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Original article

Ruminal degradation of protein of cocksfoot and perennial ryegrass as affected by various stages of growth and conservation methods

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Abstract — The behaviour of nitrogenous fractions in the rumen of sheep fed grasses at different vegetation stages and preserved by different methods was examined in this study. The measurements were made on four sheep fed forages. In Experiment 1, we used fresh cocksfoot cut at three stages of vegetation in the first growth stage (heading, first flower and full flower), two silages (one made with formic acid and the other without a preservative) and a first flower hay. In Experiment 2, we used fresh perennial ryegrass cut in the first growth stage (end of heading) and in the second growth stage at 7 week regrowth. A silage made with formic acid, two wrapped big bales harvested at 42% and 58% dry matter and a hay cut at the first growth stage (end of heading) were compared. The effective degradability of crude protein (DegN) estimated using the in situ method was lower ($P < 0.05$) with the latter vegetation stage for cocksfoot: 0.693 for the heading, 0.667 for the first flower, 0.597 for the full flower. For cocksfoot, the DegN of the silages was higher ($P < 0.05$) for silage without additive (0.770) than for silage with formic acid (0.705) and higher than that of the fresh forage at first flower (0.667). The DegN of hay was markedly lower (0.537, $P < 0.05$) than that of the original fresh forage. The DegN of silage of perennial ryegrass (0.760) and wrapped big bales harvested at 42% DM (0.739) were higher ($P < 0.05$) than that of fresh forage harvested at the end of heading (0.705). The DegN of wrapped big bales of perennial ryegrass harvested at 58% DM (0.667) and hay (0.536) were lower than those of the other forages ($P < 0.05$). Whatever the forage studied, the concentration of total nitrogen (tN), ammonia nitrogen (NH_3-N) and non-ammonia nitrogen (NAN) were high 1 or 2 h after feeding and diminished rapidly up to 7 h after feeding. Some of the solubilised nitrogen remained as proteins 1 to 2 h after feeding for fresh forage harvested at various growth stages, but no protein was found in the rumen fluid after sheep were fed the silage (with or without preservative), wrapped big bales or hay. The proportion of dietary NAN flow (relative to ingested nitrogen) escaping rumen degradation was 5.8% to 10.1% for perennial ryegrass and 10.9% to 15% for cocksfoot.

cocksfoot / perennial ryegrass / conservation method / vegetation stage / protein degradation / rumen fluid composition

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Résumé — Dégradation dans le rumen des protéines de fourrage de graminées (dactyle et ray-grass anglais) à différents stades de végétation et modes de conservation. Pour apprécier l'importance du flux d'azote solubilisé dans le rumen pouvant contribuer au flux d'azote intestinal, des mesures sur 4 moutons nourris avec des fourrages de graminées, récoltés à des stades de végétation différents ou conservés par différentes méthodes ont été réalisées. Dans l'expérience 1, un fourrage vert de dactyle récolté à 3 stades de végétation au 1er cycle (épiaison, début floraison, floraison) ainsi que 2 ensilages (avec et sans conservateur) et un foin, récoltés au stade début floraison ont été étudiés. Dans l'expérience 2, un fourrage vert de ray-grass anglais (stade épiaison) a été comparé à des repousses de 7 semaines ou à un ensilage conservé avec de l'acide formique, deux balles rondes récoltées à teneurs en matière sèche différentes (42 % et 58 %) et un foin. La dégradabilité des matières azotées dans le rumen (DegN), mesurée par la méthode des sachets de nylon diminue avec le stade de végétation: elle est de 0,693 pour le stade épiaison, 0,667 pour le stade début floraison et 0,597 pour le stade floraison. Elle est significativement ($P < 0,05$) plus élevée pour l'ensilage sans conservateur (0,770) que pour celui conservé avec de l'acide formique (0,705) et plus élevée que celle du fourrage vert récolté au même stade (0,667). La DegN du foin est significativement plus faible (0,537 $P < 0,05$) que celle du fourrage vert. Les DegN de l'ensilage de ray-grass anglais (0,760) et de la balle ronde à 42 % de matière sèche (0,739) sont significativement plus élevées que celle du fourrage vert correspondant (0,705). Les DegN de la balle ronde récoltée à 58 % de matière sèche (0,667) et du foin (0,536) sont significativement plus faibles que celles des autres fourrages de ray-grass anglais. Quel que soit le fourrage étudié, les teneurs en azote total, en azote ammoniacal, et en azote non ammoniacal dans le rumen sont maximales 1 ou 2 h après le repas et diminuent jusqu'à 7 h après le repas. Une partie des matières azotées solubilisées reste sous forme de protéines 1 ou 2 h après le repas pour les fourrages verts tandis qu'on ne retrouve pas de protéines solubilisées dans le rumen des moutons recevant des fourrages conservés (ensilages, balles rondes, foin). Le flux d'azote alimentaire non ammoniacal (exprimé par rapport à l'azote ingéré) qui échappe à la dégradation dans le rumen et transite avec le liquide ruminal représente de 5,8 % à 10,1 % pour le ray-grass anglais et de 10,9 % à 15 % pour le dactyle.

dactyle / ray-grass anglais / mode de conservation / stade de végétation / dégradation des protéines / composition du jus de rumen

1. INTRODUCTION

The in situ method is used in France to predict the quantity of protein from dietary sources escaping degradation in the rumen. Because of a shortage of data on forages, the feeding systems suggested by INRA [19] use fixed crude protein degradability values (DegN) for each of the most representative forage types, respectively fresh forages, hay, and silages with or without preservatives. Many factors influence the ruminal degradability of forage crude protein, e.g. stage of maturity, [40], forage species [20, 30] and preservation method [34].

The in situ method assumes that crude protein disappearing from bags incubated in the rumen is completely degraded to ammonia nitrogen. Studies have shown that some solubilised nitrogen is indeed converted into ammonia after a meal but some can also remain as protein or non-ammonia nitrogen (NAN) and leave the rumen with the liquid phase.

Our aim was to compare the degradability of two grasses, cocksfoot and perennial ryegrass, harvested at various growth stages or regrowth with different methods of conservation, to determine the proportion of soluble NAN escaping ruminal degradation.

2. MATERIALS AND METHODS

The different vegetation stages of forage grasses are defined by INRA in [19].

2.1. Forages

Experiment 1 was conducted (on year 1998) with fresh cocksfoot cut at three different stages during the first harvest cycle (heading, first flower and full flower). Two silages and one hay were made from the fresh forage cut at the first flower. The fresh forage was ensiled in 4-m^3 experimental silos. One silage was made without an additive and the other with formic acid (3.5 $L \cdot t^{-1}$). The hay was dried on the ground in good weather.

Experiment 2 was carried out with fresh perennial ryegrass (on year 1999) in the first growth stage (end of heading) and in the second growth stage (stemmy regrowth) at 7 week regrowth after the end of the heading stage.

One silage, two wrapped big bales and one hay were made from the fresh perennial ryegrass cut at the end of the heading stage. The silage was made without wilting and preserved with formic acid (3.5 $L \cdot t^{-1}$). The fresh forage was wilted for two and three days after cutting for wrapped big bales harvested at, respectively, 42% and 58% dry matter. 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 60 ± 3.0 kg and fitted with ruminal cannulae. During the experimental periods, the animals were housed in individual pens and allowed free access to water and a salt block.

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

The measurements were made on fresh forages at various growth stages in the spring/summer and in the autumn for the conserved forages (silages, wrapped big bales and hay). They were fed the fresh or conserved forage in a chopped form.

Each measurement period included a two-week adaptation phase and two weeks of measurements: one week for the rumen fluid kinetic sampling and rumen content measurements and then one for the in situ degradation kinetics. Forages placed in the Dacron bags were identical to those in the diet. The bags were filled at the end of the week of serial sampling and rapidly frozen to prevent modifications of forages (see Sect. 2.3).

Concurrently, the digestibility of organic matter (OMD) was measured on another group of six intact, 3 or 4 year old sheep kept in metabolism cages.

2.3. Measurements

The experiment was conducted on fresh and not frozen forages, because freezing at –20 °C modifies the characteristics of forages and their degradability [17, 23].

2.3.1. Digestibility

Digestibilities were measured according to the method described by Demarquilly and Weiss [13]. The sheep were fed 10% above the previous day's consumption, in equal portions at 08.00 h and 17.00 h, and the uneaten forage was removed before the morning allocation. Digestibility measurements were done with total collection of faeces for 6 days after a preliminary period of 15 days.

2.3.2. In situ degradation

Crude protein degradability was measured using the in situ method, as described by Michalet-Doreau et al. [28]. Dacron bags (Ankom Co., Fairport, New-York) (pore size 30 to 60 μm) of inside surface 5 × 11 cm, closed with two stitches, were used. Forage samples weighed into bags were prepared according to Dulphy et al. [15]. Fresh forages, silages and wrapped big

bales were chopped to 4-5 mm in length. The hay was ground to a mesh size of 4 mm. A quantity equivalent to 2.5 g dry matter (DM) was weighed into the bags and incubated in the rumen of the four fistulated sheep fed the same forage. Incubation times were 2, 4, 8, 16, 24 and 48 h. Two replications per sheep were used for 2, 4 and 8 h, and three replications 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, to detect any changes in the level of degradation during the experiment. The bags were removed from the rumen and kept at –20 °C until analysis. Before analysis the bags were defrosted and rinsed with cold water until the rinse water ran clear. The bags were then beaten for 7 min in a "Seward stomacher" for pummelling of particles [26], followed by further washing and finally dried at 60 °C for 48 h. Michalet-Doreau and Ould-Bah [29] showed that beating the residues in the bags in a stomacher could significantly reduce microbial contamination of the undegraded fraction of the sample. Crude protein solubility without incubation in the rumen (T0 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.

The in situ dry matter and N disappearance were fitted to the model of Ørskov and McDonald [31]

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

The effective degradability of dry matter (DegDM) or crude protein (DegN) was calculated as: $a + (b \times c)/(c+kp)$, assuming $kp=0.06 \cdot h^{-1}$ [27].

2.3.3. Ruminal fluid sampling

Rumen fluid was taken from the same sheep on two consecutive days, before the morning meal (T0 h), and 1, 2, 4 and 7 h after feeding. A 150-mL sample of rumen fluid was taken, muslin-filtered and centrifuged for 5 min at $120 \times g$ to remove dietary particles and protozoa. The supernatant was centrifuged at +4 °C and $27\,000 \times g$ for 20 min to remove dietary particles and bacteria and the supernatant was then analysed for nitrogen. The proteins were then precipitated by adding sulfosalicylic acid ($400\ g \cdot L^{-1}$) and separated after centrifuging ($20\,000 \times g$ for 10 min); the final supernatant was then analysed for nitrogen.

2.3.4. Rumen content and digesta kinetics

Total reticulo-rumen contents were determined by manually emptying the rumen before the morning meal (09.00 h) and after the evening meal (19.00h). Manual evacuations of whole rumen contents were carried out after an interval of at least 60 h to ensure normal digestion [1]. After emptying, the rumen contents were weighed, homogenised and sampled for DM determination and then put back into the rumen. The whole procedure took no longer than 30 min [10].

A 200-mL dose of a Cr-EDTA solution was introduced intraruminally at 7 h (2 h before the morning feeding). Seven samples (50 mL) were taken 2, 4, 6, 10, 26, 28 h and 30 h after administration of the marker to determine the liquid turnover rate. The samples were stored at –20 °C until analysis for Cr.

2.3.5. Chemical analyses

The total nitrogen (tN) content of the forages and bag residues and the soluble nitrogen content of the rumen fluid (before and after precipitation with sulfosalicylic acid) were determined [2]. The protein nitrogen content in the rumen fluid was calculated as the difference between total nitrogen in the rumen fluid and total nitrogen after precipitation with sulfosalicylic acid. The ammonia nitrogen (NH_3-N) values were determined on the final supernatant

(after precipitation with sulfosalicylic acid) by the Conway method [12].

NDF, ADF, and ADL concentrations in forages were determined as described by Van Soest et al. [42]. Sodium sulphite was not included in the neutral detergent solution and heat-stable α-amylase was not added to the solution before fibre analysis [42].

The fermentative characteristics of the silages were analysed (pH, NH_3-N, soluble N, volatile fatty acids and lactic acid) [14].

The Cr concentrations on centrifuged liquid were determined by absorption spectrometry using a Perkin-Elmer Model 2380 spectrophotometer [38]. The fractional turnover rate (kl) was estimated as the slope of the linear regression between the natural logarithm of Cr concentrations in the rumen fluid and the time of sampling following marker administration [18].

2.3.6. Estimation of NAN flow

The fraction of non-ammonia nitrogen (NAN) in the rumen fluid that escaped degradation in the rumen and reached the small intestine was estimated from the NAN concentrations at the different kinetics times (T0 h (before the morning meal), 1, 2, 4 and 7 h after feeding). The mean NAN concentration was calculated as mean of the 12 hourly samples. For each forage the NAN dietary flow ($g \cdot d^{-1}$) was equal to: mean NAN concentration × mean volume of liquid phase × kl.

2.3.7. Calculations and statistical analysis

In situ degradation parameters were analysed using variance analysis (SAS GLM procedure) [36], according to the following model:

$Y = M + Ai + Tj + Eij$ where M is the overall mean, and Ai is the animal effect (df = 3), Tj is the stage of vegetation or the conservation effect in each experiment.

Forage type, forage maturity and forage conservation were not treated as a factorial since the stage of maturity descriptions and method of conservation were not the same for both forage types. For cocksfoot, Tj is the stage of vegetation (heading, first flower, full flower, df = 2) or conservation effect (fresh at first flower, silage with formic acid or without a preservative and hay; df = 3), Eij is the residual error term (df = 6 or df = 9, respectively).

For perennial ryegrass, Tj is the stage of vegetation (end of heading, regrowth, df = 1) or conservation effect (fresh at the end of heading, silage, wrapped big bales and hay; df = 4), Eij is the residual error term (df = 3 or df = 12, respectively).

Fresh forages were compared first according to their vegetation stage (heading, first flower and full flower for cocksfoot, and between first and second growth stage for perennial ryegrass), and second for the same vegetation stage (first flower for cocksfoot and end of heading for perennial ryegrass) according to their mode of conservation (silages, wrapped big bales or hay). This comparison was made for in situ degradation and rumen fluid measurements.

3. RESULTS

3.1. Chemical composition

The chemical composition of the forages and their OMD are given in Table I. The crude protein (CP) content decreased with vegetation stage for cocksfoot and was higher for cocksfoot than for perennial ryegrass. For cocksfoot or perennial ryegrass, the CP content was similar for fresh forage, silages and wrapped big bales harvested at the same vegetation stage (respectively the first flower and end of heading) and lower for the hay.

The NDF content increased according to the maturity of the plant for cocksfoot

Table I. Chemical composition and OM digestibility of experimental forages.

	CP ($g \cdot kg^{-1}$ DM)	NDF ($g \cdot kg^{-1}$ DM)	ADF ($g \cdot kg^{-1}$ DM)	OMD (%)
Cocksfoot				
Heading	131.1	581	282	69.5
First flower	112.7	676	360	63.5
Flowering	76.9	697	387	60.4
Silage without additive (First flower)	117.6	587	328	68.2
Silage + formic acid (First flower)	117.3	614	343	68.0
Hay (First flower)	102.7	697	376	58.4
Perennial ryegrass				
End of heading	85.6	620	366	65.7
Stemmy regrowth (6 weeks)	116.3	513	305	77.1
Silage + formic acid (end of heading)	87.5	578	371	64.8
Wrapped big bales (42% DM) (end of heading)	96.3	558	354	66.4
Wrapped big bales (58% DM) (end of heading)	83.8	592	373	67.2
Hay (end of heading)	78.1	628	383	63.5

OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; OMD: organic matter digestibility; DM: dry matter.

(Tab. I). Furthermore, for both forages, it was lower for silages and wrapped big bales and higher for hay, than the corresponding fresh forage.

The organic matter digestibility (OMD) decreased with advanced maturity according to the increase in cell-wall content. Although the chemical compositions (CP, NDF, ADF) were similar, cocksfoot silage without formic acid was of poorer quality than that preserved with formic acid.

The pH, the acetic acid content, the NH_3-N/tN and soluble N/tN were higher for silage without an additive than for that preserved with formic acid (Tab. II). Perennial ryegrass silage preserved with formic acid was of good quality. The wrapped big bales harvested at 42% DM was of poorer quality than that harvested at 58% DM (Tab. II): the proteolysis was higher for the wrapped big bale harvested at 42% DM.

3.2. In situ degradation

3.2.1. Experiment 1 (Tab. III)

Dry matter and crude protein effective degradability (DegDM and DegN) of cocksfoot decreased progressively with the maturity of the forage ($P < 0.05$). The degradation rate (c) was not affected ($P > 0.05$) by the stage of vegetation, but 'a' and 'b' were lower ($P < 0.05$) for the first flower and flowering stages than for the heading.

The DegDM values of the silages were similar, slightly higher than that of fresh

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

	Cocksfoot silage		Perennial ryegrass		
			silage	wrapped big bales	
	no additive	formic acid	formic acid	42% DM	58% DM
pH	4.1	3.9	4.0	5.4	5.8
NH_3-N (% tN)	8.7	6.3	6.0	7.1	3.6
Soluble N (% tN)	58.3	53.8	48.1	58.5	50.1
Acids (g·kg^{-1} DM)					
Lactic	62.3	47.8	68.0	28.3	16.1
Acetic	22.6	18.0	11.0	5.0	5.6
Propionic	0.5	0.2	1.9	0.0	0.0
Butyric	0.0	10.6	0.0	0.0	0.0
Alcohols (g·kg^{-1} DM)					
Methanol	0.1	0.0	0.6	0.0	0.0
Ethanol	2.6	3.2	4.0	15.2	27.2
Propanol	0.1	0.2	0.2	0.0	0.0
Butanol	0.1	0.2	0.0	0.0	0.0

DM: dry matter; NH_3-N: ammonia nitrogen; tN: total nitrogen.

forage and significantly higher than that of hay ($P < 0.05$), while the 'a' fraction was higher and the 'b' fraction was lower than that of hay ($P < 0.05$). The DegN of silages was higher than that of fresh forage harvested at the first flower, while the DegN of hay was lower than that of fresh forage. In addition, DegN of silage without an additive was significantly higher ($P < 0.05$) than that of silage with formic acid. The 'a', 'b' fractions and 'c' coefficient were significantly different between the two silages ($P < 0.05$).

3.2.2. Experiment 2 (Tab. IV)

The DegDM for fresh forage of perennial ryegrass was lower at the first growth stage than at the second growth stage while the 'b' fraction and 'c' coefficient were not significantly different ($P < 0.05$). As with the modelling parameters, the 'a' and 'b' fractions were different ($P < 0.05$) and the DegN values for fresh forage were not significantly different ($P > 0.05$).

The DegDM value for the silage of perennial ryegrass was the highest over all ryegrass forages while the DegDM for fresh forage was not significantly different from that of hay. The DegDM value for the wrapped big bales (42% DM) was intermediate between those of silage and wrapped big bales (58% DM). The DegDM value for wrapped big bales (58% DM) was close to that of the hay.

The DegN values for silages were higher than for fresh forage ($P < 0.05$) and for wrapped big bales ($P < 0.05$), while the DegN of hay was the lowest ($P < 0.05$). The DegN value for wrapped big bales was intermediate between those of silage and hay.

Table III. In situ degradation parameters for cocksfoot (experiment 1) according to the stage of growth and the conservation method

Cocksfoot (Experiment 1)				
DM	a	b	c	Deg DM
Heading	0.261[a]	0.521[a]	0.054[a]	0.504[a]
first flower	0.199[b]	0.486[a]	0.045[a]	0.404[b]
flowering	0.181[b]	0.364[a]	0.052[a]	0.346[c]
RSD	0.0163	0.0492	0.0116	0.0178
N	a	b	c	DegN
Heading	0.286[b]	0.616[a]	0.121[a]	0.693[a]
first flower	0.355[a]	0.508[b]	0.099[a]	0.667[a]
flowering	0.302[b]	0.480[b]	0.104[a]	0.597[b]
RSD	0.0160	0.0332	0.0197	0.0190
DM	a	b	c	Deg DM
beginning flowering	0.199[b]	0.486[c]	0.045[a]	0.404[b]
Silage without additive	0.248[a]	0.504[c]	0.037[ab]	0.438[a]
Silage + formic acid	0.241[a]	0.639[b]	0.027[bc]	0.423[ab]
Hay	0.164[c]	0.808[a]	0.019[c]	0.351[c]
RSD	0.0120	0.0686	0.0080	0.0133
N	a	b	c	DegN
beginning flowering	0.355[c]	0.508[b]	0.099[a]	0.667[c]
Silage without additive	0.571[a]	0.311[d]	0.109[a]	0.770[a]
Silage +formic acid	0.523[b]	0.370[c]	0.063[b]	0.705[b]
Hay	0.324[d]	0.611[a]	0.035[b]	0.537[d]
RSD	0.0140	0.0361	0.0200	0.0135

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 the same column correspond to a significant difference ($P < 0.05$).

3.3. Rumen fluid composition (total N (tN), non-ammonia N (NAN), ammonia N (NH_3-N), protein nitrogen (protein-N))

3.3.1. Experiment 1

At all sampling times, tN contents in the rumen juice of fresh forage of cocksfoot at the heading stage were higher ($P < 0.05$) than at the first flower and full flower stage. Protein-N, NH_3-N, also followed the same trend but the differences between the vegetation stages were not always significant (Fig. 1). In contrast, at 1 h and 2 h after feeding, tN and NH_3-N contents were not significantly different between the different conservation methods (Fig. 2). Although the tN contents were lower than for fresh forages at all sampling times, the time course of the tN content in rumen fluid was rather similar with hay, silages and with fresh forage.

The NH_3-N content represented 40% tN to 60% tN at 1 h and 2 h after feeding and remained higher than 40% tN until 7 h after feeding for fresh cocksfoot forage, as well as for the silages or hay. NH_3-N contents measured 1 h or 2 h after the meal were lower for fresh forage than for silages ($P > 0.05$) but protein-N was higher ($P < 0.05$). Protein-N contents were higher 1 h and 2 h after feeding ($P < 0.05$) for fresh forages at the early vegetation stage, and represented about 20 to 30% of tN in the rumen fluid whatever the vegetation stage and

Table IV. In situ degradation parameters perennial ryegrass (experiment 2) according to the stage of growth and the conservation method.

Perennial ryegrass (Experiment 2)				
DM	a	b	c	Deg DM
End of heading (1st growth)	0.259[b]	0.505[a]	0.050[a]	0.486[b]
Stemmy regrowth	0.334[a]	0.508[a]	0.057[a]	0.580[a]
RSD	0.0128	0.022	0.0098	0.0099
N	a	b	c	DegN
End of heading (1st growth)	0.446[a]	0.366[b]	0.144[a]	0.705[a]
Stemmy regrowth	0.310[b]	0.585[a]	0.136[a]	0.716[a]
RSD	0.0112	0.0165	0.0060	0.0082
DM	a	b	c	Deg DM
End of heading (1st growth)	0.259[b]	0.505[dc]	0.050[a]	0.486[c]
Silage+formic acid	0.277[a]	0.468[d]	0.078[b]	0.542[a]
Wrapped big bales (42% DM)	0.274[a]	0.531[bc]	0.052[a]	0.516[b]
Wrapped big bales (58% DM)	0.222[c]	0.574[a]	0.047[a]	0.473[c]
Hay	0.222[c]	0.571[ab]	0.046[a]	0.470[c]
RSD	0.0093	0.0259	0.0080	0.0123
N	a	b	c	DegN
End of heading (1st growth)	0.446[b]	0.366[c]	0.144[b]	0.705[c]
Silage + formic acid	0.566[a]	0.256[d]	0.188[a]	0.760[a]
Wrapped big bales (42% DM)	0.577[a]	0.273[d]	0.089[c]	0.739[b]
Wrapped big bales (58% DM)	0.406[c]	0.441[b]	0.089[c]	0.667[d]
Hay	0.165[d]	0.686[a]	0.071[c]	0.536[e]
RSD	0.0084	0.0165	0.0127	0.0107

DM: dry matter; N: nitrogen; a: rapidly degraded fraction; b: slowly degraded fraction; c: rate of degradation; Deg: degradability = a + (bc)/(c + k); RSD: residual standard deviation; different superscripts in a same column correspond to a significant difference ($P < 0.05$).

the kinetics time. Proteins were not observed in the rumen fluid of sheep fed hay or silages. The NAN content remained high for all sampling times, between 40% tN and 60% tN whatever the vegetation stage or the conservation method. The time course and contents of the various nitrogenous forms (tN, NH_3-N, NAN) were also similar for the two silages.

3.3.2. Experiment 2

For all the kinetic times in the rumen (Fig. 3), there was little difference of tN content ($P > 0.05$) between the first growth and regrowth (except 4 h after feeding). In contrast, at the first growth, the protein-N and the NH_3-N contents were higher, respectively at 1 h and 2 h ($P < 0.05$) after feeding. The NAN contents were not significantly lower for regrowth ($P > 0.05$).

The tN, NH3-N and NAN contents were the highest in the rumen for wrapped big bales (42% DM), which had the highest crude protein content and 'a' fraction. The tN and NH_3-N contents of wrapped big bales (58% DM) were similar and lower than for silage with formic acid ($P > 0.05$). The tN and NH_3-N contents were low but not significantly different ($P > 0.05$) between fresh forages and hay (Fig. 4), which was surprising because the crude protein content of the forage, fraction 'a' and DegN of the hay were lower than for fresh forages ($P < 0.05$). On the contrary, protein-N was the highest 1 and 2 h after the meal for fresh forages.

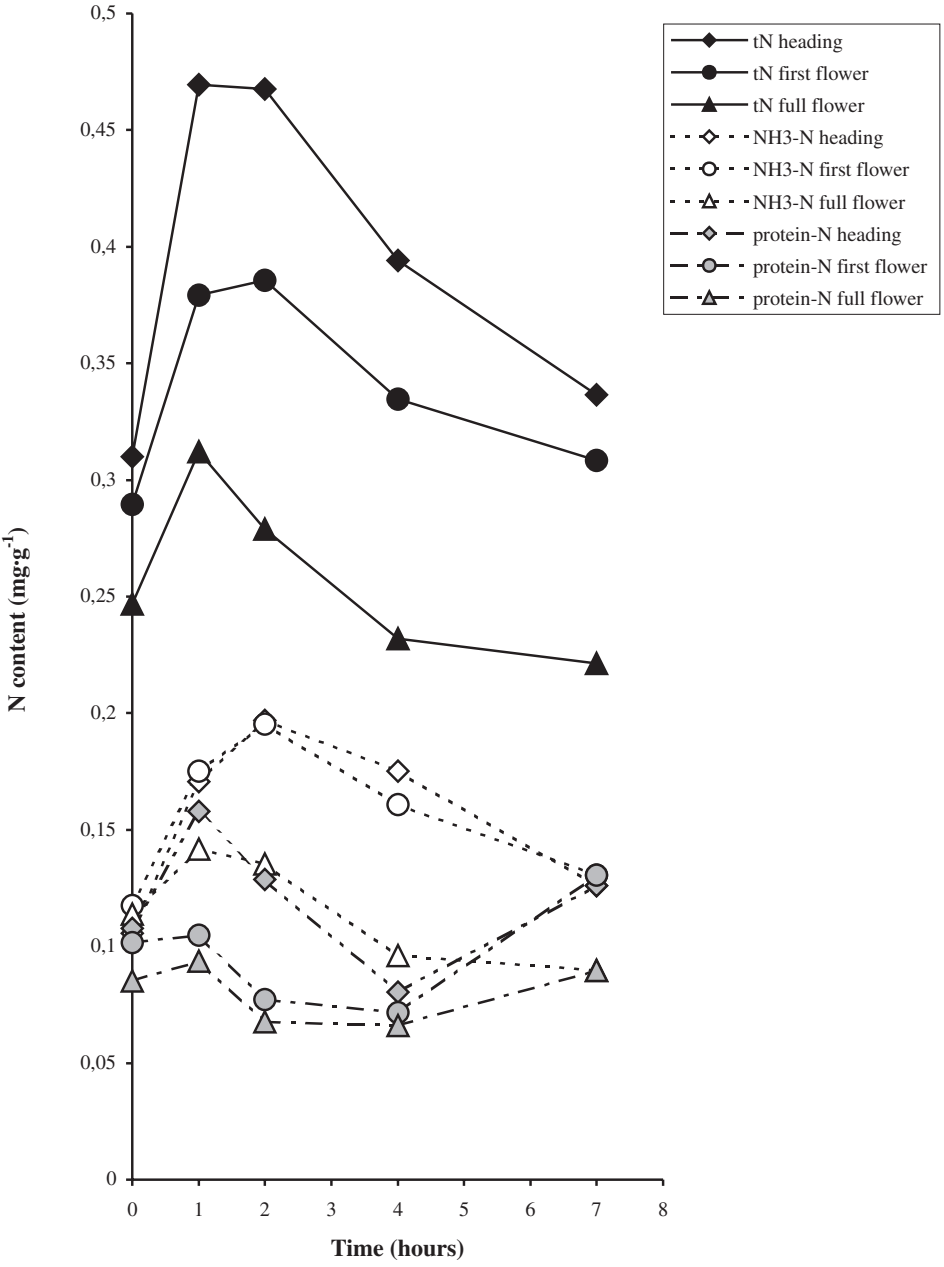

Figure 1. Evolution of the concentration (mg·g^{-1}) of tN, NH_3-N, protein-N in the rumen fluid for cocksfoot fresh forages at different vegetation stages (heading, first flower, full flower) before the morning meal (T0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

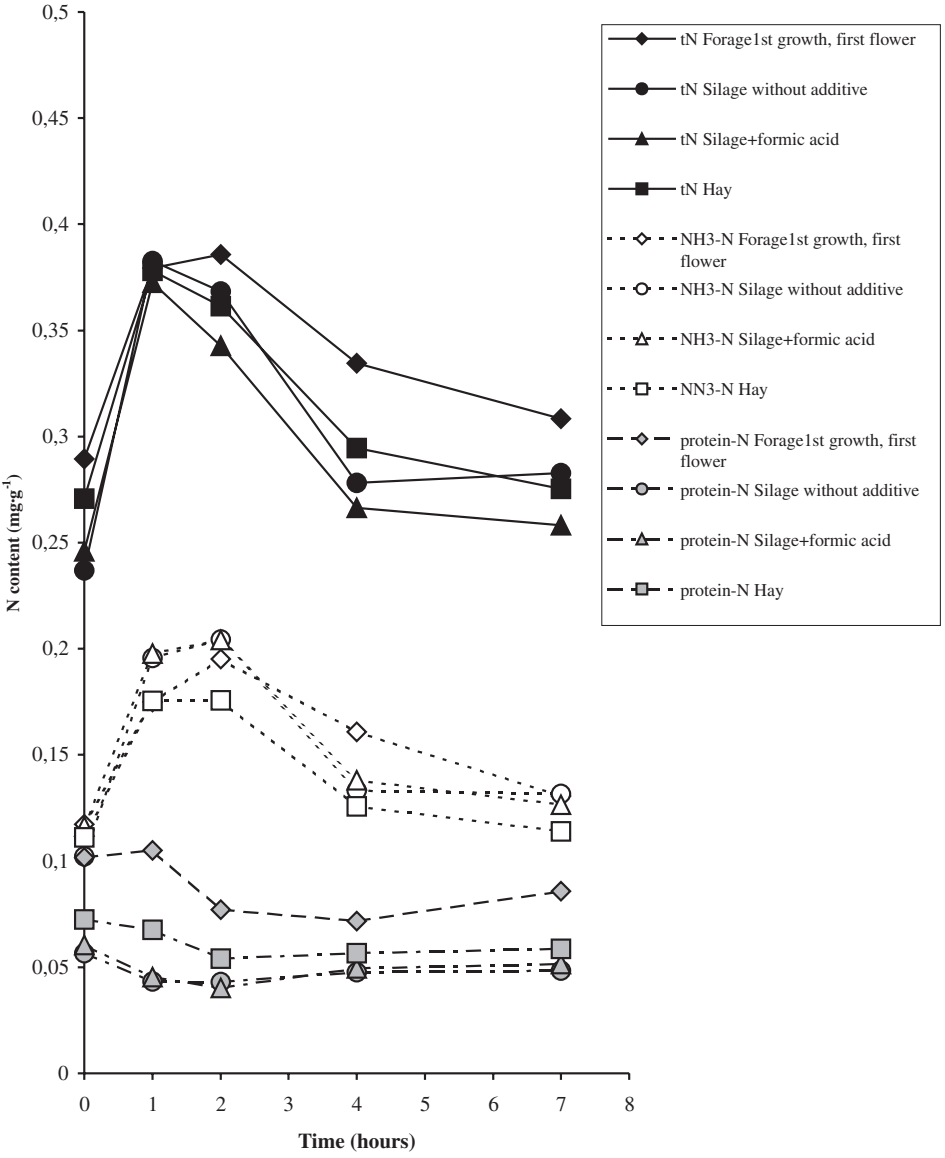

Figure 2. Evolution of the concentration ($mg \cdot g^{-1}$) of tN, NH_3-N, protein-N, in the rumen fluid for cocksfoot fresh forage, silage without additive, silage + formic acid and hay, before the morning meal (T0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

Generally the tN, NH_3-N and protein-N values were lower for perennial ryegrass than for cocksfoot, but the NH_3-N/tN ratio was always greater than 30% and represented from 48 to 56% tN for ryegrass silage conserved with formic acid and wrapped big bales (42% DM), 1 h and 2 h after feeding. Protein-N content represented about 30%

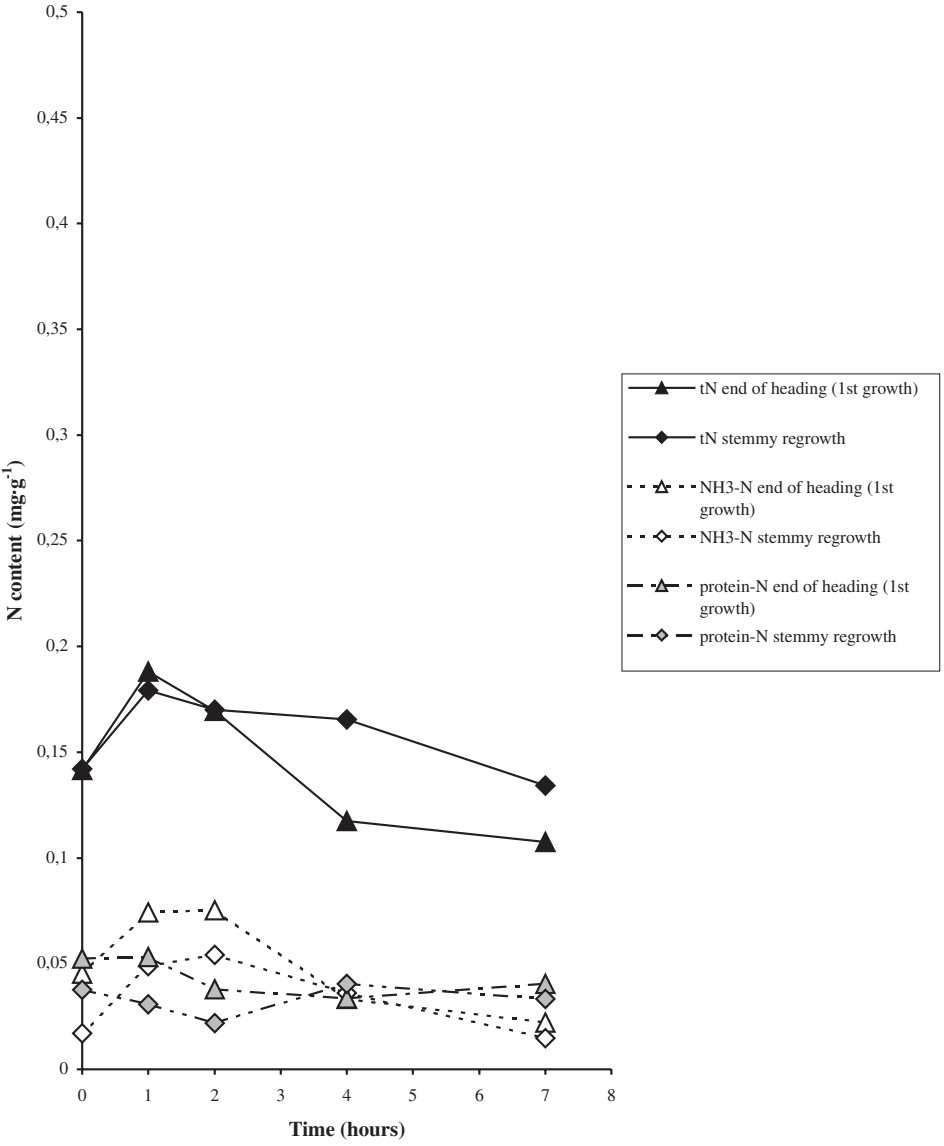

Figure 3. Evolution of the concentration ($mg \cdot g^{-1}$) of tN, NH_3-N, protein-N in the rumen fluid for perennial ryegrass fresh forages at the first vegetation stage and for regrowth, before the morning meal (T0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

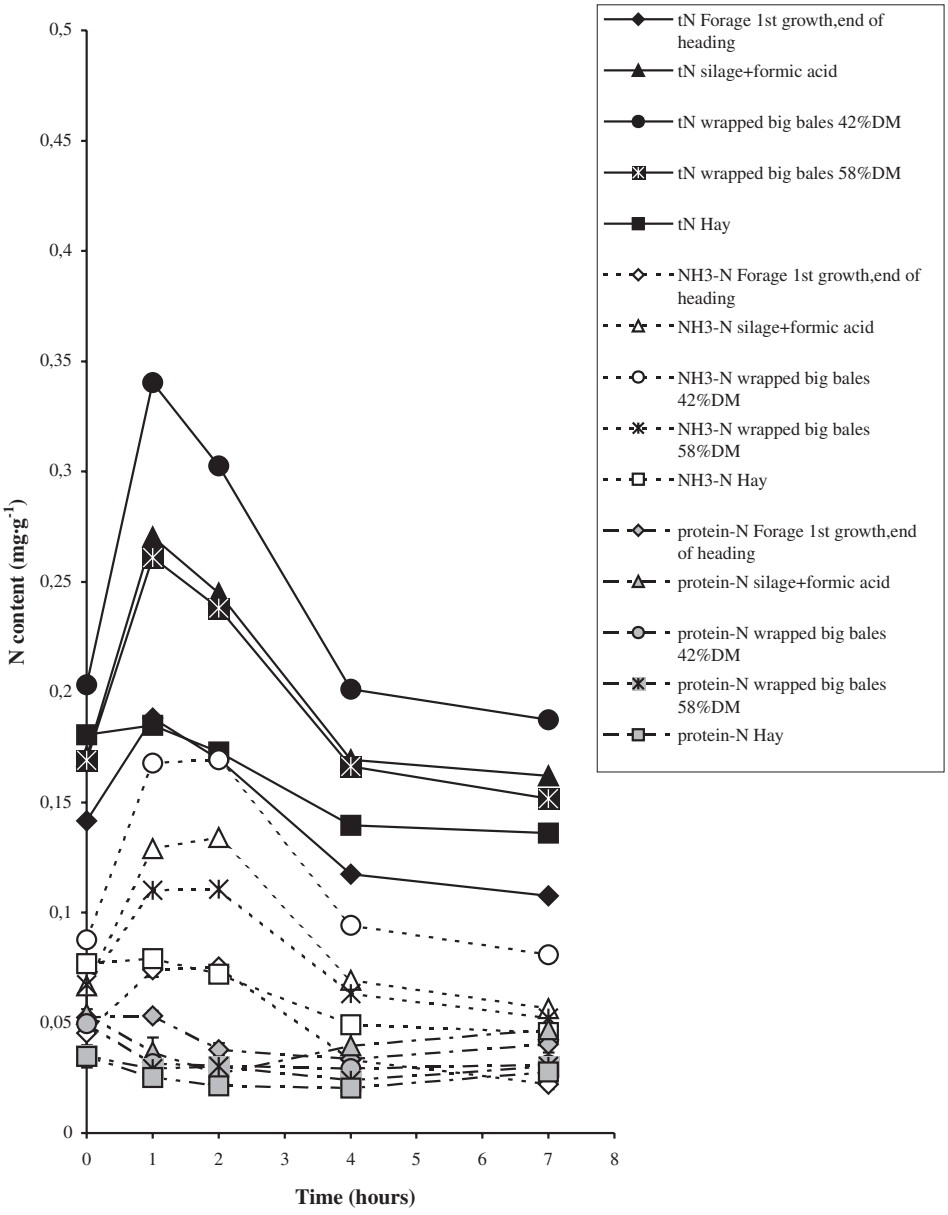

Figure 4. Evolution of the concentration (mg·g^{-1}) of tN, NH_3-N, protein-N, in the rumen fluid for perennial ryegrass fresh forage, silage + formic acid , wrapped big bale 42% DM, wrapped big bale 58% DM and hay, before the morning meal (T0 h) and 1 h, 2 h, 4 h, and 7 h after the meal.

tN for fresh forage and only 10% tN for silage, wrapped big bales and hay.

4. DISCUSSION

4.1. Chemical composition and degradability

Advancing the stage of maturity resulted in a larger depression in rumen DegDM and DegN of cocksfoot fresh forages, as in the results of Waters and Givens [45], Vanhatalo et al. [41], Balde et al. [9]. At the same vegetation stage the DegN of cocksfoot was lower than that of ryegrass, consistent with more abundant and more lignified cell walls [21]. Cocksfoot was less rich in soluble carbohydrate than perennial ryegrass, what may have resulted in a decrease in microbial synthesis and lower degradability. In the same species, at the same vegetation stage, the DegN varied according to the conservation method, as in the results of Verbic et al. [43]: the silages were more degraded than fresh forages and hays, and wrapped big bales were intermediate between silages and hay according to the dry matter at harvesting. On the contrary to the results of Vik-Mo [44], but consistent with our results on lucerne [6], the DegN was significantly lower for silage of cocksfoot with formic acid than for silage without an additive. As in our data on lucerne [3, 6], in agreement with the results obtained by Janicki and Stallings [20], Lopez et al. [22], the immediately soluble fraction 'a' of silages and wrapped big bales (42% DM) of cocksfoot and perennial ryegrass was higher than those of fresh forage and hay because of fermentation in the silo.

4.2. The various nitrogen fractions in rumen fluid

In agreement with the results of Rinne et al. [35], for cocksfoot in the rumen, tN and NH_3-N contents decreased with advancing grass maturity. They were not significantly different between the fresh forage and silage or hay harvested at the same vegetation stage (first flower) ($P > 0.05$), which was unexpected, since the DegN was different ($P < 0.05$). In addition, for perennial ryegrass, no significant differences of tN or NH_3-N contents in the rumen were observed between fresh forage and hay harvested at the first flower stage, although the NH_3-N contents were higher for silages and wrapped big bales than for fresh forages ($P < 0.05$), as observed by Siddons et al. [37]. However, as we noted on some high tN single feeds, [4, 5, 7] and lucerne [6], part of the solubilised nitrogen remained as protein in the rumen at 1 and 2 h after feeding for cocksfoot fed fresh forages. As in previous feedings with lucerne [3], no true protein content in the rumen fluid of sheep fed silage was obtained, since the proteins, especially ribulose 1-5 diphosphate carboxylase-oxygenase, were already degraded in the silo. As might be expected for hay that was less and more slowly degraded, nitrogen degraded progressively to the NH_3-N, the peptide N plus the amino acids N stages with no intermediate protein accumulation.

The dietary NAN flow that escaped degradation in the rumen and reached the small intestine was estimated (Tab. V). The proportion of NAN dietary flow (% of ingested N) that escaped rumen degradation was small (on average 10.6%) and ranged from 5.8 to 10.1% for perennial ryegrass and 10.9 to 15% for cocksfoot. As in the results of Peltekova and Broderick [32], Choi et al. [11], some soluble N escaped rumen degradation and flowed to the duodenum in the liquid phase of the digesta. However, in this study, dietary NAN flow may have been overestimated. This fraction may contain other N forms such as nucleic acids [33] that are found in low quantities [39] and other nitrogen forms (saliva N, endogenous N, microbial nitrogen) as observed by Choi et al. [11]. On the contrary to the results for

Table V. Ratio between the amount of dietary non-ammonia nitrogen (NAN) flow liable to nitrogen intake for cocksfoot and perennial ryegrass according to the vegetation stage or conservation method.

	CP	DMI	DegN	Vol	kl	NAN dietary flow/N intake	1 – DegN
	($g \cdot kg^{-1}$ MS)	($kg \cdot d^{-1}$)	(%)	(L)	(h^{-1})	($g \cdot d^{-1}$)	
Cocksfoot							
Heading	131.1	2.06	0.693	10.6	0.099	13.2	0.31
Beginning flowering	112.7	1.70	0.667	12.1	0.066	11.2	0.33
Flowering	76.9	1.73	0.597	12.8	0.071	14.0	0.40
Silage without additive	117.6	1.34	0.770	11.8	0.071	11.9	0.23
Silage + formic acid	117.3	1.16	0.705	10.5	0.070	10.9	0.30
Hay	102.7	1.41	0.537	13.9	0.064	15.6	0.40
Perennial ryegrass							
End of heading	85.6	1.60	0.705	10.1	0.075	7.6	0.30
Stemmy regrowth (7 weeks)	116.3	2.11	0.716	9.2	0.084	5.8	0.28
Silage + formic acid	87.5	1.38	0.760	6.1	0.061	7.7	0.24
Wrapped big bales (42% DM)	96.3	1.39	0.739	10.0	0.075	10	0.26
Wrapped big bales (58% DM)	83.8	1.35	0.667	9.6	0.073	10.1	0.33
Hay	78.1	1.59	0.536	10.9	0.070	9	0.46

CP: crude protein; DMI: dry matter intake; DegN: effective degradability of nitrogen (kp = 0.06 h^{-1}); Vol: mean volume of liquid phase (L); kl: daily digesta fractional turnover rates (h^{-1}); NAN: non-ammonia nitrogen.

in situ degradation of N (DegN) obtained on the same samples, and to previously reported results [8, 16, 24, 25], the effects of maturity stage or conservation method were not observed, but the NAN flow was relative to ingested N in this study.

5. CONCLUSION

The DegN in fresh cocksfoot decreased with the age of the forage. For both forages, it was higher for silages than for fresh forage and hay harvested at the same stage. The DegN of wrapped big bales of perennial ryegrass was close ($P < 0.05$) to that of silage with formic acid. The DegN of wrapped big bales (58% DM) was intermediate between those of silage and hay.

In the rumen fluid, some of the solubilised nitrogen remained as proteins 1 and 2 h after feeding for fresh forages at different stages of harvesting, while no proteins were observed after feeding in the rumen fluid of sheep that ate silage (with or without preservative) or hay. Rumen fluid contents for the various nitrogenous forms (tN, NH_3-N) were more important for perennial ryegrass than for cocksfoot. The dietary NAN flow relative to ingested nitrogen in the rumen fluid escaping degradation in the rumen was from 5.8 to 10.1% and from 10.9 to 15%, respectively, for perennial ryegrass and cocksfoot.

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