 min), then dried in a forced air oven at 80 °C for 48 h. There were 6 measurements for each point in time (3 cows x 2 replications).

Chemical analysis

The nitrogen content of the forages and of the bag residues after incubation was determined by the Kjeldahl method, and the cell wall constituents (NDF, ADF, ADL) were determined in the forages (Goering and Van Soest, 1970).

Calculations

Nitrogen disappearance in the rumen was adjusted to an exponential model (Orskov and McDonald, 1970),

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

This model supposes 3 fractions in the forage: one rapidly degradable fraction (a), one with slower degradation (b) at a rate reducing exponentially (e^{-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 (Vérité and Peyraud, 1989), forage degradability can be calculated by the following equation:

$$\% \text{ degrated N} = a + bc \,/\,(c + 0.06)$$

The microbial contamination of bag residues can induce a important bias on the measurement of nitrogen degradability. To take into account this microbial contamination, we estimated the error (ER) related to contamination from chemical composition of forages (Michalet-Doreau and Ould-Bah, 1989), according to the formula:

$$ER = 6.4 - 0.035 \, CP + 0.017 \, NDF$$

The crude protein (CP) and neutral detergent fiber (NDF) content of forages were expressed in g per kg DM.

Statistical analysis

The effect of stage of maturity on N degradation was tested for each forage by an analysis of vaiance using the SAS GLM procedure (SAS Institute, 1985) with 2 main effects: stage and animal. Vegetation stage differences were separated by the Duncan's multiple range test when the effect was significant.

RESULTS

The chemical composition of the forages is given in table I. The crude protein content decreases with vegetation stage from 15.0 to 8.6% DM for rye grass and from 28.8 to 19.5% for lucerne. The cell wall constituents content increased with vegetation stage in both forages.

Data on *in situ* N degradability are given in table II. At a similar vegetation stage, N lucerne degradability was always higher than that of rye grass, and means were respectively 73.5 and 70.7%. The higher degradability of lucerne was essentially due to higher degradation rate of legumes (table II). For both forages, there was an effect of stage of maturity on forage N degradability. For grass, a high value occurred at the beginning of growth, 72.2% on average from leafy stage to heading, but this decreased quickly between heading and end of heading by 7.6% (1.1% / d). The rapidly degraded fraction increased as the forage became more mature, values ranged from a mean of 19.9% at the vegetative stage to 39.2% at the late stages. This variation was compensated by a decrease in degradation rate and an increase in undegradable fraction. After heading, only the degradation rate continued to decrease, and consequently the N degradability also decreased. For lucerne, the decrease of N degradability was approximately linear between the vegetative stage (79.0%) and the end of budding (70.0%), corresponding to a decrease of 0.3% / d. This variation was related to a significant decrease in the rapidly degraded N fraction from 34.3 to 20.0% and of N

Table I. Chemical composition of forages.

Maturity stage	Cutting date (1990)	DM (%)	Ash	CP	NDF (g/kg DM)	ADF	ADL
Italian ray-grass							
Leafy stage	7 March	23.3	91	150	437	179	25
Leafy stage	13 March	27.5	105	140	454	196	27
Grazing stage	18 April	25.7	111	118	472	203	28
Heading	2 May	30.0	118	104	469	219	35
End of heading	9 May	33.6	119	86	524	274	51
Lucerne							
Vegetative	21 March	19.7	105	288	365	204	54
Vegetative	2 April	25.4	101	235	391	229	56
Early budding	9 April	25.8	96	214	442	273	83
Budding	17 April	26.3	96	204	442	273	76
End of budding	24 April	28.2	89	195	462	284	95

Table II. *In situ* N degradation of forages.

Maturity stage	Rapidly degraded fraction (% N)	Slowly degraded fraction (% N)	Undegraded fraction (% N)	Degradation rate/h	Uncorrected N degradability (% N)	Corrected N degradability (% N)
Italian ray-grass						
Leafy stage	20.6[a]	74.8[b]	4.7[a]	0.142[a]	72.9[a]	81.4[a]
Leafy stage	19.2[a]	76.8[a]	3.7[a]	0.140[a]	73.1[ab]	82.3[a]
Grazing stage	38.9[b]	50.6[b]	10.5[b]	0.110[b]	71.6[ab]	81.8[a]
Heading	41.4[b]	47.4[b]	11.3[b]	0.103[b]	71.3[b]	82.0[a]
End of heading	37.4[b]	50.7[b]	11.8[b]	0.070[c]	64.6[c]	76.9[b]
Lucerne						
Vegetative	34.3[a]	62.0[a]	3.7[a]	0.162[a]	79.0[a]	81.4[a]
Vegetative	27.4[b]	61.7[a]	9.5[b]	0.154[a]	73.2[b]	77.9[bc]
Early budding	24.0[b]	67.9[b]	8.1[bc]	0.152[a]	72.7[b]	79.0[c]
Budding	18.4[c]	74.0[c]	7.4[c]	0.166[a]	72.8[b]	79.5[c]
End of budding	20.0[c]	71.9[c]	8.1[bc]	0.137[b]	70.0[c]	77.4[b]

For each forage, different subscripts in a same column correspond to a significant difference ($P < 0.05$).

degradation rate which varied from 0.162 to 0.137 h^{-1}.

If the N degradability values were corrected to take into account microbial contamination, N degradability increased on average by 10.2% for grasses and by 5.5% for lucerne, and N degradability was then 80.9% and 79.0% respectively. However, this increase was not the same for all the stages of maturity. The decrease in N degradability with the stage of forage maturity was lower than for the uncorrected values, 5% between heading and end of heading against 7.6% for grass, and 4% against 9% for lucerne when the microbial contamination was taken into account.

DISCUSSION

In this trial, the mean degradability of rye grass nitrogen was 70.7%, near to the value (76.8%) calculated from the data of Van Vuuren *et al* (1990) obtained for rye grass cut at different stages of maturity. In contrast, Le Goffe (1991) reported a higher value (81.7) for a large number of fresh forage the samples (72). The differing reports can be related both to the chemical composition of the samples and to their preparation. The *CP* content of the grasses varied between 120 g in this study and 189 g/kg/DM in Le Goffe's study, whereas in the Dutch study (Van Vuuren *et al,* 1990) mean *CP* was 250 g/kg DM. Moreover, sample preparation prior to *in situ* incubation was 60 °C oven-drying and grinding over 0.8 mm in this study, freeze-drying and grinding (Le Goffe, 1991) or chopping and freezing (Van Vuuren *et al,* 1990). Compared with the fresh initial forage, over-drying involves a decrease in nitrogen degradability when the sample is chopped and has no effect when this is ground after drying. Freeze-drying followed grinding leads to an overestimation of N degradability compared with the fresh initial forage

(Michalet-Doreau, 1989; Vanhatalo and Varvikko, 1989). The nitrogen degradability of forages could have been overestimated in the Le Goffe's study. Lindberg (1988) also measured N degradation kinetics for a timothy harvested at different stages of maturity and dried at 65 °C. The mean N degradability calculated from degradation parameters was 60.9%, a much lower value than those reported from the other trials. However, when Lindberg (1988) took into account microbial contamination of bags in the rumen, the N degradability increased to a mean of 85.1%. In the present study, the degradability of rye grass N also increased from 70.7 to 80.9% if microbial contamination was taken into account. In an earlier trial on 6 grass samples harvested at different stages of maturity, the mean N degradability of the fresh forages was 75.9% (Michalet-Doreau and Ould-Bah, 1992).

On average, the lucerne N degradability was higher (80.9%) than that of rye-grass (70.7%), this difference being due to a higher degradation rate of lucerne. Osibe *et al* (1987) found a greater difference between legumes (60.9%) and grass (50.0%). When microbial contamination was taken into account, the lucerne N degradability was approximately equal to that of grass, 80.9 and 79.0% respectively.

The N degradability decreased with advancing age by 7.6% for rye grass and by 9% for lucerne during the experimental period. This decrease was linear for the lucerne, but the grass N degradability showed a plateau followed a sudden decrease after the heading. This difference in behaviour between grasses and lucerne according to maturity stage is also found for the other nutritive characteristics, such as digestibility (Demarquilly and Jarrige, 1971). Lindberg (1988) and Van Vuuren *et al* (1990) followed the N degradability of grasses over a number of weeks. The N degradability values were higher in early

cut than in late cut grass batches, and the N degradability described a curve with a plateau over a 6-wk period followed by a large decrease at the end of growth in the study of Van Vuuren *et al* (1990). In this study, the N degradability decrease was related to the decrease of the N soluble fraction, whereas in our study the N degradability decrease was accompanied by an increase in the N rapidly degraded fraction. But the measurement method of this fraction was not similar in the 2 studies, and this could be at the origin of the difference. In the study of Van Vuuren (1990), the soluble fraction was estimated as the fraction disappearing from the bags during washing (zero incubation time) and then was independent of the degradation curve. In contrast, in our study, the rapidly degraded fraction was estimated by adjustment to an exponential model. The value was dependent on the degradation curve, and when the degradation rate decreased, the rapidly degraded fraction increased. In these 2 studies, microbial contamination was not taken into account and this could introduce a bias into the results. When microbial contamination was taken into account, the decrease of N degradability with advancing age was lower, *ie* 5% for rye grass and 4% for lucerne in this experiment, and even 0% for Lindberg (1988). Messman *et al* (1992) studied the N disappearance of 2 bromegrass hays harvested at the late-boot or full-head stage of maturity. Advanced maturity decreased the degradability of microbial-corrected N by 5%. The variations in N degradability of rye grass studied by Le Goffe (1991) were similar, from 96 to 91% during the first cycle of vegetation. However the extent of the decrease can vary. In a earlier study, the degradability of microbial-corrected N of forages harvested at 2 stages of maturity decreased by 16.8% with a rye grass and only by 5.1% with the 3 other forages (Michalet-Doreau, 1990).

The results of this study suggest that stage of maturity of forage has an effect on the N degradability in the rumen, but this effect of maturation on the protein value of Italian rye grass is different from that of lucerne. In rye grass, ruminal protein degradability stays constant for some weeks, especially when it is corrected for microbial contamination, and then suddenly falls, whereas in lucerne ruminal protein degradability decreases linearly.

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