Can herbage nitrogen fractionation in *Lolium perenne* be improved by herbage management?

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Abstract

The high degradability of grass protein is an important factor in the low nitrogen (N) utilization of grazing bovines in intensive European grassland systems. We tested the hypothesis that protein degradability as measured by the Cornell Net Carbohydrate and Protein System (CNCPS) protein fractionation scheme, can be manipulated by herbage management tools, with the aim to reduce N loss to the environment. A field experiment comprising the factorial combinations of three fertilizer N application rates (0, 90 and 390 kg N ha^{-1} $year^{-1}$), three regrowth periods (2–3, 4–5, and 6–7 weeks), two perennial ryegrass (*Lolium perenne* L.) cultivars [Aberdart (high sugar content) and Respect (low sugar content)] and two cutting heights (approximately 8 and 12 cm) was conducted at Teagasc, Johnstown Castle Research Centre, Wexford, Ireland. The plots were sampled during four seasons [September/October 2002 (late season), April 2003 (early season), May/June 2003 (mid season) and September 2003 (late season)] and protein fractions were determined in both sheath and lamina material. The protein was highly soluble and on average 19% and 28% of total N was in the form of non-protein N, 16% and 19% in the form of buffer-soluble protein, 52% and 40% in the form of buffer-insoluble protein, and 12% and 13% in the form of potentially available cell wall N for lamina and sheath material, respectively. In both materials only 0.9% of total N was present as unavailable cell wall N. In general the herbage management tools investigated did not have much effect on protein fractionation. The effects of regrowth period, cultivar and cutting height were small and inconsistent. High N application rates significantly increased protein degradability, especially during late season. This is relevant, as it has been shown that enhanced protein degradation increases the potential N loss through urine excretion at a time when urine-N excreted onto pasture is prone to leaching. However, the effect

was most evident for sheath material, which forms only a small proportion of the animals' intake. It was concluded that there appears to be little scope for manipulating the herbage-N fractionation through herbage management. The consequences for modelling herbage quality could be positive as there does not seem to be a need to model the individual N fractions; in most cases the N fractions can be expressed as a fixed proportion of total N instead.

Additional keywords: cutting height, high-sugar grass, N application rate, perennial ryegrass, regrowth period

Introduction

In many parts of Europe, the diet of bovines consists mainly of grazed grass (Beever & Reynolds, 1994; Lantinga *et al.*, 1996). A large proportion of the herbage-nitrogen (N) is not utilized by the animal and is excreted via dung and urine. This contributes to environmental N pollution in the form of ammonia and nitrous oxides in the atmosphere or as nitrate in soil and ground water (Tamminga, 1992). One of the main problems in intensive European grass-based systems is the high N content of the herbage, which is generally highly soluble and therefore rapidly degraded in the rumen (Beever & Reynolds, 1994). A substantial amount of herbage-N is present in the form of non-protein N (nitrate, ammonia, amides and short-chain amino acids) (Nowakowski, 1962) or protein soluble in the cell contents, whereas only a relatively small proportion is linked to the cell wall matrix and therefore slowly degradable or unavailable in the rumen (Valk *et al.*, 1996). When the availability of readily available energy (such as water-soluble carbohydrates) is relatively low, N and energy release in the rumen may become asynchronous. This asynchrony leads to accumulation of ammonia in the rumen, which increases the risk of ammonia loss from the rumen. This ammonia is converted into urea and mainly excreted via urine (Nocek & Russell, 1988).

Decreasing the degradability of the herbage-N is one pathway through which bovine N efficiency may be improved, as it would result in an improved balance between the carbohydrate and protein supply to the rumen. Another effect of decreased protein degradability is the potential increase of the proportion of rumen undegradable protein (RUP) (Buxton, 1996). The digestible part of this RUP can be absorbed from the small intestine as free amino acids and peptides, which can be used directly by the animal (Buxton, 1996; Bohnert *et al.*, 2002). Increasing the proportion of RUP results in lower rumen ammonia levels and increased N recycling to the gut, thus subsequently decreasing urinary-N excretion (Castillo *et al.*, 2001). In an indoor-feeding situation, adjusting protein degradability is relatively straightforward. However, if the intake consists of grazed grass, the herbage quality and protein degradability could potentially be affected by a range of factors, such as weather, soil type and herbage management. Herbage management tools like N application rate and length of regrowth period have been shown to have a significant effect on herbage-N and energy content (Hoekstra *et al.*, 2007a). However, not much is known of the impact of these herbage management tools on the degradability of N (Hoekstra *et al.*, 2007b). There are indications that the degradability of N decreases with increasing length of the regrowth period, as the amount of non-structural N tends to decrease after an initial

peak at 2 weeks (Nowakowski, 1962; Peyraud & Astigarraga, 1998). However, there is very little information on the proportion of N that is allocated to the cell wall, and reported effects of length of regrowth period and N application rate are few and rather inconsistent (Wilman *et al.*, 1977; Valk *et al.*, 1996; Hoekstra *et al.*, 2007b). Licitra *et al.* (1996) developed analytical methods to divide crude protein into five fractions varying in rumen availability and intestinal digestibility. This protein fractionation scheme is used in the Cornell Net Carbohydrate and Protein System (CNCPS), a mathematical model that estimates cattle's requirements and nutrient supply based on animal, environmental, and feed compositional information for a range of production situations (Fox *et al.*, 2004).

We hypothesized that the degradability of herbage-N as measured by the CNCPS protein fractionation scheme can be manipulated by herbage management tools. The objective of the current experiment was to determine the effect of N application rate, length of regrowth period and cutting height on the fractionation of protein in the lamina and sheath material of two perennial ryegrass (*Lolium perenne* L.) cultivars (a high- and a low-sugar cultivar) throughout the growing season.

Materials and methods

Experimental design and sample collection

A field experiment was laid out at Johnstown Castle Research Centre, Wexford, Ireland. For a full description of the trial see Hoekstra *et al.* (2007a). In short, the experiment comprised three replications of the factorial combinations of two perennial ryegrass cultivars [Aberdart (high sugar content, HS) and Respect (low sugar content, LS)], two cutting heights (approximately 8 (LD) and 12 (HD) cm), three regrowth periods (2–3, 4–5, and 6–7 weeks; coded T1, T2 and T3) and three fertilizer N rates (0, 90 and 390 kg N ha^{-1} $year^{-1}$ divided over 7 split applications; coded as N). Plots measured 1.5 m × 2 m. Measurements were taken during four seasons: September/October 2002 (S1; late season), April 2003 (S2, early season), May/June 2003 (S3, mid season) and August/September 2003 (S4, late season).

At each harvest, samples of approximately 250 g herbage were taken by cutting the swards at 1 cm above ground level. The samples were manually divided into sheath (pseudo-stem material consisting of sheath, stem and new leaves within the sheath tube), lamina, inflorescence and dead material (defined as material of which > 50% of surface was dead). The samples were immediately stored in a freezer at –20 °C until later analysis. For further analytical details see Hoekstra *et al.* (2007a).

Chemical analyses

The separated fractions of pseudo-stems, laminae and inflorescences were freeze-dried and subsequently ground over a 1-mm sieve. The samples from the three replications were bulked in order to obtain sufficient material for chemical analysis.

Total N was determined by means of a Kjeldahl analyser. The N fractions in the

Table 1. Description and calculation of the protein fractions used in the Cornell Net Carbohydrate and Protein System model. Based on Licitra *et al.* (1996).

Code	Protein fraction (g per kg total N)	Description	Calculation[1, 2]
A_N	Non-protein N (NPN)	With the precipitants used (tungstic acid), peptides consisting of < 3 amino acids included. 100% available in the rumen.	1000–TPN
$B1_N$	True (buffer) soluble protein	True protein soluble in buffer solution (pH = 6.7–6.8) represents the true protein soluble in rumen solution. Fast rumen degradation, 100% digestible in intestines.	TPN–BIN
$B2_N$	ND[3] soluble N, insoluble in buffer	True protein insoluble in rumen solution. Intermediate rumen degradation rate, 100% intestinal digestibility.	BIN–NDIN
$B3_N$	ND insoluble N, soluble in AD[4]	The nitrogen associated with NDF is normally cell wall-bound protein. This protein is assumed slowly degradable in the rumen, but completely digestible in the intestines.	NDIN–ADIN
C_N	AD insoluble protein N	Used to identify unavailable protein: is assumed to have zero ruminal and intestinal digestibility.	ADIN

[1] All N fractions expressed in g per kg N.

[2] TPN = true protein nitrogen; BIN = buffer insoluble nitrogen; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen.

[3] ND = neutral detergent.

[4] AD = acid detergent.

samples were determined based on the CNCPS fractionation scheme (Table 1). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were analysed sequentially after the NDF and ADF analyses, respectively (Licitra *et al.*, 1996). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991), using a Fibertec apparatus (Tecator, Höganäs, Sweden) for the samples collected in the season S1 and an ANKOM200 Fiber Analyser (Ankom Technology Corporation Macedon, New York, USA) for the samples collected in the seasons S2–S4. True protein nitrogen (TPN) and borate buffer insoluble nitrogen (BIN) were analysed as described by Licitra *et al.* (1996).

In order to check the reproducibility of the analyses, a subset of 24 samples was sent to Cornell University for re-analysis. Here the same methods were used, with the exception of ADIN and NDIN, which were determined using a gravimetric method (Van Soest *et al.*, 1991) (similar to Fibertec, but less automated) instead of the ANKOM200 Fiber Analyser.

The mass fractions of the different N fractions were expressed both in g per kg DM (subscript DM) and in g per kg N (subscript N); the abbreviations for the fractions are explained in Table 1.

Statistical analyses

The correlations between the analytical results of the samples analysed at Wageningen University (WU) and Cornell University (CU) were determined. If the regression coefficients differed significantly ($P < 0.05$) from 1 (corresponding to a statistically significant bias of the residuals) or the mean residual differed significantly ($P < 0.05$) from 0, this was taken as an indication of a relative or absolute bias, respectively.

Analysis of variance was carried out using the SAS GLM procedure (SAS Enterprise Guide version 8.2) to determine the effect of grassland management tools on the N fractions in the herbage samples. The bulking of the material for chemical analysis resulted in single values for all factorial treatment combinations. The main effects (N = N application rate, T = regrowth period, D = cutting height, C = cultivar) and two-way interactions were included in the model (n = 36, d.f. error = 16). The analysis of variance was conducted separately for the four seasons and for lamina and sheath material.

Results

Comparison of analytical results

The WU and the CU results of the chemical analyses are compared in Table 2. The results for TPN and BIN showed a very strong correlation (98.7% and 97.6%, respectively; $P < 0.0001$). However, there was a small, but statistically significant bias

Table 2. Correlation between the analytical results from Wageningen University (WU) and Cornell University (CU).

Analysis[1]	Mean (g per kg DM)		R^2	SE	P [2]	Correction equation	Bias
	WU	CU	(%)				
N-total	17.9	17.2	99.8	0.3	****	–0.11 + 0.97WU	absolute + relative
NDF	474.8	481.5	88.5	17.2	****	–34.6 + 1.09WU	no bias
ADF	253.3	253.4	82.5	12.5	****	44.8 + 0.82WU	relative
TPN	13.2	14.5	98.7	0.8	****	0.55 + 1.06WU	absolute + relative
BIN	11.5	11.7	97.6	0.9	****	0.34 + 0.99WU	no bias
NDIN [3]							
All	4.5	2.4	88.2	0.4	****	0.54 + 0.41WU	absolute + relative
L	6.5	3.2	70.6	0.4	***	0.49 + 0.41WU	absolute + relative
S	2.5	1.6	81.4	0.2	****	–0.82 + 0.98WU	absolute
ADIN	1.3	0.1	3.1	0.1	ns		

[1] NDF = neutral detergent fibre; ADF = acid detergent fibre For the other abbreviations see Table 1.

[2] Statistical significance: *** = $P < 0.001$; **** $P < 0.0001$; ns = not significant.

[3] Correlation for NDIN was calculated for the whole sample (All) and for the lamina (L) and sheath (S) samples separately.

for TPN between the results from WU and CU. For NDIN the correlation was strong (R^2 = 88.2%, $P < 0.0001$), but there was a strong bias (both absolute and relative). The higher NDIN values with the ANKOM200 system employed by WU may be related to incomplete rinsing of the sample bags after the NDR procedure (Bovera *et al.*, 2003). The difference between the WU results and the CU results for NDIN was much less for the sheath material than for the lamina material. This may be related to the presence of macromolecule clusters containing N that were not washed out from the bags (or are more resistant to washing out) and were more abundant in the lamina than in the sheath, such as chlorophyll. Therefore, the correction equations differed for lamina and sheath material.

The values for ADIN were very low and no statistically significant correlation was found between the WU and the CU values, whereas there was up to an 8-fold difference between the two. The variation between duplicates often exceeded 50%, indicating that the analytical variation was relatively large compared with the treatment variation.

Where a statistically significant bias was found (TPN, NDIN and ADIN), the results from CU were more comparable with literature values than the WU results (Wilman *et al.*, 1977; Valk *et al.*, 1996; Boudon & Peyraud, 2001; Smith *et al.*, 2002). Moreover, the CU results for NDIN and ADIN were in good agreement with the values determined at WU for season S1, when the Fibertec was used (comparing S1 with S4, Table 3). So we

Table 3. Mean protein fractions for lamina and sheath material during the four seasons. Standard error[1] in parentheses.

Protein fraction[2]	Season				Mean
	S1	S2	S3	S4	
	----------	----------	(g per kg DM)	----------	----------
Lamina material					
A_{DM}	37 (1.6)	31 (1.8)	21 (1.6)	32 (2.3)	30 (1.1)
$B1_{DM}$	29 (1.7)	28 (1.5)	23 (1.0)	23 (1.0)	26 (0.7)
$B2_{DM}$	93 (4.0)	84 (4.0)	76 (5.0)	81 (2.5)	83 (2.1)
$B3_{DM}$	21 (0.7)	19 (1.1)	18 (1.0)	20 (0.5)	20 (0.4)
C_{DM}[3]	2.4 (0.21)	0.5 (0.52)	1.1 (0.81)	1.5 (0.40)	1.4 (0.43)
Sheath material					
A_{DM}	27 (1.9)	25 (1.8)	15 (1.6)	20 (1.7)	22 (1.0)
$B1_{DM}$	13 (0.6)	13 (0.6)	13 (0.8)	14 (0.5)	13 (0.3)
$B2_{DM}$	30 (1.2)	33 (1.5)	27 (1.7)	24 (0.7)	28 (0.7)
$B3_{DM}$	13 (0.4)	7 (0.5)	7 (0.8)	12 (0.3)	9 (0.4)
C_{DM}[3]	1.6 (0.47)	0.3 (0.35)	0.7 (0.35)	1.2 (0.37)	1.0 (0.30)

[1] For the abbreviations see Table 1.

[2] n = 34 for S1; n = 36 for S2–S4.

[3] Mean values (n = 4) based on WU Fibertec analysis for S1 and CU analyses for S2–S4.

corrected the WU NDIN (S2–S4) and TPN values using the equations in Table 2, before further calculations were made and statistical analyses were carried out. For the ADIN we used the CU values rather than the WU values for S2–S4, because the correlation between WU and CU values was not statistically significant.

Effect of herbage management tools on N fractionation

On dry matter basis

The mass fractions of the five protein fractions in lamina and sheath material were on average 26, 19, 56, 15 and 1.2 g per kg dry matter (DM) for A_{DM}, $B1_{DM}$, $B2_{DM}$, $B3_{DM}$ and C_{DM}, respectively (based on Table 3). Mass fractions of A_{DM}, $B2_{DM}$ and $B3_{DM}$ tended to follow the total protein mass fraction (Hoekstra *et al.*, 2007a, Table 2 and Figure 1): the mass fractions were significantly ($P < 0.001$) higher at higher N application rates and at shorter regrowth periods ($P < 0.01$, except for lamina material during S1 and S4) (data not shown). At longer regrowth periods the effect of N application rate was less strong, resulting in a statistically significant N × T interaction in most cases. In some cases the LS cultivar contained significantly higher mass fractions of A_{DM}, $B2_{DM}$ and $B3_{DM}$. There was no consistent effect of cutting height (data not shown).

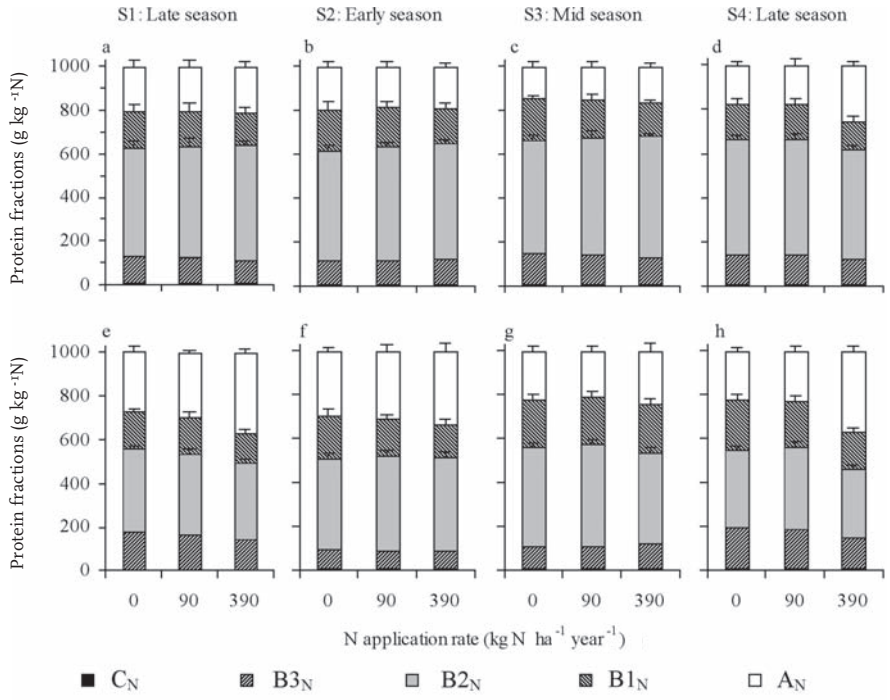

Figure 1. The effect of N application rate (kg N ha^{-1} $year^{-1}$) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N mass fraction (g per kg N) of perennial ryegrass lamina (a–d) and sheath (e–h) material for late season 2002 (a, e), early season 2003 (b, f), mid season 2003 (c, g) and late season 2003 (d, h). Bars represent 2× standard error (n = 12). C_N is the average value over all treatments.

For $B1_{DM}$, there was a statistically significant effect of N application rate and length of regrowth period ($P < 0.05$ and $P < 0.01$, respectively) during S1 and S3, and for lamina material during S2, but no significant effects were found in the other seasons and plant parts. The mass fraction of C_{DM} was very small and there were no statistically significant effects of herbage management tools or significant differences between lamina and sheath material or between seasons (Table 3).

On nitrogen basis

On average, 186 and 280 g per kg N was in the form A_N, 164 and 186 g per kg N in $B1_N$, 519 and 396 g per kg N in $B2_N$, 121 and 130 g per kg N in $B3_N$ and only 9 g per kg N in C_N for lamina and sheath material, respectively (Figures 1–3). The mass fraction of A_N was higher for sheath than for lamina material (280 and 186 g per kg N, respectively), whereas the opposite was found for $B2_N$ (396 and 519 g per kg N for sheath and lamina material, respectively).

The mass fraction of A_N was lower during S3 than during the other seasons (188 and 228 g per kg N, respectively). For sheath material, the $B3_N$ mass fraction increased

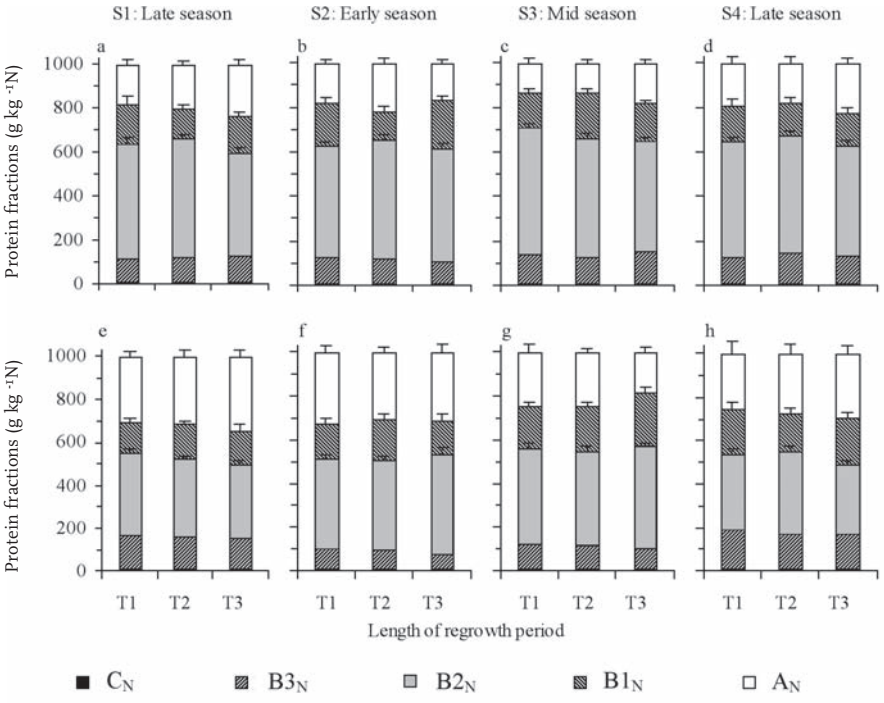

Figure 2. The effect of length of regrowth period (T1 = 2–3, T2 = 4–5, and T3 = 6–7 weeks) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N mass fraction (g per kg N) of perennial ryegrass lamina (a–d) and sheath (e–h) material for late season 2002 (a, e), early season 2003 (b, f), mid season 2003 (c, g) and late season 2003 (d, h). Bars represent 2× standard error (n = 12). C_N is the average value over all treatments.

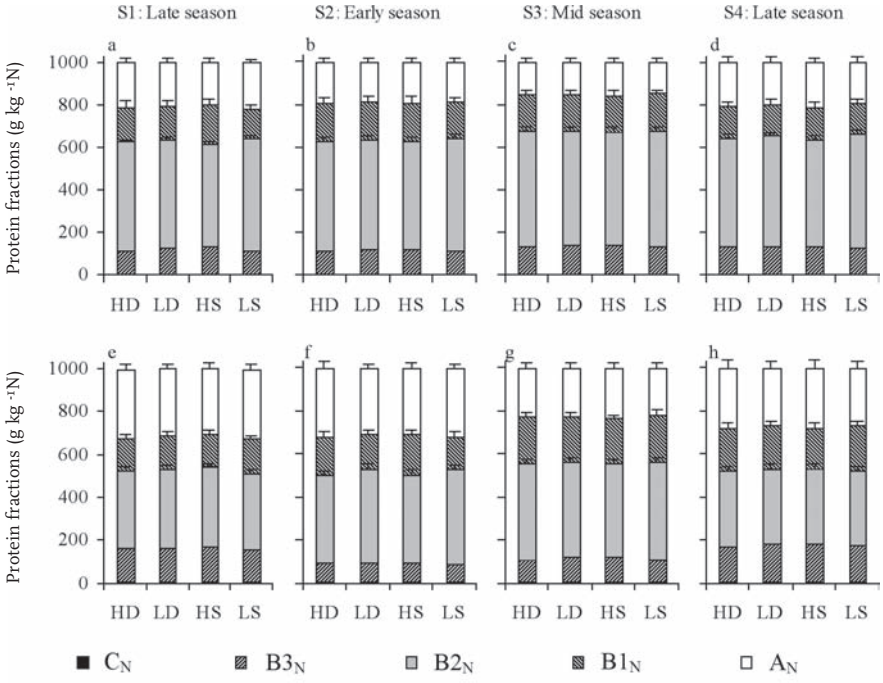

Figure 3. The effect of cutting height (LD = low cutting height, HD = high cutting height) and cultivar (LS = low-sugar cultivar, HS = high-sugar cultivar) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N mass fraction (g per kg N) of perennial ryegrass lamina (a–d) and sheath (e–h) material for late season 2002 (a, e), early season 2003 (b, f), mid season 2003 (c, g) and late season 2003 (d, h). Bars represent 2× standard error (n = 18). C_N is the average value over all treatments.

with progressing season (86, 108 and 162 g per kg N for early, mid and late season, respectively).

N application rate had the strongest effect on sheath material, where it significantly ($P < 0.05$) affected the N fractionation in most cases during late season and in some cases during mid season (Figure 1). The mass fraction of A_N and $B1_N$ tended to be higher at high N application rates, whereas for $B3_N$ and $B2_N$ the opposite was true. For lamina material the effect was only significant ($P < 0.01$) during late season when $B3_N$ was lower and A_N (not during S1) was higher at high N application rates.

In some cases there was a statistically significant ($P < 0.05$) effect of regrowth period (Figure 2). During early season the effect was very inconsistent. For sheath material during mid season there was an increase in $B1_N$ and $B2_N$ at the expense of A_N. In sheath material (and to a lesser extent in lamina material) during late season, A_N tended to increase and $B2_N$ and $B3_N$ tended to become smaller at longer regrowth lengths.

There were only few statistically significant ($P < 0.05$) effects of cultivar and cutting height but the effects were small and inconsistent (Figure 3).

Discussion

N fractionation of herbage

In line with expectations we found that the protein in grass was highly soluble. Nearly a quarter of the protein was in the form of non-protein N (A_N), which is within the range of values reported in literature (Reid & Strachan, 1974; Boudon & Peyraud, 2001). The true protein in the cell contents consisted mostly of $B2_N$ (458 g per kg N) (insoluble in the buffer solution, representing rumen liquid) and the remainder formed the $B1_N$ fraction (175 g per kg N). We found only one other publication in which the B1 and B2 fractions were measured in herbage (Rinne *et al.*, 1997). However, the subject of this study was timothy grass (*Phleum pratense*) rather than perennial ryegrass, and the results do not appear to be comparable, as timothy grass has a higher cell wall nitrogen content. Our results showed that the cell wall protein consisted mainly of $B3_N$ (125 g per kg N), which is potentially degradable in the rumen. Only a very small fraction was in the form of C_N (9 g per kg N), which is assumed undegradable. This is in agreement with values reported in the literature (Wilman *et al.*, 1977; Wilman & Wright, 1978; Valk *et al.*, 1996; Smith *et al.*, 2002).

Effect of herbage management on N fractionation

N application rate, length of regrowth period and to a lesser extent cultivar had a statistically significant effect on the protein fractions if expressed on a DM basis, but this was mainly a reflection of the changes in the total N mass fraction. Therefore, when the protein fractions were expressed in g per kg of total N, most of the effects were small or non-significant. Under the current experimental conditions (3–7 weeks regrowth with no extreme growth conditions) the grass plant apparently is very conservative in the way it deals with N, as the distribution of N over the different N fractions is fairly constant, irrespective of developmental stage.

N application rate tended to have most effect for sheath material and during late season (S1 and S4), when it increased the proportion of non-protein N (A_N) at the expense of the true protein N (B_N). This effect is in agreement with the results of other studies (Nowakowski, 1962; Wilman *et al.*, 1977; Valk *et al.*, 1996; Peyraud & Astigarraga, 1998). During late season, factors other than N availability (like temperature and sunlight) may have been limiting plant growth. This would result in an accumulation of non-protein N in the plant, which cannot be converted into amino acids and proteins for plant growth due to a lack of available energy (in the form of water-soluble carbohydrates) (Hoekstra *et al.*, 2007b). Apparently, during early and mid season, the N availability was not in excess of the (relatively high) crop demand at the N application rates used in this experiment.

Some effect would have been expected of the length of the regrowth period on A_N, as there tends to be an initial surge of N uptake just after N application. The non-protein N is subsequently converted into plant protein (Nowakowski, 1962), resulting in a decrease in A_N mass fraction with increasing regrowth period. In this experiment, no such decrease was found (an opposite trend was observed instead), implying that

the non-protein N of the initial boost had already been converted into plant protein before weeks 2–3. The effect of length of the regrowth period on the other protein fractions was also inconsistent. In some studies the cell wall N (NDIN) was reported to increase with length of the regrowth period (Cone *et al.*, 1996; Boudon & Peyraud, 2001), which tends to be related to the increased proportion of cell wall material in the plant. However, in an earlier study (Hoekstra *et al.*, 2007a) changes in NDF mass fraction as a result of length of the regrowth period were relatively small and other studies have shown inconsistent effects (Wilman *et al.*, 1977).

There was not much effect of cultivar on N fractionation. Similarly, the results of Smith *et al.* (2002) show no relation between NDIN mass fraction and low- or high-sugar cultivar either. However, the lack of effect of cultivar in our experiment does not exclude the potential for cultivars with different protein fractionation, as our cultivars were selected for their difference in water soluble carbohydrates (WSC) mass fraction rather than protein degradability. Also cutting height had no consistent effect on N fractionation.

There is virtually no information available on differences in N fractionation between lamina and sheath material in perennial ryegrass. On the whole, herbage management tended to affect N fractionation in the sheath material more than in the lamina material. The A_N fraction was much larger in sheath material than in lamina material, which may indicate that excess N is stored in the sheath before it is converted into protein. Similarly, sheaths also form the main site for storage of carbohydrate reserves in the form of WSC, which is an important feature for recovery and initial regrowth after cutting (Fulkerson & Donaghy, 2001).

Potential for impact on bovine N utilization

During late season (S1 and S4), high N-application rates resulted in higher protein degradability. High protein degradability is likely to result in a lower bovine-N utilization, resulting in increased N losses via urine (Nocek & Russell, 1988). This is especially relevant in grazing systems, as urine-N excreted onto the pasture during late season is very prone to leaching (McGechan & Topp, 2004; Schulte *et al.*, 2006). However, the effect of N application rate was strongest for sheath material, which forms only a small portion of the total intake (Brereton *et al.*, 2005).

Rumen undegradable protein (RUP) is mainly related to cell wall material, and the range in $B3_N$ is relatively small (max 20% increase between 0 and 390 N application rate). Therefore the herbage management tools studied in this experiment do not appear to be effective for manipulating the RUP mass fraction. However, studies based on *in sacco* degradation (Van Vuuren *et al.*, 1991; Valk *et al.*, 1996) did indicate a slight increase in RUP for longer regrowth periods and lower N application rates. This may indicate that the actual situation in the rumen differs from laboratory analysis. Therefore the dynamics of degradation of the cell walls in the rumen may affect N degradation.

The finding that the ratio between nitrogen fractions is rather constant and that all fractions seem to follow the total N content of herbage (with the exception of cuts under high N application rates during late season) could have positive implications for

modelling herbage quality. For example, the CNCPS model requires N fractionation as an input to calculate protein available for production. The lack of response of N fractionation to herbage management would allow the use of standard 'feed library values' for the N fractions as a proportion of total N for different seasons.

Conclusions

- The protein in perennial ryegrass was highly soluble with on average 23% non-protein N, 12% in potentially available cell wall N and only 0.9% unavailable.
- The protein fraction expressed on a DM basis responded strongly to herbage management. However, the contents mainly followed the changes in total N. Consequently, if expressed as proportion of total N, there was not much effect of herbage management.
- During late season, high N application rates increased the protein degradability (higher A_N and lower B_N). This potentially increases the N loss through urine excretion at a time when urine-N excreted onto pasture is very prone to leaching.
- Changes in sheath material tended to be more pronounced than changes in lamina material, limiting the effect on the N fractionation of the intake, as sheath material only forms a small portion of the intake during grazing.
- There appears to be limited scope for the manipulation of herbage-protein fractionation through herbage management.
- The consequences for modelling herbage quality could be positive as there does not seem to be a need to model the individual N fractions; in most cases the N fractions can be expressed as a fixed proportion of total N instead.

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References

Beever, D.E. & C.K. Reynolds, 1994. Forage quality, feeding value and animal performance. Paper presented to the Grassland and Society. In: L. 'T Mannetje & J. Frame (Eds), Grassland and Society. Proceedings of the 15th General Meeting of the European Grassland Federation, 6–9 June 1994, Wageningen. Wageningen Pers, Wageningen, pp. 48–60.

Bohnert, D.W., C.S. Schauer & T. DelCurto, 2002. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: Cow performance and efficiency of nitrogen use in wethers. *Journal of Animal Science* 80: 1629–1637.

Boudon, A. & J.L. Peyraud, 2001. The release of intracellular constituents from fresh ryegrass (*Lolium*

perenne L.) during ingestive mastication in dairy cows: effect of intracellular constituent, season and stage of maturity. *Animal Feed Science and Technology* 93: 229–245.

Bovera, F., M. Spanghero, G. Galassi, F. Masoero & A. Buccioni, 2003. Repeatability and reproducibility of the Cornell Net Carbohydrate and Protein System analytical determinations. *Italian Journal of Animal Science* 2: 41–50.

Brereton, A.J., N.M. Holden, D.A. McGilloway & O.T. Carton, 2005. A model describing the utilization of herbage by cattle in a rotational grazing system. *Grass and Forage Science* 60: 367–384.

Buxton, D.R., 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* 59: 37–49.

Castillo, A.R., E. Kebreab, D.E. Beever, J.H. Barbi, J.D. Sutton, H.C. Kirby & J. France, 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *Journal of Animal Science* 79: 247–253.

Cone, J.W., A.H. Van Gelder & H.J.P. Marvin, 1996. Influence of drying method and ageing on chemical and physical properties and in vitro degradation characteristics of grass and maize samples. *Journal of Agricultural Science, Cambridge* 126: 7–14.

Fox, D.G., L.O. Tedeschi, T.P. Tylutki, J.B. Russell, M.E.V. Amburgh, L.E. Chase, A.N. Pell & T.R. Overton, 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology* 112: 29–78.

Fulkerson, W.J. & D.J. Donaghy, 2001. Plant-soluble carbohydrate reserves and senescence – key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* 41: 261–275.

Hoekstra, N.J., P.C. Struik, E.A. Lantinga & R.P.O. Schulte, 2007a. Chemical composition of lamina and sheath of *Lolium perenne* as affected by herbage management. *NJAS – Wageningen Journal of Life Sciences* 55: 55–73.

Hoekstra, N.J., R.P.O. Schulte, E.A. Lantinga & P.C. Struik, 2007b. Pathways to improving the N efficiency of grazing bovines. *European Journal of Agronomy* 26: 363–374.

Kebreab, E., J. France, D.E. Beever & A.R. Castillo, 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems* 60: 275–285.

Lantinga, E.A., N. Gaborcik & B.O.M. Dirks, 1996. Ecophysiological aspects of herbage production in grazed and cut grassland. In: G. Parente, J. Frame & S. Orsi (Eds), Proceedings of the 16th General meeting of the European Grassland Federation, 15–19 September 1996, Grado. Agenzia Regionale per lo Sviluppo Regionale (ERSA), Gorizia, pp. 151–161.

Licitra, G., T.M. Hernandez & P.J. Van Soest, 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* 57: 347–358.

McGechan, M.B. & C.F.E. Topp, 2004. Modelling environmental impacts of deposition of excreted nitrogen by grazing dairy cows. *Agriculture, Ecosystems and Environment* 103: 149–164.

Nocek, J.E. & J.B. Russell, 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial protein synthesis and milk production. *Journal of Dairy Science* 71: 2070–2107.

Nowakowski, T.Z., 1962. Effects of nitrogen fertilizer on total nitrogen, soluble nitrogen and soluble carbohydrate content in grass. *Journal of Agricultural Science, Cambridge* 59: 387–392.

Peyraud, J.L. & L. Astigarraga, 1998. Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: consequences on animal nutrition and N balance. *Animal Feed Science and Technology* 72: 235–259.

Reid, R.L. & N.H. Strachan, 1974. The effects of a wide range of nitrogen rates on some chemical

constituents of the herbage from perennial ryegrass swards with and without white clover. *Journal of Agricultural Science, Cambridge* 83: 393–401.

Rinne, M., S. Jaakkola & P. Huhtanen, 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. *Animal Feed Science and Technology* 67: 1–17.

Schulte, R.P.O., K. Richards, K. Daly, I. Kurz, E.J. McDonald & N.M. Holden, 2006. Agriculture, meteorology and water quality in Ireland: a regional evaluation of pressures and pathways of nutrient loss to water. *Biology and Environment* 106: 117–133.

Smith, K.F., R.A. Culvenor, M.O. Humphreys & R.J. Simpson, 2002. Growth and carbon partitioning in perennial ryegrass (*Lolium perenne*) cultivars selected for high water-soluble carbohydrate concentrations. *Journal of Agricultural Science, Cambridge* 138: 375–385.

Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. *Journal of Dairy Science* 75: 345–357.

Valk, H., I.E. Kappers & S. Tamminga, 1996. In sacco degradation characteristics of organic matter, neutral detergent fibre and crude protein of fresh grass fertilized with different amounts of nitrogen. *Animal Feed Science and Technology* 63: 63–87.

Van Soest, P.J., J.B. Robertson & B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74: 3583–3597.

Van Vuuren, A.M., S. Tamminga & R.S. Ketelaar, 1991. *In-sacco* degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows. *Journal of Agricultural Science, Cambridge* 116: 429–436.

Wilman, D. & P.T. Wright, 1978. The proportions of cell content, nitrogen, nitrate-nitrogen and water-soluble carbohydrates of three grasses in early stages of regrowth after defoliation with and without applied nitrogen. *Journal of Agricultural Science, Cambridge* 91: 381–394.

Wilman, D., M. Daly, A. Koocheki & A.B. Lwoga, 1977. The effects of interval between harvests and nitrogen application on the proportion and digestibility of cell wall, cellulose, hemicellulose, and lignin and on the proportion of lignified tissue in leaf cross-section in two perennial ryegrass varieties. *Journal of Agricultural Science, Cambridge* 89: 53–63.