Pathways for improving the nitrogen efficiency of grazing bovines

Promotor: Prof. dr. ir. P.C. Struik
Hoogleraar gewasfysiologie, Wageningen Universiteit

Co-promotoren: Dr. ir. E.A. Lantinga
Universitair hoofddocent bij de leerstoelgroep Biologische landbouwsystemen, Wageningen Universiteit
Dr. ir. R.P.O. Schulte
Head of Environmental Research Department, Teagasc, Johnstown Castle Research Centre, Wexford, Ireland

Promotiecommissie:
Prof. dr. ir. H. van Keulen, Wageningen Universiteit
Dr. P. O'Kiely, Teagasc, Grange Beef Research Centre, Ireland
Dr. ir. A.M. van Vuuren, Animal Sciences Group, Lelystad
Dr. ir. J. Dijkstra, Wageningen Universiteit

Dit onderzoek is uitgevoerd binnen de C.T. de Wit onderzoekschool: Production Ecology and Resource Conservation.

Pathways for improving the nitrogen efficiency of grazing bovines

N.J. Hoekstra

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff,
in het openbaar te verdedigen
op woensdag 19 december 2007
des namiddags te vier uur in de Aula.

Nyncke Jitske Hoekstra
Pathways for improving the nitrogen efficiency of grazing bovines
2007

PhD Thesis, Wageningen University, The Netherlands
With references - With summaries in English and Dutch

ISBN: 978-90-8504-783-4

Abstract

Hoekstra, N.J., 2007. Pathways for improving the nitrogen efficiency of grazing bovines. PhD thesis, Wageningen University, Wageningen, The Netherlands. With references - With summaries in English and Dutch, 160 pp.

Livestock production has been identified as a major source of nitrogen (N) losses in agro-ecosystems. N excreted in dung and urine contributes to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water. Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal. Bovine N utilisation can be increased substantially through changing the composition of the diet. In many parts of Europe, a large proportion of the bovine's diet consists of grass taken up by grazing. Manipulating the nutritional composition of grazed grass poses a complex challenge, since it is hard to control the diet under grazing as this depends on grassland management and environmental factors.

The objective of this research was to investigate the efficacy of grassland management tools for manipulating herbage quality and to assess the subsequent effect on the N efficiency of grazing cows.

In the literature review, three pathways were identified through which more efficient N utilisation by grazing bovines can be achieved by manipulation of the chemical composition of the grass forage: 1) matching protein supply to animal requirements, 2) balancing and synchronising carbohydrate and N supply in the rumen, and 3) increasing the proportion of rumen undegradable protein (RUP). Under grazing conditions, grassland management tools, such as the length of the regrowth period, defoliation height, fertiliser N application rate, and growing high-sugar grass cultivars, are the main tools to manipulate herbage quality and subsequent bovine N efficiency.

A field experiment was carried out to study the effect of those grassland management tools on the chemical composition of lamina and sheath material. These results were used to design a model for predicting the efficacy of herbage management tools for affecting the quality of herbage ingested by cattle under strip-grazing management. This model was validated and connected to the Cornell Net Carbohydrate and Protein System model in order to evaluate the effectiveness of a) the grassland management tools and b) the herbage quality pathways on the N utilisation of grazing dairy cows.

Within the modelled scenario the concentration of crude protein (CP) in the ingested dry matter (DM) was the main factor affecting N utilisation. Model predictions indicated that herbage should be managed to achieve a CP concentration of 130−150 g / kg DM in order to maximise the efficiency of N utilisation for milk production and minimise the proportion of N excreted in urine. Both N application rate and rotation length were shown to be effective tools for affecting the CP concentration of the intake and subsequent cow N utilisation. However, there was no effect of the high-sugar cultivar and defoliation height on cow N utilisation. Assessment of the effectiveness of the three herbage quality pathways for improving bovine N utilisation resulted in the following conclusions: 1) N utilisation is strongly related to the daily N intake (g / day), however, this seems more connected to the N concentration of the ingested DM (g / kg DM), rather than the actual daily N intake. Therefore, the effect is more related to the balance between energy and N (pathway 2). 2) The balance between N and energy is the most important herbage quality factor for improving bovine N utilisation. In contrast, the synchronisation between the release of energy and N seems to have little effect. 3) The proportion of protein in the form of RUP is not much affected by the herbage management tools, and is therefore not an effective pathway for improving the N utilisation of grazing cows.

It is recommended that the model will be extended to include a herbage yield and intake component. This would allow the model to be used to design herbage management systems to optimise N utilisation on a yearly basis.

Additional key words: cow nutrition, defoliation height, grassland management, herbage quality, high-sugar cultivars, *Lolium perenne*, nitrogen application rate, nitrogen utilisation, perennial ryegrass, regrowth period, water-soluble carbohydrates.

Table of contents

1

General introduction

General introduction

Context

Grassland covers approximately 25% of the world area (FAO, 2007). Of the 4.3 million hectares used for agriculture in Ireland, around 90 percent is under grassland, the majority of which is permanent pasture. The dairy sector is the most important sector of Irish agriculture, accounting for 28 percent of the value of agricultural output. It is estimated that up to 85% of the milk produced in Ireland comes from grazed grass (Dillon & Stakelum, 1999).

Problem statement

Livestock production has been identified as a major source of nitrogen (N) losses in agro-ecosystems. N excreted in dung and urine contributes to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water (Tamminga, 1992). Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal (Jonker *et al*., 1998). In general, the N utilisation of grazing cows for milk production is often lower than 25% of N intake (Tas *et al*., 2006), although the theoretical maximum of efficiency of N utilisation may reach 40 to 45% of N intake (Van Vuuren & Meijs, 1987).

Indoor feeding experiments have provided evidence that it is possible to increase the bovine N utilisation substantially through changing the composition of the diet, resulting in a significant reduction of N losses to the environment (Jonker *et al.*, 1998; James *et al*., 1999; Kröber *et al*., 2000). Pathways for increasing animal N-efficiency using mixed diets of herbage and supplements have been reviewed by

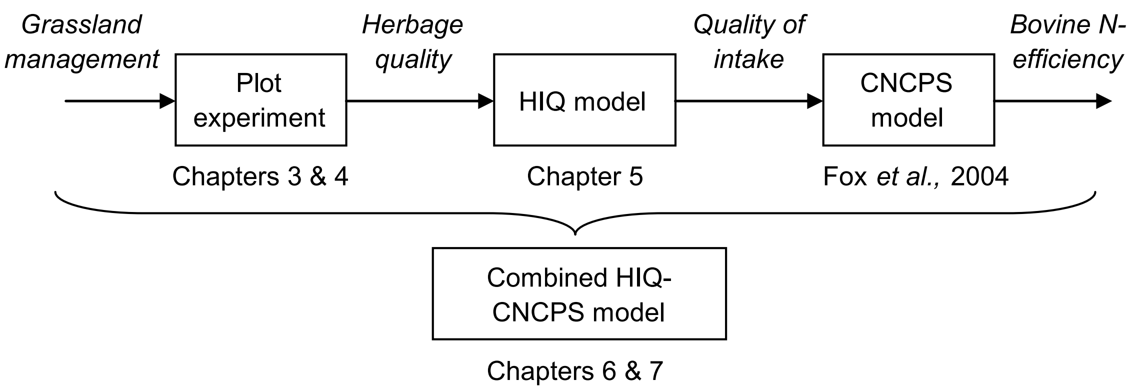

Figure 1 Overview of the structure of the thesis, in which field experiments, animal experiments and modelling work are combined to address the research objective: to assess the efficacy of grassland management tools for manipulating the herbage quality and to assess the subsequent effect on the N efficiency of grazing cows.
HIQ = herbage intake quality; CNCPS = Cornell Net Carbohydrate and Protein System.

Castillo *et al.* (2000; 2001a; b). However, in many parts of Europe, a large proportion of the bovine's diet consists of grass taken up by grazing (Beever & Reynolds, 1994; Lantinga *et al.*, 1996). Specifically, in Ireland, increasing the proportion of grazed grass in the cow's diet is seen as an important way of improving farm profitability, resulting in increasingly longer grazing seasons (Kennedy *et al.*, 2007). Manipulating the nutritional composition of grazed grass poses a more complex challenge, since it is much harder to control the diet under grazing as this depends on grassland management and environmental factors.

Objectives

The objectives of this research were:

- to investigate the efficacy of grassland management tools for manipulating a) herbage quality, and b) the quality of the herbage ingested during grazing; and
- to assess the subsequent effect on the N efficiency of grazing cows.

The research consisted of a combination of literature review, plot experiments, animal experiments and modelling work (Fig. 1). In the literature review (Chapter 2) the research questions and hypotheses are further outlined and these form the basis of the following Chapters 3−7.

Outline of thesis

First, a literature review was conducted in order to assess the main pathways for improving the nitrogen efficiency of grazing bovines (Chapter 2). The review first identifies the main pathways through which more efficient N utilisation by grazing bovines can be achieved by manipulating the grass quality. Subsequently, the spatial and temporal variation of the herbage chemical composition and the main processes involved are described. This knowledge is then used to describe how this grass quality may be manipulated by grassland management tools, i.e. length of the regrowth period, the residual sward height and length of grazing period, fertiliser N application rate and high-sugar grass cultivars, with the objective to assess the potential effect of these herbage management tools on bovine N efficiency.

The effect of grassland management on herbage quality

Based on the findings of the literature review a field experiment with small cut plots was designed and carried out in which the effects of length of regrowth period, defoliation height, N application rate and high-sugar grass cultivar on the water-soluble carbohydrates (WSC), the neutral detergent fibre (NDF), the acid detergent fibre (ADF) and the total N content of lamina, sheath, and inflorescence material were measured (Chapter 3). Chapter 4 presents the results of the same experiment for N fractionation, with the hypothesis that protein degradability as measured by the Cornell Net Carbohydrate and Protein System (CNCPS) protein fractionation scheme can be manipulated by grassland management tools.

The effect of grassland management tools on the quality of herbage ingested during grazing

The herbage quality of the ingested herbage is not equivalent to the quality of the standing herbage. It is well known that animals graze selectively, not only between plant species, but also intra-specifically between plant organs (Curll *et al*., 1985; Grant *et al*., 1985). The prediction of this quality of herbage intake is further complicated by the spatial heterogeneity of herbage quality and grass morphology in the sward, which originates largely from the deposition of animal faeces (Lantinga *et al.*, 1987). Therefore, in Chapter 5, the results from the cut plot experiment and a grazing experiment are used to develop, validate and apply a herbage intake quality model (HIQ-model) to assess the efficacy of herbage management tools on the WSC, N and NDF concentration of herbage intake during grazing.

The effect of grassland management tools on the nitrogen efficiency of grazing bovines

The link with bovine N efficiency is established in Chapter 6, in which this HIQ model is linked to the Cornell Net Carbohydrate and Protein System (CNCPS) model, a mathematical model to evaluate diet and animal performance that was developed from basic principles of rumen function, microbial growth, feed digestion and passage, and animal physiology. This is followed by a validation of the combined model.

In the synthesis chapter (Chapter 7), the HIQ-CNCPS model and the results of the previous chapters are applied to evaluate the effectiveness of a) the grassland management tools and b) herbage quality pathways identified in Chapter 2 on the N efficiency of grazing dairy cows.

This thesis contributes to the analysis of nitrogen flows in the soil-plant-animal system of permanent grasslands grazed by bovines by describing in quantitative terms the impact of different management tools on herbage quality, herbage intake quality and nitrogen utilisation by the animal.

2

Pathways for improving the nitrogen efficiency of grazing bovines

N.J. Hoekstra, R.P.O. Schulte, P.C. Struik & E.A. Lantinga, 2007. *European Journal of Agronomy* 26, 363-374

Abstract

Livestock production has been identified as a major source of nitrogen (N) losses. Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal. The objective of this chapter is to identify pathways for producing grass-based diets that maximise bovine N utilisation during grazing, based on literature on the interface of plant and animal sciences. The focus is on Western-European perennial ryegrass-based systems under rotational grazing and both beef and dairy production systems are considered.

Three pathways have been identified through which more efficient N utilisation by grazing bovines can be achieved by manipulation of the chemical composition of the grass forage: 1) Matching protein supply to animal requirements, 2) balancing and synchronising carbohydrate and N supply in the rumen, and 3) increasing the proportion of rumen undegradable protein (RUP).

Matching the diet requirements of grazing bovines through herbage manipulation encompasses the manipulation of carbon (C) and nitrogen (N) contents of growing herbage. These C and N contents vary both spatially within the grass sward and over time. Under grazing conditions, grassland management tools, such as the length of the regrowth period, grazing intensity, fertiliser N application rate and herbage cultivar are the main pathways to manipulate C and N dynamics. Regrowth length, N application rate and high-sugar cultivars were shown to be the most promising grassland management tools with respect to manipulating herbage quality and subsequent bovine N efficiency. However, these management tools are interrelated and may show adverse effects on production.

Due to the complex nature of interactions, modelling is essential in order to quantify and predict the effect of any combination of herbage management tools under specific circumstances.

Areas in which additional research is required are the fractionation of N compounds in herbage as affected by herbage management, and the effect of high-sugar cultivars on bovine N efficiency under a range of herbage management combinations.

Keywords: grassland management, herbage management, herbage quality, high-sugar cultivars, nitrogen utilisation

1. Introduction

Livestock production has been identified as a major source of nitrogen (N) losses. N excreted in dung and urine contributes to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water (Tamminga, 1992). Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal (Jonker *et al*., 1998).

Indoor feeding experiments have provided evidence that it is possible to increase the bovine N utilisation substantially through changing the composition of the diet, resulting in a significant reduction of N losses to the environment (Jonker *et al*., 1998; James *et al*., 1999; Kröber *et al*., 2000). Pathways for increasing animal N-efficiency using mixed diets of herbage and supplements have been reviewed by Castillo *et al*. (2000; 2001a; b). However, in many parts of Europe, a large proportion of the bovine's diet consists of grass taken up by grazing (Beever & Reynolds, 1994; Lantinga *et al*., 1996). Specifically, in Ireland and UK, livestock production systems are increasingly dependent on grass-only diets, which is accompanied by increasingly longer grazing seasons. Manipulating the nutritional composition of this grazed grass poses a more complex challenge than changing the diet when animals are kept indoor, since 1) it is much harder to control the diet under grazing compared to stable feeding, and 2) it is difficult to reconcile the optimum composition with the imperative of productivity, as the high N fertiliser application levels required for high grass production levels reduce N utilisation.

One of the major challenges associated with diets consisting of grazed grass is the low efficiency of protein utilisation. This can be largely attributed to impaired rumen function due to 1) the relatively high concentration of soluble protein, and 2) the imbalance in the supplies of carbohydrate and protein (Beever & Reynolds, 1994; Lantinga & Groot, 1996). When supplies of readily available energy (mainly water-soluble carbohydrates) in the rumen are sufficiently high, amino acids taken up by the microbes can be incorporated into microbial protein. However, when the availability of water-soluble carbohydrates is relatively low, either amino acids or structural carbohydrates of the plant are used by rumen microbes for the bulk of their energy supply. These compounds are relatively slowly degradable and, as a result, there can be a lack of both balance and synchronisation of N and energy release in the rumen. This leads to ammonia accumulation in the rumen, which is absorbed across the rumen wall and subsequently converted into urea (Nocek & Russell, 1988; Miller *et al*., 2001). This urea is mainly excreted through the urine and rapidly converted to ammonia, which is highly prone to volatilisation (Jarvis *et al*., 1989), and to nitrates, which can either be used by crops or lost through leaching (Smith & Frost, 2000).

The objective of this chapter is to identify pathways for producing grass-based diets that maximise bovine N utilisation during grazing, based on literature on the interface of plant and animal sciences. The chapter consists of the following topics: 1)

Evaluation of the optimum diet composition required for maximum bovine N utilisation, 2) Short overview of N and C dynamics in perennial ryegrass during growth, 3) Review of individual grassland management tools aimed at producing herbage for maximum bovine N efficiency, and 4) Integration of these tools at farm scale, i.e. strategies at farm system level.

Throughout the chapter the focus will be on Western-European perennial ryegrass-based long term grassland under rotational grazing and both beef and dairy production systems will be considered.

2. Pathways for improving bovine N efficiency

Throughout this chapter bovine N efficiency is defined as N utilisation, i.e. the N output in milk and meat, divided by the N intake through diet. In addition to N efficiency, the partitioning of the N loss over urine and faeces is important, as N in urine is a much more important source of pollution than faecal N (Van Horn *et al.*, 1996). Three pathways have been identified through which more efficient N utilisation by grazing bovines can be achieved by manipulating the chemical composition of the grass forage: 1) Matching protein supply to animal requirements, 2) Balancing and

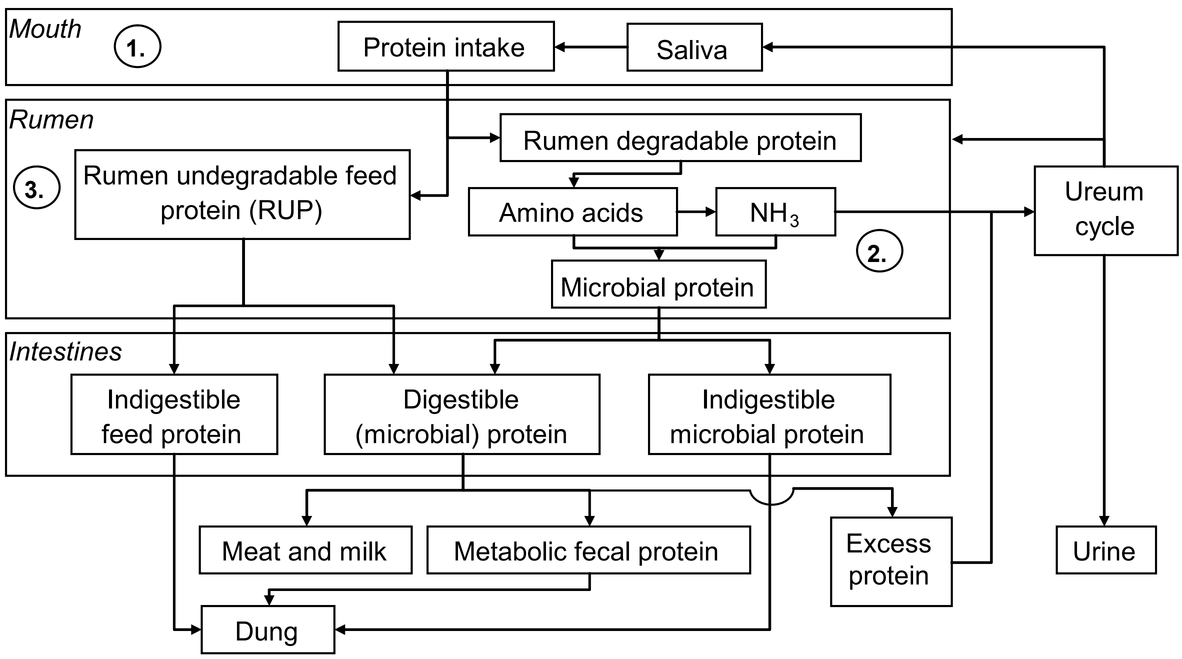

Figure 1 Simplified schedule of nitrogen digestion by bovines and pathways to improve nitrogen efficiency. ① Matching protein supply to animal requirements; ② Balancing and synchronising carbohydrate and N supply in the rumen; ③ Increasing the proportion of rumen undegradable protein (RUP).

synchronising carbohydrate and N supply in the rumen, and 3) Increasing the proportion of rumen undegradable protein (RUP).

2.1 Matching protein supply to animal requirements

Matching the herbage protein content to animal requirements is an important way to improve bovine N efficiency (Fig. 1,①) Animal requirements for N change with physiological state and may vary from 11 g / kg of dry matter (DM) intake for maintenance to up to 32 g / kg DM for lactation and growth (Thompson & Poppi, 1990). The minimum N concentration in pasture DM required in order to avoid impaired ruminal digestion is approximately 24 g N / kg pasture DM (Thompson & Poppi, 1990; Tamminga & Verstegen 1996). Herbage protein concentrations vary depending on environmental variables and herbage management, resulting in either excess or shortage of forage protein supply to the animal. N consumed in excess of animal requirement is excreted in faeces and urine (Kirchgessner *et al.*, 1994). A number of studies have shown that decreasing N intake not only reduces total N excretion, but also the proportion of urinary N (James *et al.*, 1999; Kröber *et al.*, 2000; Castillo *et al.*, 2001b; Kebreab *et al.*, 2001), which should in turn reduce the potential for ammonia emissions (Table 1, Fig. 2).

On the other hand, shortage of protein will limit ruminal microbial yield and animal performance (Beever & Reynolds, 1994; Tamminga & Verstegen, 1996). Dietary protein intakes below the animal requirements for maintenance may even result in negative N efficiencies in terms of product N / input N (Bohnert *et al.*, 2002), if animals start to use body reserves. In addition, Minson (1990) stated that when forage N concentrations fall below 11.2 g N / kg DM, there is a rapid fall in voluntary intake, which has a direct effect on animal performance.

2.2 Balancing and synchronising rumen carbohydrates and N supply

A second pathway through which animal N efficiency may be improved is through balancing and synchronising carbohydrates and protein supply to the rumen (Fig. 1,②). This implies the optimisation of both the amounts of rumen available carbohydrates and protein and their respective degradation rates in the rumen.

Leaf N content in grazed grass is generally high, the N is highly soluble and therefore rapidly degraded in the rumen (Beever & Reynolds, 1994). In order to capture this N, an adequate source of readily available energy is required. Water-soluble carbohydrates are released into the rumen after disruption of the plant cell, e.g. through mastication by the ruminant, and are immediately available for the rumen microorganisms. Cell walls are more resistant to microbial attack and their rate of degradation may be too low to assure a carbohydrate supply for microbial growth and

Table 1

Effect of concentrate based diet N content on N utilisation of bovines.

Diet	N intake		N output (g / kg N intake)			Ammonia N losses	Milk yield	Source [a]
	(g N / kg DM)	(g N / day)	Milk	Faeces	Urine	(g / kg N intake)	(kg / day)	
Low N	19.8	412	357	434	133	61	30.5	1
Mid N	23.5	512	314	400	400	111	32.5	
High N	28.0	604	267	325	325	147	32.9	
Low N	15.4	111		410	560	396		2
High N	17.4	129		353	592	475		
Low N	23.3	422	261	318	361	nd [b]	23.1	3
High N	29.3	516	237	287	433	nd	24.5	

[a] 1 = Kröber *et al*., 2000; Early-lactation Brown Swiss cows, concentrate based diet, N-losses calculated based on 7 weeks slurry storage; 2 = James *et al*., 1999; Holstein heifers, total mixed ration; 3 = Castillo *et al*., 2001b; Holstein/Friesian cows in early to mid lactation, fed on silage and concentrates.

[b] nd = not determined.

efficient N utilisation (Beever & Reynolds, 1994; Lantinga & Groot, 1996). Increasing the WSC content has been advocated as being an important way to improve N utilisation of forages, through the improved balance and synchronisation of release of C and N compounds (Beever & Reynolds, 1994; Lantinga & Groot, 1996; Miller *et al*., 2001). Inversely, synchronisation may be increased by reducing the degradability of N compounds, that is, by increasing the proportion of N related to the cell wall at the expense of soluble N and especially non-protein N (Beever & Reynolds, 1994).

The need to balance daily inputs of ruminally available energy and N is well recognised (Nocek & Russell, 1988). However, studies that have aimed to synchronise the ruminal degradation of N and energy in mixed diets have produced conflicting results.

Yields of microbial N increased when diets were designed to match the amount and pattern of degradation of degradable energy with those of N (Herrera-Saldana *et al*., 1990; Aldrich *et al*., 1993; Sinclair *et al*., 1993, 1995; Kolver *et al*., 1998b), but the effect on milk production remains unclear (Herrera-Saldana & Huber, 1989; Aharoni *et al*., 1993; Aldrich *et al*., 1993; Kolver *et al*., 1998b). Henning *et al*. (1993) and Newbold & Rust (1992) concluded that merely synchronising energy and N release rates in the rumen does not necessarily increase microbial yield, partly because of N recycling from the plasma urea pool back into the rumen during periods of N shortage.

They suggested that responses in microbial growth ascribed to improved energy and N synchronisation may instead have been the result of an improved overall balance of energy and N supply to the rumen.

2.3 Increasing the proportion of rumen undegradable protein (RUP)

Another effect of decreased protein degradability is the increase of the proportion of rumen undegradable protein (RUP) (Fig. 1,③). 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, or are deaminated by the liver into urea N (Buxton, 1996; Bohnert *et al*., 2002). Increasing the proportion of RUP could result in lower rumen ammonia levels, increased N recycling to the gut (due to lower ruminal ammonia levels), and could subsequently decrease urinary N excretion (Bohnert *et al*., 2002). This concept is supported by results from both Dinn *et al*. (1998) and Castillo *et al*. (2001b), who found that increasing the proportion of RUP in the diet significantly decreased the urinary N excretion with no or only a small decrease in milk yield (Table 2).

However, at feed protein levels in excess of animal requirement, increasing the proportion of RUP may not increase animal N efficiency, as excess protein absorbed from intestines is deaminated and excreted through urine (Metcalf *et al*., 1996; Mishra & Rai, 1996; Bohnert *et al*., 2002). Studies by Metcalf *et al*. (1996) showed that increasing the digestible rumen-undegradable protein in the diet increased the supply of amino acids to the udder but did not result in corresponding increases in milk protein yield, implying that factors other than essential amino acid supply limit the milk protein synthesis in the udder.

There is some controversy on the significance of RUP in perennial ryegrass: in

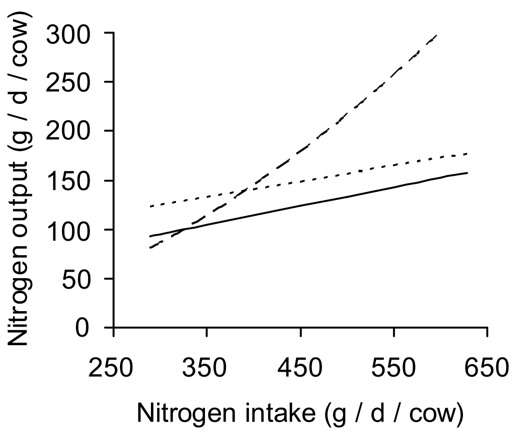

Figure 2 Relationship between total N intake and faecal, milk and urinary N outputs for diets varying in protein content (Based on Kebreab *et al*., 2001). Fitted lines represent —— Milk N, - - - Faecal N and — — Urine N.

Table 2

Effect of diet protein degradability on milk yield and animal N utilisation.

Diet	N intake		N output (g / kg N intake)			Milk yield	Source
	(g N / kg DM)	(g N / day)	Milk	Faeces	Urine	(kg / day)	
High N content	29.3	680	258	235	389	34.2	Dinn *et al.*, 1998 [a]
Medium N + Rumen prot. AA	26.7	580	293	268	340	32.8	
Low N + Rumen prot. AA	24.5	530	331	290	310	32.8	
High N degradability	26.7	472	246	277	459	23.8	Castillo *et al.*, 2001b [b]
Medium N degradability	26.5	469	255	307	385	23.4	
Low N degradability	26.0	465	245	323	346	24.1	

[a] Rations formulated to meet AA (amino acid) requirements of the cows (Based on CNCPS model). Medium and low rations incorporated rumen protected Lys and Met to assist in supplying optimal dietary AA profiles at lower dietary percentages of CP.

[b] Silage based diets with three levels of protein degradability in the concentrate (estimated digestible rumen undegraded protein of 887, 1073, and 1249 g / day for the high, medium and low degradability diets, respectively).

the data reviewed by Beever & Siddons (1986), the contribution of RUP to the small intestinal protein supply comprised only c. 30−50 g / kg of the total digestible protein. However, more recent data based on *in sacco* studies indicate that this percentage of RUP in perennial ryegrass ranges from 20% (Van Vuuren *et al.*, 1992) and c. 33% (Peyraud *et al.*, 1997) to up to 44% (Valk *et al.*, 1996). In grazed herbage, RUP is largely associated with the protein fraction linked to the cell wall, where it is more or less protected from microbial attacks. Values for cell wall protein-N in perennial ryegrass are in the order of 10% of N-total (see Tables 3 and 4), which is lower than RUP determined in *in sacco* studies. This implies that some of the non cell wall protein-N may not be degraded in the rumen. There have been some efforts to fractionate non cell wall protein-N into different fractions according to solubility (e.g. Licitra *et al.*, 1996), but results for such soluble N fractions have not been reported for perennial ryegrass. Therefore, it is unclear to what extent this proportion of RUP in grass can be manipulated.

Additionally, increasing the proportion of less degradable (cell wall) protein may increase the fraction of undegradable protein in the intestines, resulting in an increase in N excretion through dung (Buxton, 1996; Valk *et al.*, 1996). In this case, the bovine N efficiency may not be improved, but as N excreted through dung is far less prone to volatilisation and leaching than N excreted through urine (Van Horn *et al.*, 1996), losses to the environment may still be lowered.

3. Spatial and temporal variation of C and N fractions in herbage

Matching the diet requirements of grazing bovines through herbage manipulation encompasses the manipulation of carbon (C) and nitrogen (N) contents of growing herbage. These C and N contents vary both spatially within the grass sward and over time.

3.1 Spatial variation of C and N fractions

The chemical composition of the various plant components, i.e. lamina, sheath, stem and inflorescence, may differ significantly (Wilman *et al*., 1976d; Smith *et al*., 2002). Therefore, whole grass crop chemical composition depends on the distribution of dry matter over the various plant components. Additionally, the chemical composition of animal intake at grazing is different from the whole crop chemical composition, due to the process of selective grazing of plant components (Tharmaraj *et al*., 2003).

The main function of the lamina is photosynthesis. Relatively high levels of lamina N are required for maximum photosynthesis (Grindlay, 1997), as a result of which most of the plant N can be found in the laminae (Wilman *et al*., 1976d; Sanderson & Wedin, 1989; Smith *et al*., 2002). The sheath has a more structural role in lifting the lamina up towards the light, which results in relatively higher levels of structural material (cell wall content) (Lantinga *et al*., 2002; Smith *et al*., 2002), with a relatively low protein content (Sanderson & Wedin, 1989; Gastal & Lemaire, 2002). Also, cell wall digestibility is generally lower for sheaths than for laminae (Wilman *et al*., 1976d; Groot, 1999). Furthermore, grass sheaths function as the main site for storage of carbohydrate reserves. Therefore, levels of WSC in sheaths are usually higher than in laminae (Smith *et al*., 2002), this difference increasing with age (Fulkerson & Slack, 1995; Fulkerson & Donaghy, 2001). During generative growth, the stems form the major component of the plant, consisting mainly of structural tissue with a high cell wall content, which is low in N content and WSC content and has a low digestibility (Minson, 1990). In this context, the use of cultivars with different heading dates will affect the seasonal pattern of herbage quality.

3.2 Temporal variation of C and N contents during growth

Changes in herbage chemical composition during regrowth are the result of changes in composition of individual plant parts, changes in proportion of plant parts in DM, and the formation of new tillers.

3.2.1 Carbon

During regrowth, water-soluble carbohydrate (WSC) levels in the sheath follow a Ushaped curve of depletion and restoration (Davies, 1965). Just after defoliation the plant relies (partly) on WSC reserves for its regrowth and these can be replenished only as the photosynthetic capacity is restored (Fulkerson & Slack, 1995; Fulkerson & Donaghy, 2001). The maximum WSC levels primarily depend on the time of the day, the season and on weather conditions, but also on the rate of N application and ryegrass cultivar. High levels of irradiance increase the production of WSC through photosynthesis, whereas high temperatures increase respiration rates, resulting in lower levels of WSC to be stored (Robson *et al*., 1988; Fulkerson & Donaghy, 2001). During generative regrowth, WSC concentrations tend to be lower than during late summer and autumn, due to the high growth rate during that time (Pollock & Jones, 1979).

Structural carbohydrates are the main component of cell walls. In general, the cell wall content of DM increases with ageing (Wilman *et al*., 1977; Lindberg & Lindgren, 1988; Groot, 1999), because of cell wall thickening or lower contents of protein and other cell solubles (Wilson, 1994). Immediately after synthesis, cell wall polymers are completely digestible, but the indigestible fraction of the cell wall increases with time, due to the increasing proportion of lignin and the cross-linking of cell wall polymers (Wilman *et al*., 1977; Groot, 1999). The resulting decrease in digestibility during regrowth is more pronounced in sheath and especially stem material, compared to lamina material (Terry & Tilley, 1964; Wilman *et al*., 1976d; Minson, 1990; Groot, 1999). Therefore, the decline in total herbage digestibility with regrowth is associated with the decreased digestibility of green leaf, sheath and particularly stem, and the increase in the proportion of dead leaf, sheath and stem (Wilman *et al*., 1976d).

3.2.2 Nitrogen

The non-structural N (both protein and non-protein N) content as a percentage of DM generally decreases with age (Nowakowski, 1962; Wilman *et al*., 1976b; Lindberg & Lindgren, 1988). The initial uptake of N is driven by N supply and not by growth, resulting in a surge of non-protein N content shortly after fertiliser application (Nowakowski, 1962; Peyraud & Astigarraga, 1998). In an experiment by Nowakowski (1962), the nitrate-N content (as a fraction of both total N and DM) reached a peak 2 weeks after fertiliser N application (0.8% and 0.9% of DM for 63 and 126 kg N / ha applied per cut, respectively) and was thereafter strongly reduced with increasing duration of regrowth to 0.08% and 0.44% of DM, respectively, after 6 weeks. The non-structural N content is further reduced during growth, due to an increasing proportion of supportive material (stem and sheath) (Gastal & Lemaire, 2002).

Table 3
Effect of length of regrowth period on the total N content, the Neutral Detergent Insoluble Nitrogen (NDIN) and the Acid Detergent Insoluble Nitrogen (ADIN) for several grass species.

	Regrowth period (weeks)		Total-N (%DM)		NDIN (%N)		ADIN (%N)		Source [a]
	Y [b]	O	Y	O	Y	O	Y	O	
Italian ryegrass	1	6	5.8	3.6					1
Perennial ryegrass	3	6	3.6	2.6	8.5	9.0			2
Timothy	4	9	3.8	1.6	40.0	35.0	2.5	5.6	3
Timothy, 1984	2	8	2.5	1.0	8.7	18.2	1.6	15.1	4
Timothy, 1985	2	8	5.0	2.3	6.1	15.0	0.9	4.1	4
Orchardgrass	2	5	3.2	2.0	33.4	31.5	1.2	3.0	5
Perennial ryegrass	3	6	3.6	1.5	15.1	20.4			6

[a] 1 = Nowakowski, 1962; 2 = Wilman *et al*., 1977; 3 = Lindberg & Lindgren, 1988; 4 = Sanderson & Wedin, 1989; 5 = Balde *et al*., 1993; 6 = Cone *et al*., 1996.
[b] Y = Young, O = Old.

There is very little information on the temporal changes in the proportion of N allocated to the cell wall in perennial ryegrass and trends found for several grass species are inconsistent. Decreasing (Wilman *et al*., 1977; Lindberg & Lindgren, 1988; Balde *et al*., 1993) and increasing (Sanderson & Wedin, 1989; Cone *et al*., 1996) proportions of N have been reported to be in the cell wall with increasing age (Table 3). Additionally, not much is known about the effect of ageing on the amount of undegradable protein (protein linked to lignin and cellulose) in perennial ryegrass. It has the tendency to increase with increasing length of regrowth, but its proportion in grass is generally small (Lindberg & Lindgren, 1988; Sanderson & Wedin, 1989; Rinne *et al*., 1997) (Table 3).

4. Grassland management tools for manipulation of diets under grazing

Under grazing conditions, grassland management tools, such as the length of the regrowth period, grazing intensity, fertiliser N application rate and herbage cultivar are the main pathways to manipulate C and N dynamics, and therefore herbage chemical composition. It is important to assess in what way these grassland management tools affect the herbage chemical composition and where the main possibilities to optimise the chemical composition of the intake lie with respect to animal N efficiency in light of the three pathways for improving animal N efficiency identified in the above section: 1) Matching protein supply to requirements, 2) Balancing and synchronising carbohydrates and N supply, and 3) Increasing the proportion of RUP.

4.1 Regrowth period

The length of the regrowth period of herbage before defoliation is an important determinant of herbage yield and herbage quality (Buxton, 1996), as it determines at what stage the processes of morphological and physiological development are interrupted.

4.1.1 Effect on bovine N-efficiency

Increasing the length of the regrowth period reduces the amount of dietary N and increases the balance of N and energy in the rumen, whereas the effect on RUP is unclear. However, the reduced OM digestibility may decrease energy availability with high maturity, reducing animal productivity. Publications that describe the effect of length of regrowth period on bovine N-efficiency are scarce. Van Vuuren *et al*. (1991), using the in sacco technique, estimated that although the dietary N content decreased by 33% between week 1 and 8, the amount of degradable N entering the small intestines was reduced by only 25% (Fig. 3). This was attributed to an increase in water soluble carbohydrate content and thus a more efficient microbial synthesis, which compensated for the decrease in fermentable organic matter (Van Vuuren *et al*., 1991). Similarly, Mambrini & Peyraud (1994) reported that the quantity of apparently

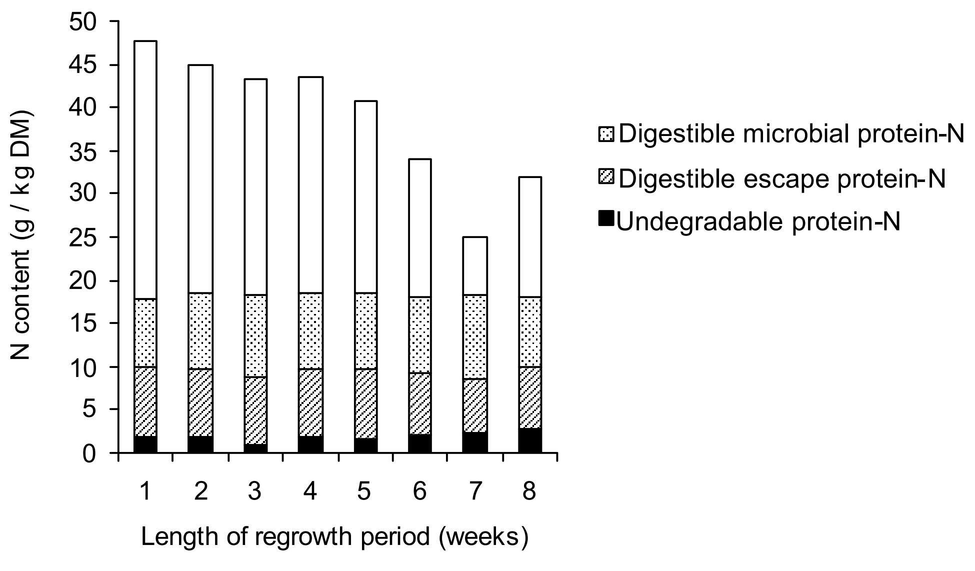

Figure 3 Effect of length of regrowth period (weeks) of perennial ryegrass plants on fractions of undegradable protein-N, digestible escape protein-N, and digestible microbial protein-N entering the small intestine of dairy cows, based on *in sacco* degradation. Full bar represents total feed N content, and by difference, the open part of the bar represents ammonia N not captured in the rumen (based on Van Vuuren *et al*., 1991).

digestible non-ammonia N of perennial ryegrass declined by 5.7 g / kg DM during regrowth from 28 to 50 days (grass N content of 24 and 16 g / kg DM, respectively).

As a result, losses of ammonia-N from the rumen and subsequent excretion through urine are decreased with longer regrowth periods.

4.2 Grazing management: Residual sward height and length of grazing period

Residual sward height (RSH) is an important factor in grazing management and is determined by the combination of pre-grazing herbage mass, stocking rate and the length of the grazing period (days spent grazing on one area). There are two pathways through which residual sward height (RSH) may affect animal N-efficiency. 1) RSH may affect sward chemical composition either directly, through changed chemical composition of individual plant organs, or indirectly, through changes in the proportions of plant organs and, 2) RSH may alter the proportion of different plant organs in animal intake.

4.2.1 Quality of grass

The literature on the effect of defoliation height on the chemical composition of regrowing perennial ryegrass plant parts is inconclusive. There is some evidence that the digestibility of lamina in cocksfoot is lower at high initial sward heights due to a decrease in leaf appearance rate (Duru & Ducrocq, 2002). However, studies on the effect of initial sward height on leaf appearance rate of perennial ryegrass have yielded conflicting results (Grant *et al*., 1981; Van Loo, 1993; Hernández Garay *et al*., 2000). Generally, the lamina:sheath-ratio in regrowths in vegetative swards is not related to the initial sward height (Davies, 1988; Hume & Brock, 1997; Hernández Garay *et al*., 2000), which can be explained through the highly significant positive relation between sheath and lamina length (Grant *et al*., 1981; Van Loo, 1993). However, dead material, sheaths, and during generative regrowth, stem material, may accumulate with high residual sward heights (Mayne *et al*., 1987; Hoogendoorn *et al*., 1992; Hernández Garay *et al*., 2000), which may impact significantly on herbage quality.

4.2.2 Effect on bovine N-efficiency

RSH has a distinct impact on the chemical composition of the diet selected by grazing ruminants (Woodward, 1998; Delagarde *et al*., 2000; Hutchings & Gordon, 2001). The chemical composition of a rotationally grazed sward shows very large variations from the top to the base of the sward (Delagarde *et al*., 2000), which fundamentally reflect the decrease of the proportion of blades and the increase of the proportion of sheaths, stems and dead tissue with increasing depth (Milne *et al*., 1982). This results in a decrease in CP-content and DM digestibility and an increase in WSC, NDF and lignin contents with increasing depth in the sward (Delagarde *et al*., 2000; Hutchings & Gordon, 2001; Yin *et al*., 2003).

No studies on the effect of RSH on animal N-efficiency were found in the literature. The almost antagonistic effect of RSH on sward composition and actual animal intake makes it difficult to anticipate the effects. E.g. maintaining high RSH will result in higher proportion of stems and dead material in the total sward. However, the composition of the actual intake may be affected to a much lesser extent, as the animals graze less deep and therefore have a higher proportion of lamina in intake.

Therefore, it is unclear what the overall effect of residual sward height and length of grazing period is on bovine N utilisation.

4.3 Fertiliser N application

4.3.1 Quality of grass

The application of fertiliser N has a positive effect on herbage growth (Holliday & Wilman, 1965; Le Clerc, 1976; Wilman *et al*., 1976a; Lantinga *et al*., 1999a; Nevens & Reheul, 2003). Increasing N fertilisation brings about a strong increase in grass CP content, which is nearly linear up to very high levels of applied N (Nowakowski, 1962; Reid & Strachan, 1974; Wilman *et al*., 1976b) (Table 4) (for an extensive review on the effects of N application on chemical composition and nutritive value of fresh herbage, see Peyraud & Astigarraga, 1998). Plants show a rapid uptake of N immediately after N application, and the rate of uptake increases with the level of fertilisation. The increased growth rate with higher N application rates, results in a higher subsequent rate of dilution of the N content during regrowth (Wilman *et al*., 1976b; Peyraud & Astigarraga, 1998).

The proportion of plant N present as true protein is decreased with increasing levels of fertiliser N application. The amount of cell wall N as percentage of DM increases with higher N fertilisation, but to a lesser extent than total N, so the proportion of total N bound to cell wall material actually falls slightly (Wilman *et al*., 1977; Wilman & Wright, 1978; Lindberg & Lindgren, 1988; Valk *et al*., 1996) (Table 4). There is not much literature on the effect of N application rate on the proportion of undegradable N, and no consistent trends have been reported (Lindberg & Lindgren, 1988; Valk *et al*., 1996) (Table 4).

The negative effect of N application rate on the WSC content has been widely documented (Smith, 1973a; Reid & Strachan, 1974; Wilman & Wright, 1978; Mc Grath, 1992; Valk *et al*., 1996), and can be attributed to the increased crop growth rate, combined with an increase in utilisation of carbon for protein synthesis and for the production of energy required for the nitrate reduction before protein synthesis (Reid & Strachan, 1974). In contrast, the structural carbohydrate content is practically

Table 4

Effect of fertiliser N application rate on the total N content, the Neutral Detergent Insoluble Nitrogen (NDIN) and the Acid Detergent Insoluble Nitrogen (ADIN) for several grass species.

	N application (kg N / ha)		Total-N (%DM)		NO_3 (%N)		NDIN (%N)		ADIN (%N)		Source [a]
	Low	High	Low	High	Low	High	Low	High	Low	High	
Perennial ryegrass (June 1992)	150	450	2.2	3.7			11.0	8.5	1.0	0.9	1
Perennial ryegrass (July 1992)	150	450	3.1	4.1			12.5	9.6	1.0	1.2	1
Perennial ryegrass (3 wks)	0	525	3.0	3.9		4.5	10.4	8.5			2
Perennial ryegrass (6 wks)	0	525	2.3	3.0			9.2	9.0			2
Perennial ryegrass	0	140	2.8	4.5	0.4	7.0	11.1	9.0			3
Italian ryegrass	0	126/cut	2.7	5.2	1.0	8.7					4
Timothy	90	270	2.2	3.8			46.0	43.0	2.4	1.8	5

[a] 1 = Valk *et al*., 1996; 2 = Wilman *et al*., 1977; 3 = Wilman & Wright, 1978; 4 = Nowakowski, 1962; 5 = Lindberg & Lindgren, 1988.

unchanged by increasing levels of N fertilisation, observed variations being inconsistent and minor (Waite, 1970; Wilman *et al*., 1977; Valk *et al*., 1996; Peyraud & Astigarraga, 1998).

4.3.2 Effect on bovine N efficiency

The strong effect of fertiliser N application rate on herbage N content makes this an important tool for matching animal N requirements with the herbage content. Decreasing the N application rate is likely to decrease losses of rumen ammonia and subsequent urinary N excretion (Peyraud & Astigarraga, 1998). The decline in the CP content and solubility, combined with an increase in the WSC content, is likely to result in a better balance and synchronisation of C and N in the rumen. Decreasing the N application rate will increase the proportion of RUP (Van Vuuren *et al*., 1991; Valk *et al*., 1996), but may also increase the proportion of undegradable protein (Fig. 4).

The studies reviewed by Peyraud & Astigarraga (1998) all showed that reduced levels of fertilisation caused only a small (i.e., 5% on average) reduction in the amount of non-ammonia N entering the intestine despite a much lower CP content and a slightly lower CP digestibility in less fertilised grass (Van Vuuren *et al*., 1992; Peyraud *et al*., 1997; Delagarde *et al*., 1997) (Fig. 4). This resulted in a decreased output in urinary N, whereas N excretion through dung was hardly affected (Peyraud & Astigarraga, 1998). However, when CP content falls below animal requirements, a reduction in both herbage intake and protein intake may result in a decreased animal production level (Minson, 1990; Peyraud & Astigarraga, 1998).

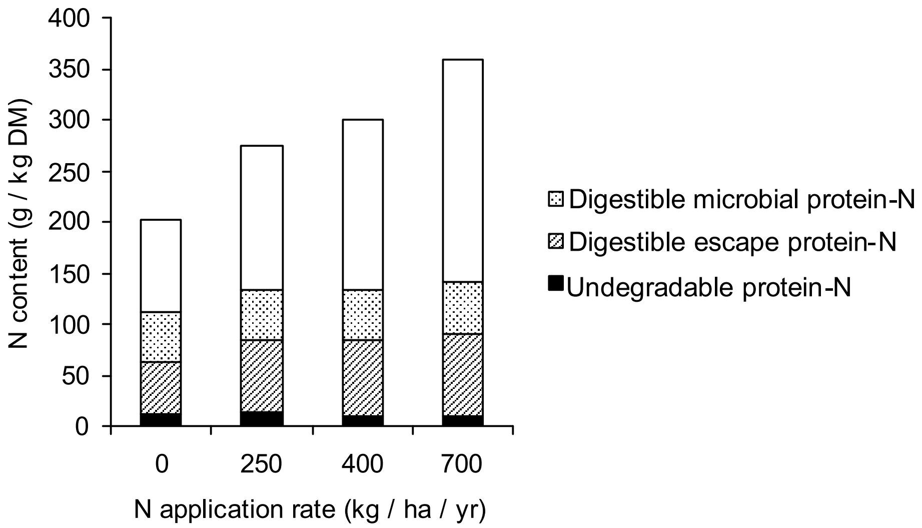

Figure 4 Effect of N application rate (kg / ha / yr) on fractions of undegradable protein-N, digestible escape protein-N, and digestible microbial protein-N in 7-day old perennial ryegrass plants entering the small intestine of dairy cows. Full bar represents total feed N content, and by difference, the open part of the bar represents ammonia N not capture in the rumen (based on Van Vuuren *et al.*, 1991).

Therefore, lowering the N fertilisation of fresh forage is an efficient means of reducing N losses through urine excretion of bovines with little or no modification in their performance.

4.4 High-sugar grass cultivars

4.4.1 Quality of grass

Advances in conventional plant breeding have resulted in the development of grasses with increased levels of WSC, compared to conventional commercial cultivars (Humphreys 1989a, b). No satisfactory evidence has been found yet as to the mechanism by which these higher WSC concentrations could be maintained (Smith *et al.*, 2002). When growth rates are limited while maintaining a high level of photosynthesis, accumulation of WSC can occur. Extra carbon may potentially be diverted for storage as WSC if synthesis of other compounds is reduced and particularly if there is less synthesis of complex compounds (e.g. lignin), which require more energy to synthesize (Penning de Vries *et al.*, 1974; Smith *et al.*, 2002). Although in some of the high-sugar cultivars tested by Smith *et al.* (2002) a decrease in the root/shoot ratio was detected, this was not consistent for all the high-sugar cultivars.

However, in several (field) trials it has been shown that high-WSC genotypes accumulate higher WSC concentrations while growing at equivalent rates (Smith *et al.*, 1998; Conaghan *et al.*, 2002; Smith *et al.*, 2002; Smit *et al.*, 2005). However, Gilliland *et al*., 2002 reported higher WSC yields for tetraploid perennial ryegrasses compared to the high-sugar grass Aberdart, even though the WSC concentration of Aberdart was higher.

Selection for high WSC concentrations has no consistent effect on N content, with higher (Smith *et al*., 1998, 2001), equal (Lee *et al*., 2001; Smith *et al*., 2002) and lower (Miller *et al*., 2001) concentrations reported, confirming that the negative correlation between WSC and N does not apply across different genetic lines (Humphreys, 1994). There is no indication for differences in N fractionation for high-sugar cultivars: Smith *et al*. (2002) found similar proportions of cell wall and cell content protein for several cultivars, and Miller *et al*. (2001) reported that the N-digestibility was not significantly different.

The cell wall content is generally lower in high-sugar cultivars (Smith *et al*., 1998; Lee *et al*., 2001; Miller *et al*., 2001; Smith *et al*., 2001; 2002; Smit *et al*., 2005), and in a few cases the same was found for the proportion of indigestible cell wall (Miller *et al*., 2001; Smith *et al*., 2001). However, Taweel *et al*. (2005b) found no difference in degradation rate of the cell wall using both in vivo and in vitro techniques. Combined with the higher level of WSC content, this results in a higher DM digestibility for the high-sugar cultivars (Humphreys, 1989b; Smith *et al*., 1998; Lee *et al*., 2001; Miller *et al*., 2001; Smith *et al*., 2001; Smit *et al*., 2005).

Table 5

Effect of high-sugar perennial ryegrass on herbage intake, milk yield and bovine N utilisation.

Source	WSC content (g / kg DM)		Herbage intake (kg DM / day)		Milk yield (kg / day)		Milk N (g / g N intake)		Urine N (g / kg N intake)	
	HS	LS	HS	LS	HS	LS	HS	LS	HS	LS
Lee *et al*., 2002 [a,b]	242	161	9.3	6.7	na	na	na	na	nd	nd
Miller *et al*., 2001 [b,c]	165	126	11.6	10.7	15.3	12.6	0.30	0.23	0.25	0.35
Moorby *et al*., 2006a [b,d]	243	161	15.3	13.1	32.7	30.4*	0.36	0.37*	0.20	0.27
Tas *et al*., 2006 [e,f]									Milk NH_3-N (mg / L)	
2002	144	110	18.0	15.6	28.8	26.0	0.25	0.25*	144	156*
2003	131	87	18.4	17.4	25.7	25.2*	0.20	0.19*	149	165
Taweel *et al*., 2005a [e]										
Latin square a	181	157	16.2	16.6*	23.8	24.7*	0.26	0.26*	76	109
Latin square b	180	149	16.2	17.1*	25.1	26.6*	0.28	0.28*	108	125

HS: high-sugar cultivar; LS: low-sugar cultivar; na: not aplicable; nd: not determined; *: no significant difference
[a] Hereford × Friesian steers
[b] HS herbage harvested early in the morning and LS in afternoon to maximise difference in WSC
[c] Holstein-Friesian dairy cows in late lactation
[d] Holstein-Friesian dairy cows in early lactation
[e] Holstein-Friesian dairy cows in mid lactation
[f] Grazing

4.4.2 Effect on bovine N efficiency

High-sugar grasses have no direct effect on the herbage N content, but may increase the total N intake through an increased intake rate, because of its higher palatability (Jones & Roberts, 1991), lower resistance to physical breakdown during chewing (Lantinga & Groot, 1996) and higher digestibility. The increase in WSC content is likely to improve balance and synchronisation of the N and C supply to the rumen, whereas no effect on the N fractionation or the proportion of RUP has been reported.

Even though high-sugar grasses in theory have a beneficial effect on bovine N efficiency, there is little experimental evidence to support this (Miller *et al*., 2001; Lee *et al*., 2002; Taweel *et al*., 2005a; Moorby *et al*., 2006a; Tas *et al*., 2006) (Table 5). In three experiments (Miller *et al*., 2001; Lee *et al*., 2002; Moorby *et al*., 2006a) voluntary herbage intake was significantly higher for the high-sugar cultivar. However, in these experiments the dry matter and NDF content of the high- and low-sugar grasses were significantly different (partly due to the harvesting regime aimed at maximising the difference in WSC content), making it difficult to explain the cause of the observed differences. Both Miller *et al*. (2001) and Moorby *et al*. (2006a) found that a smaller proportion of diet N was excreted through urine, but only the former reported a significant increase in milk yields. Urinary N was not determined by Lee *et al*. (2002) and Taweel *et al*. (2005a) and Tas *et al*., (2006), but decreased levels of rumen NH_3 and milk NH_3, respectively, might indicate that urinary N was also lower in these experiments (Jonker *et al*., 1998) (Table 5).

Lee *et al*. (2002) found no improvement in rumen microbial protein synthesis in the rumen and the flow of non-ammonia N to the intestines and concluded that the results provided little evidence that an increase in the synchrony between energy and N release in the rumen improved microbial protein synthesis in the rumen.

In conclusion, in theory, high sugars are a promising means of improving bovine N utilisation. However, animal trials so far have not shown a consistent increase in N utilisation, although urinary N excretion tends to be reduced.

5. Farm scale strategies for improving N-efficiency at grazing system level

Above established links between grassland management tools and bovine N efficiency suggest that N-losses can be minimised through a combination of high-sugar perennial ryegrass cultivars, low N application rates and long regrowth periods. However, in practice, implementation of such management systems is subject to the following constraints:

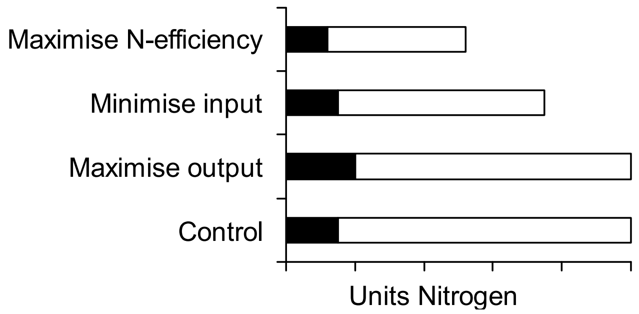

Figure 5 Generic representation of strategies for improving N-efficiency.
■ = N-output, □ = N-loss, contour represents N-input. For more detail, see text.

1. In practice it is not possible to implement a random combination of management tools, as they are interrelated. The rotation length is a function of N application rate, residual sward height and stocking rate (O'Riordan *et al*., 1998), and can therefore not be manipulated independently, vice versa.
2. Above suggested combination may have a negative impact on both herbage and animal production, as the reduced herbage growth rates at low N application rates may not be compensated for by increased regrowth length. Additionally, the reduced OM digestibility at long regrowth periods (Van Vuuren *et al.*, 1991) may reduce animal production. Since N-efficiency is defined as N output in animal products proportionate to the dietary N intake, such a reduction of output is likely to result in lower efficiency, unless it is balanced by a sufficient reduction of input. Different strategies with respect to improving bovine N efficiency can be recognised, depending on whether the emphasis is on optimising N input, N output or N efficiency itself (Fig. 5). If the emphasis is on output, N efficiency can be improved by a higher N output in animal products at the same (diet) N inputs. Alternatively, N efficiency may be improved through reduced N inputs while maintaining the same animal output N. Maximising N efficiency may require a reduction in both input and output.
3. Interactions cannot be presumed to be negligible. For example, N content of grass increases with higher N application rates, however, the difference in N content between two contrasting N application rates decreases with longer regrowth periods (Wilman *et al*., 1976b). Some interactions have been studied in quite some detail (e.g. N and regrowth period, Wilman *et al.*, 1976a,b,d), whereas others have been neglected. Additionally, results obtained in zero-grazing studies may not necessarily apply to the grazing situation, due to animal-sward interactions (Woodward, 1998; Lantinga *et al*., 1999a). To complicate matters further, effects and interactions are site specific and may vary from year to year (e.g. Wilman *et al*., 1976b; Sanderson & Wedin, 1989; Wilkins *et al.*, 2000; Gustavsson & Martinsson, 2001), which necessitates experiments in which such interactions can

be quantified.

Due to the complexity and number of interactions, modelling is essential for understanding and predicting processes involved for different objectives and conditions. A model on such a complex system would have to include both herbage (growth, morphology, chemical composition) and animal (herbage intake, physiology, N excretion) components.

Combining disjunctive models is difficult and often impossible due to the lack of compatibility of software and of input and output factors. For example, animal models require detailed fractionation of herbage composition, either based on herbage chemical analyses (e.g. CNCPS, from Russell *et al.*, 1992 to Fox *et al*., 2004) or in sacco degradation studies (e.g. Dijkstra *et al*., 1992). Up to date, herbage models have not predicted these variables in sufficient detail. Therefore, it is not yet possible to predict animal N efficiency under grazing from current modelling studies.

6. Conclusions

- In livestock farming, there is scope to reduce N-losses to air and water by maximising the N-efficiency of grazing bovines. This requires the production of herbage that satisfies the temporal patterns of the dietary N and energy requirements for bovines.
- Longer regrowth periods, reduced N application rates and use of high-sugar cultivars were identified as the most promising grassland management tools to produce herbage chemical composition that meets these dietary N and energy requirements.
- Due to the complex nature of interactions, modelling is essential in order to quantify and predict the effect of any combination of herbage management tools under specific circumstances.
- Areas in which additional research is required are the fractionation of N compounds in herbage as affected by herbage management and the effect of high-sugar cultivars on bovine N efficiency under a range of herbage management combinations.

3

Chemical composition of lamina and sheath of *Lolium perenne* as affected by herbage management

N.J. Hoekstra, P.C. Struik, E.A. Lantinga & R.P.O. Schulte, 2007. NJAS – Wageningen Journal of Life Sciences, 55: 55-73

Abstract

The quality of grass in terms of form and relative amounts of energy and protein affects both animal production per unit of intake and nitrogen (N) utilisation. Quality can be manipulated by herbage management and choice of cultivar. The effects of N application rate (0, 90 or 390 kg N / ha / yr), duration of regrowth period (2−3, 4−5, or 6−7 weeks), and cutting height (8 or 12 cm) on the concentrations of nitrogen (N), water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and ash in lamina and sheath material of a high-sugar (Aberdart) and a low-sugar (Respect) perennial ryegrass (*Lolium perenne*) cultivar, were studied in a factorial field experiment during four seasons in 2002 and 2003. Expressing NDF and ADF concentrations in g / kg WSC-free dry matter (DM) increased the consistency of treatment effects. The high-sugar cultivar had generally higher WSC concentrations than the low-sugar cultivar, especially during the late season. Moreover, the relative difference in WSC concentration between the two cultivars tended to be higher for the lamina material than for the sheath material, which suggests that the high-sugar trait may be more important under grazing conditions, when lamina forms the bulk of the intake, than under mowing regimes. Longer regrowth periods and lower N application rates increased WSC concentrations and decreased N concentrations; interactions between regrowth period and N application rate were highly significant. The concentrations of NDF and ADF were much less influenced. The NDF concentration in terms of g / kg WSC-free DM tended to be higher at lower N application rates and at longer regrowth periods. The effect of cutting height on herbage chemical composition was unclear. In conclusion, high-sugar cultivars, N application rate and length of the regrowth period are important tools for manipulating herbage quality.

Keywords: cutting height, feeding value, herbage quality, high-sugar cultivar, nitrogen application rate, perennial ryegrass, regrowth period

1. Introduction

In many parts of Europe, a large proportion of the bovine's diet consists of grazed grass (Lantinga *et al*., 1996). The quality of the grass intake affects both animal production and nitrogen (N) utilisation (Rearte, 2005). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations are important parameters of herbage quality as they affect dry matter intake and digestibility. Protein is an essential nutrient, but the N concentration of temperate pasture grazed at an immature stage is usually in excess of animal requirements. The water-soluble carbohydrates (WSC) are relevant as the main source of energy. However, the WSC concentration is generally too low to balance the high concentration of highly degradable protein. This imbalance results in large losses of N from the rumen and in a low N utilisation by the grazing cow (Rearte, 2005).

The main tools to manipulate grass quality are regrowth duration, N application rate and cutting height, which have been the subject of many studies. Additionally, in the UK perennial ryegrass cultivars have been developed that have higher WSC concentrations (Humphreys, 1994). The use of these cultivars has led to increased animal production (Lee *et al.*, 2001; Miller *et al.*, 2001) and reduced urinary N output (Miller *et al.*, 2001).

Designing management systems aimed at optimizing grass quality requires the prediction of grass chemical composition as affected by management tools and their interactions. Some interactions have been studied in great detail [e.g., those between N application rate and regrowth period, by Wilman *et al.* (1976c)], other ones not. For example, little is known about the performance of the high-sugar cultivars under different herbage management regimes. Moreover, the studies tended to focus on the whole crop, rather than on individual plant parts. This makes it hard to apply the results obtained from cutting trials to grazing situations, as the lamina material forms the bulk of the intake under grazing (Brereton *et al.*, 2005).

The objective of this study was to quantify in detail the effect of herbage management tools and their interactions on the chemical composition of lamina as well as sheath fractions of a low or normal and a high-sugar perennial ryegrass cultivar throughout the growing season. The selection of management tools was based on a review paper by Hoekstra *et al.* (2007a), who identified the most relevant management tools for manipulating the carbon and nitrogen concentrations in grass herbage.

2. Materials and methods

2.1 Experimental design

The 36 factorial combinations of 2 cultivars of perennial ryegrass (*Lolium perenne* L.), 2 cutting heights, 3 regrowth periods and 3 fertilizer-N application rates were compared in a field experiment of the split-split-plot design, replicated 3 times, with cultivars as main factor, the factorial combinations cutting height × regrowth period as split factor, and the fertilizer-N application rates as split-split factor. Per cultivar and per replication, the six combinations of cutting height × regrowth period were arranged in strips, so as to be able to cut the grass mechanically at the same height and on the

Table 1
Sampling dates and weather data for the four measurement periods during the four seasons

Season		Date start period (T0)	Regrowth period (days) T1	T2	T3	Mean max. temp. (ºC)	Mean min. temp. (ºC)	Mean rainfall (mm / day)
S1	Late season	05/09/2002	15	29	49	16.0	9.6	4.1
S2	Early season	14/03/2003	19	33	47	12.4	5.4	2.0
S3	Mid season	08/05/2003	20	34	48	16.0	9.4	2.4
S4	Late season	24/07/2003	22	34	48	20.3	12.6	1.6

same day with a plot harvester (Haldrup, Logstor, Denmark). The smallest experimental unit measured 1.5 m × 2 m.

A high-sugar (Aberdart) and a low-sugar cultivar (Respect) were used (coded HS and LS), both diploids with similar heading dates [27 and 23 May, respectively (Anon., 2001)]. The cutting heights were about 8 or 12 cm (coded LD and HD, respectively), the regrowth periods 2–3, 4–5, or 6–7 weeks (coded T1, T2 and T3, respectively) and the N application rates 0, 90 or 390 kg N / ha / yr (coded N) split over seven equal applications. Measurements were taken during four seasons: September / October 2002 (late season; S1), April 2003 (early season; S2), May / June 2003 (mid season; S3) and August / September 2003 (late season; S4) (Table 1). To enable the grass to recover from the sampling (see below), each replication (block) consisted of two similar sub-blocks, each comprising the 36 treatment combinations. One sub-block was harvested only during the seasons S1 and S3, the other one only during the seasons S2 and S4. This layout resulted in a total of 216 experimental units (2 cultivars × 2 cutting heights × 3 regrowth periods × 3 N application rates × 3 replicates × 2 sub-blocks to allow harvesting in the four seasons).

The two cultivars were sown on 12 June 2002 at a seeding rate of 4 g m^{-2}, using a plot fertilizer spreader (Probe, Fiona, Denmark). The seed was worked in by hand, using a rake. At the 3-leaf stage a basal NPK (10:10:20) fertilizer dressing was applied equivalent to 50, 50 and 100 kg N, P and K per ha. After the first cut (8 weeks after sowing), the plots were cut every four weeks, using the plot harvester. Within four days after each cut the plots were fertilized with the assigned N rates (0, 13 or 56 kg N / ha per cut), using the plot fertilizer spreader. At the start of the four seasons the plots were cut at the assigned cutting height and sampling took place after 2–3, 4–5, or 6–7 weeks, according to the regrowth period.

The experiment was located on a clay soil at Johnstown Castle Research Centre, Wexford, Ireland. Minimum and maximum daily temperatures and daily rainfall were recorded at the centre's weather station (Table 1). The seasons S1 and S3 were similar in temperature conditions, but S2 was cooler and S4 warmer and dryer.

2.2 Sampling

At each harvest, fresh grass samples of approximately 250 g were taken by cutting the plants at 1 cm above ground level from the middle of the plots, using electric shears (Wolf-Garten, Accu 75 Professional). Each sampling event started at the same time of the day (09:00 h) to avoid effects of diurnal changes in WSC concentration (Donaghy & Fulkerson, 1998). The samples were stored immediately at 4 ^{o}C. They were manually divided into sheath (sheath, stem and new leaves within the sheath tube), lamina, inflorescence and dead material (defined as leaves and sheaths of which more than 50% of the surface was dead). After all samples had been dissected, i.e., within three days after storing at 4 ^{o}C, the fractions were stored in a freezer. Earlier research to test the methodology had shown that our procedure had little absolute effect on the chemical composition and was very unlikely to influence treatment effects (N.J. Hoekstra, unpublished results).

During the last week of S1 not all the treatments could be harvested due to calves that had entered some of the plots.

2.3 Chemical analyses

The separated fractions of sheaths, laminae and inflorescences were freeze-dried and subsequently ground over a 1-mm sieve. To obtain sufficient material for chemical analysis the lamina and sheath material from the three replications was bulked per treatment. Bulking of the inflorescence material was done over the two cutting height treatments and the three replications. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin concentrations were determined according to Van Soest *et al*. (1991). The samples were analysed for total N using a Kjeldahl-N analyser. Water-soluble carbohydrates (WSC) were determined colorimetrically with an automatic analysing device (Technicon autoanalyzer 2, Technicon Instruments Corporation, Tarrytown, NY, USA), using ferricyanide (Struik, 1983).

N, ADF and NDF concentrations were expressed in g / kg dry matter (DM) and converted into g / kg WSC-free DM, using the following equation:

$$\frac{\text{Fraction}\left(\text{g / kg DM}\right)}{1000\left(\text{g / kg DM}\right)-\text{WSC}\left(\text{g / kg DM}\right)}\times 1000\left(\text{g / kg DM}\right)$$

Detailed chemical analysis of the different fractions of total nitrogen was also carried out but will be reported in another paper (Hoekstra *et al*., accepted).

2.4 Statistical analyses

Analysis of variance was carried out using the SAS GLM procedure (SAS Enterprise

Guide version 8.2). Analysis of variance was done separately for the four seasons and for lamina and sheath material. The bulking of the material for chemical analyses resulted in single values for all factorial treatment combinations. All main effects and two-factor interactions were included in the model, resulting in 36 treatment combinations (d.f. error = 16).

3. Results

3.1 Lignin and ash

The acid detergent lignin concentration was 11.1 g / kg DM on average, and fluctuated

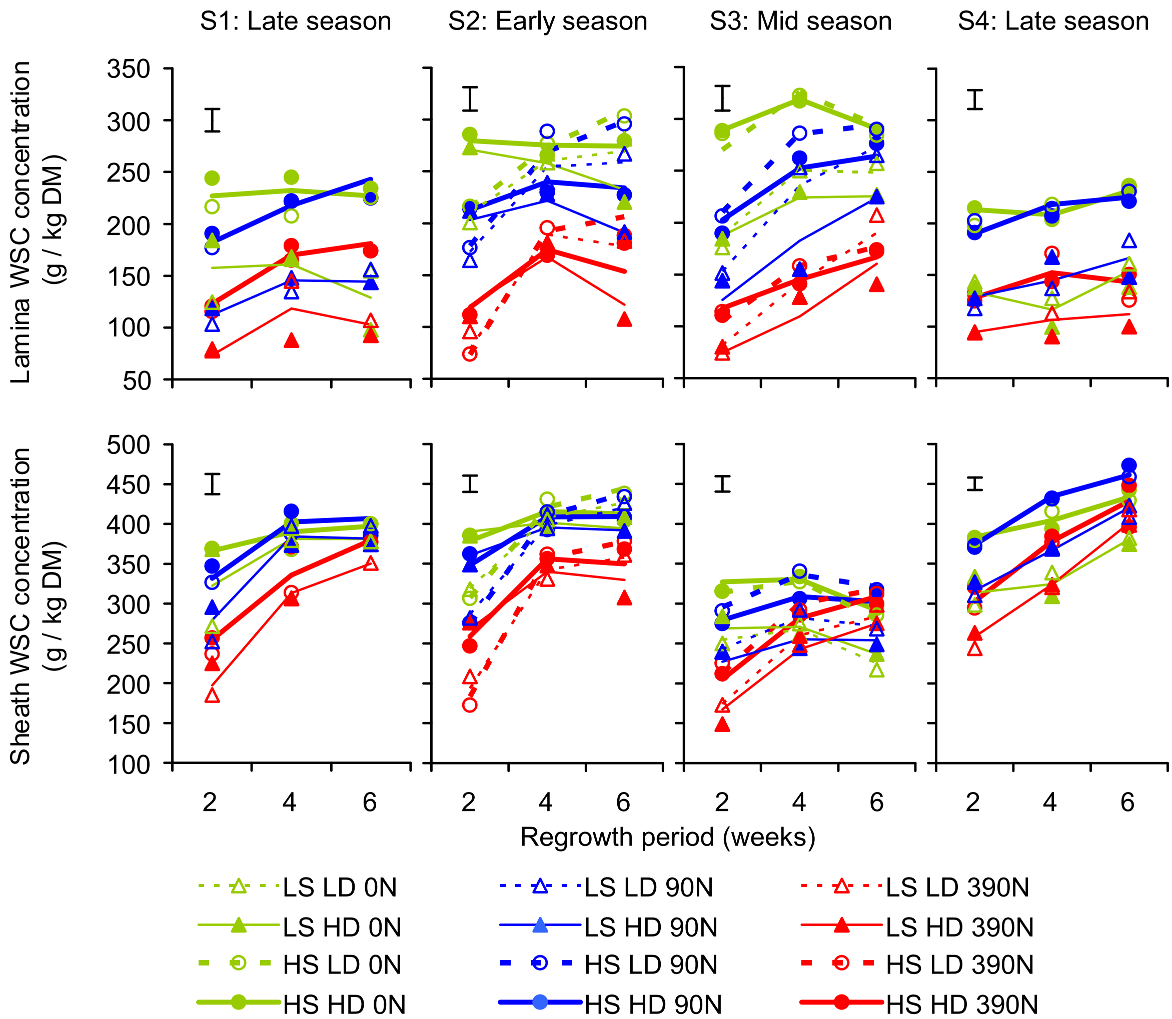

Figure 1 WSC concentration of lamina and sheath material as affected by cultivar (HS = high-sugar; LS = low-sugar), N application rate (0, 90 and 390 kg / ha / year), regrowth period (about 2, 4 and 6 weeks) and cutting height (LD = low height; HD = high height), for late season 2002 (S1), early season 2003 (S2), mid season 2003 (S3) and late season 2003 (S4). Lines represent the ANOVA model, averaged over non-significant effects. Error bars are 2 × standard error for the comparison of individual model points.

strongly, which appeared to be due to analytical errors (which were large in comparison to the total variation) rather than treatment effects (data not shown).

The ash concentration averaged 82.9 and 73.1 g / kg for lamina and sheath material, respectively (data not shown). Variation appeared to be influenced by artefacts (soiling) rather than treatment *per se*.

3.2 Water-soluble carbohydrates

The water-soluble carbohydrates (WSC) concentration varied strongly, ranging from 74 to 323 g / kg DM in the lamina and from 149 to 473 g / kg DM in the sheath material (Fig. 1). The WSC concentration of the lamina material tended to be higher during S2 and S3 (212 and 207 g / kg DM, respectively) than during S1 and S4 (160 and 159 g / kg DM, respectively), whereas the WSC concentration of the sheath material was lower during S3.

There was a statistically significant ($p < 0.01$) positive effect of the HS cultivar on the WSC concentration in all treatment combinations, except for sheath material during S2 (Table 2). For lamina material during S2, the difference was statistically

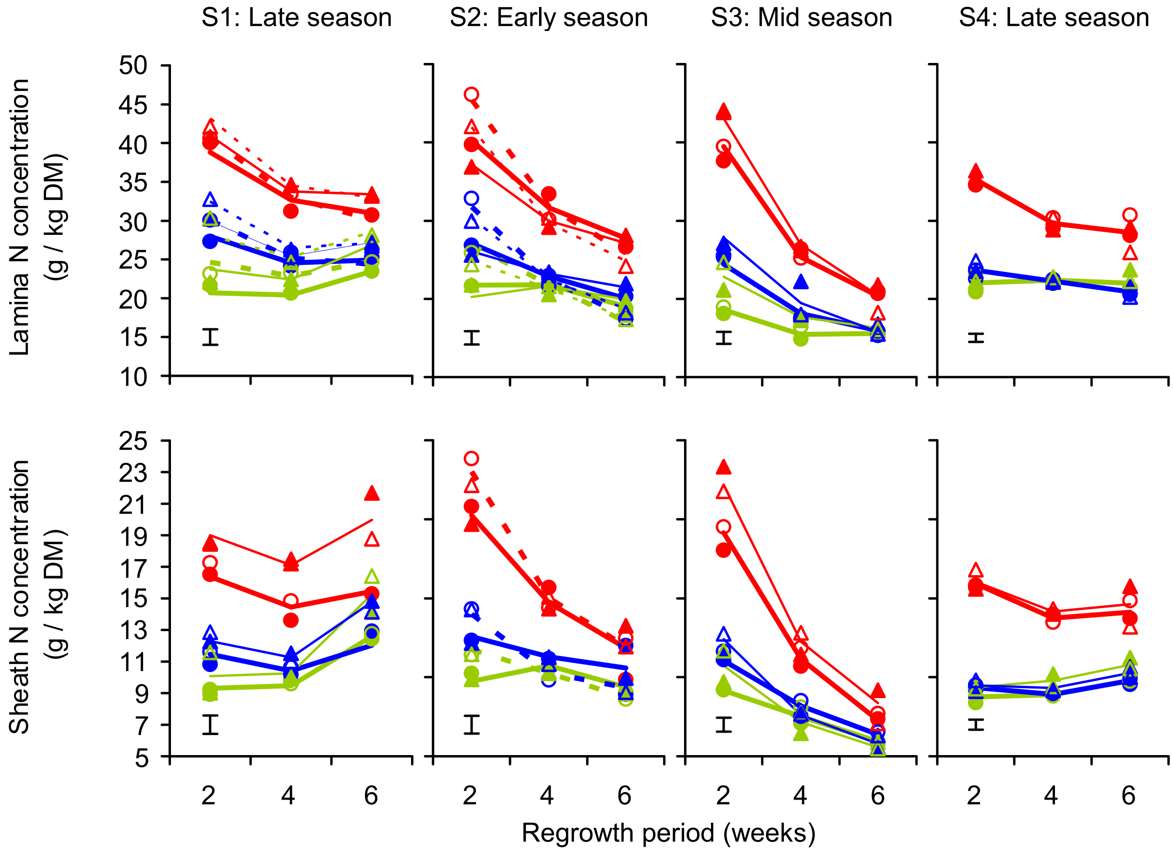

Figure 2 N concentration of lamina and sheath material as affected by cultivar, N application rate, regrowth period and cutting height, for four seasons (S1-S4). Lines represent the ANOVA model, averaged over non-significant effects. Error bars are 2 × standard error for the comparison of individual model points. See Fig. 1 for legend.

Table 2

Statistical significance[a] of effects and the two-factor interactions[b] of grassland management tools[c] on WSC, N and NDF concentrations (g / kg DM) and the WSC-free NDF and ADF concentrations (g / kg WSC-free DM) of lamina and sheath material during four seasons (S1-S4). The R^2_{adj} is the percentage variance accounted for by the ANOVA model.

	Lamina										Sheath									
	R^2_{adj}	N[b]	T	C	D	NT	NC	ND	TC	TD	R^2_{adj}	N	T	C	D	NT	NC	ND	TC	TD
WSC (g / kg DM)																				
S1	0.89	***	**	***		*					0.89	***	***	**		*				
S2	0.94	***	***	**		*			*	***	0.96	***	***		**	**			*	***
S3	0.95	***	***	***	**	**	**		*		0.91	***	***	***		***		*		
S4	0.89	***	**	***			**				0.95	***	***	***		***				
N (g / kg DM)																				
S1	0.96	***	***	***	**	***					0.97	***	***	***		*				
S2	0.97	***	***	*		***				***	0.97	***	***			***				**
S3	0.98	***	***	***		***			**		0.99	***	***	*		***	*		**	
S4	0.95	***	***			***					0.98	***	**	*		***				
NDF (g / kg DM)																				
S1	0.76			***							0.93	***	***	***	*	**			*	**
S2	0.73	***	*	*						*	0.90	***	***			*			**	***
S3	0.91	***	***	***	***	***	***				0.87	**	***	***	***	***	***			*
S4	0.75		**	***							0.97	***	***	***		***		*	*	*
NDF (g / kg WSC-free DM)																				
S1	0.67	***		*		*					0.78	***	***							
S2	0.61	**	***					**			0.91	***	***		***					
S3	0.91	***	***			***					0.96	***	***	**	***	***	**			**
S4	0.66	***	***								0.72	***	***	*						
ADF (g / kg WSC-free DM)																				
S1	0.24	*									0.78	*	***	*						
S2	0.88	***	***	*		**		*			0.78		***							
S3	0.56		***								0.66	*	***		*					
S4	ns[d]										0.94	***	*	***	***	*			***	

[a] Levels of statistical significance: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$
[b] The interaction C × D was never significant and is therefore not included in the table.
[c] N = N application rate, T = Rotation length, C = Cultivar, D = Defoliation height
[d] ns = not statistically significant

significant only at longer regrowth periods (a significant ($p < 0.05$) T × C interaction). For laminae during S3 and S4 there was a statistically significant N × C interaction ($p < 0.05$) indicating that the difference between HS and LS cultivars was smaller at 390 than at 0 and 90 kg N / ha / yr.

In all treatment combinations, WSC concentration was significantly ($p < 0.01$) reduced by an increase in N application rate, although in some cases the difference between the 0 and 90 kg N / ha / yr application rate was not statistically significant. For all seasons there was a statistically significant ($p < 0.01$) positive effect of the the length of the regrowth period (T) on WSC concentration. In the sheath material this average increase was stronger at the high N application rate [N × T interaction statistically significant ($p < 0.05$)].

The effects of cutting height on WSC concentration were inconsistent: they were statistically significant in a few cases only. During S3, the low cutting height significantly ($p < 0.01$) increased the WSC concentration of the laminae. In contrast, the WSC concentration was higher for the high cutting height for T1 and lower for T3 due to a statistically significant ($p < 0.001$) T × D interaction for S2 lamina and sheath material.

The relative increase in WSC concentration for the HS cultivar compared with the LS cultivar [(HS - LS) / LS ×100%] tended to be larger during S1 and S4 than during S2 and S3 and larger for lamina than for sheath material (Table 3).

3.3 Dry matter versus WSC-free dry matter

All concentrations on the basis of DM are by definition mutually correlated because they are expressed in relative terms. The range in WSC concentration as affected by the treatments was large, resulting in a strong effect on the other fractions. In order to eliminate this effect, the N, NDF and ADF concentrations were also expressed on the basis of WSC-free DM. If expressed in this way the treatment effects on NDF and ADF became much more consistent. As to N concentration there was not much difference between the two methods (data not shown). So in the remainder of this

Table 3

Average relative increase[a] in WSC concentration (%) in the HS cultivar compared to the LS cultivar for lamina and sheath material during four seasons. Mean of all herbage management treatments, standard error between parentheses (S1, n = 16; S2−4, n = 18).

	S1		S2		S3		S4		Mean	
Lamina	60.8	(7.5)	9.2	(4.1)	29.8	(4.9)	49.5	(5.4)	36.7	(3.6)
Sheath	11.3	(3.1)	1.4	(1.8)	21.0	(2.1)	16.6	(1.7)	12.6	(1.4)

[a] Calculated as [(HS − LS) / LS] × 100%

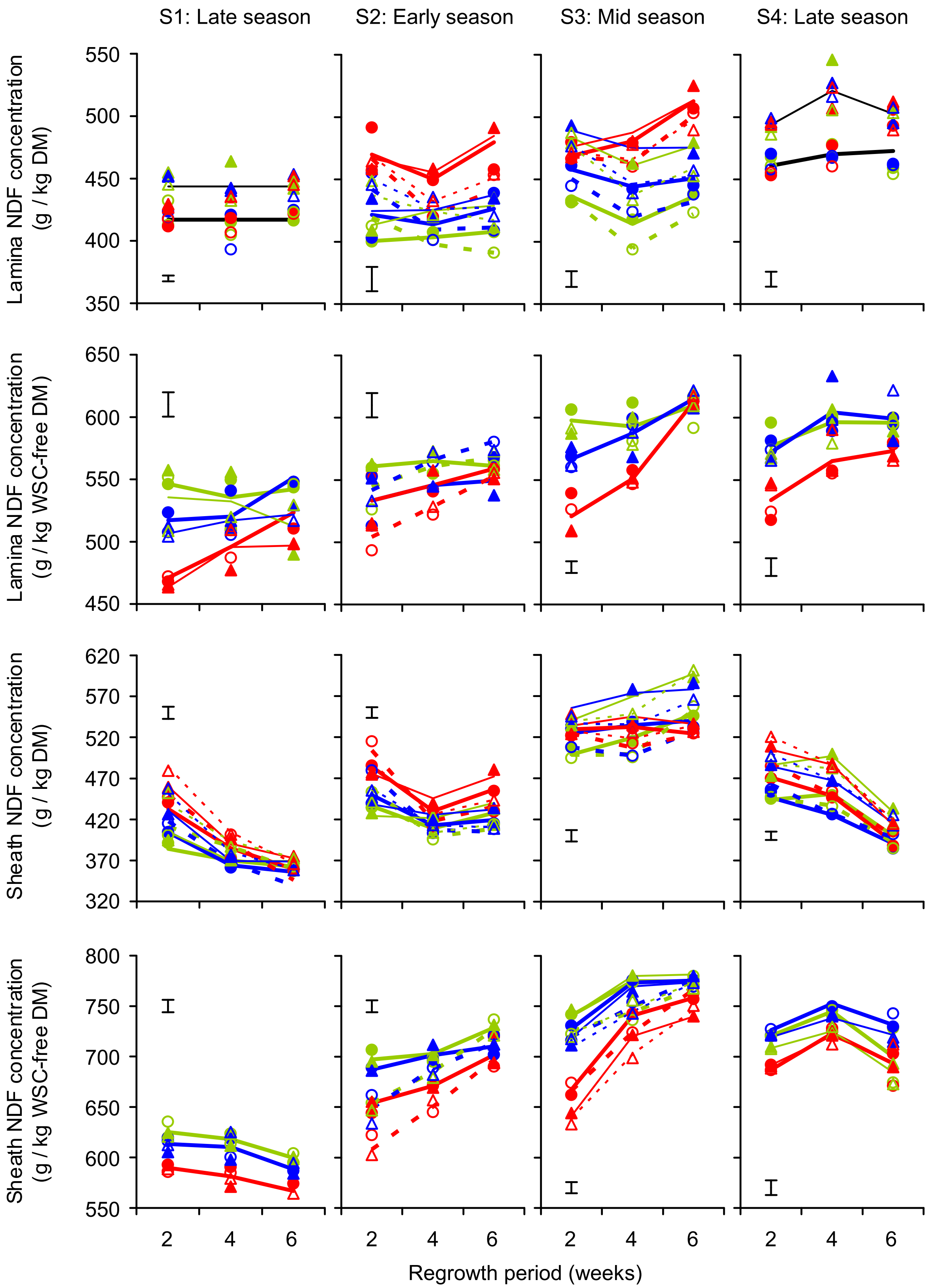

Figure 3 NDF concentration of lamina and sheath material expressed as g / kg DM or g / kg WSC-free DM as affected by cultivar, N application rate, regrowth period and cutting height during four seasons (S1-S4). Lines represent the ANOVA model, averaged over non significant effects. Error bars are 2 × standard error for the comparison of individual model points. See Fig. 1 for legend.

chapter NDF and ADF concentrations will be expressed in g / kg WSC-free DM, unless stated otherwise.

3.4 Nitrogen

The average N concentration did not vary much between the seasons, but was much lower for sheath than for lamina material: 11.8 and 25.6 g / kg DM, respectively) (Fig. 2).

There was a statistically significant effect of N application rate ($p < 0.001$) on the N concentration for all periods and for both lamina and sheath material (Fig. 2 and Table 2). However, the difference in N concentration among N application rates tended to be smaller with longer regrowth periods (T), resulting in a statistically significant ($p < 0.001$; S1 lamina $p < 0.05$) N × T interaction.

During S2, but especially during S3, N concentration of lamina and sheath material decreased strongly and significantly ($p < 0.001$) with T. During S1 and S4 the effect of T was still statistically significant, but less pronounced at low N application rates.

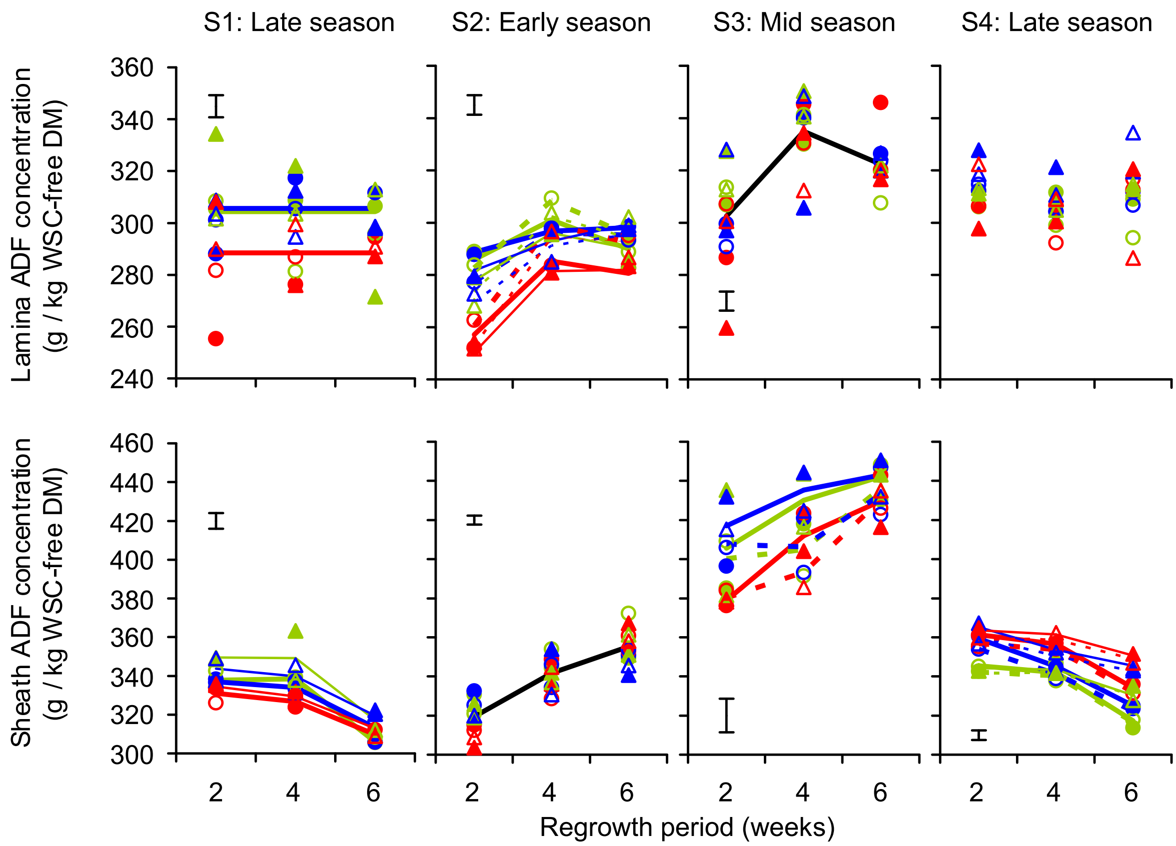

Figure 4 ADF concentration of lamina and sheath material as affected by cultivar, N application rate, regrowth period and cutting height, for four seasons. Lines represent the ANOVA model, averaged over non significant effects. Error bars are 2 × standard error for the comparison of individual model points. For legend see Fig. 1.

During S3 and S1, the low-sugar cultivar generally had a significantly ($p < 0.05$) higher N concentration. For S1 this was mainly during the longer regrowth period, whereas during S3 the difference was only statistically significant for the short regrowth period.

In most cases, N concentration was not significantly affected by cutting height, except during S2 when a statistically significant ($p < 0.01$) T × D interaction was found. During S1 the N concentration of the lamina was slightly but significantly ($p < 0.01$) higher for the lower cutting height.

3.5 Neutral detergent fibre

The neutral detergent fibre (NDF) concentration tended to be lower in lamina than in sheath material (on average 558 and 687 g / kg WSC-free DM, respectively) and higher for S3 than for the other seasons (Fig. 3).

N application rate had a statistically significant ($p < 0.01$) negative effect on NDF concentration (g / kg WSC-free DM) in all cases (Table 2). During S3 and for lamina material during S1 there was a statistically significant N × T interaction, the increase in NDF with T becoming larger as N application rate increased. There was a positive statistically significant ($p < 0.001$) effect of T on the NDF concentration in all cases, except for lamina material during S1. However, for sheath material during S1 and S4 the NDF concentration tended to decrease rather than increase with a longer regrowth period.

There was no statistically significant cultivar effect except for sheath material during S3 and S4 and for lamina material during S1, where the HS cultivar tended to have slightly higher NDF concentrations with higher N application rates.

During S2 and S3, cutting height had a statistically significant ($p < 0.001$) effect on the NDF concentration of the sheath material. During S3 the difference in the NDF concentration of the sheath material between HD and LD decreased with T, resulting in a statistically significant ($p < 0.01$) T × D interaction.

3.6 Acid detergent fibre

The acid detergent fibre (ADF) concentration ranged from 252 to 451 g / kg WSC-free DM and tended to be higher during S3, especially for the sheath material (Fig. 4). The effect of N application rate was small and the ADF concentrations either significantly ($p < 0.05$) decreased (lamina material during S1 and S2, and sheath material during S1 and S3) or significantly increased (sheath material during S4) with increasing N rate (Table 2).

During S2 and S3, ADF concentrations (g / kg WSC-free DM) significantly (p

Table 4

Chemical composition of inflorescence material (g / kg dry matter).

	Average[a]	SD[b]	n
N total	17.3	2.5	22
WSC	137.0	21.5	25
NDF	589.4	22.8	22
Lignin	30.3	4.0	17
Ash	45.8	2.7	29

[a] Average over all treatments
[b] SD = standard deviation of the mean

< 0.001) increased with T. However, during S1 and S4 no effect of T was found on NDF concentration of the lamina material, whereas it was slightly but significantly ($p < 0.001$) negative for the sheath material.

Cultivar had a small negative effect on NDF concentration in sheath material during S1 and S4 due to a lower ADF concentration of the HS cultivar. There was a statistically significant effect ($p < 0.05$) of cutting height for sheath material during S3 and S4, as ADF concentration was slightly lower at the lower cutting height.

3.7 Inflorescences

Due to a shortage of material, the inflorescences could not be analysed for all treatment combinations and the number of missing values was too large for analysis of variance. Therefore, only average concentrations of NDF, ash, N, and WSC in inflorescence material are presented in Table 4. These values are included to complete the picture of the chemical analysis of the herbage material.

4. Discussion

4.1 Analysis gap

The concentrations of the different chemical constituents did not always add up to 100%. We therefore asked other laboratories to re-run our analyses. These laboratories confirmed our findings. The gap cannot be explained by the fatty acids, which were not included in the analyses: fatty acids usually have low concentrations. However, the gap was correlated with the concentrations of the water-soluble carbohydrates (data not shown), suggesting that especially long-chain carbohydrates could have contributed. Also other studies have reported gaps in the analyses, and have suggested that this unexplained component is likely to have consisted of a mixture of lipids, organic acids, pectins, and other carbohydrate compounds (Van Soest, 1982; Smith *et al*., 2002). Attempts to analyse these non-structural poly-carbohydrates were not successful (data not shown).

4.2 Lignin and ash

The concentrations of acid detergent lignin could not be assessed with high accuracy. But as the concentrations were low the analytical error in assessing the lignin concentration did not interfere with the other results.

Average levels and variations in ash concentrations were considerably larger than those in the concentration of lignin, but were more related to soiling than to herbage management treatments. Given the fact that the ash concentrations were fluctuating, sometimes even considerably, it was an option to express concentrations of chemical constituents on the basis of the organic matter instead of the dry matter. However, this was not considered to provide more insight than our current approaches of expressing concentrations on either DM basis or WSC-free DM basis.

4.3 Dry matter versus water-soluble carbohydrates-free dry matter

When NDF and ADF were expressed as a proportion of WSC-free DM, treatment effects became much more consistent, which yielded some interesting insights. For example, when NDF was expressed in g / kg DM, no consistent effect of N application rate on NDF concentration was found, which is in agreement with the studies of Wilman *et al*. (1977), Valk *et al*. (1996) and Peyraud & Astigarraga (1998). However, when expressed in terms of WSC-free DM, higher N application rates significantly ($p < 0.001$) reduced the NDF concentration. In that case the higher N application rate resulted in a higher concentration of N, present mainly in the cell contents. As a result the amount of cell wall is relatively lower. Apparently, in terms of g / kg DM, this decrease in cell wall concentration is negated by the accompanied decrease in WSC. This phenomenon can also be derived from the data by Valk *et al*. (1996).

Generally the NDF and ADF concentrations of grass are reported to be lower for high-sugar than for low-sugar cultivars (Smith *et al*., 1998; 2001; 2002; Lee *et al*., 2001; Miller *et al*., 2001). However, when NDF and ADF were expressed on a WSC-free DM basis, the cultivar effect largely disappeared, indicating that the increase in WSC concentration of the HS cultivar was partly obtained at the expense of NDF and ADF (less WSC converted into cell wall material).

In our study the expected increase in NDF and ADF concentrations with longer regrowth periods and in all seasons was not observed when the concentrations were expressed in g / kg DM. However, when expressed in g / kg WSC-free DM, it became apparent that this lack of effect was partly due to the relatively strong increase in WSC with the length of the regrowth period. This is in agreement with Wilson (1994), who stated that although the NDF concentration usually increases with the length of the regrowth period, this effect may be negated by an increase in storage of WSC components.

Expressing ADF and ADL on the basis of NDF instead of DM or WSC-free DM can provide detailed insight into the effects of herbage management on cell-wall digestibility, but such an approach did not serve our general objective of obtaining insight into the chemical composition of the dry matter.

4.4 Effects of the high-sugar cultivar

The higher WSC concentration of the HS cultivar compared with the LS cultivar was 43 g / kg DM, which is comparable to or lower than the concentrations found in other studies (Humphreys, 1994; Miller *et al*., 2001). The difference between HS and LS was most apparent during the mid and late seasons (S1, S3 and S4, Table 3). This would be an important feature as N losses from grazing tend to be larger during the late season, due to the higher N concentrations of the grass (Beever *et al*., 1978) and the increased risk of leaching. However, other studies have reported varying trends in the difference in WSC concentration between high and low-sugar cultivars over the seasons (Jones & Roberts, 1991; Radojevic *et al*., 1994).

On average, the WSC concentration of the HS cultivar compared with the LS cultivar tended to be higher in the lamina material than in the sheath material, especially during the late seasons (Table 3). This would imply that the high-sugar trait of the plant material taken in by the ruminant may be more pronounced under grazing, when lamina material forms the bulk of the intake (Brereton *et al*., 2005). It is questionable, however, whether the ruminant may benefit from this trait as nutritional quality and intake by grazing dairy cows did not differ significantly among high-sugar and low-sugar cultivars (Smit *et al*., 2005; Moorby *et al*., 2006b). We confirmed this in our model studies (Hoekstra *et al*., submitted b).

Conversely, in three other studies that have measured the WSC concentration of the different crop fractions, the relative WSC concentration tended to be higher in the 'stem' material (Smith *et al*., 2001; 2002; Turner *et al*., 2001). However, the methods these authors used were different from our methods as their experiments were performed under controlled environmental conditions, using different cultivars and seedlings (only up to 42 days of age) rather than mature plants.

For other recent information on the effects of grazing on or feeding of grass with an elevated concentration of water-soluble carbohydrates we refer to Moorby *et al*. (2006a), Shewmaker *et al.* (2006), Smit *et al.* (2006) and Taweel *et al*. (2005a, 2006). In general, also these papers report surprisingly little effect of the high-sugar trait on chemical composition, intake and nutritional quality, although dairy cattle obviously prefer the high-sugar grass when offered a choice.

In some cases N concentration tended to be slightly higher in the low-sugar cultivar, but this tendency was not consistent. This trend is confirmed in the literature, where higher (Smith *et al*., 1998; 2001), equal (Lee *et al*., 2001; Smith *et al*., 2002) and lower (Miller *et al*., 2001) concentrations are reported for high-sugar cultivars compared with control cultivars. This would confirm that the sink-source related negative correlation between WSC and N does not apply across different genetic lines (Humphreys, 1994).

4.5 Effects of length of the regrowth period

The WSC concentration usually increases with the length of the regrowth period, as was also the case in our experiment. Immediately after cutting, the plant partly relies on WSC reserves for its regrowth and these can be replenished only if the photosynthetic capacity is restored (Fulkerson & Donaghy, 2001). The increase in WSC with time tended to be strongest in sheath material, whereas in the lamina, especially during the late season, hardly any increase took place. This can be explained by the fact that the sheath is the main storage site for WSC. The increase in WSC concentration of the sheath increased with N application rate to such an extent that at the last harvest date there was no difference in WSC level among the different N application rates. Apparently, with longer regrowth periods, the increased photosynthetic capacity as a result of the increased growth rates at higher N application rates compensates for the amount of WSC that is used as a source of energy for the extra growth.

Generally, the NDF concentration increases with the regrowth period due to the maturation of the plant (Wilman *et al*., 1977; Wilson, 1994; Groot, 1999). In our experiment the changes in NDF concentration (g / kg WSC-free DM) with regrowth period were relatively small, but the concentration tended to increase with time in all cases except for sheath material during the late season. The negative effect during the late season of 2002 may be explained by the fact that the young sward was still developing, resulting in an increased tillering rate. So while individual sheaths were ageing, the formation of new sheaths decreased the relative age, which resulted in a decrease of NDF material with time. This may also have been the case during the late season of 2003, as tillering rates during this part of the year are likely to be higher in order to compensate for tiller losses after generative regrowth. The effect of the length of the regrowth period on ADF concentration was very similar to the effect on NDF.

The observed decrease in N concentration with increasing length of the regrowth period is widely documented (Nowakowski, 1962; Wilman *et al*., 1976c).

The initial uptake of N is driven by N supply and not by growth, resulting in a high level of N shortly after fertilizer application, which is diluted as the herbage mass increases with time. The N × T interaction that was found can be explained by the higher dilution rate at high N application rates, as both the initial boost in N uptake and growth rate are increased. So the difference in N concentration among the different N application rates becomes smaller as the periods of regrowth become longer. During the late season, the decrease in N concentration with regrowth period was less pronounced, and the N concentration of the sheath even increased. The lower dilution rates can be explained by the reduced growth rates during this season. As explained above, the increase in N concentration of sheath material during the late season may be the result of increased tillering rates.

4.6 The effect of N application rate

The strong negative effect of a higher N application rate on WSC concentration found in this study has been widely documented in earlier studies (Reid & Strachan, 1974; McGrath, 1992; Valk *et al.*, 1996). It can be attributed to an increased crop growth rate in combination with an increase in utilisation of carbon for protein synthesis and for the production of energy required for the nitrate reduction preceding protein synthesis (Reid & Strachan, 1974).

N fertilisation strongly increases the herbage N concentration (Nowakowski, 1962; Reid & Strachan, 1974; Wilman *et al.*, 1976c). However, as discussed above, because of the statistically significant N × T interactions, the difference in both WSC and N concentration as affected by N application rate becomes smaller with longer regrowth periods.

4.7 The effect of cutting height

The effect of cutting height on herbage chemical composition was unclear, with effects being inconsistent and often relatively small. Also the literature on the effect of cutting height is inconclusive. There is some evidence that the digestibility of lamina in cocksfoot (*Dactylis glomerata*) is lower at high initial sward heights due to a decrease in leaf appearance rate (Duru & Ducrocq, 2002), reflecting higher NDF concentrations. However, studies on the effect of initial sward height on leaf appearance rate of perennial ryegrass have yielded conflicting results (Grant *et al.*, 1981; Hernández Garay *et al.*, 2000). Moreover, the effect on leaf appearance rate is based on the difference in initial sheath length as affected by cutting height. The cutting height in our experiment may not have been low enough to cut off sheaths, which would explain the lack of a direct effect.

4.8 Lamina versus sheath material

Distinct differences were observed in chemical composition between lamina and sheath material so that their relative proportions in the intake have a pronounced effect on diet composition. Consistent with literature, the N concentration was higher (Grindlay, 1997) and the WSC concentration was lower (Smith *et al*., 2002) for lamina than for sheath material. Generally, the NDF and ADF concentrations are higher for sheath than for lamina due to the more structural role in lifting the lamina up towards the light (Lantinga *et al*., 2002; Smith *et al*., 2002). When expressed in g / kg WSC-free DM this was also found in the current experiment. However, when expressed in g / kg DM, NDF and ADF concentrations of the sheath were equal to or lower than those of the lamina during all seasons except mid season. This can be partly explained by the sheath material during these seasons, which comprises the sheath tube including the young leaves developing within the tube. During mid season the sheath fraction consisted mainly of real stem material, which has a higher NDF concentration compared with sheath material (Minson, 1990).

4.9 Seasonal effects

In our experiment the results for the late seasons of 2002 and 2003 were very similar, suggesting that the observed effects were not just typical for newly sown swards or a particular year.

The sheath material had lower N and WSC and higher ADF and NDF concentrations during mid season than during the other seasons, which could be attributed to the formation of real stems. In contrast to studies of Beever *et al*. (1978), the average N concentration in the current experiment was not higher during the late season.

5. Conclusions

- When NDF and ADF concentrations were expressed in g / kg WSC-free DM, treatment effects became much more consistent than when expressed in g / kg DM, which yielded interesting insights. We therefore recommend this method for interpreting NDF and ADF concentrations.
- As expected, the high-sugar cultivar generally had a higher WSC concentration than the low-sugar cultivar (on average 25% higher). However, the difference between the two cultivars was especially pronounced during the late season (35%), which would be an important feature to reduce the relatively high N losses from grazing during this period. Moreover, the higher WSC concentration of the high-

sugar cultivar tended to be higher in lamina material (37% increase) than in sheath material (13% increase). This implies that the high-sugar trait may be utilized more effectively under grazing conditions, when lamina forms the bulk of the intake.

- Longer regrowth periods and lower N fertilizer rates increased WSC concentrations and decreased N concentrations; interactions between regrowth period and N application rate were highly significant. NDF and ADF concentrations were less affected. The NDF concentration in terms of g / kg WSC-free DM tended to be higher at low N application rates and at longer regrowth periods.
- The prevalence of statistically significant interactions stresses the importance of evaluating the effects of N application, regrowth period and grass cultivar in factorial experiments, rather than in isolation.
- The effect of cutting height on herbage chemical composition was unclear. The effect probably depends more on the proportion of stem, lamina and dead material in the sward than on the changes in chemical composition of the individual components.

Acknowledgements

The authors wish to thank Hennie Halm for the chemical analysis, and E.J. Bakker for his advice on the statistical analysis. The first author was supported under the Teagasc Walsh Fellowship Scheme.

4

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

N.J. Hoekstra, P.C. Struik, E.A. Lantinga, M.E. Van Amburgh & R.P.O. Schulte,
accepted for publication by NJAS – Wageningen Journal of Life Sciences

Abstract

The high degradability of grass protein is an important factor in the low nitrogen (N) utilisation 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 / yr), 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.

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

1. 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 in 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 decreasing urinary N excretion (Castillo *et al*., 2001b). 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*., 2007b). However, not much is known of the impact of these herbage management tools on the degradability of N (Hoekstra *et al*., 2007a). 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*., 2007a). 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.

2. Materials and methods

2.1 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.* (2007b). 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 / yr 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 minus 20 ^{o}C until later analysis. For further analytical details see Hoekstra *et al.* (2007b).

2.2 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 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 ANKOM^{200} 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

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	Fraction (g / kg total N)	Description	Calculation [a, b]
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 [c] 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 [d]	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

[a] All N fractions expressed in g / kg N
[b] TPN = true protein nitrogen; BIN = buffer insoluble nitrogen; NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen
[c] ND = neutral detergent
[d] AD = acid detergent

ANKOM^{200} Fiber Analyser.

The concentrations of the different N fractions were expressed in g / kg DM (subscript DM) as well as in g / kg N (subscript N); the abbreviations for the fractions are explained in Table 1.

2.3 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.

3. Results

3.1 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 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 ANKOM^{200} 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

Table 2

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

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

[a] For abbreviations see Table 1.
[b] Statistical significance. *** = p < 0.001; **** p < 0.0001; ns = not significant.
[c] Correlation for NDIN was calculated for the whole sample (All) and for the lamina (L) and sheath (S) samples separately.

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 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.

3.2 Effect of herbage management tools on N fractionation

3.2.1 On dry matter basis

The concentrations of the five protein fractions in lamina and sheath material were on average 26, 19, 56, 15 and 1.2 g / kg dry matter (DM) for A_{DM}, $B1_{DM}$, $B2_{DM}$, $B3_{DM}$ and

Table 3
Mean protein fractions (g / kg DM) for lamina and sheath material during the four seasons. Standard error[a] between brackets.

Protein fraction	Period S1	S2	S3	S4	Mean
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}[b]	2.4 (0.21)	0.5 (0.52)	1.1 (0.81)	1.5 (0.4)	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}[2]	1.6 (0.47)	0.3 (0.35)	0.7 (0.35)	1.2 (0.37)	1.0 (0.30)

[a] n = 34 for S1; n = 36 for S2–S4.
[b] Mean values (n = 4) based on WU Fibertec analysis for S1 and CU analyses for S2–S4.

C_{DM}, respectively (based on Table 3). Concentrations of A_{DM}, $B2_{DM}$ and $B3_{DM}$ tended to follow the total protein concentration (Hoekstra *et al*., 2007b, Table 2 and Fig. 1): the concentrations 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 concentrations of A_{DM}, $B2_{DM}$ and $B3_{DM}$. There was no consistent effect of cutting height (data not shown).

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 concentration 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).

3.2.2 On nitrogen basis

On average, 186 and 280 g / kg N was in the form A_N, 164 and 186 g / kg N in $B1_N$, 519 and 396 g / kg N in $B2_N$, 121 and 130 g / kg N in $B3_N$ and only 9 g / kg N in C_N for lamina and sheath material, respectively (Fig. 1–3). The concentration of A_N was

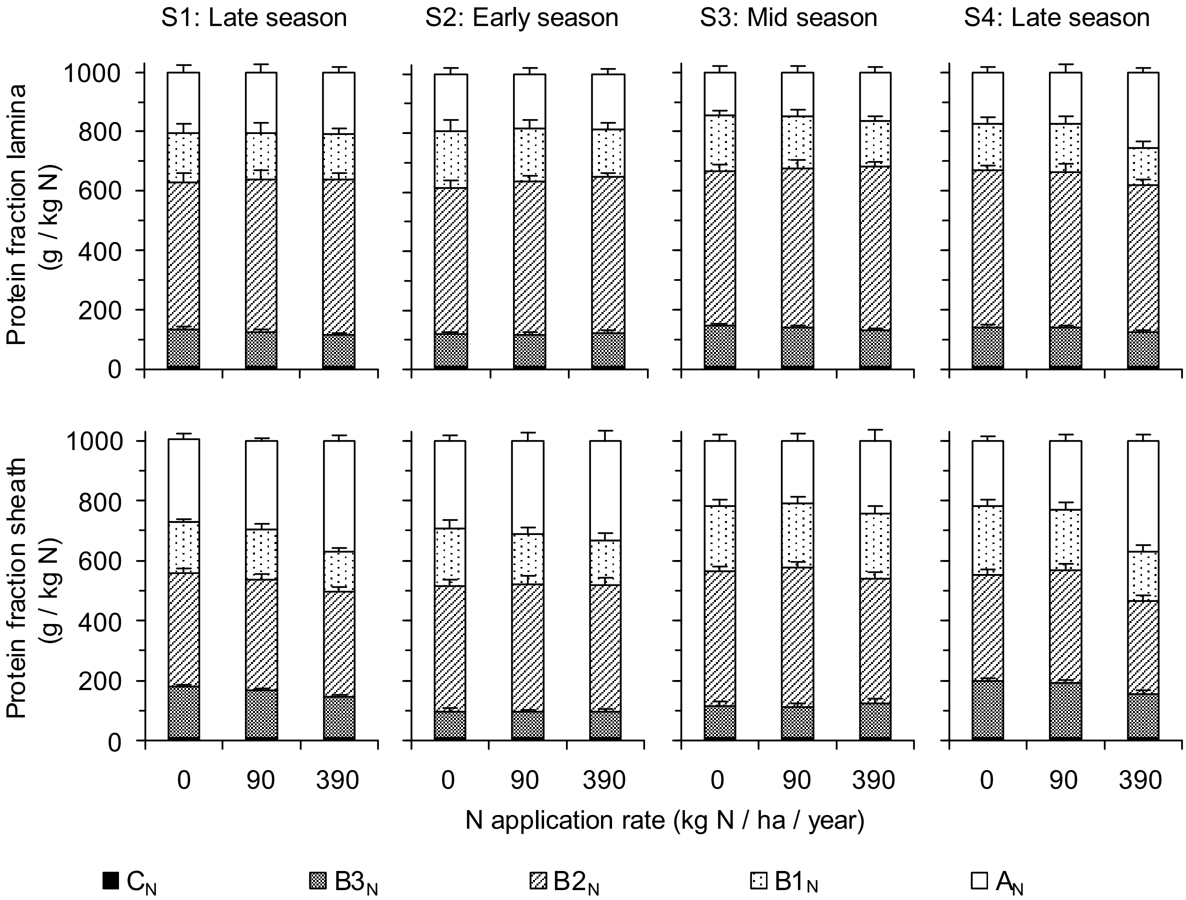

Figure 1 The effect of N application rate (kg N / ha / year) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N concentration (g / kg N) of perennial ryegrass lamina and sheath material for late season 2002, early season 2003, mid season 2003 and late season 2003. Bars represent 2× standard error (n = 12). C_N is the average value over all treatments.

higher for sheath than for lamina material (280 and 186 g / kg N, respectively), whereas the opposite was found for $B2_N$ (396 and 519 g / kg N for sheath and lamina material, respectively).

The concentration of A_N was lower during S3 than during the other seasons (188 and 228 g / kg N, respectively). For sheath material, the $B3_N$ concentration increased with progressing season (86, 108 and 162 g / 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 (Fig. 1). The concentration 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 (Fig. 2). During early season the effect was very inconsistent. For sheath

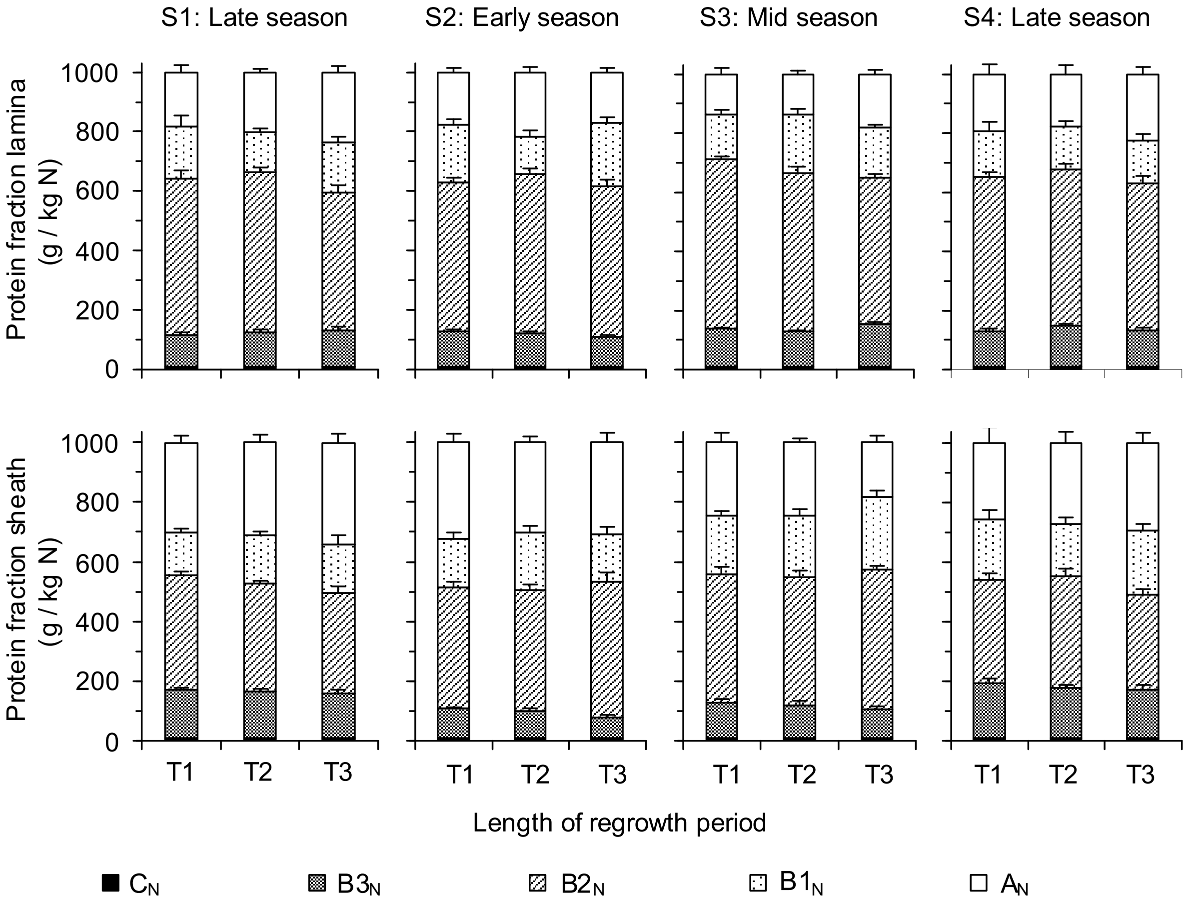

Figure 2 The effect of length of regrowth period (T1 = 2–3, T2 = 4–5, and T3 = 6–7 weeks) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N concentration (g / kg N) of perennial ryegrass lamina and sheath material for late season 2002, early season 2003, mid season 2003 and late season 2003. Bars represent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

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 (Fig. 3).

4. Discussion

4.1 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 / kg N) (insoluble in the buffer solution, representing rumen liquid) and the remainder formed the $B1_N$ fraction (175 g / 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 / kg N), which is potentially degradable in the rumen. Only a very small fraction was in the form of C_N (9 g / 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).

4.2 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

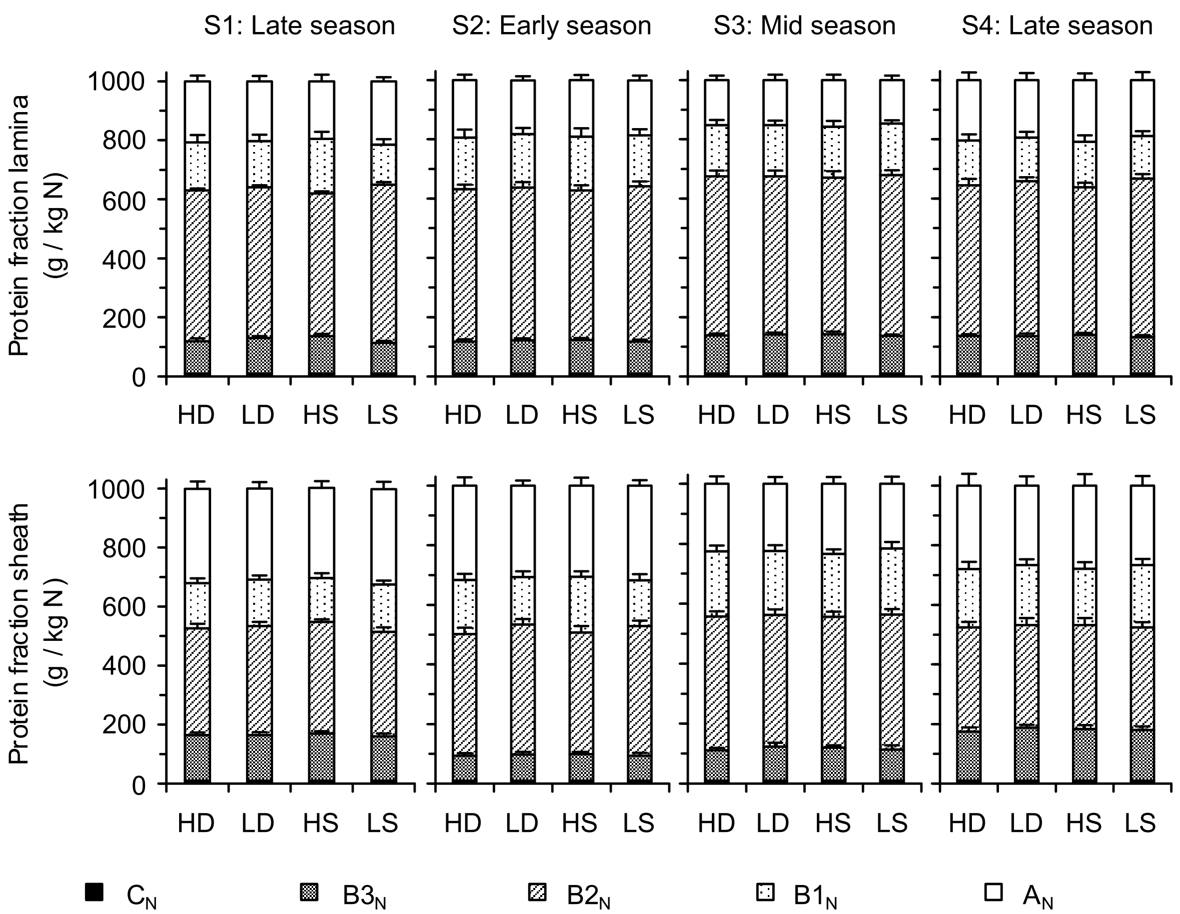

Figure 3 The effect of cutting height (LD = low cutting height, HD = high cutting height) and cultivar (LS = low-sugar cultivar, HS = high-sugar cultivar) on the A_N, $B1_N$, $B2_N$, $B3_N$ and C_N concentration (g / kg N) of perennial ryegrass lamina and sheath material for late season 2002, early season 2003, mid season 2003 and late season 2003. Bars represent 2× standard error (n=18). CN is the average over all treatments.

this was mainly a reflection of the changes in the total N concentration. Therefore, when the protein fractions were expressed in g / 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*., 2007a). 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 concentration 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 week 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*., 2007b) changes in NDF concentration 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 concentration 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) concentration 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).

4.3 Potential for impact on bovine N utilisation

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 utilisation, 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 concentration. 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 modeling 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.

5. 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.

Acknowledgements

The authors wish to thank Hennie Halm, Wageningen University, for his contribution to the chemical analyses. The first author was supported under the Teagasc Walsh Fellowship Scheme.

5

Modelling the concentrations of nitrogen and water-soluble carbohydrates in grass herbage ingested by cattle under strip-grazing management

N.J. Hoekstra & R.P.O. Schulte, accepted for publication by Grass and Forage Science

Abstract

Grazing by bovines is accompanied by localised deposition of nitrogen (N) in urine and dung patches, which has been associated with risks of losses of N to water and air. There is scope to reduce N excretion by increasing the N efficiency of grazing bovines through manipulation of the energy and N contents of the diet. The impact of grassland management on herbage quality has been well described, but due to asymmetric grazing of animals between individual plant organs, it has not yet been established how this translates into the N and water-soluble carbohydrate (WSC) concentrations of the intake.

In this chapter a model is presented with the objective to assess the efficacy of individual grassland management tools in manipulating the WSC and N concentration of the herbage intake of strip-grazing bovines throughout the growing season. The grassland management tools assessed were ryegrass cultivar, N application rate, rotation length and defoliation height. This model was calibrated and independently evaluated for three distinct periods during the grazing season, i.e. early (April), mid (June, generative growth phase) and late (September) season. There was a good correlation between predicted and observed WSC concentration in the intake (R^2 = 0.78, $p < 0.001$). The correlation between predicted and observed NDF concentrations in the intake was reasonable (R^2 = 0.49, $p < 0.05$) with a small absolute bias. Differences in the N concentration between laminae and sheaths and between clean patches and fouled patches were adequately simulated, though N concentration of the intake was over-predicted in early season and under-predicted during the late season. However, this did not affect the ability of the model to assess the efficacy of grassland management tools for manipulating the WSC and N concentration in the intake of strip-grazing bovines.

Model application showed that reduced N application rates and longer rotation lengths were effective tools for manipulating herbage quality during early and mid season. During late season, the large proportion of area affected by dung and urine reduced the impact of N application rate on herbage quality. In contrast, relative differences between high-sugar and low-sugar cultivars were largest during this period. This suggests that high-sugar cultivars may be an important tool to increase bovine N-efficiency during late season, when risks of N losses to water are largest. The model output showed that defoliation height affects the chemical composition of the intake of both the current and the subsequent period, but the two effects are opposite. When defoliation height is the same at the start and end of the grazing period, the chemical composition of the intake tended to be similar for the low and high defoliation height.

Keywords: defoliation height, herbage quality, high-sugar, intake quality, rotation length, water-soluble carbohydrates, herbage morphology

1. Introduction

In large parts of Europe, a substantial fraction of the bovine's diet consists of grazed grass (Beever & Reynolds, 1994; Lantinga *et al*., 1996). Grazing is accompanied by localised deposition of nitrogen (N) in urine and dung patches. Grazing at times that grass growth rates and hence nitrogen uptake are low has been associated with increased risks of losses of N to water and air (e.g. McGechan & Topp, 2004; Schulte *et al*., 2006). The quality of the grass intake has a pronounced effect on both animal production and N utilisation (Rearte, 2005). Therefore, there is scope to reduce N excretion by increasing the N efficiency of grazing bovines through manipulation of the energy and N concentrations of the diet, as reviewed by Hoekstra *et al*. (2007a). N utilisation may be improved by 1) reducing the relatively high concentration of protein in grazed grass (Tamminga & Verstegen, 1996), and 2) increasing the ratio of carbohydrate to N supplies in the diet (Beever & Reynolds, 1994; Lantinga & Groot, 1996). This reduces ammonia accumulation in the rumen, and subsequent excretion of urea through urine (Nocek & Russell, 1988; Miller *et al*., 2001).

Hoekstra *et al*. (2007b), demonstrated how the levels of water-soluble carbohydrates (WSC) and N of perennial ryegrass (*Lolium perenne*) (two cultivars: high-sugar and low-sugar) may be manipulated by herbage management. In general, they found that the high-sugar cultivar had higher WSC concentration than the low-sugar cultivar, with differences tending to be larger during the late season. Additionally, the relative difference in WSC concentration between the two cultivars tended to be higher in the lamina material than in the sheath. Both the length of the regrowth period and N application rate strongly affected the WSC and N concentrations, and interactions were highly significant. The effect of defoliation height on the chemical composition of lamina and sheath material remained unclear, as effects were small and inconsistent.

However, it remains difficult to predict the WSC and N concentrations of the intake of grazing bovines, as this depends not only on the WSC and N concentrations of lamina and sheath material in the sward, but also on the proportions of laminae and sheaths in the intake. It is well known that animals graze selectively, not only between plant species, but also intra-specifically between plant organs (Curll *et al*., 1985; Grant *et al*., 1985). As a result, diet composition cannot be equated to sward composition. Grassland management also impacts on the proportions of lamina and sheath in the intake, and effects may vary between years and seasons. Therefore, manipulation of bovine N efficiency through grassland management requires the prediction of the quality of the herbage intake (as opposed to the quality of the standing herbage) as a function of grassland management.

The prediction of this quality of herbage intake is complicated by the spatial heterogeneity of herbage quality and grass morphology in the sward, which originates largely from the deposition of animal faeces. Dung and urine both have pronounced effects on local sward growth, herbage quality and grazing patterns. The area affected by dung and urine patches can range from zero at the start of the grazing season to over 50% at the end of the grazing season (Lantinga *et al*., 1987; Haynes & Williams, 1993). The herbage surrounding fouled patches is partially rejected by grazing bovines (Bosker *et al*., 2002). This is partly because of differences in morphological composition of the herbage around dung pats, with fouled areas generally containing a higher proportion of pseudo-stems. Research by both Garcia *et al*. (2003) and Brereton *et al*. (2005) suggests that clean and fouled patches are each grazed down to heights at which the lamina:sheath ratios in the upper layer are identical, resulting in higher stubble heights for fouled patches.

In order to predict bovine N-efficiency from herbage quality, and identify grassland management tools that optimise this efficiency, an intake quality model is required that takes account of dung and urine patches. There is a large variety of intake models for grazing ruminants (e.g. Hutchings & Gordon, 2001; Baumont *et al*., 2004; Brereton *et al.,* 2005), most of which simulate the total intake quantity during the defoliation of progressive sward layers down to a minimum sward height, i.e. the stubble layer. Most of these models take account of vertical heterogeneity, but few include explicit descriptions of clean and fouled patches, with the exception of the model by Brereton *et al*. (2005).

In this study, we simplified the approach of Brereton *et al.* (2005), to predict the total daily proportion of lamina and sheath material of strip-grazing bovines. We connected this simplified intake model to the empirical herbage quality model based on data by Hoekstra *et al*. (2007b), to create an intake quality model. The objective of this study was to assess the potential efficacy of individual grassland management tools in manipulating the WSC and N concentration of the herbage intake of strip-grazing bovines throughout the growing season. The grassland management tools assessed were ryegrass cultivar, N application rate, rotation length and defoliation height.

In this chapter, the intake quality model is outlined first, and its input variables calibrated using a controlled, simulated grazing experiment, after which model predictions are verified using grazing observations from four experimental farmlets. Subsequently, the efficacy of combinations of grassland management tools in manipulating N and WSC intake by grazing bovines is evaluated in scenario analyses.

2. Model description

2.1 Model overview

An overview of the model is presented in Fig. 1 (for abbreviations see Appendix A).

The objective of the model is to predict the WSC, N and neutral detergent fibre (NDF) concentration of perennial ryegrass intake during grazing as a function of 1) herbage morphology, specifically the relative proportion of lamina, sheath and inflorescence material in the sward and their maximum heights, and 2) chemical composition of lamina, sheath and inflorescence material as a function of grassland management tools, including perennial ryegrass cultivar (high- versus low-sugar perennial ryegrass), fertiliser N application rate, rotation length and defoliation height.

The model distinguishes two patch types: clean patches and patches affected by dung and urine ("fouled patches"). The model simulates three discrete periods of the growing season (early, mid and late season). It is assumed that all the herbage above the stubble height is ingested by the grazing animal. This simplification limits the model applicability to a strip-grazing situation.

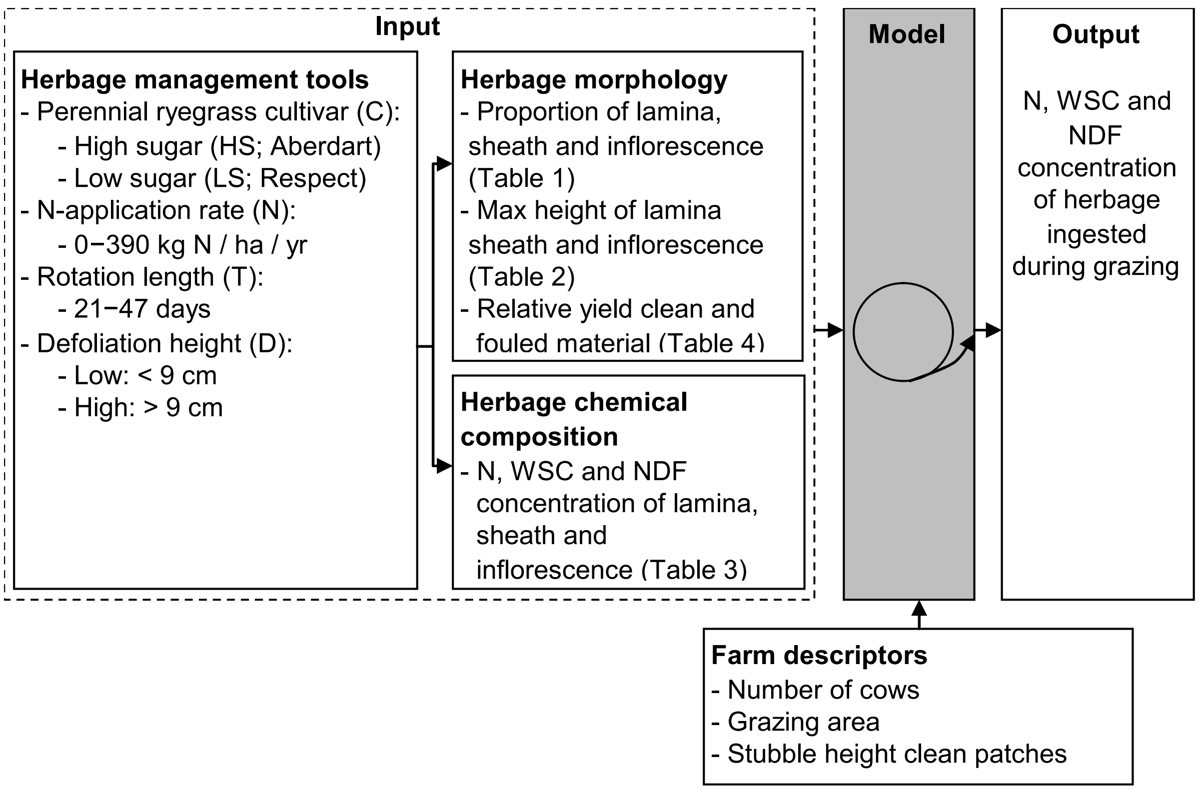

Figure 1 Overview of model structure.

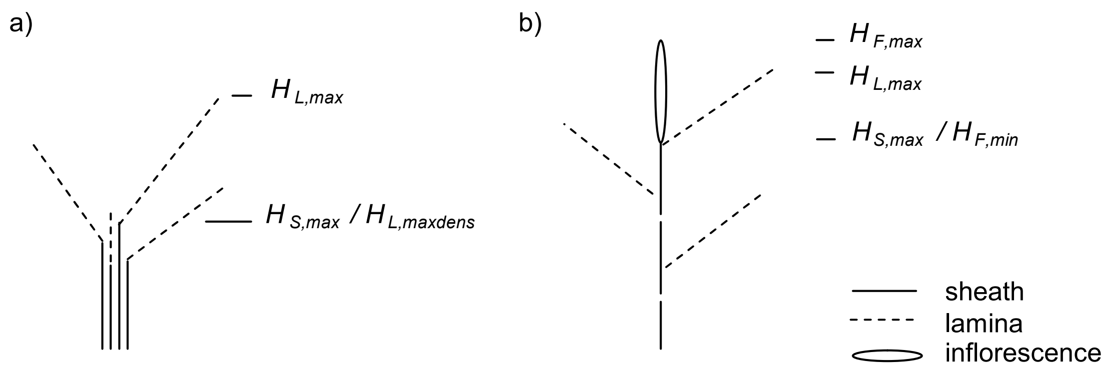

Figure 2 Schematic representation of the structure of a grass tiller during vegetative (a) and generative (b) regrowth. For abbreviations see text and Appendix A.

2.1.1 Sward profile

It is assumed that the sward consists of three fractions (plant organs): lamina, sheath and inflorescence (the latter only during mid season) material. The proportions of lamina (L_{Sward}), sheath (S_{Sward}) and inflorescence (F_{Sward}) in the sward are determined by the plant morphological development which is affected by both season and grassland management (Table 1, as discussed below).

The vertical distribution of these herbage fractions is based on a generic representation of plant morphology as visualised in Fig. 2.

The vertical distribution of the relative lamina density can be represented by a triangular function with total surface area of one (Fig. 3a) (Lantinga *et al*., 1999b). The proportion of the lamina above the stubble (L_{Intake}) (no dimensions) is equal to the surface of the marked area in Fig. 3a, which can be calculated by integration of the relative lamina density from H_{St} to $H_{L,max}$. When the stubble height exceeds the height of the maximum lamina density, this equates to:

$$L_{Intake} = 0.5 \cdot D_{L,\max} \cdot \frac{\left(H_{L,\max} - H_{St}\right)^2}{\left(H_{L,\max} - H_{L,\max dens}\right)}; H_{St} > H_{L,maxdens} \qquad (1a)$$

When the stubble height is lower than the height of the maximum lamina density, the equation is:

$$L_{Intake} = 0.5 \cdot D_{L,\max} \cdot \left(H_{L,\max} - \frac{H_{St}^{\ 2}}{H_{L,\max dens}}\right); 0 \leq H_{St} \leq H_{L,maxdens} \qquad (1b)$$

where

$H_{L,max}$ = the maximum lamina height (mm) (input variable, Table 2)

$H_{L,maxdens}$ = the height (mm) of maximum relative lamina density which is assumed to be equal to the maximum sheath height (mm) (Table 2) (Schulte &

Lantinga, 2002).

H_{St} = the stubble height (mm) (input variable)

$D_{L,max}$ = the maximum relative lamina density (mm^{-1}), which can be calculated from (as deducted from Fig. 3a, assuming a triangle with height $D_{L,max}$, base $H_{L,max}$ and triangle surface area of 1):

$$D_{L,\max} = \frac{2}{H_{L,\max}} \qquad (2)$$

Similarly, the vertical distribution of the relative sheath density is approximated by a triangle (Fig. 3b), where the relative sheath density is highest at ground level and decreases linearly with sward height. The proportion of the sheath above the stubble (S_{Intake}) (no dimensions) is calculated by computing the surface of the marked triangle, which results in:

$$S_{Intake} = \frac{(H_{S,\max} - H_{St})}{H_{S,\max}} \cdot \left(1 - \frac{H_{St}}{H_{S,\max}}\right);\ 0 \le H_{St} < H_{S,max} \qquad (3)$$

Where $H_{S,max}$ is the maximum sheath height (mm) (input variable, Table 2).

The relative density distribution of the inflorescence material is simulated analogue to that of the laminae, with this difference that the triangle starts at the minimum inflorescence height, $H_{F,min}$ (mm, Table 2). Again, the proportion of inflorescence above the stubble (F_{Intake}) (no dimensions) is calculated by computing the surface of the marked area. For stubble heights between the height of maximum relative inflorescence density and the maximum inflorescence height, F_{Intake} is:

$$F_{Intake} = 0.5 \cdot D_{F,\max} \cdot \frac{(H_{F,\max} - H_{St})^2}{(H_{F,\max} - H_{F,\max dens})};H_{F,maxdens}<H_{St}<H_{F,max} \qquad (4a)$$

For stubble heights between the minimum inflorescence height and the height of maximum relative inflorescence density, F_{Intake} is:

$$F_{Intake} = 0.5 \cdot D_{F,\max} \cdot \left((H_{F,\max} - H_{F,\min}) - \frac{(H_{St} - H_{F,\min})^2}{(H_{F,\max dens} - H_{F,\min})} \right);$$

$$H_{F,min}<H_{St} \le H_{F,maxdens} \qquad (4b)$$

When the stubble height is lower than the minimum inflorescence height all the inflorescence material is above the stubble:

$$F_{Intake} = 1 \,;\, H_{St} < H_{F,min} \tag{4c}$$

where

$H_{F,max}$ = the maximum inflorescence height (mm) (input variable, Table 2)

$H_{F,min}$ = the minimum inflorescence height (mm) (input variable, Table 2)

$H_{F,maxdens}$ = the height of maximum relative inflorescence density (mm) which is assumed to be equal to the average of $H_{F,max}$ and $H_{F,min}$

$D_{F,max}$ = the maximum relative inflorescence density (mm^{-1}) which can be calculated from:

$$D_{F,\max} = \frac{2}{\left(H_{F,\max} - H_{F,\min}\right)} \tag{5}$$

The proportion of total sward material above the stubble (T_{Intake}) (no dimensions) is computed as:

$$T_{Intake} = \left(L_{Intake} \cdot L_{Sward}\right) + \left(S_{Intake} \cdot S_{Sward}\right) + \left(F_{Intake} \cdot F_{Sward}\right) \tag{6}$$

Subsequently, the proportion of the intake consisting of lamina material (I_L) is calculated as:

$$I_L = \frac{L_{Intake} \cdot L_{Sward}}{T_{Intake}} \tag{7}$$

Similar equations apply for the proportion of intake consisting of sheath (I_S) and inflorescence (I_F) material.

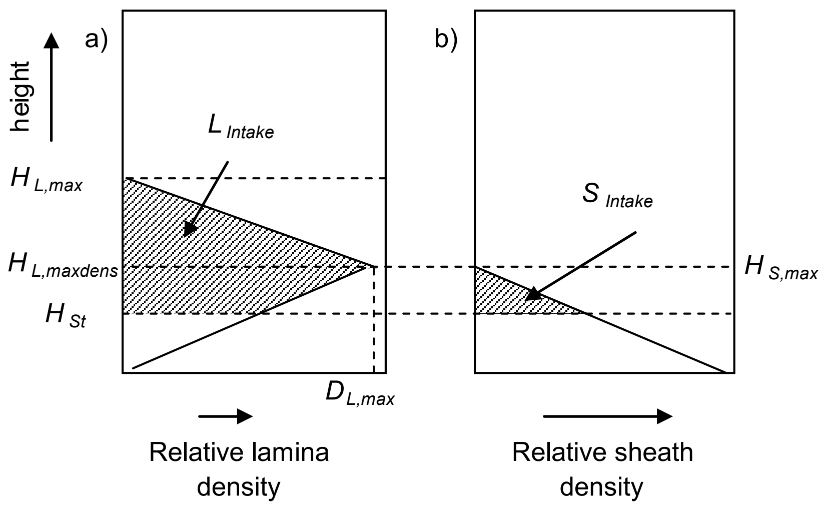

Figure 3 Modelled vertical distribution of the relative lamina (a) and sheath (b) density. For equations and abbreviations see text and Appendix A.

Table 1

Summarising data and regression equations describing the effect of grassland management on the proportions of sheath (S_{Sward}), lamina (L_{Sward}) material in the sward during early, mid and late season (n = 108).

Season	Regression equation[a]		R^{2b}	RMSE	Min	Max	Mean
Early	S_{Sward} =	$0.24 - 0.06D + 0.004T + 0.0001DN + 0.002DT$	0.79	0.034	0.27	0.57	0.39
Mid	S_{Sward} =	$0.63 - 0.003T - 0.001N - 0.06C + 0.07D + 0.0003CN + 0.0002TN$	0.70	0.043	0.35	0.69	0.51
	L_{Sward} =	$0.35 + 0.001N + 0.12C - 0.10D - 0.001CN - 0.00002TN$	0.75	0.058	0.13	0.64	0.41
Late	S_{Sward} =	$0.27 + 0.03C + 0.02CD + 0.00000TN$	0.60	0.022	0.22	0.37	0.30

[a] C = Cultivar (1 = high sugar, 0 = low sugar), N = N application rate (kg / ha / yr), T = Rotation length (days) and D = Defoliation height (1 = high, 0 = low)
[b] All R^2 values are significant at $p < 0.0001$.
Early and late season: $L_{Sward} = 1 - S_{Sward}$; Mid season: $F_{Sward} = 1 - (S_{Sward} + L_{Sward})$

Table 2

Summary of data and regression equations describing the effect of grassland management on the maximum height (mm) of lamina ($H_{L,max}$), sheath ($H_{S,max}$) and inflorescence ($H_{F,max}$) material and the minimum height of inflorescence material ($H_{F,min}$) during early, mid and late season (n = 108).

Season		Regression equation[a]	$R^{2\,b}$	RMSE	Min	Max	Mean
Early	$H_{L,max}$ =	$66.3 + 2.52T + 1.43DT + 0.005TN$	0.83	26.8	103	400	198
	$H_{S,max}$ =	$0.88 + 1.062T + 0.64DT + 0.002TN$	0.66	18.4	23	184	57
Mid	$H_{L,max}$ =	$160.6 + 3.17T + 61.8D + 0.24CN + 0.008TN$	0.74	58.5	160	657	351
	$H_{S,max}$ =	$95.6 + 3.68T + 57.4D + 0.22CN + 0.004TN$	0.64	56.2	115	521	271
	$H_{F,max}$ =	$-97.3 + 13.3T + 0.32CN + 3.4DT$	0.84	81.9	84	808	443
	$H_{F,min}$ =	$41.9 + 2.57T + 1.23CT + 0.12DN$	0.57	41.5	58	330	151
Late	$H_{L,max}$ =	$125.2 + 0.94T + 0.26N + 0.066DN$	0.88	19.4	120	355	205
	$H_{S,max}$ =	$11.6 + 0.30T + 3.76C + 4.44D + 0.081N - 0.023CN$	0.86	5.0	17	79	37

[a] C = Cultivar (1 = high sugar, 0 = low sugar), N = N application rate (kg / ha / yr), T = Rotation length (days) and D = Defoliation height (1 = high, 0 = low)
[b] All R^2 values are significant at $p < 0.0001$

2.1.2 Herbage chemical composition

The N, WSC and NDF concentration of the intake is calculated by multiplying the N, WSC and NDF concentration of the lamina, sheath and inflorescence material with their respective proportions in the intake. For example, the WSC concentration of the intake is calculated as:

$$WSC_{Intake} = \left(I_L \cdot WSC_L\right) + \left(I_S \cdot WSC_S\right) + \left(I_F \cdot WSC_F\right) \quad (8)$$

where

WSC_{Intake} = the WSC concentration in the intake (g / kg DM)

WSC_L, WSC_S and WSC_F = the WSC concentrations (g / kg DM) of the lamina, sheath and inflorescence material, respectively (input variable, Table 3).

Similar equations apply for the N and NDF concentration of the intake.

2.1.3 Dung and urine patches

The grazing area affected by dung and urine patches during each period is calculated as the product of the number of cows, the number of excretions per day, the area affected per excretion and the cumulative number of days spent on the grazing area.

Cows tend to urinate and to defecate on average twelve times per day (for dairy cows it is assumed that about 10 urinations and defecations per day are made onto the pasture). The area of grass affected per excretion (dung and urine combined) has been estimated as 0.93 m^2 (0.68 m^2 for urine + 0.25 m^2 for dung) (Lantinga *et al.*, 1987). The fraction of the grazing area affected by excreta is calculated by dividing the total excretion area by the total grazing area. The Poisson distribution is used to correct this fraction of the area affected for overlap, assuming that the distribution of excreta was not concentrated in specific areas (Haynes & Williams, 1993).

Lantinga *et al.* (1987) estimated that the average rate of N applied to an area affected by a urination or defecation is 500 kg N / ha and 2000 kg N / ha, respectively (at a daily intake of 16 kg herbage DM with an N concentration of 40 g / kg DM). However, the N that is actually available for plant growth depends on weather and soil conditions and modelling the processes involved is beyond the scope of this study. Therefore, the effect of the deposition of dung and urine patches on the sward structure and herbage chemical composition is simulated by assuming that the total available N load on these patches is 390 kg N / ha / yr, above which rate no agronomic response is expected (Coulter, 2004).

In this model, the stubble height for clean patches is an input variable. The stubble height for the fouled patches is then calculated based on the concept that fouled patches are grazed down to heights at which the lamina:sheath ratio in the upper layer is identical to the clean fraction (Garcia *et al.*, 2003; Brereton *et al.*, 2005).

Table 3

Regression equations describing the effect of grassland management on the WSC, N and NDF concentration (g / kg DM) of lamina and sheath material during early mid and late season (n = 36).

		Regression equation [a, b]	R^2	RMSE	Min	Max	Mean
Lamina							
WSC	Early	145.0 − 0.28N + 3.5T + 108.7D − 3.5TD	0.84	26.2	74	304	212
	Mid	139.5 − 0.24N + 2.39T + 72.2C − 0.14NC	0.88	25.8	74	322	207
	Late	111.6 − 0.09N + 0.92T + 79.4C − 0.11NC	0.88	16.8	90	237	159
Total N	Early	32.7 + 0.06N − 0.34T − 9.1D − 0.001NT + 0.25TD	0.95	1.68	17.4	46.1	25.9
	Mid	24.5 + 0.08N − 0.20T − 0.001NT	0.92	2.34	14.9	44.1	22.4
	Late	21.2 + 0.05N − 0.001NT	0.90	1.51	20.2	36.4	25.2
NDF	Early	415.1 + 0.11N	0.53	17.6	391	492	432
	Mid	458.1 − 38.7C + 0.002NT + 0.09NC + 0.37TD	0.76	14.7	394	525	460
	Late	505.8 − 38.0C	0.65	14.4	453	546	486
Sheath							
WSC	Early	211.7 − 0.22N + 5.36T + 134.7D − 3.67TD	0.83	28.3	173	438	360
	Mid	260.8 − 0.42N + 49.1C + 0.01NT	0.80	20.0	149	340	272
	Late	268.3 − 0.32N + 2.61T + 54.9C + 0.007NT	0.88	20.7	244	473	373
Total N	Early	13.6 + 0.04N − 0.10T − 3.49D − 0.0007NT + 0.095TD	0.94	1.03	8.6	23.8	12.5
	Mid	12.3 + 0.04N − 0.14T − 0.0008NT	0.93	1.28	5.5	23.3	9.9
	Late	9.0 + 0.01N	0.85	1.02	8.4	16.8	11.3
NDF	Early	458.1 + 0.09N − 1.23T + 0.30TD	0.50	19.4	396	515	437
	Mid	513.9 + 1.37T − 47.0C + 13.4D − 0.003NT + 0.11NC	0.81	12.2	495	601	538
	Late	561.5 − 2.76T − 34.4C	0.85	14.7	385	520	449

[a] C = Cultivar (1 = high sugar, 0 = low sugar), N = N application rate (kg / ha / yr), T = Rotation length (days) and D = Defoliation height (1 = high defoliation, 0 = low defoliation)
[b] All R^2 values are significant at $p < 0.0001$

In order to compute the chemical composition of the combined intake of clean and fouled material, the chemical composition of the clean and fouled intake is multiplied by the weighted average of intake from clean and fouled patches. This weighted average is defined by the proportion of the area that is fouled, the relative proportion of clean and fouled material above the stubble and the relative yields of clean and fouled material (no units) (input variable, Table 4 as discussed below).

2.1.4 Periods of the growing season

The model sequentially simulates three discrete periods of the growing season: early

(April/May), mid (June, generative regrowth) and late (August/September) season. The three periods are connected through their stubble heights: the initial sward height or defoliation height (D) of period "t" is equal to the post-grazing stubble height (H_{St}) of the previous period "t−1". If the stubble height (t−1) is higher than 90 mm, the defoliation height (t) is "high", if it is lower than 90 mm, the defoliation height is "low".

3. Determination of input variables

The input variables for the model were based on a cutting experiment designed to assess the effect of grassland management on the morphology and physiology of perennial ryegrass. The experiment was carried out at Johnstown Castle Research Centre, Wexford, Ireland (latitude 52º N, longitude 6º W) during 2003.

3.1 Materials and methods

3.1.1 Experimental design

The experiment with plots of 1.5 m × 2 m comprised all combinations of two perennial ryegrass (*Lolium perenne* L.) cultivars (high and low sugar content), two initial cutting heights (approximately 8 and 12 cm), three regrowth periods (approximately 2, 4, and 6 weeks) and three fertiliser N application rates (0, 90 and 390 kg N / ha / yr applied in 7 equal splits, one before each regrowth). The experiment was laid out in three replications and measurements were made April/May, June (generative regrowth) and August/September 2003. The two *Lolium perenne* L. cultivars were Aberdart (high-sugar content) and Respect (normal to low sugar content), both diploids with similar heading dates (27th and 23rd of May, respectively) (Anon., 2001; Gilliland *et al.*, 2002). For a more detailed description see Hoekstra *et al*. (2007b).

3.1.2 Measurements

One day before each harvest, 40 tillers per plot were cut off at soil surface. Whole plants were selected, to give an equitable representation of both small and large tillers. The lengths of the sheaths and leaves of all green leaves of each of the 40 tillers were measured.

At each harvest, grass samples of c. 250 g were taken, by cutting the grass plants at 1 cm above ground level using electric sheers (Wolf-Garten, Accu 75 Professional). They were manually divided into sheath (sheath, stem and new leaves within the sheath tube), lamina, inflorescence and dead material (defined as leaves and sheaths of which >50% of surface was dead) and the fresh weight of each fraction was recorded. The separated fractions of sheaths, laminae and inflorescences were freeze-dried and weighed to determine the dry matter content, before being ground to pass a 1 mm sieve. The three replicates of these fractions were bulked and analysed for NDF, WSC and N. For details on the methods see Hoekstra *et al*. (2007b).

After sampling, the plots were mown at 1 cm above soil surface using a lawnmower, the grass was weighed and a sample oven-dried for dry matter determination. Total yield was determined as the sum of the lawnmower yield and the weight of the sample.

3.1.3 Statistical analyses

The data were analysed through SAS Enterprise Guide v. 8.2 using the general linear model backward elimination procedure (Mead *et al*., 1998). The full model contained all the main variables (N application rate (N), regrowth period (T), cultivar (C) and defoliation height (D)) and their two-way interactions. N and T were quantitative variables, whereas C and D were nominal variables with two levels. Variables were eliminated from the full model (starting with variable with highest value for p) until all the remaining variables were significant at $p < 0.01$. Regression analyses were done separately for the three periods and for lamina and sheath material.

3.2 Results

3.2.1 Proportion of herbage fractions in the sward

The proportion of lamina, sheath and inflorescence material in the sward was calculated from the dry matter weights of the separated organs. The regression equations describing the effect of grassland management on the proportion of lamina (L_{Sward}), sheath (S_{Sward}), and inflorescence (F_{Sward}) material in the whole sward during three periods are presented in Table 1.

3.2.2 Maximum and minimum height of lamina, sheath and inflorescence material

The maximum heights of lamina, sheath and inflorescence material were based on the length of the longest lamina, sheath and inflorescence (measured from soil surface) of each of 40 tillers per replicate. Of these 40 lengths, the 95 percentile was taken in order to obtain the maximum length while excluding any outliers. In order to convert lamina length to lamina height, the lamina length was multiplied by factor 0.91 to correct for the leaf angle (Schulte & Lantinga, 2002, their Table 1). The minimum height of inflorescence material was assumed to be equal to the 5 percentile of the maximum sheath length. The linear regression equations predicting the effect of grassland management on maximum and minimum heights are presented in Table 2.

Table 4

Summary of data and regression equations describing the effect of grassland management on the relative yield (kg / kg) of clean compared to fouled patches during early, mid and late season.

Season	Regression equation[a]	R^{2b}	RMSE	Min	Max	Mean
Defoliation height clean = defoliation height fouled (n = 36)						
Early	1 − 0.0068T + 0.000017NT	0.83	0.082	0.58	1.00	0.87
Mid	0.74 + 0.0007N − 0.0055T + 0.00001TN	0.97	0.069	0.38	1.00	0.74
Late	0.67 + 0.00085N	0.95	0.056	0.56	0.86	0.71
Defoliation height clean = low; fouled = high (n = 18)						
Early	0.56 + 0.00001TN	0.61	0.072	0.41	0.83	0.63
Mid	0.40 + 0.0009N	0.86	0.065	0.32	0.84	0.55
Late	0.59 + 0.0008N	0.87	0.054	0.51	0.92	0.71

[a] N = N application rate (kg / ha / yr) and T = Rotation length (days)
[b] All R^2 values are significant at $p < 0.0001$.

3.2.3 Relative yield of clean and fouled patches

The relative yield of the clean patches compared to the fouled patches was calculated based on the yields from the cutting experiment (data not shown) and separate calculations/equations were presented for two occasions, depending on their initial sward heights (defoliation height):

a) When the defoliation height (t−1) of the clean material was equal to the defoliation height of fouled material, the relative yield was calculated by dividing the yields at N application rates of 0 and 90 kg N / ha / yr, by the yields at the 390 kg N / ha / yr application rate at similar grassland management treatments.
b) In some cases the defoliation height (t−1) of the clean fraction may be low (<9 cm), and the defoliation height of the fouled fraction high. In this case, the relative yield was calculated by dividing the yields at low cutting height and 0, 90 and 390 kg N / ha / yr by the yields at high cutting height at 390 kg N / ha / yr.

The calculated relative yields were subjected to multiple regression analyses and the resulting regression equations are presented in Table 4.

3.2.4 N, WSC and NDF concentration of lamina, sheath and inflorescence material

The regression equations describing the effect of grassland management on the N, WSC and NDF concentration of lamina and sheath material during the three periods are presented in Table 3 (data presented and discussed in Hoekstra *et al.* (2007b)).

For the inflorescence material average values of 17.3, 589.4 and 137.0 g / kg DM were taken for the N, NDF and WSC concentration, respectively (Hoekstra *et al*., 2007b, their Table 4).

4. Model validation

The model was subjected to a sensitivity analysis in which the effects of changes in input variables (maximum lamina and sheath length, the proportion of lamina in the sward, the relative yield and the absolute level of N, WSC or NDF in the lamina or sheath material (intercept in regression equations, Table 3)) on changes in chemical composition (N, WSC and NDF concentration) of the intake were calculated.

A model validation was carried out, to establish the model's ability to assess the potential efficacy of individual grassland management tools in manipulating the WSC and N concentration of the herbage intake of strip-grazing bovines throughout the growing season.

For the validation, the model was run with the inputs from a purpose designed validation experiment (see below). These included the number of animals, the grazing area, herbage cultivar, nitrogen application rate, rotation length and post-grazing stubble heights of the clean patches (Table 5). The correlation between the predicted and observed WSC, NDF and N concentration (g / kg DM) in the intake was determined. If the slope was significantly ($p < 0.05$) different from one (corresponding to a significant bias of the residuals) or the mean residual was significantly ($p < 0.05$) different from zero, this indicated a relative or absolute bias, respectively.

4.1 Materials and methods validation experiment

4.1.1 System design

During the grazing season of 2004 a total of four grassland management systems consisting of 15 animals each, were applied on the beef and dairy farms of Johnstown Castle, Teagasc, Ireland. On each farm, one system was aimed at obtaining optimal bovine N efficiency through a combination of high-sugar grass, lower N application rate and longer rotation length, whereas the other system was a control, that is, managed "as usual" (Table 5). On both the dairy and the beef farms, in September 2002, an area of approximately 6.5 ha was ploughed and reseeded with *Lolium perenne*, cv. Aberdart. During 2003, the fields were grazed in order to establish a good grazed sward. P and K fertilisation was applied as required per soil test, and the N-fertiliser application rate was 200 kg N / ha / yr on both farms. The control fields were older swards which had been managed for grazing in previous years. The botanical composition of the paddocks as determined by the dry-weight-rank method ('t Mannetje & Haydock, 1963) with yield correction (Jones & Hargreaves, 1979) is presented in Table 5.

Table 5
Validation experiment: Weather data, botanical composition and grassland management for the dairy and beef high-sugar (HS, managed for optimum bovine N efficiency) and low-sugar (LS, managed as usual) systems during early, mid and late season.

	Dairy HS			Dairy LS			Beef HS			Beef LS
	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Late
Average weather data										
Max temperature (°C)	13.3	18.2	19.4							
Min temperature (°C)	6.0	9.7	12.9							
Rain (mm/day)	1.8	0.9	3.5							
Botanical composition										
% *Lolium perenne*		97			78			88		65
% *Agrostis stolonifera*					14					29
% *Trifolium repens*					2			6		
% Other		3			6			6		6
Grassland management										
Date	02-05	12-06	04-09	02-05	12-06	04-09	08-05	20-06	11-09	11-09
Total area (ha)	6.2			5.4			4.4			4.9
Total grazing area (ha)	3.7	4.8	6.2	4.2	4.8	5.4	2.2	3.0	4.4	4.9
N application rate (kg / rotation)	30	25	20	40	40	30	30	20	20	30
Rotation length (days)	30	27	30	28	30	27	29	25	30	21
Defoliation heights clean (mm)	67	82	76	76	83	66	78	77	68	64

The systems were closely monitored during three periods of 5 consecutive days each in the year 2004, i.e. during early, mid and late season (only during late season for the beef control system, Table 5). The grazing regime was strip grazing; during the monitoring weeks, this was a strict one-daily strip-grazing regime, whereas it was less strict throughout the remaining grazing season.

4.1.2 Measurements

During the monitoring periods, every morning the pre- and post-grazing herbage masses were determined using the double sampling method (Davies *et al*., 1993), which entails two steps:

1. Establishing the relation between herbage height and herbage dry matter mass: Before each monitoring period, the heights of 30 patches per system, ranging from low to high sward height, were measured using a plate meter (430 g; 10 dm^2), after which these patches were harvested with a lawnmower at approximately 5 cm above ground level for DM weight determination. Subsequently, linear regression

equations of DM weight against sward height were derived for each farm and each period.

2. During the monitoring periods, the average height of 50 clean and 50 fouled patches were measured both pre- and post-grazing for each of the five strips, using a plate meter. The herbage masses of the clean and fouled area were calculated by using the regressions derived above.

During the monitoring period, herbage samples were cut at soil surface level from both fouled and clean patches both pre- and post-grazing on each strip. Part of the pre-grazing samples of day 2 and day 4 were stored in the freezer for morphological analyses, which entailed measuring maximum leaf, sheath and inflorescence length of 40 tillers per treatment. The remainder of each pre-grazing sample was immediately stored at 4 ˚C and was separated into pseudo-stem, lamina, dead material and inflorescence (mid season only) within 8 hours after sampling. The dissected fractions were stored in the freezer, before freeze-drying and grinding through a 1 mm sieve. The fractions were bulked over the five days and DM, N-total, NDF and WSC concentrations were determined as described in Hoekstra *et al*. (2007b). The post-grazing samples were immediately stored in the freezer and were separated into fractions, before oven-drying at 100 ˚C overnight for DM analyses.

4.1.3 Calculations

The proportion of lamina, sheath and inflorescence in the sward was calculated from the DM weight of the separated fractions for both pre- and post-grazing swards. The maximum lamina, sheath and inflorescence heights were based on the leaf length measurements and calculations were as described before.

The proportion lamina in the intake (I_L) was calculated as follows:

$$I_L = \frac{\left(Y_{pre} \cdot L_{Sward,pre}\right) - \left(Y_{post} \cdot L_{Sward,post}\right)}{(Y_{pre} - Y_{post})} \qquad (10)$$

where Y_{pre} and Y_{post} are the total dry matter yield above ground level (kg / ha) pre- and post-grazing calculated using the regression equations of DM weight against sward height. $L_{Sward,pre}$ and $L_{Sward,post}$ are the proportion of lamina material in the sward pre- and post-grazing, respectively.

Similar equations apply for the proportion of sheath and inflorescence in intake (I_S and I_F, respectively).

In cases where the amount of sheath pre-grazing was slightly smaller than post-grazing, (resulting in negative values for I_S), I_S was assumed to be zero and the other fractions were recalculated accordingly.

The chemical composition of the clean and fouled intake was calculated by multiplying the chemical composition of lamina, sheath and inflorescence material of the clean and fouled area with their proportions in the intake. By multiplying the chemical composition of the clean and fouled material with their relative proportion in the total intake, the chemical composition of the total intake was calculated.

4.2 Results and discussion

4.2.1 Sensitivity analysis

When the maximum lamina height ($H_{L,max}$) was increased or decreased by 50%, this had little or no effect on the chemical composition of the intake (less than 1%). However, a similar change in maximum sheath height ($H_{S,max}$) resulted in a change of up to 10%. This can be explained from the fact that lamina always forms a large proportion of the intake, but the proportion of sheath in the intake depends very much on the difference between stubble height and $H_{S,max}$.

The model was relatively insensitive to changes the proportion of lamina (L_{Sward}) in the sward (which automatically changes S_{Sward}) and the relative yield of clean and fouled patches. A 50% change in these input variables resulted in a maximum change in chemical composition of 20% and 10%, respectively. The model was very sensitive to changes in the absolute concentration of N in sheath and especially lamina material, and a 50% change in both resulted in a change of maximum 50% in chemical composition of the intake.

4.2.2 Model evaluation

When the model was run with the predicted maximum leaf heights, no significant correlation was found between the predicted and observed values of the WSC, N and NDF concentration in the intake. This was largely due to the difference in maximum

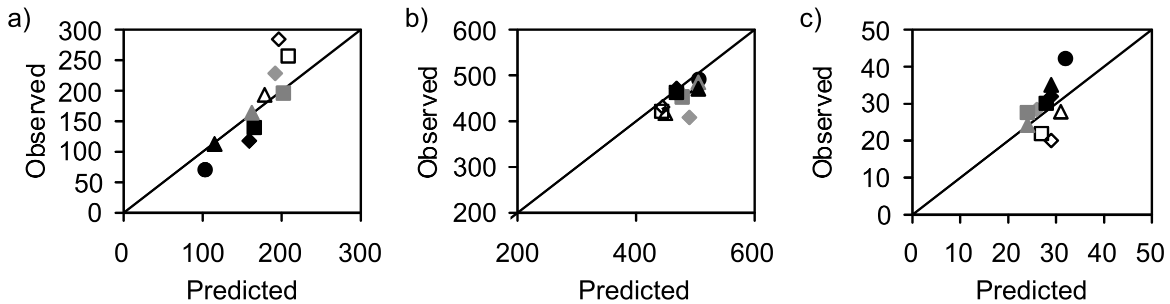

Figure 4 Correlation between model prediction and observed values of the a) WSC concentration ($R^2 = 0.78$; $p < 0.001$; RMSE = 34), b) NDF concentration ($R^2 = 0.49$; $p < 0.05$; RMSE = 24) and c) N concentration ($R^2 = 0.28$; p = NS; RMSE = 6) in the total intake for the four systems (Beef HS ◆; Dairy HS ■; Beef LS ●; Dairy LS▲) during early (open symbols), mid (grey symbols) and late (solid symbols) season.

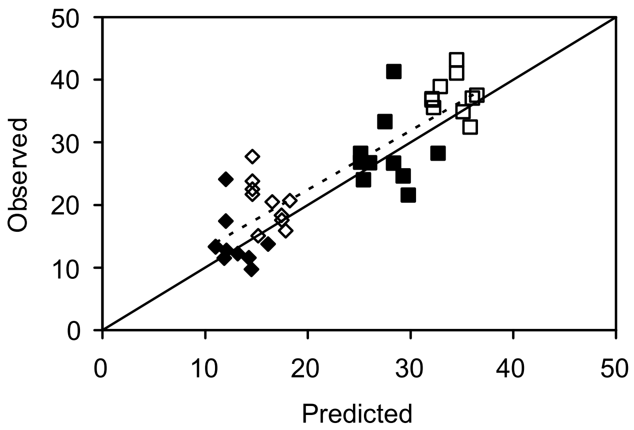

Figure 5 Correlation between model prediction and observed values of the N concentration (g / kg DM) in lamina (■) and sheath (◆) material for clean (closed symbols) and fouled (open symbols) material (R^2 = 0.75; $p < 0.0001$, RMSE = 5).

leaf heights during the cutting experiment and the validation experiment, as the maximum lamina and especially sheath heights measured in the validation experiment were out of phase with the heights from the cutting plot experiment (data not shown). Therefore, the model validation was performed with the observed maximum leaf heights, instead.

In this case, the correlation between the predicted and observed WSC concentration (g / kg DM) in the intake was good (R^2 = 0.78; $p < 0.001$, Fig. 4a), with no significant relative or absolute bias. The correlation between predicted and observed NDF concentration (g / kg DM) was reasonable (R^2 = 0.49; $p < 0.05$, Fig. 4b), with no significant ($p < 0.05$) relative bias, and a small absolute bias resulting in a slight overprediction of the NDF concentration in the intake. However, the range in NDF levels in the intake was rather small (c. 420–500 g / kg DM). There was no significant correlation between the predicted and observed N concentration (g / kg DM) in the intake (Fig. 4c). The N concentration in the intake tended to be over-estimated during the early and under-estimated during the late season. The prediction of the percentage of clean material in the total intake was reasonable (R^2 = 0.47; $p <$ 0.05) and could not explain above-mentioned lack of correlation. Therefore, this lack of correlation was expected to be caused by a discrepancy in the prediction of the N concentration of lamina and sheath material.

There was a strong and very significant correlation (R^2 = 0.75; $p < 0.001$; small absolute bias of 2 g / kg DM) between predicted and observed values for the N concentration of lamina and sheath material for individual clean and fouled patches (Fig. 5). This indicated that the differences in N concentration between lamina and sheath were predicted accurately. Additionally, it showed that assumptions with respect to the N concentration of fouled patches were reasonable. However, the prediction of the N concentration within the different organs and clean and fouled fractions was much less accurate. During the early season, the N concentration in lamina and sheath material tended to be over-estimated, whereas during the late season, the model under-estimated the N concentration. Especially, the N concentration of the LS beef system during the late season was higher than expected, which may have been partly due to the fact that the interval between fertilisation and grazing on this sward was relatively short (less than 2 weeks).

Generally, the herbage N concentration is highly site specific and season specific. Therefore, it is not surprising that the herbage N concentrations of the validation experiment were different from the cutting experiment. Soil and weather conditions are not part of the model and therefore the model cannot account for variation due to these factors. However, the difference between lamina and sheath material and clean and fouled patches was predicted satisfactorily.

The absolute level of N concentration during individual seasons is directly affected by the intercept of the regression equations (Table 3) as shown in the sensitivity analysis. However, changing these intercepts does not change the effect of the grassland management tools on the N concentration of the intake. Therefore, even though the model tended to overpredict the N concentration during early season and to underpredict the N concentration during late season, this only affected the absolute level of N during those seasons, but not the ability of the model to predict the efficacy of the grassland management tools for manipulating the N and WSC concentration.

5. Model application: The efficacy of grassland management tools for manipulating N and WSC concentration in the intake

The model was applied with the objective of assessing the efficacy of grassland management tools on manipulating the N and WSC concentration and the WSC:N ratio of the intake, which are directly related to bovine N utilisation. For the model application runs, the farm descriptors (grazing area, number of cows and dates) from the Dairy HS system in the validation experiment were used (Table 5). All other input variables were based on the cutting experiment (Tables 1−4).

In Fig. 6, the modelled effects of N application rate, rotation length and herbage cultivar on the N and WSC concentration (g / kg DM) and WSC:N-ratio of the intake of clean and fouled patches combined during three periods are presented.

5.1 Rotation length

Rotation length was shown to be an important tool for improving the herbage quality with respect to bovine N utilisation (Fig. 6). The N concentration of the intake was decreased and the WSC concentration was increased with increasing rotation lengths, which is in line with other studies (Davies, 1965; Wilman *et al.*, 1976b; Fulkerson & Donaghy, 2001). During early and late season, this effect reflected the changes in the N and WSC concentration of lamina material, as the intake during these periods

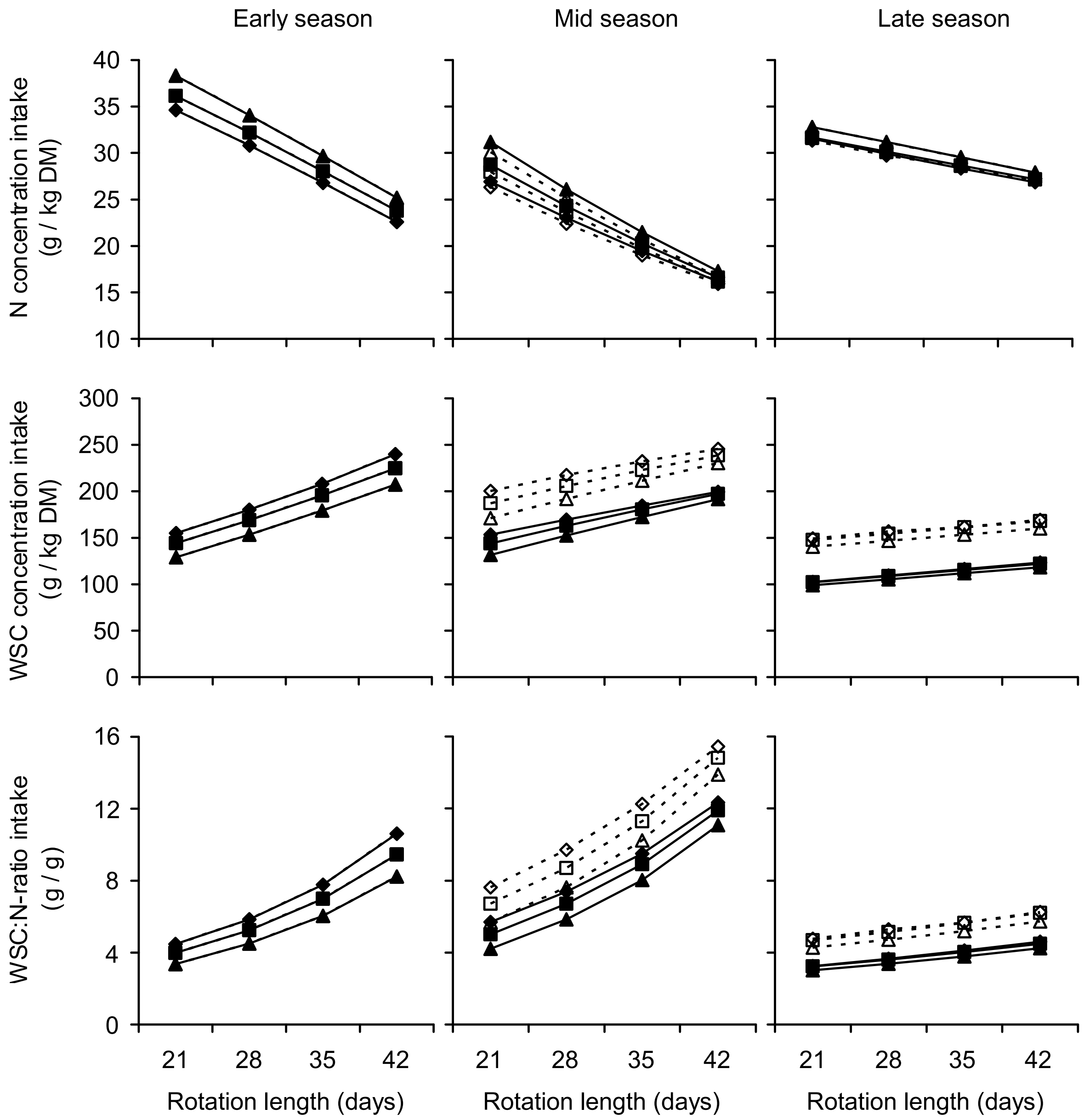

Figure 6 Effect of rotation length (days), N application rate (◆ 0, ■ 200 and ▲300 kg N / ha / yr) and cultivar (HS; open symbols, dashed lines and LS; solid symbols and lines) on the N and WSC concentration (g / kg DM) and the WSC:N-ratio of the herbage intake during early, mid and late season.

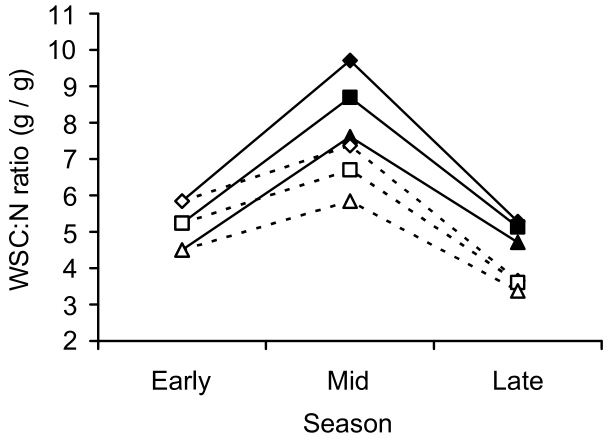

Figure 7 The effect of N application rate (◆0, ■200, ▲300 kg / ha / yr) on the WSC:N-ratio of the intake for two cultivars (LS; open symbols, dashed lines and HS; solid symbols and lines) during early, mid and late season during which periods the proportion of the area affected by dung and urine was 11, 24 and 37%, respectively (T=28 days; low defoliation height; H_{St} = 60 mm).

consisted of lamina only. During mid season, longer rotation lengths resulted in a lower proportion of lamina material in the intake. This impacted on the WSC and N concentration in the intake, as the WSC concentration of lamina material is lower and its N concentration is higher compared to sheath and inflorescence material (Hoekstra *et al*., 2007b).

5.2 N application rate

N application rate had a large impact on both WSC and N concentration during early and mid season, with WSC concentration of the intake decreasing and N concentration increasing with higher N application rates. This is in agreement with other studies (Wilman *et al.*, 1976b; Peyraud & Astigarraga, 1998). However, during the late season, the effect of N application rate was very small (Fig. 7). This could be explained from the fact that with progressing season, the proportion of the grazing area that is affected by dung and urine (A_F) increased from approximately 10% during the early season to 40% during the late season. As A_F increased, the proportion of fouled material in the intake increased as well. This fouled material had a relatively high N concentration and a low WSC concentration and WSC:N-ratio, compared to clean material, due to the N released from the dung and urine. Therefore, during the late season the WSC concentration was actually lower and the N concentration higher than expected on the basis of grassland management alone. Additionally, the differences between clean and fouled patches were larger at low compared to high N application rates: at low N application rates, the effect of the extra N from the dung and urine was relatively stronger than at high N application rates. Therefore, the effect of N application rate on the WSC and N concentrations in the intake became very small towards the end of the growing season. As a result, during the late season, reducing N application rates does not appear to be a very effective tool for increasing bovine N-efficiency. This reduced effect of N application rate was not anticipated based on data from other studies as most studies examining the effect of N application rate are based on cutting experiments rather than grazing experiments, and would therefore not include the effect of urine and dung patches.

5.3 High-sugar cultivar

The effect of the high-sugar cultivar was most pronounced during the late season. This was affected by two factors: 1) the difference in WSC concentration between high and low-sugar cultivars tended to increase with growing season, and 2) the difference was larger for lamina material than for sheath material (Hoekstra *et al*., 2007b). Therefore, during the late season, the difference between high and low-sugar cultivars became even more pronounced as the intake consisted of lamina only. As a result, the high-sugar cultivar was most effective during the period when it is needed most to increase N efficiency. However, as discussed in Hoekstra *et al*. (2007b) contrasting results have been reported by other authors (Radojevic *et al*., 1994; Smith *et al*., 2002)

5.4 Defoliation height

Little information is available on the effect of defoliation height on the chemical composition of the intake during grazing, and results are inconsistent, because of the effect of defoliation height both on the current intake as on the subsequent regrowth (as highlighted by Hoekstra *et al*., 2007a). In the intake quality model these two effects were quantified as follows:

1) The defoliation height at the current grazing (stubble height, t) had a direct impact on the chemical composition of the intake as it influenced the proportion of plant organs in the intake, with lower stubble heights resulting in a higher proportion of sheath in the intake. Therefore, the concentration of WSC was higher and the concentration of N in the intake was lower at lower stubble heights (Fig. 8).

2) The defoliation height of the previous period affected the regrowth of the subsequent period. The effect of defoliation height (t−1) on the chemical composition of lamina and sheath was found to be small and inconsistent (Hoekstra *et al*., 2007b). However, defoliation height had a distinct impact on herbage morphology and subsequent sward structure. High defoliation height generally resulted in an increase in the maximum sheath height (Table 2) and an increase of the proportion of sheath in the sward (Table 1). Therefore, the proportion of sheath material in the intake increased with lower defoliation heights, resulting in higher concentrations of WSC and lower

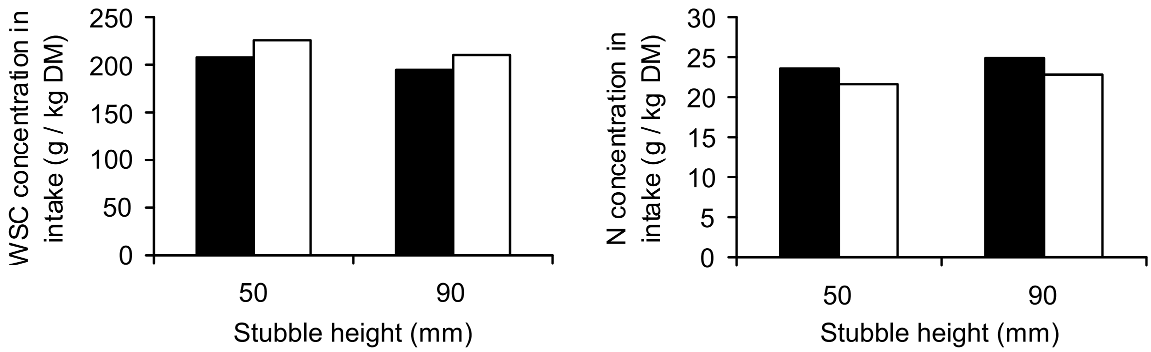

Figure 8. Effect of stubble height (t) (50 and 90 mm) and defoliation height (t−1) (■ low defoliation, < 90 mm; □ high defoliation, > 90 mm) on a) the WSC concentration of the intake and b) the N concentration of the intake (T = 28 days, N = 200 kg N / ha / yr).

concentrations of N in the intake.

As a result, this effect of defoliation height on the subsequent period was opposite to the effect of stubble height at the current period. In fact, when the defoliation height was similar at the start and the end of the grazing period (Stubble height (t) = 50 mm and defoliation height (t−1) = low, or stubble height (t0) = 90 mm and defoliation height (t−1) = high), the two effects of defoliation height tended to negate each other resulting in similar N and WSC concentrations of the intake (Fig. 8). Therefore, the model suggested that defoliation height had little or no effect on the quality of the intake when maintained at a constant height throughout the season.

6. Conclusions

- The model validation showed that the prediction of the WSC concentration in the intake was adequate. The N concentration tended to be under-estimated during late season and over-estimated during early season, which was probably due to differences in site and weather conditions, which are not part of the model. However, this did not affect the ability of the model to assess the efficacy of grassland management for manipulating the N and WSC concentration in the intake of strip-grazing bovines.
- Model application shows that reduced N application rates and longer rotation lengths are important tools for adjusting the diet N and WSC concentration of strip-grazed herbage during early and mid season, which has potential for improving the bovine N efficiency. Towards the end of the season, the large proportion of area affected by dung and urine resulted in a reduced effect of N application rate. This stresses the need to incorporate the effect of dung and urine when modelling the intake quality under grazing.

- In contrast, the relative difference in WSC concentration between high and low-sugar cultivars was largest in the late season. This suggests that high-sugar cultivars may be an important tool to increase bovine N-efficiency during the late season, when the risk of N-losses to the environment is largest.
- The model output showed that defoliation height affects the chemical composition of the intake of both the current and the subsequent period, but the two effects are opposite. When defoliation height is the same at the start and end of the grazing period, the chemical composition of the intake was similar for the low and high defoliation height.

Acknowledgement

N.J. Hoekstra was supported under the Teagasc Walsh Fellowship Scheme.

Appendix A: Model outputs and variables

Symbol	Definition	Unit	Eqn/ Table
Model outputs			
L_{Intake}	Proportion of lamina material above the stubble	-	Eqn 1
S_{Intake}	Proportion of sheath material above the stubble	-	Eqn 3
F_{Intake}	Proportion of inflorescence material above the stubble	-	Eqn 4
T_{Intake}	Proportion of total sward material above the stubble	-	Eqn 6
I_L	Proportion of intake consisting of lamina material	-	Eqn 7
I_S	Proportion of intake consisting of sheath material	-	Eqn 7
I_F	Proportion of intake consisting of inflorescence material	-	Eqn 7
WSC_{Intake}	WSC concentration of intake during grazing	g / kg DM	Eqn 8
N_{Intake}	N concentration of intake during grazing	g / kg DM	Eqn 8
NDF_{Intake}	NDF concentration of intake during grazing	g / kg DM	Eqn 8
Input variables			
L_{Sward}	Proportion of lamina in the sward	-	Table 1
S_{Sward}	Proportion of sheath in the sward	-	Table 1
I_{Sward}	Proportion of inflorescence in the sward	-	Table 1
H_{St}	Stubble height	mm	
$H_{L,max}$	Maximum lamina height	mm	Table 2
$H_{S,max}$	Maximum sheath height	mm	Table 2
$H_{F,max}$	Maximum inflorescence height	mm	Table 2
$H_{F,min}$	Minimum inflorescence height	mm	Table 2
RY	Relative yield	-	Table 4
WSC_L	WSC concentration of the lamina material	g / kg DM	Eqn 8
WSC_S	WSC concentration of the sheath material	g / kg DM	Eqn 8
WSC_F	WSC concentration of the inflorescence material	g / kg DM	Eqn 8
Internal variables			
$D_{L,max}$	Maximum relative lamina density	mm^{-1}	Eqn 2
$D_{F,max}$	Maximum relative inflorescence density	mm^{-1}	Eqn 5
$H_{L,maxdens}$	Height of maximum relative lamina density which is assumed to be equal to the maximum sheath height	mm	Eqn 1
$H_{F,maxdens}$	Height of maximum relative inflorescence density which is assumed to be equal to the average of $H_{F,max}$ and $H_{F,min}$.	mm	Eqn 4

6

Predicting the nitrogen utilisation of grazing dairy cows: model description and validation

N.J. Hoekstra, E.A. Lantinga, P.C. Struik & R.P.O. Schulte, Submitted to Agricultural Systems

Abstract

Nitrogen (N) excreted in dung and urine by grazing cows is associated with losses of N to the environment either as ammonia and N oxides in air, or as nitrate in soil and ground water. Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal. The quality of the grass ingested has a pronounced effect on both production and N utilisation by the animal. N fertiliser application rate, length of the regrowth period and high-sugar cultivars have been shown to be effective tools to manipulate the N or energy contents of grass.

The objective of this study was to develop a tool for predicting efficacy of these herbage management tools for improving the N utilisation of grazing dairy cows by linking the recently developed herbage intake quality (HIQ) model to the Cornell Net Carbohydrate and Protein System (CNCPS) model.

Firstly, the HIQ model was extended to predict the input required by the CNCPS model. Subsequently the combined HIQ-CNCPS model was validated using data from a grazing experiment with two farmlets consisting of 15 dairy cows each, during three distinct periods in the grazing season. The correlations between the observed and predicted values of milk production and the N partitioning over milk, faeces, life weight change and urine were highly significant, and predictions were reasonably accurate (R^2 0.52 to 0.77 on individual cow basis; R^2 0.77 to 0.97 on herd basis) with some relative biases but only one absolute bias (5% under-prediction of faeces N). Sensitivity analyses of the CNCPS model showed that the herbage CP content was the most important herbage quality factor for improving bovine N efficiency.

The HIQ-CNCPS model can be used to assess the efficacy of herbage management tools for manipulating the N utilisation of strip-grazing dairy cows, and this is the subject of further publications.

Keywords: cow nutrition, herbage intake, high-sugar cultivar, *Lolium perenne*, N application, perennial ryegrass, quality, regrowth period

1. Introduction

Grazed grass forms the main component of cow diets in large parts of Europe (Beever & Reynolds, 1994; Lantinga *et al.*, 1996). Grazing is accompanied by localised deposition of nitrogen (N) in urine and dung patches. N excreted in dung and urine may contribute to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water (Tamminga, 1992). Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal (Jonker *et al*., 1998).

The quality of the grass intake, in particular the proportion and form of protein and energy in the ingested dry matter (DM), has a pronounced effect on both animal production and N utilisation. Controlling N utilisation through diet composition has been the subject of numerous animal studies (Castillo *et al*., 2001a,b; Rearte, 2005; reviewed by Hoekstra *et al*., 2007a) and modelling work (e.g. Dijkstra *et al*., 1992; Kebreab *et al*., 2002; Fox *et al*., 2004).

In turn, herbage quality can be manipulated by grassland management. N fertiliser application rate, extending the length of the regrowth period length and growing high-sugar cultivars have been shown to be effective tools to manipulate the N and energy contents of grass in a range of agronomic studies (e.g. Wilman *et al*., 1976a; Fulkerson & Donaghy, 2001; Smith *et al*., 2002; Hoekstra *et al*., 2007b).

However, herbage quality of the standing biomass is not synonymous with quality of the ingested diet of grazing animals, as animals graze selectively, not only selecting amongst plant species, but also intra-specifically amongst plant organs (Curll *et al*., 1985; Grant *et al*., 1985). As a result, diet composition cannot be equated to sward composition.

In order to ascertain how animal N utilisation can be manipulated through herbage management, we must predict the quality of the ingested herbage from the quality of the standing herbage mass. This prediction is complicated by the spatial heterogeneity of grass morphology in the sward, largely originating from the deposition of animal excreta. Dung and urine have pronounced local effects on sward growth, herbage quality and grazing patterns (Lantinga *et al*., 1987). The proportion of the area affected by dung and urine patches can range from 0% at the start of the grazing season to over 50% at the end of the grazing season (Lantinga *et al*., 1987; Haynes & Williams, 1993).

Hoekstra & Schulte (accepted) developed a herbage intake quality (HIQ) model to predict the quality of ingested herbage, by simulating horizontal and vertical variability of the herbage quality. This allowed them to assess the efficacy of several herbage management tools (N application rate, length of regrowth period, defoliation height, and high-sugar cultivars) on the water-soluble carbohydrates (WSC), N and neutral detergent fibre (NDF) concentrations in the ingested herbage of strip-grazing bovines throughout the grass growing season.

In order to assess the impact of these management tools on bovine N utilisation, in this study, we have connected this HIQ model to the Cornell Net Carbohydrate and Protein System (CNCPS) model (Fox *et al*., 2004) for evaluating herd nutrition and nutrient excretion (Fig. 1). In this model, estimates of cattle requirements and nutrient

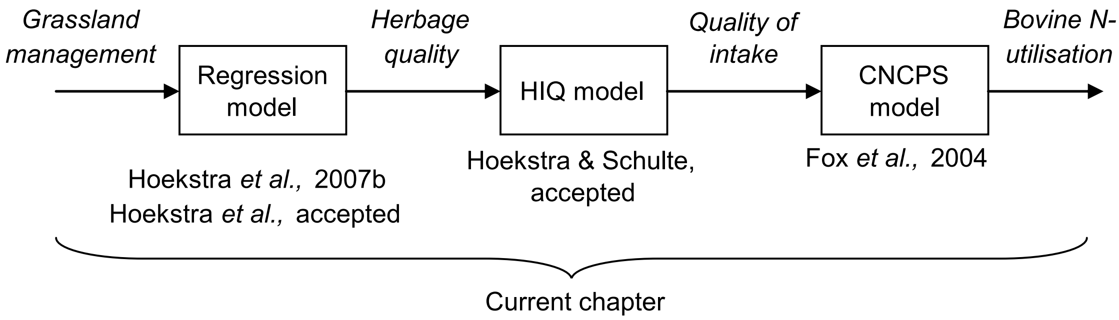

Figure 1 Overview of the data and models used to develop a model for assessing the effect of herbage management tools on the N utilisation of grazing cows.

supply are based on animal, environmental and feed compositional information. It has been used successfully on beef and dairy farms to evaluate diets (Kolver *et al.*, 1998a; Ruiz *et al*., 2002; Fox *et al*., 2004).

Firstly, in this chapter, we briefly describe the HIQ and CNCPS models and the process of connecting these two models. Subsequently, we describe the validation experiment and its main outcomes. This is followed by a sensitivity analysis of the CNCPS model, and validation and evaluation of the combined HIQ-CNCPS model. Finally, we discuss the applicability of this new model.

2. Model theory

2.1 The herbage intake quality model

The herbage intake quality (HIQ) model is described in detail by Hoekstra & Schulte (accepted). In summary, the objective of the HIQ model is to predict the efficacy of herbage management tools including perennial ryegrass cultivar (high- versus low-sugar perennial ryegrass), fertiliser N application rate, length of the regrowth period and herbage defoliation height on the WSC, N and NDF concentrations the ingested herbage DM of strip-grazing cows. The model is divided into three modules: 1) Simulation of the vertical sward structure, 2) Simulation of the horizontal sward structure, and 3) Prediction of the chemical composition of plant parts.

The vertical sward structure is predicted from the relative proportions of lamina, sheath and inflorescence material in the sward and the maximum height of lamina, sheath and inflorescence material as a function of herbage management tools. The vertical distribution of plant components is used to calculate the proportion of lamina, sheath and inflorescence material in the intake.

Urine and dung excreted onto the pasture have a large effect on the sward and the quality of the ingested herbage during grazing (Lantinga *et al*., 1987; Haynes &

Williams, 1993). Therefore, the model distinguishes two patch types: clean patches and patches affected by dung and urine ("fouled patches"), which affect both horizontal and vertical sward structure and the chemical composition of plant parts. The N, WSC and NDF concentrations in lamina, sheath and inflorescence material on clean and fouled patches are predicted as a function of herbage management.

The model was calibrated and independently evaluated for three distinct periods during the grazing season, i.e. early (April), mid (June, reproductive growth phase) and late (September) season. There was a good correlation between predicted and observed WSC concentration in the intake ($R^2 = 0.78$, $p < 0.001$). The correlation between predicted and observed NDF concentrations in the intake was reasonable ($R^2 = 0.49$, $p < 0.05$) with a small absolute bias. Differences in the N concentration between laminae and sheaths and between clean patches and fouled patches were adequately simulated, although N concentration of the intake was over-predicted for the early season and under-predicted for the late season. However, this did not affect the ability of the model to assess the efficacy of grassland management tools for manipulating the WSC and N concentrations in the intake of strip-grazing bovines (Hoekstra & Schulte, accepted).

2.2 The Cornell Net Carbohydrate and Protein System model

2.2.1 General description

An overall description of the Cornell Net Carbohydrate and Protein System (CNCPS) model has been given by Fox *et al*. (2004). Briefly, the CNCPS model is a mathematical model to evaluate diet and animal performance that was developed from basic principles of rumen function, microbial growth, feed digestion and passage, and animal physiology. The factors used to estimate requirements of dairy cows are the calculated maintenance requirements, predicted growth requirements, pregnancy requirements and requirements for lactation computed based on the amount and composition of the milk. Factors that influence the supply of nutrients include carbohydrate and protein characteristics as described by neutral detergent fibre (NDF), lignin, crude protein (CP), protein fractionation and non-structural carbohydrate components of the diet, as discussed below (Section 2.2.2). Ruminal carbohydrate digestion and microbial protein production are predicted from the growth of micro-organisms that ferment either structural or non-structural carbohydrates and the ruminal degradation of protein. Microbial protein production and metabolisable energy and protein (ME and MP) supplies available for milk production are the outcomes of these feed and animal characteristics. Intestinal digestion and metabolism of nutrients are determined using either empirical equations or transfer coefficients.

2.2.2 Feed description

In order to predict rumen fermentation and escape, carbohydrates and proteins are divided into fractions. The carbohydrates (CHO) are categorized into A, B1, B2 and C. The CHO-A fraction consist mainly of sugars and the CHO-B1 fraction is made up of starch and pectins. The CHO-B2 fraction is composed of potentially available NDF. The CHO-C pool is an indigestible fraction, and it is computed as NDF × lignin × 24 (g / kg DM) (Fox *et al.*, 2004). In this study the CHO-A and CHO-B1 fractions were combined into one pool, the non-structural carbohydrates (NSC) (A. Pell, 2003, personal communication). This pool is rapidly fermentable and calculated as dry matter (DM) minus the sum of NDF (adjusted for neutral detergent insoluble protein), crude protein (CP = N × 6.25), fat and ash (Fox *et al*., 2004).

Protein fractions (expressed as a percentage of CP) are described similarly to

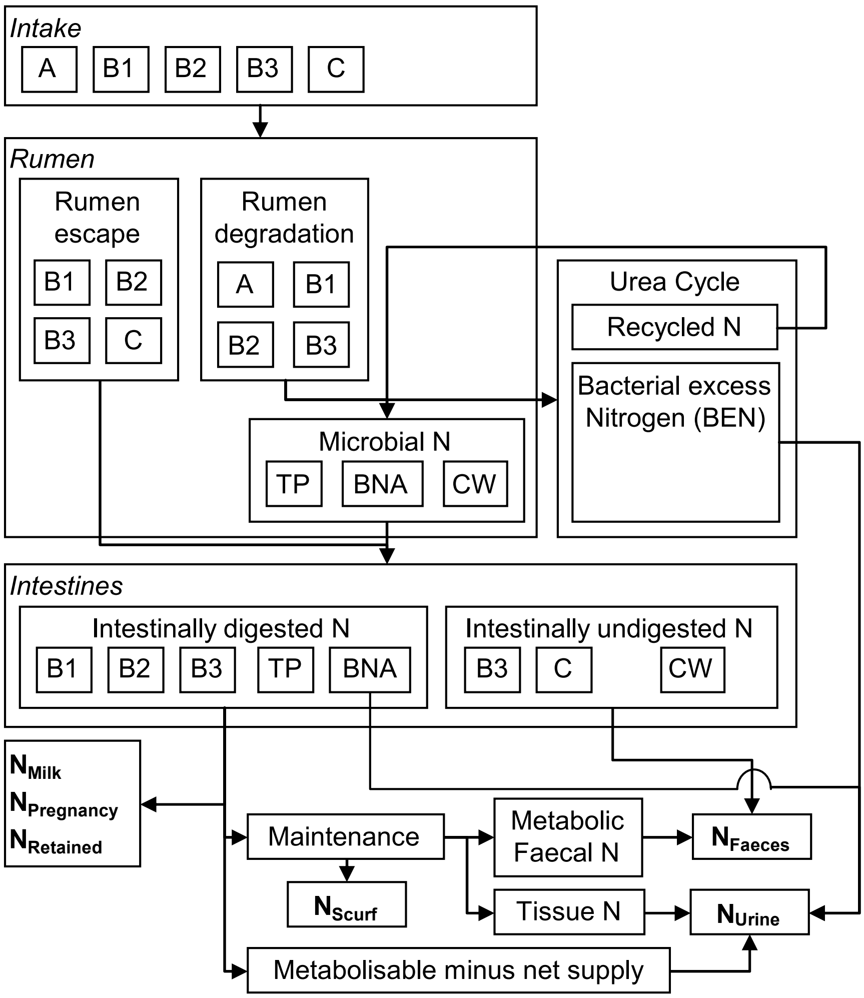

Figure 2 Schematic representation of the nitrogen intake, rumen degradation, intestinal digestion and excretion as modelled by the CNCPS model. A, B1, B2, B3 and C are the protein fractions differing in ruminal and intestinal degradability (see text). Microbial N is divided into true protein (TP) (60% of microbial N), Bacterial nucleic acids (BNA) (15% of microbial N, is non-protein N) and cell wall (CW) nitrogen (25% of microbial N).

carbohydrates (Fig. 2). Protein fraction A is non-protein N that enters the ruminal ammonia pool directly. Protein fraction B1 is true protein that has a rapid ruminal degradation rate and is nearly completely degraded in the rumen. The protein fraction C is acid detergent insoluble protein (ADIP) and is assumed to be unavailable. Protein fraction B3 is slowly degraded protein and is determined by subtracting the ADIP from the neutral detergent insoluble protein (NDIP). The protein fraction B2, which is partly degraded in the rumen, makes up the remainder of the protein (calculated as 100 minus the other protein fractions) (Fox *et al.*, 2004).

The CNCPS model requires ruminal degradation rates (K_d, %/hr) for the individual carbohydrate and protein fractions (Fox *et al.*, 2004). Intestinal digestibility is assumed to be 100%, 100%, 80% and 0% for the protein fractions B1, B2, B3, and C, respectively (Fig. 2).

2.2.3 Prediction of milk N output and N excretion

Fig. 2 gives an overview of the partitioning of feed and bacterial N over maintenance, pregnancy, milk N production, N retained, and urine and faecal N excretion.

The milk production depends on the energy and protein available for milk production and is predicted from the minimum of metabolisable protein (MP) and metabolisable energy (ME) allowable milk production. Milk CP concentration is predicted from peak milk production and days since calving and is therefore not sensitive to changes in the diet. Part of the available protein is assigned to pregnancy and maintenance, which in turn is split into scurf (skin, hair, horn and detritus), tissue N, and metabolic faecal N (Fig. 2).

Faecal nitrogen excretion (N_{Faeces}, g / day) is calculated as (Fox *et al.*, 2003) (Fig. 2):

$$N_{Faeces} = FFN + BFN + MFN \qquad (1)$$

where FFN is faecal N from indigestible feed (part of protein B3 and C), BFN is bacterial faecal N, primarily bacterial cell wall (CW), and MFN is metabolic faecal N. Urinary N excretion (N_{Urine}, g / day) is calculated from (Fox *et al.*, 2003) (Fig. 2):

$$N_{Urine} = BEN + BNA + MNS + TN \qquad (2)$$

where

BEN is available rumen N in excess of bacterial N requirement, which is zero unless the excess rumen ammonium N minus the recycled N is larger than zero. BNA is bacterial nucleic acids (non protein N) which are assumed to be intestinal-digestible but unavailable for utilisation. MNS (metabolisable to net supply) is the metabolisable N supply available for production minus N used (i.e., unavoidable losses of N during conversion to milk protein, and protein in pregnancy and live weight gain). The efficiency factors for N utilisation may range from 1 for maintenance and 0.7 for milk protein production to 0.3 and 0.5 for pregnancy and gain, respectively. TN is degraded tissue N (Fig. 2).

The N-component of the CNCPS model is a mass-balance model; as a result, the remainder of the N balance consists of $N_{Retained}$, which is the N retained (or lost) by the animal.

2.3 Linking the models - feed inputs

The herbage intake quality model as described by Hoekstra & Schulte (accepted) was linked to the CNCPS model, thus allowing direct modelling of the effect of herbage management on animal production and N efficiency during grazing.

In addition to the model outputs of the original herbage intake quality model (N, NDF and WSC concentration of intake), the CNCPS required more feed quality inputs. In order to accommodate these inputs, the original herbage intake quality model was extended with the following components:

1. Hoekstra *et al*. (2007b) found no significant consistent effects of herbage management tools on the ash and lignin concentrations of lamina, sheath and inflorescence material. Therefore mean values for these components in lamina, sheath and inflorescence material were used (Hoekstra *et al*., 2007b) and the concentrations in the herbage intake were calculated as described by Hoekstra & Schulte (accepted).
2. The effect of herbage management on the protein fractionation of lamina and sheath material was reported by Hoekstra *et al*. (accepted). The results were subjected to linear regression analyses to describe the effect of herbage management tools on the protein fractions (as described in Hoekstra & Schulte (accepted)), and these equations were added to the herbage intake quality model.
3. The fat concentration of the herbage intake was estimated as 29 g / kg DM for mid season and 39 g / kg DM for early and late season, based on values by Gilliland *et al*. (2002).
4. We assumed a K_d of 13 % / h for the CHO-B2, which Kolver *et al.* (1998a) used for a highly digestible grass sward in their CNCPS validation. The K_d of the NSC-pool (CHO-A/B1) was assumed to be 2 times faster than the K_d of CHO-B2 based on data from the feed library (Fox *et al*., 2003). The degradation rate of the cell wall protein (protein fraction B3) was assumed to be equal to the K_d of the CHO-B2 fraction (A. Pell, 2003, personal communication) and the K_d

of protein fractions B2 and B1 were based on the feed library (20 and 200 % / hr, respectively) library (Fox *et al*., 2003).

5. From the validation in the HIQ model paper it became clear that the herbage model over-predicted the N concentration in spring and under-predicted the N concentration in summer (Hoekstra & Schulte, accepted). Even though this did not affect the model predictions of the efficacy of herbage management for manipulating the N concentration, this bias will affect the predictions of the CNCPS model. Therefore, the combined HIQ-CNCPS model was recalibrated for this specific experiment, by fitting the predicted N concentration with the observed N concentration in lamina and sheath material, minimising the residual sum of squares. The experiment-specific intercepts of the N prediction regression equations for N concentration in both lamina and sheath material were 114% and 84% during early and late season, respectively, of the values reported in a cutting experiment by Hoekstra & Schulte (accepted).

3. Materials and Methods

First, the CNCPS model was subjected to a sensitivity analysis in which the effects of a 10% change in feed composition on the ME- and MP-allowable milk production and rumen N balance, N_{Faeces}, N_{Urine}, $N_{Retained}$ and N_{Milk} during early, mid, and late season were evaluated.

Subsequently, the combined HIQ-CNCPS model was validated with herbage and animal data by evaluating and comparing the predicted and observed milk yields (kg / day), milk N outputs (N_{Milk}; g N / day), faecal N excretion rates (N_{Faeces}; g N / day), urine N excretion rates (N_{Urine}; g N / day), and changes in N in live weight (N_{LWC}; g N / day). It was assumed that the model predicted $N_{Retained}$ (g N / day) was equivalent to the measured N in live weight change. If the slope of a linear regression between predicted and observed values was significantly ($p < 0.05$) different from one (corresponding to a significant bias of the residuals) or the mean residual was significantly ($p < 0.05$) different from zero, this indicated a relative or absolute bias, respectively.

3.1 Experimental design

During the grazing season of 2004, two grassland farmlets with 15 animals each, were applied on the dairy farm of Johnstown Castle Research Centre, Teagasc, Ireland. One farmlet was aimed at obtaining optimal bovine N efficiency (HNE) through a combination of high-sugar grass, relatively low N application rate and long regrowth

Table 1

Validation experiment: herbage management and chemical composition of ingested herbage for the high N efficiency system (HNE) and control N efficiency system (CNE) during early, mid, and late season.

	Early		Mid		Late	
	HNE	CNE	HNE	CNE	HNE	CNE
Herbage management						
N application rate (kg / rotation)	30	40	25	40	20	30
Length of regrowth period (days)	30	28	27	30	30	27
Defoliation height non-fouled area (mm)	67	76	82	83	76	66
Chemical composition of ingested herbage						
NDF (g / kg DM) [a]	422	417	454	485	462	471
Lignin (g / kg NDF) [a]	28	27	34	37	24	23
CP (g / kg DM) [a]	137	174	173	151	188	219
Protein fractions (g / kg CP) [b]						
A (non protein N)	180	184	167	166	255	244
B1 (buffer soluble protein N)	133	116	109	137	95	71
B2 (buffer insoluble protein N)	559	585	564	576	516	551
B3 (NDIN – ADIN)	118	106	150	112	125	125
C (ADIN) [c]	9	9	9	9	9	9
Ash (g / kg DM) [a]	86	91	90	77	99	94
Fat (g / kg DM) [d]	39	39	29	29	39	39
NSC (g / kg DM) [e]	317	279	254	247	212	177
WSC (g / kg DM) [a]	256	193	194	164	139	113

[a] Method of analysis as described in Hoekstra *et al.*, 2007b
[b] Method of analysis as described in Hoekstra *et al.*, accepted
[c] Average value based on Hoekstra *et al.*, accepted
[d] Based on Gilliland *et al.*, 2002
[e] Non-structural carbohydrates = 1000–NDF–CP–Fat–Ash

period, whereas the other farmlet (CNE) was a control, that is, managed “as usual” (Table 1) (for more details, see Hoekstra & Schulte (accepted)). The farmlets were closely monitored during three periods of 5 consecutive days in the year 2004, i.e. during early season (April/May), mid season (June, reproductive growth), and late season (September) (Table 1).

Thirty multiparous Frisian Holstein dairy cows were blocked according to stage of lactation and milk yield, and allocated at random from among blocks to the farmlets. The average lactation number was 3 and average daily milk yield during March was 28.7 kg milk / cow / day.

The grazing regime was strip grazing; during the monitoring weeks, this was a strict one-daily strip-grazing regime, whereas it was less strict throughout the remaining grazing season. During the spring period the dairy cows received 2.0 kg concentrates per day in order to prevent the occurrence of grass tetanus. No concentrates were fed during the remaining periods.

3.2 Herbage DM intake: the alkane technique

During the sampling periods, the herbage intake was estimated using the alkane double-indicator technique (Berry *et al.*, 2000) employing controlled-release capsules (CRC, type MCM, Captec Ltd, Auckland, New Zealand). The capsules were dosed nine days prior to the sampling period. During the sampling period, a dung sample from each cow was taken from freshly dropped dung pats or rectally, before morning and afternoon milking (7.00 and 15.00 hrs, respectively). In order to determine the alkane release rate of the CRC's, dung samples were taken from one cow from each herd, once daily after the sampling had stopped until after the stated end point of release of alkanes (c. 20 days).

Samples were immediately frozen at –20 °C and later 10 g of each sample was bulked per animal. Of this bulked sample, a small sub-sample was taken for determination of N concentration; the rest was oven-dried at 60 °C for 48 hours. Grass samples mimicking the grazing pattern of the cattle were taken by taking 50 'snips' from each paddock following a W-shape pattern with snip depth depending on the observed grazing height (from the previously grazed paddock) for both clean and fouled patches. Samples were taken every morning, starting one day prior to the start and ending one day before the end of faecal sampling. These samples were immediately frozen at –20 °C and chopped and sub-sampled before being freeze-dried and ground to pass a 1 mm sieve. When applicable, concentrate samples were taken and frozen at –20 °C, before freeze drying and grinding the samples. The herbage, dung and concentrate samples were analysed for DM and ash. The n-alkane concentrations were determined as described by Stakelum & Dillon (1990). The fresh dung samples (bulked over 5 days) were analysed for total N concentration.

Additional pre- and post-grazing herbage samples to determine herbage quality of the intake were taken and analysed as described in Hoekstra & Schulte (accepted).

3.3 Milk yields and live weight changes

Milk yields were recorded for every milking. Two composite milk samples were collected daily for each cow during the sampling week. One sample was stored in a cold room (4 ºC, for two days maximum) before crude protein and fat concentration determination (Infrared analysis, Lactoscope, Delta instruments, Drachten, The Netherlands). During early and late season, the second sample was stored at –20 ˚C and was later analysed for urea-N concentration (UV-method, Boehringer Mannheim, Mannheim, Germany).

Cows were weighed every two to four weeks throughout the grazing season, to provide an estimate of changes in body weight.

3.4 Calculations

The daily herbage intake (I, kg DM / day) was calculated from the following formula given by Dove & Mayes (1991):

$$I = \left(\frac{Fi}{Fj} \cdot \left(Dj + Ic \cdot Cj \right) - Ic \cdot Ci \right) \Big/ \left(Hi - \frac{Fi}{Fj} \cdot Hj \right) \qquad (3)$$

where Fi, Ci and Hi are respective concentrations (mg / kg DM) of the 'natural alkane' C_{33} in faeces, concentrate and forage. Fj, Cj and Hj are respective concentrations (mg / kg DM) of 'unnatural alkane' C_{32}. Dj is the batch release rate for the CRC, calculated as the amount of alkane in the capsule divided by the number of days until a 50% drop in the alkane concentration in the dung had occurred. The stated batch release rate was 360 mg / day, but calculated values varied from 400 mg / day during early and mid season to 360 mg / day during late season. Deviations from the stated release rate were previously reported by Ferreira *et al*. (2004). Ic is the intake of concentrate (kg / day DM).

The faecal output (O, kg / day) is calculated by:

$$O = \frac{Dj + I \cdot Hj + Ic \cdot Cj}{Fj} \cdot frf \qquad (4)$$

where frf is the faecal recovery factor, assuming a faecal recovery of the C_{32} alkane of 0.928 (Ferreira *et al*., 2004). Formulae for cows consuming no concentrate are similar, but omitting Ci, Cj and Ic.

The N intakes and outputs were calculated by multiplying the DM intake, milk yield, dung excretion and live weight changes with their respective N concentrations. For live weight change was assumed that every kg of live weight change contained 23.7 g N. Urine N excretion (g N / day) was calculated by difference:

$$N_{Urine} = N_{Intake} - N_{Milk} - N_{Faeces} - N_{LWC} \qquad (5)$$

3.5 Observed patterns

The average chemical composition of the ingested herbage for the two dairy systems is presented in Table 1, and the results for DM intake, milk production, and N partitioning are presented in Table 2. The herbage DM intake declined from 18.4 kg / day to 14.9 kg / day for the CNE herd during early and late season, respectively, which is in line with expectations for dairy cows with similar milk production. The CP concentration of the intake ranged from 13.7 to 21.9 g / kg DM and the N_{Intake} ranged from 401 to 556 g / day. The CP concentration was higher for the HNE, except during mid season, which may be related to the higher proportion of stems in the CNE sward (data not shown) which was also accompanied by a higher NDF concentration (Table 1). N_{Milk} varied from 112 to 166 g N / day, which equated to 214 (CNE, late season) to 354 (HNE, early season) g N_{Milk} / kg N_{Intake}. N_{Faeces} ranged from 140 to 179 g / day which was 267 to 43 g / kg N_{Intake}. On average, N_{Faeces} was 9.5 g / kg DM intake, which

Table 2

Results for the dairy high N efficiency system (HNE) and the control N efficiency system (CNE) during early, mid, and late season (n = 15, SD in parentheses).

	Early season		Mid season		Late season	
	HNE	CNE	HNE	CNE	HNE	CNE
Intake						
Herbage intake (kg DM / day)	17.8 (2.8)	18.4 (3.7)	16.7 (2.3)	16.6 (2.7)	16.4 (2.1)	14.9 (1.8)
N_{Intake} (g N / day)	433 (61)	556 (102)	462 (63)	401 (65)	494 (63)	522 (64)
Milk						
Milk yield (kg / day)	31.3 (4.1)	33.7 (3.3)	26.5 (2.9)	26.2 (3.5)	21.3 (3.1)	20.6 (3.2)
Milk CP concentration (g/kg milk)	31.4 (1.8)	31.5 (1.3)	31.0 (2.1)	30.6 (1.8)	34.8 (1.6)	34.6 (2.1)
Milk Urea-N concentration (mg / dl)	6.8 (1.1)	11.2 (2.1)	nd[a]	nd	14.8 (2.4)	20.9 (2.1)
N_{Milk} (g N / day)	153 (17)	166 (18)	129 (16)	126 (18)	116 (16)	112 (15)
Live weight change (LWC)						
N_{LWC} (g N / day)	–25 (15)	–17 (12)	–9 (20)	12 (15)	9 (5)	7 (5)
Faeces						
Faecal excretion (kg DM / day)	4.3 (0.6)	4.3 (0.8)	4.9 (0.7)	5.5 (0.8)	4.0 (0.6)	3.7 (0.4)
N_{Faeces} (g N / day)	176 (25)	179 (30)	175 (33)	175 (70)	154 (23)	140 (16)
Urine						
N_{Urine} (g N / day)	129 (36)	227 (49)	168 (44)	89 (49)	215 (35)	264 (45)
N partitioning (g / kg N_{Intake})						
N_{Milk}	354 (39)	299 (42)	278 (48)	313 (48)	235 (28)	214 (25)
N_{Faeces}	405 (17)	322 (19)	378 (48)	436 (107)	311 (16)	267 (21)
N_{Urine}	298 (62)	410 (51)	362 (78)	222 (98)	436 (39)	506 (35)
N_{LWC}	–56 (33)	–31 (25)	–19 (44)	29 (39)	18 (11)	13 (11)

[a] nd = not determined

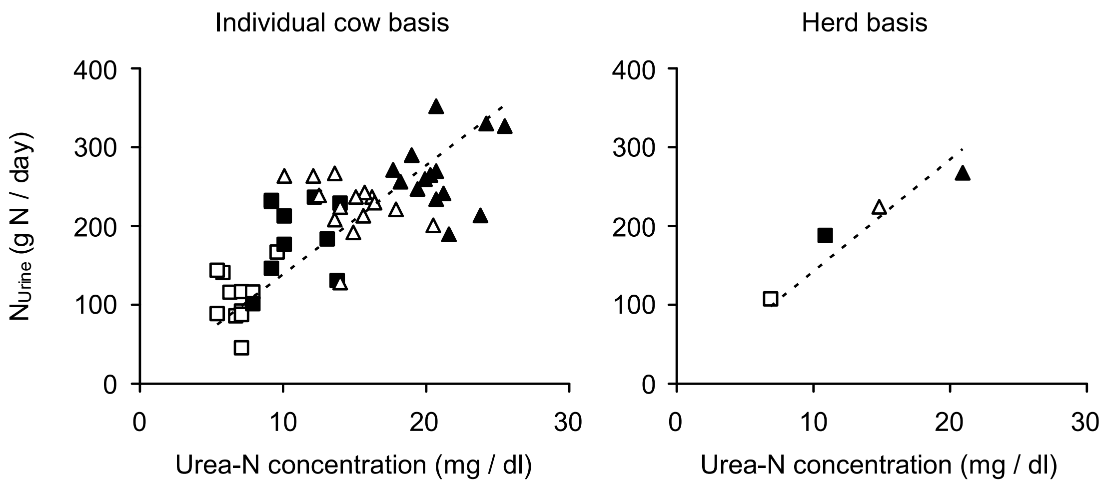

Figure 3 Correlation between urine N excretion (N_{Urine}; g / day) and milk urea-N concentration (MUN; mg / dl) on individual cow basis and herd basis, for the HNE (open symbols) and CNE (closed symbols) systems during early (■/□) and late (▲/△) season.

is close to the value of 8 reported by Lantinga *et al.* (1987). Changes in live weight gain showed considerable within herd variation and ranged from –1.0 kg per day (HNE, early season) to 0.5 kg per day (CNE, mid season), which corresponded to –24 and 12 g N per day, respectively (less than 60 g / kg N_{Intake}). The resulting urinary N excretions varied from 92.6 to 261 g / day, which amounts to 236 and 502 g / kg of N_{Intake}, respectively. These efficiency values were within the range of values reported in other grazing studies (Peyraud *et al.*, 1997; Astigarraga *et al.*, 2002).

The milk urea-N (MUN) concentration ranged from 6.8 mg / dl to 20.9 mg / dl and the correlation with urine N excretion was reasonably strong ($R^2 = 0.58$, $p < 0.001$; Fig. 3a). The slope of the equation ($N_{Urine} = 13.9 \times MUN$) was close to the formula given by Kauffman & St. Pierre (2001) ($N_{Urine} = 0.0259 \times$ Body weight $\times$ MUN) which works out at 14.5 for cows weighing 560 kg. The correlation became much stronger when it was based on the herd average, rather than individual cows ($R^2 = 0.83$, $p < 0.05$, $N_{Urine} = 14.2 \times MUN$, Fig. 3b) which is in line with other studies who have found that the MUN is a better predictor on herd rather than cow level (Trevaskis & Fulkerson, 1999).

4. Results

4.1 Sensitivity analysis of the CNCPS model

The sensitivity analysis was based on the HNE herd average and initial feed values are reported in Table 1.

There was a direct response of all the parameters to an increase in DM intake,

Table 3

Sensitivity analysis of the CNCPS model based on HNE herd averages: Percentage change in ME and MP allowable milk, rumen N balance, N_{Faeces}, N_{Urine} and N_{Milk} as a result of a 10% increase in the indicated feed component from to the initial value, during early, mid, and late season.

		Adapted feed components (%)					
	Initial value	DM intake	NDF	CP	NDIN	K_d CHO A/B1	K_d CHO B2
Early season							
ME-allowable milk (kg / day)	36	13	–2	0	0	0	0
MP-allowable milk (kg / day)	31	9	–3	2	0	–1	2
Rumen N balance (%)	105	1	1	7	0	1	–2
N_{Faeces} (g N / day)	177	9	1	–1	0	0	1
N_{Urine} (g N / day)	149	8	–2	0	0	–1	1
$N_{Retained}$ (g N / day)	–58	5	–10	–62	1	–5	10
N_{Milk} (g N / day)	150	9	–3	2	0	–1	1
N_{Milk} (g / kg N_{Intake})	36	0	–3	–7	0	–1	1
Mid season							
ME-allowable milk (kg / day)	28	15	–3	–1	0	0	0
MP-allowable milk (kg / day)	27	12	–3	3	0	–2	1
Rumen N balance (%)	133	0	2	12	0	1	–2
N_{Faeces} (g N / day)	152	10	1	–1	0	0	1
N_{Urine} (g N / day)	182	9	1	24	0	1	–2
$N_{Retained}$ (g N / day)	–4	14	7	–38	11	8	7
N_{Milk} (g N / day)	133	12	–2	3	0	–1	1
N_{Milk} (g / kg N_{Intake})	29	1	–2	–7	0	–1	1
Late season							
ME-allowable milk (kg / day)	24	15	–3	–2	0	0	0
MP-allowable milk (kg / day)	23	12	–2	3	0	–3	1
Rumen N balance (%)	154	0	1	15	0	1	–1
N_{Faeces} (g N / day)	145	10	1	–1	0	0	1
N_{Urine} (g N / day)	229	9	0	21	0	1	–1
$N_{Retained}$ (g N / day)	–6	–3	–10	7	–4	–1	–9
N_{Milk} (g N / day)	123	11	–2	4	0	–2	1
N_{Milk} (g / kg N_{Intake})	25	1	–2	–6	0	–2	1

but because the composition of the intake was the same, there were no changes in the N use efficiency for milk (N_{Milk} in g / kg N_{Intake}) (Table 3).

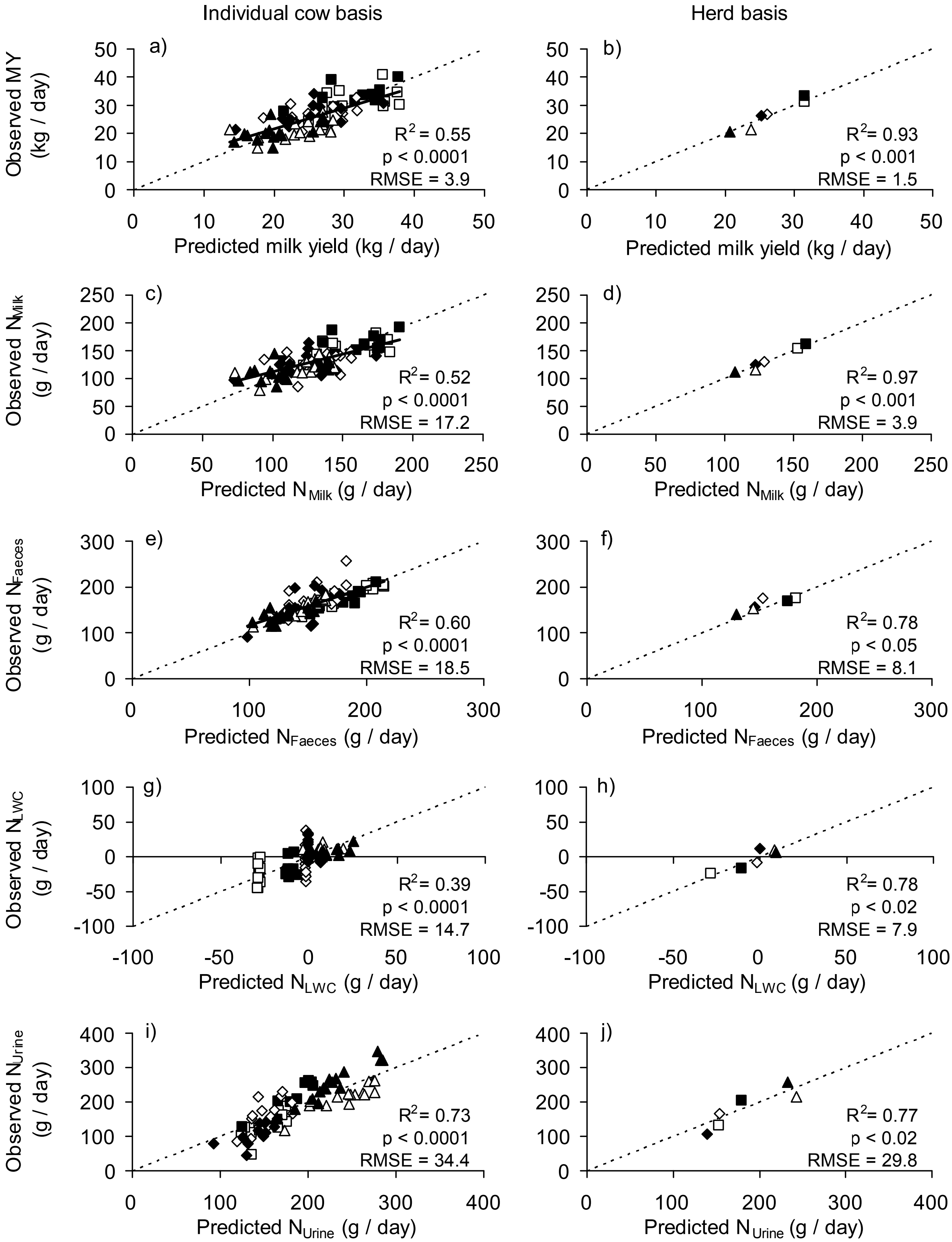

Figure 4 Correlation between observed and predicted values of milk yield (MY) (a,b), N_{Milk} (c,d), N_{Faeces} (e,f), N_{LWC} (g,h) and N_{Urine} (i,j) on individual cow basis (a,c,e,g,i) and herd basis (b,d,f,h,j) for the HNE (open symbols) and CNE (closed symbols) systems during early (■/□), mid (◆/◇) and late (▲/△) season.

An increase in NDF resulted in a decrease in the NSC concentration of the intake (approximately –20%), so the degradation rate of the energy intake decreased, while the total energy intake remained the same. ME and MP allowable milk were reduced by 2 to 3% because of reduced microbial growth. This resulted in a slightly higher rumen N balance and N_{Urine}, and a slight reduction in the Milk N efficiency.

An increase in the CP concentration in the intake slightly reduced the ME allowable milk production due to less NSC supply (CP increased at the expense of NSC) and increased ME maintenance requirements (urea cost), whereas the MP allowable milk was increased with 2 to 3%. There was little effect on faecal N and therefore the remainder of the change in CP intake was either partitioned to urine (25% increase during mid and late season) or $N_{Retained}$ (68% reduction in negative $N_{Retained}$, i.e. increase from –58 to –22 g N / day during early season).

Changing the proportion of NDIN (% of CP) in the diet had very little effect, because the concentration was relatively small (only 10% of CP). Increasing the K_d of CHO-A/B1 and CHO-B2 increased the rumen availability of energy and hence ME and MP allowable milk, resulting in a lower rumen N balance and N_{Urine}, but again the effect was rather small.

4.2 Observed and predicted values

The correlation between observed and predicted milk yield was reasonable on cow level (Fig. 4a, $R^2 = 0.55$; $p < 0.0001$) with no significant absolute bias, but a significant relative bias (slope = 0.73). When using herd level data the correlation was very good (Fig. 4b, $R^2 = 0.93$; $p < 0.001$) with no significant bias. The prediction of the milk N output on cow level was slightly less accurate (Fig. 4c) with no absolute bias, but a significant relative bias (slope = 0.64), however, on herd level the correlation was very strong (Fig. 4d, $R^2 = 0.97$, $p < 0.001$, no significant bias).

The correlation between observed and predicted faecal N excretion on cow level was good (Fig. 4e, $R^2 = 0.60$; $p < 0.0001$), with a small relative (slope = 0.83) and absolute (7.5 g / day, 5% under-prediction) bias. This was improved on herd level where the R^2 increased to 0.78 with no significant bias (Fig. 4f).

The correlation between observed and predicted N_{LWC} was highly significant but rather weak on individual cow level (Fig. 4g, $R^2 = 0.39$, $p < 0.0001$), but the correlation was stronger on herd level (Fig. 4h, $R^2 = 0.78$, $p < 0.02$). This is not surprising, because live weight gain is hard to measure accurately, and the low R^2 may be the result from inaccuracies in the measurements rather than the model.

There was a strong significant correlation between observed and predicted urine N excretion both on cow and herd level (Fig. 4 i–j; $R^2 = 0.73$ and 0.77 respectively), with no significant bias.

5. Discussion

The model predictions of milk production, N partitioning over milk, faeces, LWC and urine were reasonably accurate (R^2 0.52 to 0.77 on cow level and R^2 0.77 to 0.97 on herd level), with some relative biases but only one absolute bias (5% under-prediction of N_{Faeces}). Parts of the CNCPS model have been validated previously. In the studies of Kolver *et al*. (1998a), the CNCPS under-predicted ME allowable milk by 2.5 and 6.8% in total mixed ration and pasture fed groups, respectively. In a study on 10 dairy farms in the UK, ADAS (1998) reported that the CNCPS predicted milk yield to within 2.5 and 5% when ME or MP was limiting, respectively. Ruiz *et al*. (2002) found that 68% of the variation in MP allowable milk (MPM) production was accounted for by the CNCPS model (including N adjustment for ruminal N deficiency) with no significant absolute bias. We have been unable to find any validation of urine and faecal N excretion with this model. The validation of the faecal N excretion in the current study was satisfactory, with just a small under-prediction bias. Even though we did not directly measure the urine N excretion, the recorded changes in live weight allowed us to calculate the urine N excretion and the correlation with the CNCPS predictions was strong, with no significant biases ($p < 0.05$).

The model error is the result of the combined error of the HIQ model and the CNCPS model and measurement errors. The sensitivity analysis (Table 3) showed that the model is very sensitive to the CP concentration of the intake. It is very hard to accurately predict the precise level of CP in the intake from generalised model parameters, as this is affected by site-specific factors such as weather and soil conditions (Hoekstra & Schulte, accepted). However, in their sensitivity analysis, Hoekstra & Schulte (accepted) showed that even though the HIQ model did not accurately predict the differences in N level between seasons (over-estimation during early season, under-estimation during late season), this did not affect the model ability to predict the effects of herbage management tools on changes in N concentration. For the model validation in the current chapter, the intercepts of the algorithms predicting the N concentration in lamina and sheath material were calibrated to the levels of N during the three seasons, thus allowing successful model validation.

The newly linked model uniquely combines herbage and animal components to allow the prediction of bovine N efficiency as affected by grassland management. Other models combining grass and animal components, such as NCYCLE-IRL (Del Prado *et al*., 2006), Dairy-Sim (Fitzgerald *et al*., 2005) and the model of herbivore-plant interactions on grasslands (Hutchings & Gordon, 2001) have been previously developed. However, these models were each designed for other specific purposes. Generic models, such as NCYCLE and Diary-Sim are too generalised to account for detailed changes in grassland management. By contrast, other models (such as

Hutchings & Gordon, 2001) are very detailed and require site specific calibration of multiple parameters that are difficult to measure in the field. The current model is specifically formulated to assess the effect of grassland management tools for maximising the N efficiency of grazing bovines and requires just one parameter (which is easy to measure) to be calibrated site-specifically.

To date, experimental studies have assessed the impact of individual grassland management tools on bovine N utilisation (e.g. Van Vuuren *et al*., 1992; Valk *et al*., 1996; Peyraud *et al*., 1997). The new model takes interactions into account and allows these tools to be assessed in a realistic system context. Therefore, the model is a valuable tool for designing integrated grassland management strategies that maximise the N utilisation of grazing dairy cows, and this is the subject of further publications.

6. Conclusions

- We successfully developed a tool for predicting the effect of grassland management tools on the N utilisation of grazing cows by linking the herbage intake quality model and the CNCPS model.
- The model validation showed that the combined model satisfactorily predicted the effect of herbage management on cow N utilisation
- Sensitivity analysis of the CNCPS model showed that the herbage CP concentration was the main herbage quality factor for improving bovine N efficiency.
- The HIQ-CNCPS model can be used to assess the efficacy of herbage management tools for manipulating the N utilisation of strip-grazing dairy cows.

Acknowledgements

The authors would like to thank the ‘CNCPS team’ (Mike Van Amburgh, T.P. Tylutki, D.G. Fox and others) for providing an Excel version of their model to facilitate the connection between the two models. We are grateful to Michael O’Donovan in Teagasc, Moorepark Research Centre, for his help with the alkane technique. We thank Rioch Fox and John Murphy in Teagasc Johnstown Castle Research Centre, Ireland, and Hennie Halm, Wageningen University, The Netherlands for their technical assistance in the field and the lab. We would also like to thank Pádraig O’Kiely, Teagasc, Grange Livestock Research Centre, for his helpful comments on the manuscript. N.J. Hoekstra was supported under the Teagasc Walsh Fellowship Scheme.

7

Grassland management tools for improving the nitrogen efficiency of grazing dairy cows

N.J. Hoekstra, E.A. Lantinga, R.P.O. Schulte, P. O'Kiely & P.C. Struik, submitted to European Journal of Agronomy

Abstract

Grazing is accompanied by localised deposition of nitrogen (N) in urine and dung patches, which can contribute to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water.

In a previous review of the literature, we identified three pathways through which more efficient N utilisation by grazing bovines can be achieved by manipulating the chemical composition of the grass forage: 1) matching protein supply to animal requirements, 2) balancing and synchronising carbohydrate and N supply in the rumen, and 3) increasing the proportion of protein in the form of rumen undegradable protein (RUP). Additionally, four grassland management tools were identified, which potentially affect these pathways: N fertiliser application rate, length of regrowth period, defoliation height and high-sugar grass cultivars. In the current chapter we apply the results of a major research programme to evaluate the effectiveness of these grassland management tools and herbage quality pathways on the N utilisation of grazing dairy cows.

Within the modelled scenario the concentration of crude protein (CP) in the ingested dry matter (DM) was the main factor affecting N utilisation. Model predictions indicated that herbage should be managed to achieve a CP concentration of 130−150 g / kg DM in order to maximise the efficiency of N utilisation for milk production and minimise the proportion of N excreted in urine. Both N application rate and rotation length were shown to be effective tools for affecting the CP concentration in ingested herbage by grazing dairy cows and subsequent cow N utilisation. However, there was no effect of the high-sugar cultivar and defoliation height on cow N utilisation. Assessment of the effectiveness of the three herbage quality pathways for improving bovine N utilisation resulted in the following conclusions: 1) N utilisation is strongly related to the daily N intake (g / day), however, the effect is more related to the CP concentration of the ingested DM (g / kg DM), rather than the actual daily N intake. Therefore, the effect is more related to the balance between energy and N (pathway 2), than to the quantity of ingested N. 2) Because of the strong negative correlation between the energy and N concentration in grass as affected by herbage management tools, the balance between N and energy is the most important herbage quality factor for improving bovine N utilisation. In contrast, the synchronisation between the release of energy and N seemed to have little effect. 3) The proportion of protein in the form of RUP was not much affected by the herbage management tools, and was therefore not an effective pathway for improving the N utilisation of grazing cows.

It is recommended that the model will be extended to include a herbage yield and an intake component. This would allow the model to be used to design herbage

management systems to optimise N utilisation on a yearly basis.

Keywords: cow nutrition, herbage quality, high-sugar cultivar, *Lolium perenne*, N application rate, perennial ryegrass, regrowth period

1. Introduction

In Ireland and large parts of Europe, grazed grass forms the main component of cow diets (Beever & Reynolds, 1994; Lantinga *et al*., 1996). Grazing is accompanied by localised deposition of nitrogen (N) in urine and dung patches. N excreted in dung and urine can contribute to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water (Tamminga, 1992). Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal (Jonker *et al.*, 1998).

In general, the N utilisation of grazing cows for milk production is often lower than 25% (Tas *et al*., 2006), although the theoretical maximum of efficiency of N utilisation may reach 40 to 45% of N intake (Van Vuuren & Meijs, 1987). It is possible to increase the bovine N utilisation substantially through changing the composition of the diet, resulting in a significant reduction of N losses to the environment (Jonker *et al*., 1998; James *et al*., 1999; Kröber *et al*., 2000). In indoor feeding systems, this diet composition can be controlled by using protein and carbohydrate supplements (Castillo *et al*., 2000; 2001a; b). However, manipulating the nutritional composition of grazed grass poses a more complex challenge, since it is much harder to control the diet under grazing.

To date, a number of experimental studies have assessed the impact of individual grassland management tools such as N application rate (e.g. Van Vuuren *et al*., 1992; Valk *et al*., 1996; Peyraud *et al*., 1997; Astigarraga *et al*., 2002), length of regrowth period (e.g. Mambrini & Peyraud, 1994; Van Vuuren *et al*., 1991), and high-sugar cultivars (e.g. Miller *et al*., 2001; Tas *et al*., 2006) on bovine N utilisation, as reviewed by Hoekstra *et al*. (2007a). In this literature review, three pathways were identified through which more efficient N utilisation by grazing bovines can be achieved by manipulating the chemical composition of the grass forage:

1) Matching the herbage protein content to animal requirements is an important way to improve bovine N efficiency as most N ingested in excess of animal requirement is excreted in faeces and particularly urine (Kirchgessner *et al*., 1994; Castillo *et al*., 2001b; Kebreab *et al*., 2001) (Fig. 1a).
2) Animal N efficiency may be improved through balancing and synchronising carbohydrate and protein supply to the rumen (Beever & Reynolds, 1994). In order

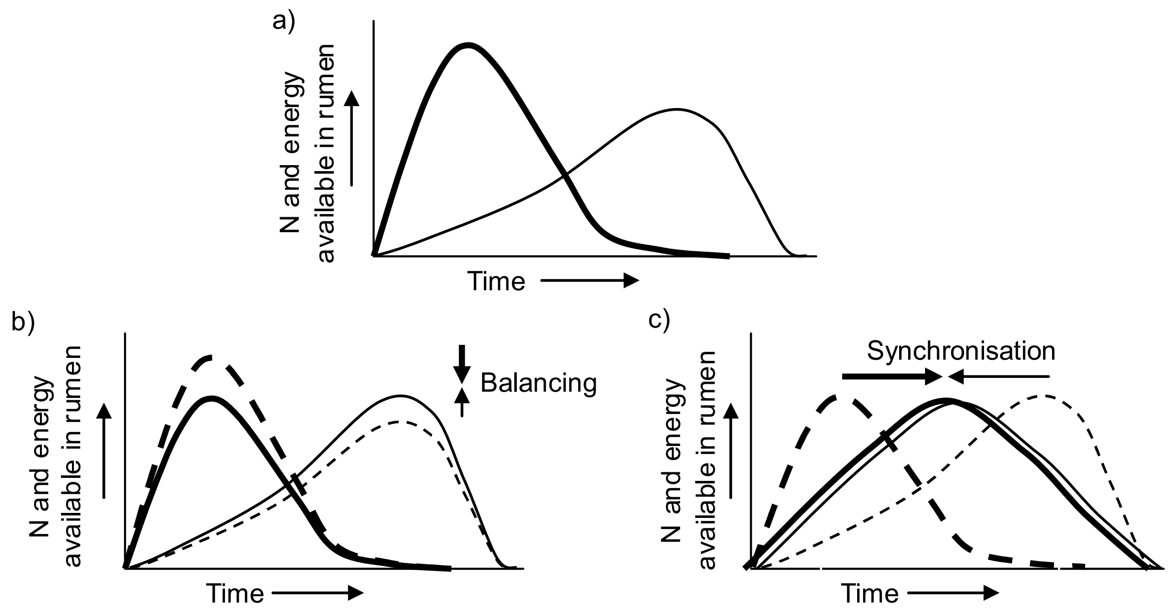

Figure 1 Schematic representation of N (▬▬) and energy (——) availability in the rumen. a) typical scenario for grazed grass, b) balancing of the amount of available N and energy, and c) synchronisation of N and energy.

for the rumen microorganisms to utilise the available N, an adequate source of readily available energy is required. Therefore it is important to ensure an optimum balance between available N and energy in the ingested herbage (Fig. 1b). In addition to the amounts of N and energy, the synchronisation of the release in the rumen is an important aspect (Fig. 1c). N in herbage is generally highly soluble and rapidly available in the rumen. Water soluble carbohydrates (WSC) are released into the rumen after disruption of the plant cell, and are immediately available for the rumen microorganisms, whereas cell walls are more resistant to microbial attack (Beever & Reynolds, 1994; Lantinga & Groot, 1996). Therefore, increasing the WSC concentration has been advocated as being an important way to improve N utilisation of forages (Beever & Reynolds, 1994; Lantinga & Groot, 1996; Miller *et al.*, 2001). Inversely, synchronisation may be increased by reducing the degradability of N compounds, that is, by increasing the proportion of N related to the cell wall at the expense of soluble N and especially non-protein N (Beever & Reynolds, 1994).

3) Another effect of decreased protein degradability is the increase of the proportion of N in the form of rumen undegradable protein (RUP). The digestible part of this RUP can be absorbed from the small intestine and used directly by the animal (Buxton, 1996; Bohnert *et al.*, 2002). Increasing the proportion of RUP could result in lower rumen ammonia levels, increased N recycling to the gut (due to lower

ruminal ammonia levels), and could subsequently decrease urinary N excretion (Bohnert *et al*., 2002). We refer to Hoekstra *et al*. (2007a) for a more detailed description of these phenomena.

In their review, Hoekstra *et al.* (2007a) identified four grassland management tools with the potential to affect these pathways: N fertiliser application rate, length of regrowth period, defoliation height and high-sugar grass cultivars (Fig. 2). On foot of this review, we undertook a major research programme to investigate the efficacy of these grassland management tools for manipulating the three herbage quality pathways and to assess the subsequent effect on the N utilisation of grazing cows. This programme combined plot experiments, animal experiments and modelling.

The impact of the selected grassland management tools and their interactions on herbage quality was quantified in a plot experiment (Hoekstra *et al*., 2007b; accepted) and significant effects of N application rate, length of regrowth period and high-sugar grass cultivar were reported. However the effect of defoliation height was found to be small and inconsistent. The resulting impact on the quality of the intake during grazing was modelled in the herbage intake quality (HIQ) model (Hoekstra & Schulte, accepted). The subsequent bovine N utilisation was predicted by connecting this HIQ model to the Cornell Net Carbohydrate and Protein System (CNCPS) model (Hoekstra *et al*., submitted a). Both the HIQ model and the CNCPS-HIQ model were successfully validated and evaluated by Hoekstra & Schulte (accepted) and Hoekstra *et al*. (submitted a), respectively.

In the current chapter we apply the results of these studies to evaluate the effectiveness of A) the grassland management tools and B) herbage quality pathways identified in the original review paper on the N utilisation of grazing cows (Fig. 2).

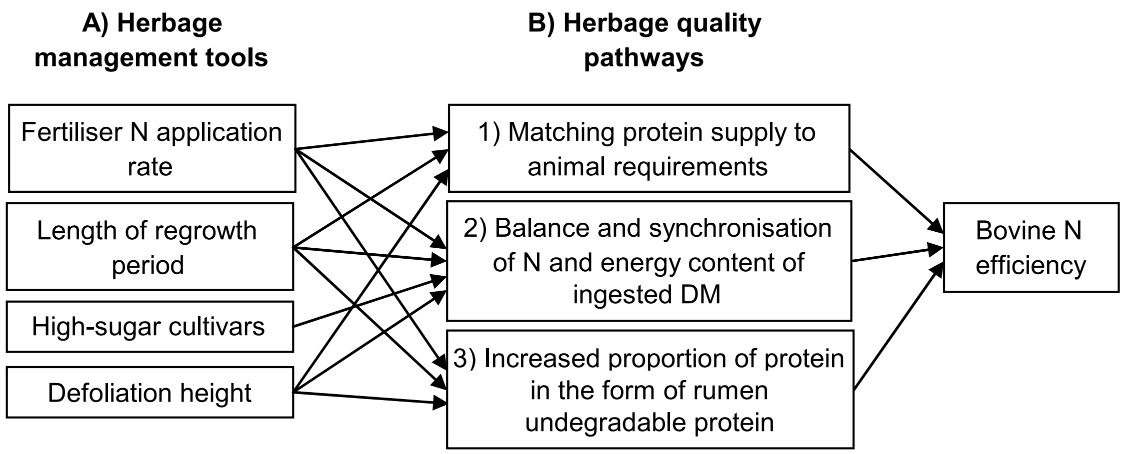

Figure 2 Schematic diagram of the effect of grassland management tools on the three herbage quality pathways for improving the N efficiency of grazing cows.

2. Materials and methods model application

The herbage intake quality (HIQ) model is described in detail by Hoekstra & Schulte (accepted). In summary, the objective of the HIQ model is to predict the efficacy of herbage management tools including perennial ryegrass cultivar (high- versus low-sugar perennial ryegrass), fertiliser N application rate, grazing rotation length and herbage defoliation height on the WSC, N and neutral detergent fibre (NDF) concentrations of herbage intake of strip-grazing cows.

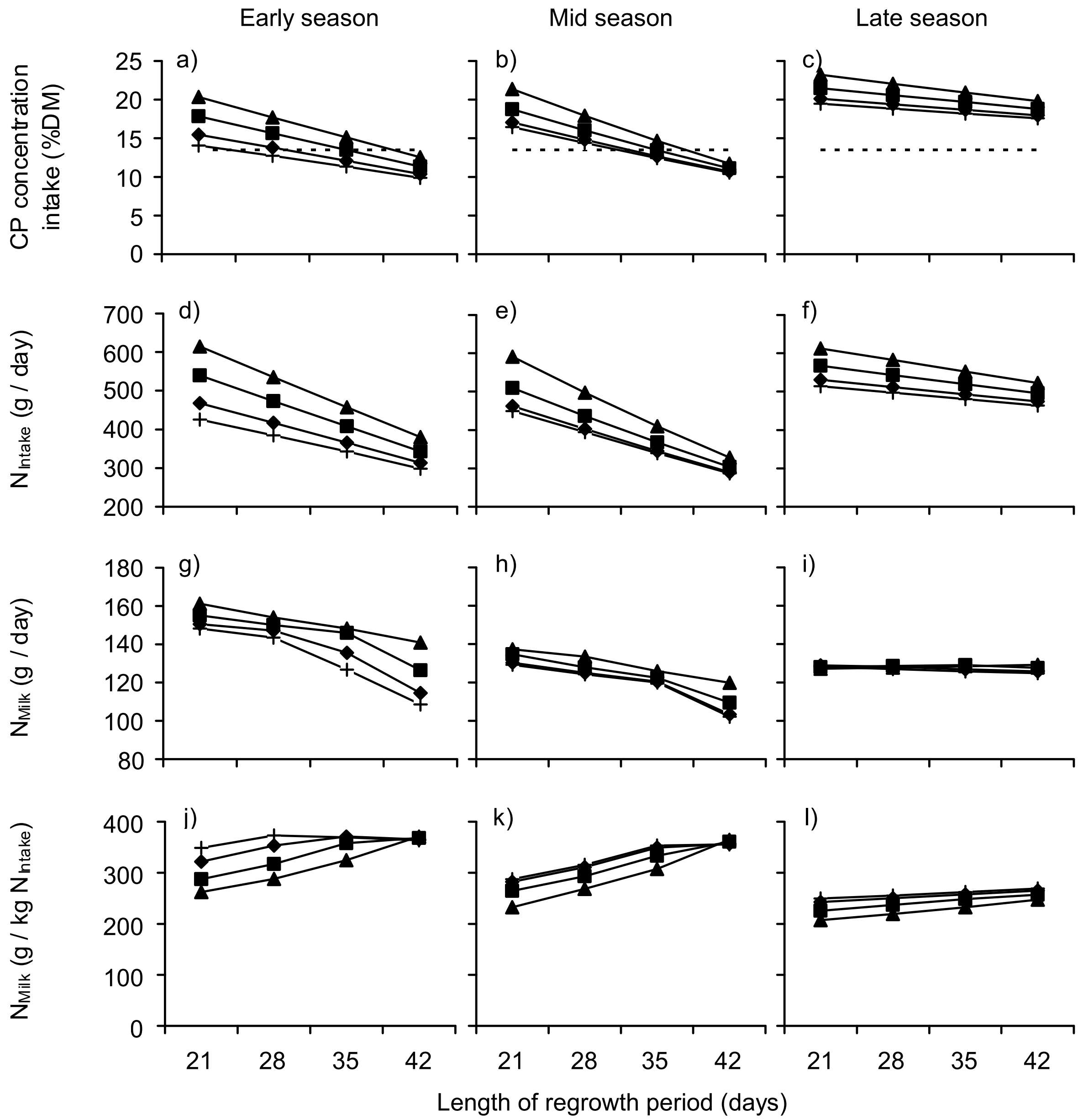

Figure 3 Modelled effect of N application rate (+ 0, ◆ 14, ■ 36 and ▲ 56 kg N / ha / rotation) and length of regrowth period (3, 4, 5 or 6 weeks) on the CP concentration of the intake (%DM), N_{Intake} (g / day), N_{Milk} (g / day) and N_{Milk} (g N / kg N_{Intake}) during early, mid and late season. a−c: --- represents the critical CP concentration (13%).

An overall description of the Cornell Net Carbohydrate and Protein System (CNCPS) model has been given by Fox *et al*. (2004). Briefly, the CNCPS is a mathematical model to evaluate diet and animal performance that was developed from basic principles of rumen function, microbial growth, feed digestion and passage, and animal physiology. The N component of the CNCPS model is a mass-balance model and the N intake is partitioned over maintenance, pregnancy, milk N production, urine and faecal N excretion and N retained, with N retained forming the remainder of the N balance (Hoekstra *et al*., submitted a).

Hoekstra *et al*. (submitted a) linked the HIQ model to the CNCPS model, thus allowing direct modelling of the effect of herbage management on animal production and N efficiency during grazing. In addition to the model outputs of the original herbage intake quality model (N, NDF and WSC concentration of intake), the HIQ model was extended to accommodate the CNCPS requirement for additional feed inputs (Hoekstra *et al*., submitted a).

The combined HIQ-CNCPS model was successfully validated (Hoekstra *et al*., submitted a) and in the current study we applied the model to assess the efficacy of grassland management tools for improving the N utilisation of grazing cows. Therefore, the model was run with all combinations of the following herbage management tools: N application rate (0, 14, 36 and 56 kg N / ha / rotation), rotation length (3, 4, 5, and 6 weeks) and perennial ryegrass cultivar (low sugar and high sugar). Defoliation height was not used as a model variable, as previous studies showed that the effect on herbage quality was small and inconsistent (Hoekstra *et al.*, 2007b). The model cow was a Friesian Holstein dairy cow in its 3rd lactation, with a weight of 574 kg and calving date on the 8th of March. The DM intake was fixed and was assumed to be the average of the HNE (high N efficiency farmlet in the validation experiment: 17.8 (+2 kg concentrates), 16.8 and 16.4 kg DM / day for early, mid, and late season, respectively (Hoekstra *et al*., submitted a, their Table 3).

3. Results

3.1 The effect of N application rate and rotation length

The effects of N application rate and rotation length on the N in the intake (N_{Intake}), milk (N_{Milk}), faeces (N_{Faeces}), urine (N_{Urine}) and live weight change (N_{LWC}) (g N / day) are presented in Fig. 3 and 4 and are discussed below.

3.1.1 N_{Intake}

The effect of N application rate, rotation length on the CP concentration of the herbage intake is presented in Fig. 3 a–c. The CP concentration was lower at lower N application rates and longer rotation lengths, with a significant interaction. For the selected combination of N application rate and rotation length the herbage CP concentration ranged from 100 to 230 g / kg DM. As the herbage DM intake was fixed, the herbage N_{Intake} followed the N concentration of the herbage and ranged from 300 to 600 g N / day (Fig. 3 d–e). During late season, the CP concentration of the intake was much higher, whereas the effect of N application rate on the CP concentration of the intake was relatively small (Fig. 3c). This is related to the large proportion of the grazing area that is affected by dung and urine patches towards the end of the grazing season (Hoekstra & Schulte, accepted).

3.1.2 N_{Milk}

N_{Milk} ranged from 100 to 160 g N / day and decreased as the season progressed (Fig. 3 g–i), due to lower milk production at the later stage of lactation, even though the milk protein concentration increased slightly (3.1, 3.2 and 3.4% during early, mid, and late season, respectively).

During early and mid season, available protein was limiting for milk production in most cases, and N_{Milk} was slightly reduced at lower N application rates and higher rotation lengths. However, the reduction in N_{Intake} was larger than the reduction in N_{Milk}, so in most cases the efficiency of N utilisation for milk (N_{Milk} in g / kg N_{Intake}) was increased (Fig. 3 j–l). At combinations of low N application rates and long rotation lengths, the decrease in milk N output became more pronounced, and the milk N efficiency stabilised. This "cut-off" point corresponds to the point where the rumen N balance becomes lower than 100%. Closer examination of the model output showed that this corresponded to a CP concentration of the intake of approximately 130 g per kg DM, regardless of season or herbage management (Fig. 5 a). Up to this cut-off point, both milk yield and N_{Milk} increase with higher N intakes, whereas after this point the response decreases (Fig. 5 c). During late season, the CP concentration was always higher than 130 g per kg, and the milk yield was limited by energy rather than protein. Therefore a decrease in N_{Intake} did not affect N_{Milk} (g N / day) (Fig. 3 i).

3.1.3 N_{Faeces}

The range in predicted N_{Faeces} (g / day) was less than 20 g N / day within seasons (Fig. 4 a–c) as there was very little effect of herbage management tools. This is in line with other studies which have shown that the faecal N excretion is little affected by diet composition (Castillo *et al.*, 2001; Kebreab *et al.*, 2001), but closely related to total herbage DM intake (Lantinga *et al.*, 1987), which decreased during the course of the grazing season.

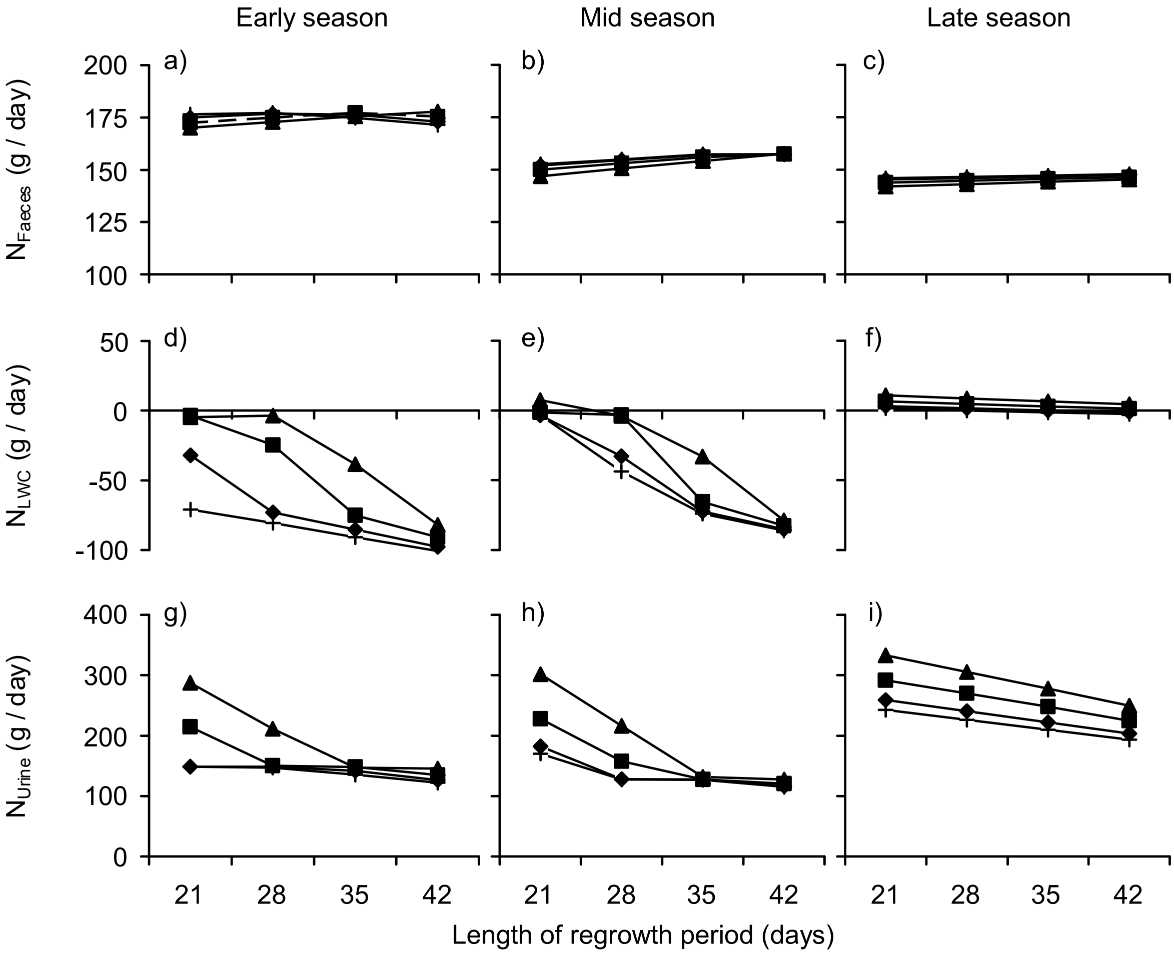

Figure 4 Modelled effect of N application rate (+ 0, ◆ 14, ■ 36 and ▲ 56 kg N / ha / rotation) and length of regrowth period (3, 4, 5 or 6 weeks) on N_{Faeces}, N_{LWC} and N_{Urine} (g / day), during early, mid and late season.

3.1.4 N_{LWC}

During early and mid season N_{LWC} was strongly negative (down to –100 g N / day), especially at low N application rates and long rotation lengths, indicating that body reserves were being used (Fig. 4 d–f). During late season N_{LWC} was slightly positive and not much affected by herbage management. This trend in live weight change is typical for a calving cow (Roche *et al.*, 2006).

3.1.5 N_{Urine}

Long rotation lengths and low N application rates strongly decreased the urine N excretion and N_{Urine} varied from 350 to 120 g / day (Fig. 4 g–i). During early and mid season the response levelled off at a given combination of N application rate and rotation length after which the urine N excretion did not decrease much further. The model prediction of N_{Urine} is based on four components. The amount of bacterial nucleic acids (BNA) and especially degraded tissue N (TN) in the urine are unlikely to be affected by herbage management tools. NEU (metabolisable N supply minus net N use, i.e., inefficiency of use) follows milk production (higher production results in higher production inefficiency losses) (Fox *et al.*, 2003). When rumen N is limiting for bacterial growth, the bacterial excess N (BEN) is zero, but it is directly increasing with excess N. Similar to rumen N balance the bacterial excess N was strongly related to CP concentration in the intake (Fig. 5b), and the cut-off point is at circa 150 g / kg DM. This is higher than the cut-off point for N_{Milk} (130 g / kg DM) and the difference is due to the N recycling to the rumen.

3.2 The effect of high-sugar grass cultivar

Model application showed very little effect of high-sugar cultivar on the N utilisation of grazing cows. The original HIQ model expresses the rapidly degradable carbohydrates in terms of WSC, whereas the CNCPS model uses non-structural

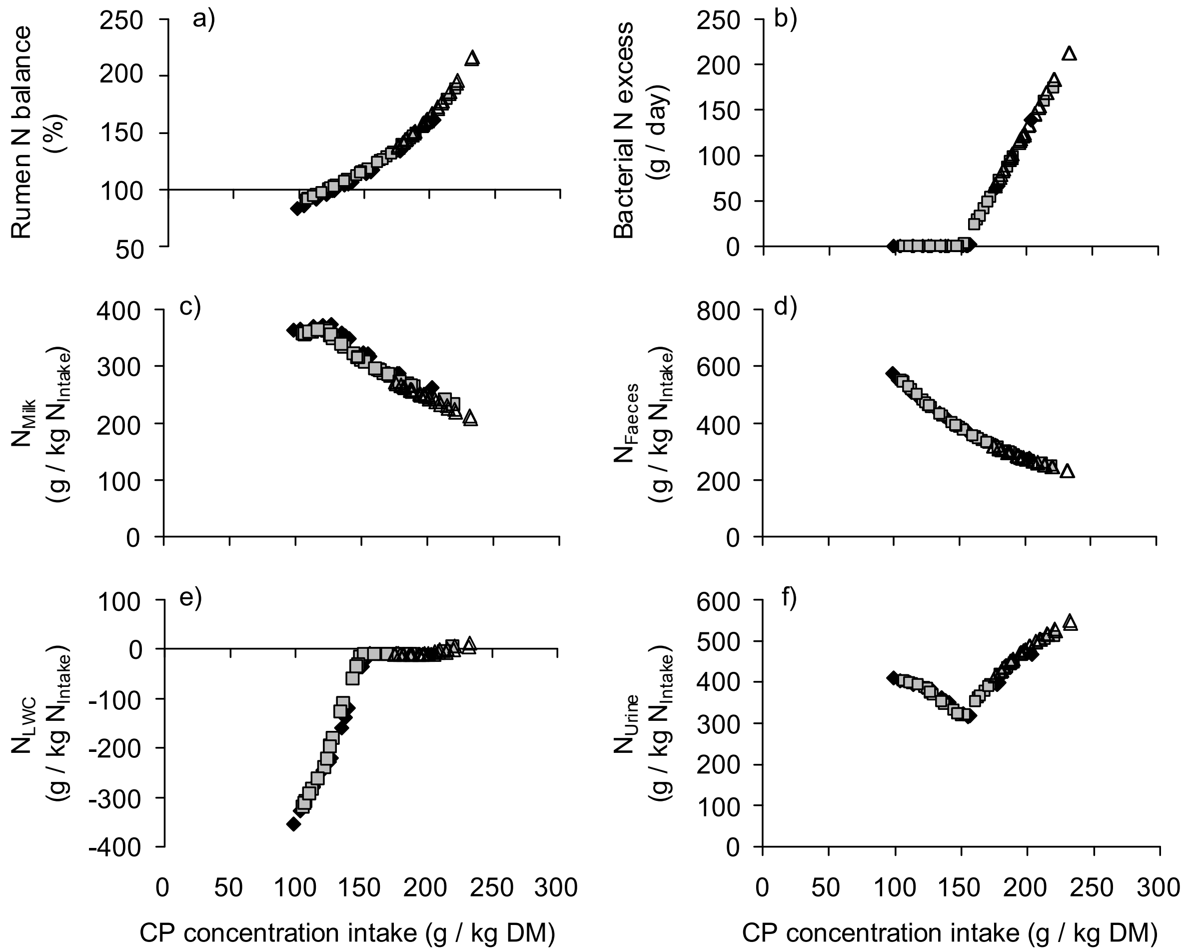

Figure 5 Modelled relation between the CP concentration in the intake (g / kg DM) and a) rumen N balance (%), b) rumen bacterial N excess (g / day), c) N_{Milk} (g / kg N_{Intake}), d) N_{Faeces} (g / kg N_{Intake}), e) N_{LWC} (g / kg N_{Intake}) and f) N_{Urine} (g / kg N_{Intake}) during early (◆), mid (■) and late (△) season.

carbohydrates (NSC). The NSC fraction is calculated as dry matter minus the sum of NDF (adjusted for neutral detergent insoluble protein), crude protein (CP = N × 6.25), fat and ash (Fox *et al.*, 2004). WSC accounted for approximately 70% of NSC (data not shown), the remainder consisting of organic acids (main component), pectins and starch (Van Soest, 1982).

During early season, the HIQ model predicted no or only a very small difference in the non-structural carbohydrate (NSC) concentration between the two cultivars (Hoekstra & Schulte, accepted), and therefore no effect on cow N utilisation could be expected. During mid season and especially late season, the model did predict a higher NSC concentration for the high-sugar cultivar (7% and 22% higher, respectively, data not shown). However, even during mid and late season the difference in N_{Urine} between the high-sugar and low-sugar cultivar was less than 3 g N / day (less than 2% difference) (data not shown).

4. Discussion

The model showed that the CP concentration in ingested herbage DM was the most important factor affecting bovine N utilisation, as the partitioning of ingested N to milk, urine, dung and retained was directly related to this variable (Fig. 5). Figure 5 shows that the 'optimum' CP concentration at which N_{Milk}/N_{Intake} was highest and the N_{Urine}/N_{Intake} was lowest is around 130−150 g / kg DM, across the different seasons. This is slightly lower than the value given by Tamminga & Verstegen (1996) who suggested a minimum CP concentration in pasture of 150 g CP / kg DM in order to avoid impaired ruminal digestion. However, other studies have found that milk production was maintained down to CP concentrations as low as 125 g / kg DM (Peyraud *et al.*, 1997).

4.1 Evaluation of the herbage management tools

4.1.1 N application rate and length of regrowth period

N application rate had a strong effect on the CP concentration in the ingested DM and this interacted with rotation length. The effect was most pronounced during early season when for the three-weekly regrowth the difference in CP concentration between the 0 and 60 N application rates was c. 65 g / kg DM whereas after 6 weeks the difference was less than 30 g / kg DM (Fig. 3 a). During early and mid season, the CP concentration in the ingested DM was limiting for milk production, and a reduction in N application rate from 60 to 0 kg N / ha / rotation slightly reduced the N_{Milk} by 15 and 10 g / day, respectively, in case of the three-weekly regrowth. During late season the reduction of N_{Intake} had little or no effect on the N_{Milk}, as the protein level in the ingested DM remained above 130 g / kg DM. The reduction in N_{Intake} translated directly into reduced N_{Urine}, which decreased from 300 g / day to less than 150 g / day for the three-weekly regrowth in early season. This is in line with the studies reviewed by Peyraud & Astigarraga (1998), which showed that reduced levels of N fertilisation caused only a small (i.e., 5% on average) reduction in milk yield, whereas urinary N excretion was reduced markedly (Peyraud *et al.*, 1997; Astigarraga *et al.*, 2002). Peyraud *et al.* (1997) found that reducing the N application rate from 80 to 0–20 kg N / ha / rotation caused no or only slight reductions in the N_{Milk} and N_{Faeces}, whereas the N_{Urine} was reduced drastically. This resulted in a higher proportion of the N_{Intake} being excreted in milk and faeces (25 → 35%) and a marked reduction in the proportion of N_{Intake} in N_{Urine} (45 → 26%), which is very similar to our model predictions.

4.1.2 High-sugar grass cultivars

In the high-sugar grass cultivar used in this model (cv. Aberdart) the WSC concentration is elevated at the expense of cell wall compared to normal cultivars. This is in contrast to a 'normal' increase in WSC, which is usually at the expense of the N concentration of the herbage (Smith *et al*., 2002; Hoekstra *et al*., 2007b). During mid season and especially late season the model predicted a substantially higher NSC concentration for the high-sugar cultivar, however, during early season, there was no or only a very small difference in the NSC concentration between the two cultivars, and therefore no effect on cow N utilisation could be expected.

It has been hypothesised that the increased WSC concentration in high-sugar grass improves the balance and synchronisation of the N and C supply to the rumen (Miller *et al*., 2001) resulting in a higher N_{Milk} and reduced N_{Urine}. Initial trials seemed promising as some studies showed increased animal production (Lee *et al*., 2001; Miller *et al*., 2001) and reduced urinary N output (Miller *et al*., 2001). More recent studies have shown no effect on milk production, and in some cases a small decrease in urinary N output (Lee *et al*., 2002b; Taweel *et al*., 2005a; Moorby *et al.*, 2006a; Tas *et al.*, 2006, as summarised by Hoekstra *et al.* (2007a), their Table 5).

The sensitivity analysis of the CNCPS model (Hoekstra *et al*., submitted a) showed that a 10% increase in the NDF concentration (corresponding to a drop of c. 20% in NSC concentration, respectively) had indeed very little effect on the rumen N balance. The shift to more rapidly degradable carbohydrates increased the carbon availability for the rumen microbial biomass, allowing for better utilisation of the available protein, thus reducing the rumen N balance. However, the effect was rather small (1 to 2%; Hoekstra *et al*., submitted a, their Table 3) and resulted in a marginal increase in the predicted ME and MP-allowable milk yield. Therefore, the increase in carbohydrate degradability due to a 20% increase in NSC concentration does not appear strong enough to increase milk production and reduce urinary N excretion significantly, under the modelled conditions. Miller *et al.* (2001) reported a slight increase in milk protein concentration, however in the CNCPS model the milk protein concentration is not related to the feed composition.

4.1.3 Defoliation height

We did not cover the effects of defoliation height in the current study, as previous work showed that the effects of defoliation height on the quality of the ingested DM were small, particularly when this defoliation height was maintained at a relatively constant height throughout the growing season (Hoekstra & Schulte, accepted).

4.2 Evaluation of the effectiveness of the herbage quality pathways

4.2.1 Pathway 1: Matching protein supply to animal requirements

The first herbage quality pathway for affecting bovine N efficiency was to match the protein supply, to animal requirements. As in the model simulation the amount of DM ingested was fixed within seasons, the relationship between ingested N and the N utilisation follows the relationship between the CP concentration (g / kg DM) and N utilisation. However, this results in separate lines for each season, as the amount of DM ingested was different for each season. Therefore, under the modelled conditions, N utilisation appears more closely connected to the N concentration of the ingested DM (g N / kg DM) than to the daily intake of N (g N / day). Additionally, model simulations in which the daily N intake was altered by varying the quantity of DM ingested (at constant CP concentration of the ingested DM) showed very little effect of the daily N intake (protein supply) on N utilisation (see also the sensitivity analysis in Hoekstra *et al*., submitted a). However, this may be a model artefact, as the literature suggests that at lower than optimal DM (and therefore N) intakes, a larger proportion of the N would be used for maintenance, resulting in a lower proportion of N used for milk production at lower intakes and thus reduced production levels (Coulon *et al*., 1989). Most studies report the N utilisation as a function of daily N intake (Castillo *et al*., 2000; Kebreab *et al*., 2001; Tas *et al*., 2006), however, recommendations for optimum dietary crude protein requirements are invariably expressed in terms of protein concentrations in ingested DM (g / kg DM), rather than CP ingested (g CP / day) indicating that this is a more generally applicable way of controlling N utilisation.

4.2.2 Pathway 2: balance and synchronisation of energy and N in ingested DM

Generally, in herbage, there is a strong and negative correlation between the CP concentration and the WSC or NSC concentration (Wilman & Wright, 1978; Valk *et* *al*., 2000) as affected by season and herbage management. Therefore, it is hard to tell whether the improved N utilisation is the result of an improved energy and N balance and/or also the result of improved synchronisation of the release of C and N in the rumen (Figs 1 b and c). However, the model predictions for the effect of the high-sugar cultivar show that an increase in NSC concentration, at the expense of NDF rather than CP causing an increased degradation rate of the energy resulting in a synchronisation of release of N and energy in the rumen, has very little effect on N utilisation. When the N_{Milk}/N_{Intake} (g / kg) is plotted against the NSC concentration in the ingested DM, two lines are formed during late and mid season, where different NSC concentrations (high-sugar and low-sugar cultivars) result in a similar N utilisation (data not shown). Therefore, N utilisation appears to be more affected by the improved balance rather than synchronisation. This is in line with the findings of Henning *et al.* (1993) and Newbold & Rust (1992), who concluded that merely synchronising energy and N release rates in the rumen does not necessarily increase microbial yield, partly because of N recycling from the plasma urea pool back into the rumen during periods of N shortage. They suggested that responses in microbial growth ascribed to improved energy and N synchronisation may instead have been the result of an improved overall balance of energy and N supply to the rumen. Because of the strong inverse correlation between the energy and N concentration in grass as affected by herbage management tools, the CP concentration is a good indicator of the balance between N and energy (assuming no soil contamination).

Reducing the degradability of the N in the ingested DM was identified as another pathway to improve the synchronisation. However, Hoekstra *et al*. (accepted) have shown that the effect of herbage management tools on herbage N fractionation was very small. Therefore, we did not find any effect on bovine N efficiency.

4.2.3 Pathway 3: increased proportion of rumen undegradable protein

As the effect of herbage management tools on the N fractionation and thus the proportion N in the form of RUP in the ingested DM was very limited, it was not surprising that this third pathway had no effect on bovine N utilisation.

4.3 Recommendations for further research

The HIQ model does not predict the actual herbage yield and intake, and the current model application did not take into account the effect of herbage management on herbage yield. However, N application rate and length of rotation period have a strong effect on herbage yield. Currently, the model application assumes that changes in herbage yield will be managed by adjusting the stocking rate to allow the same intake per cow. However, in order to be able to extrapolate the results from a per cow basis to a farm or area basis, it is essential to include herbage growth and intake components in the model.

The model could then be used to design a dynamic system in which the grassland management tools are integrated on a yearly basis. The N utilisation is lowest and the N excretion through urine is highest during late season, when the risk of losses through leaching is highest (Schulte *et al.*, 2006). Therefore, the objective of such a management system would be to minimise the N concentration in the ingested DM during late season by minimising the N application rate and maximising the rotation length, within limits to prevent a degradation of herbage quality in terms of NDF and lignin concentration and potential lodging. We hypothesise that the overall bovine N utilisation in grazing only systems (no use of supplements) could be improved by maximising the share of silage in the first cut and minimising the second cut silage would allow for a build up of grass cover for the late season, thus allowing for low N fertiliser application rates and longer durations of regrowth during the autumn. Further experimental work is required to test this hypothesis.

5. Conclusions

- Within the modelled scenario the concentration of CP in the ingested DM was the main factor affecting N utilisation by grazing dairy cows. Model predictions indicated that herbage should be managed to achieve a CP concentration of 130−150 g / kg DM in order to minimise the proportion of N_{Intake} excreted in urine and to optimise the proportion utilised for milk production without affecting milk yield per cow.
- Both N application rate and rotation length were shown to be effective tools for affecting the CP concentration in ingested DM by dairy cows and subsequent cow N utilisation. However, there was no effect of the high-sugar cultivar and defoliation height on cow N utilisation.
- Assessment of the effectiveness of the three herbage quality pathways for improving bovine N utilisation, resulted in the following conclusions:
    1) N utilisation is strongly related to the daily N intake (g / day), however, this seems more connected to the N concentration in the ingested DM (g / kg DM), rather than the actual N intake. Therefore, the effect is more related to the balance between energy and N (pathway 2) than to the quantity of ingested N.
    2) Because of the strong inverse correlation between the energy and N concentration in grass as affected by herbage management tools, the balance between N and energy (expressed in terms of CP concentration in ingested DM) is the most important herbage quality factor for improving bovine N utilisation.

In contrast, the synchronisation between the release of energy and N seemed to have little effect.

3) The proportion of protein in the form of RUP was not much affected by the herbage management tools, and was therefore not an effective pathway for improving the N utilisation of grazing cows.

- It is recommended that the model will be extended to include a herbage yield and intake component. This would allow the model to be used to design herbage management systems to optimise N utilisation on a yearly basis.

Acknowledgements

N.J. Hoekstra was supported under the Teagasc Walsh Fellowship Scheme.

References

Authors and affiliations

References

ADAS, 1998. On farm validation of the Cornell Net Carbohydrate and Protein System. ADAS Bridgets Research Centre Research Report. Martyr Worthy, Winchester, Hampshire, England.

Aharoni, Y., A. Arieli & H. Tagari, 1993. Lactational response of dairy cows to change of degradabitliy of dietary protein and organic matter. *Journal of Dairy Science* 76, 3514–3522.

Aldrich, J.M., L.D. Muller & G.A. Varga, 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance in dairy cows. *Journal of Dairy Science* 76, 1091–1105.

Anonymous, 2001. Herbage varieties: Perennial ryegrasses, Italian ryegrasses and white clover. Irish recommended list 2001/2002. Dublin: Department of Agriculture, Food and Rural Development, 12 p.

Astigarraga, L., J.L. Peyraud & L. Delaby, 2002. Effect of nitrogen fertiliser rate and protein supplementation on the herbage intake and the nitrogen balance of grazing dairy cows. *Animal Research* 51, 279–293.

Balde, A.T., J.H. Vandersall, R.A. Erdman, J.B. Reeves & B.P. Glenn, 1993. Effect of stage of maturity of alfalfa and orchardgrass on in situ dry matter and crude protein degradability and amino acid composition. *Animal Feed Science and Technology* 44, 29–43.

Baumont, R., D. Cohen-Salmon, S. Prache & D. Sauvant, 2004. A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. *Animal Feed Science and Technology* 112, 5–28.

Beever, D.E., R.A. Terry, S.B. Cammell & A.S. Wallace, 1978. The digestion of spring and autumn harvested perennial ryegrass by sheep. *Journal of Agricultural Science (Camb.)* 90, 463–470.

Beever, D.E., M.S. Dhanoa, H.R. Losada, R.T. Evans, S.B. Cammell & J. France, 1986. The effect of forage species and stage of harvest on the process of digestion occurring in the rumen of cattle. *British Journal of Nutrition* 56, 439–454.

Beever, D.E. & C.K. Reynolds, 1994. Forage quality, feeding value and animal performance. Grassland and Society. Proceedings of the 15th General Meeting of the European Grassland Federation, Wageningen, pp. 48–60.

Berry, N.R., M.R.L. Scheeder, F. Sutter, T.F. Kröber & M. Kreuzer, 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling cows. *Annals of Zootechnology* 49, 3–13.

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.

Bosker, T., N.J. Hoekstra & E.A. Lantinga, 2002. The influence of feeding strategy on growth and rejection of herbage around dung pats and their decomposition. *Journal of Agricultural Science (Camb.)* 139, 213–221.

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. France, 2000. A review of efficiency of nitrogen utilisation in dairy cows and its relationship with environmental pollution. *Journal of Animal Feed Science* 9, 1–32.

Castillo, A.R., E. Kebreab, D.E. Beever, J.H. Barbi, H.C. Kirby & J. France, 2001a. The effect of energy supplementation on nitrogen in lactating dairy cows fed grass silage. *Journal of Animal Science* 79, 240–246.

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

Conaghan, P., P. O'Kiely, F.P. O'Mara & P.J. Caffrey, 2002. Yield and chemical composition of lines of *Lolium perenne* L. selected for high water-soluble carbohydrate concentration. Agricultural Research Forum, Tullamore, Ireland, pp. 39.

Cone, J.W., A.H.V. 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 (Camb.)* 126, 7–14.

Coulon, J.B., A. Hoden, P. Faverdin & M. Journet, 1989. Dairy cows. In: Jarrige, R. (ed.) Ruminant Nutrition – recommended allowances and feed tables: Paris: INRA, pp. 73–91.

Coulter, B.S., 2004. Nutrient and trace element advice for grassland, tillage, vegetable and fruit crops. Ireland: Teagasc, 96 p.

Curll, M.J., R.J. Wilkins, R.W. Snaydon & V.S. Shanmugalingam, 1985. The effects of stocking rate and nitrogen fertiliser on a perennial ryegrass-white clover sward. 1. Sward and sheep performance. *Grass and Forage Science* 40, 129–140.

Davies, A., 1965. Carbohydrate levels and regrowth in perennial rye-grass. *Journal of Agricultural Science (Camb.)* 65, 213–221.

Davies, A., 1988. The regrowth of grass swards. In: Jones, M.B. & A. Lazenby (eds.), The Grass Crop, London: Chapman and Hall, pp. 85–117.

Davies, A., R.D. Baker & S.A. Grant, 1993. Sward measurement handbook. Reading: British Grassland Society.

Del Prado, A., L. Brown, R. Schulte, M. Ryan & D. Scholefield, 2006. Principles of development of a mass balance N cycle model for temperate grasslands: an Irish case study. *Nutrient Cycling in Agroecosystems* 74, 115–131.

Delagarde, R., J.L. Peyraud & L. Delaby, 1997. The effect of nitrogen fertilization level and protein supplementation on herbage intake, feeding behaviour and digestion in grazing dairy cows. *Animal Feed Science and Technology* 66, 165–180.

Delagarde, R., J.L. Peyraud, L. Delaby & P. Faverdin, 2000. Vertical distribution of biomass, chemical composition and pepsin-cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. *Animal Feed Science and Technology* 84, 49–68.

Dijkstra, J., H.D.S.C. Neal, D.E. Beever & J. France, 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239–2256.

Dillon, P. & G. Stakelum, 1999. Spring milk production. In: Murphy, J. (ed.) A new agenda for dairying, Ballsbridge, Dublin, Ireland: Teagasc, p. 3–7.

Dinn, N.E., J.A. Shelford & L.J. Fisher, 1998. Use of the Cornell Net Carbohydrate and Protein System and rumen-protected lysine and methionine to reduce nitrogen excretion from lactating dairy cows. *Journal of Dairy Science* 81, 229–237.

Donaghy, D.J. & W.J. Fulkerson, 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. *Grass and Forage Science* 53, 211–218.

Dove, H. & R.W. Mayes, 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A Review. *Australian Journal of Agricultural Research* 42, 913–952.

Duru, M. & H. Ducrocq, 2002. A model of lamina digestibility of Orchardgrass as influenced by nitrogen and defoliation. *Crop Science* 42, 214–223.

FAO, 2007. Food and Agricultural Organization of the United Nations. http://www.fao.org/ag/grassland.htm, last accessed September 2007.

Ferreira, L.M.M., M. Oliván, M.A.M. Rodrigues, K. Osoro, H. Dove & A. Dias-Da-Silva, 2004. Estimation of feed intake by cattle using controlled-release capsules containing n-alkanes or chromium sesquioxide. *Journal of Agricultural Science (Camb.)* 142, 225–234.

Fitzgerald, J.B., A.J. Brereton & N.M. Holden, 2005. Assessment of regional variation in climate on the management of dairy cow systems in Ireland using a simulation model. *Grass and Forage Science* 60, 283–296.

Fox, D.G., T.P. Tylutki, L.O. Tedeschi, M.E.V. Amburgh, L.E. Chase, A.N. Pell, T.R. Overton & J.B. Russell, 2003. The net carbohydrate and protein system for evaluating herd nutrition and nutrient excretion, Model documentation CNCPS version 5.0. Animal Science Mimeo 213, Department of Animal Science, Cornell University, Ithaca, New York, 237 p.

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. & K. Slack, 1995. Leaf number as a criterion for determining defoliation time for *Lolium perenne*: 2. Effect of defoliation frequency and height. *Grass and Forage Science* 50, 16–20.

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.

Garcia, F., P. Carrere, J.F. Soussana & R. Baumont, 2003. How do severity and frequency of grazing affect sward characteristics and the choices of sheep during the grazing season? *Grass and Forage Science* 58, 138–150.

Gastal, F. & G. Lemaire, 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. *Journal of Experimental Botany* 53, 789–799.

Gilliland, T.J., P.D. Barrett, R.L. Mann, R.E. Agnew & A.M. Fearon, 2002. Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science (Camb.)* 139, 257–273.

Grant, S.A., G.T. Barthram & L. Torvell, 1981. Components of regrowth in grazed and cut *Lolium perenne* swards. *Grass and Forage Science* 36, 155–168.

Grant, S.A., D.E. Suckling, H.K. Smith, L. Torvell, T.D.A. Forbes & J. Hodgson, 1985. Comparative studies of diet selection by sheep and cattle: the hill grasslands. *Journal of Ecology* 73, 987–1004.

Grindlay, D.J.C., 1997. Review: Towards and explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *Journal of Agricultural*

Science (Camb.) 128, 377–396.

Groot, J.C.J., 1999. Modelling grass digestibility, on the basis of morphological and physiological plant characteristics, PhD Thesis, Wageningen University, The Netherlands, 120 p.

Gustavsson, A.M. & K. Martinsson, 2001. Analysis of growth and nutrition value in timothy using a dynamic model. *Agricultural and Forest Meteorology* 107, 83–101.

Haynes, R.J. & P.H. Williams, 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy* 49, 119–199.

Henning, P.H., D.G. Steyn & H.H. Meissner, 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. *Journal of Animal Science* 71, 2516–2528.

Hernández Garay, A.H., C. Matthew & J. Hodgson, 2000. The influence of defoliation height on dry-matter partitioning and CO_2 exchange of perennial ryegrass miniature swards. *Grass and Forage Science* 55, 372–376.

Herrera-Saldana, R. & J.T. Huber, 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. *Journal of Dairy Science* 72, 1477–1483.

Herrera-Saldana, R., R. Gomez-Alarcon, M. Torabi & J.T. Huber, 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial synthesis. *Journal of Dairy Science* 73, 142–148.

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

Hoekstra, N.J., P.C. Struik, R.P.O. Schulte & E.A. Lantinga, 2007b. 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, accepted. Modelling the concentrations of nitrogen and water-soluble carbohydrates in grass herbage ingested by cattle under strip-grazing management. *Grass and Forage Science*.

Hoekstra, N.J., P.C. Struik, E.A. Lantinga, M.E.V. Amburgh & R.P.O. Schulte, accepted. Can herbage nitrogen fractionation in *Lolium perenne* be improved by herbage management? *NJAS - Wageningen Journal of Life Sciences*.

Hoekstra, N.J., E.A. Lantinga, R.P.O. Schulte & P.C. Struik, submitted a. Predicting the nitrogen utilisation of grazing dairy cows: model description and validation. *Agricultural Systems*.

Hoekstra, N.J., E.A. Lantinga, R.P.O. Schulte P. O'Kiely & P.C. Struik, submitted b. Grassland management tools for improving the nitrogen efficiency of grazing dairy cows. *European Journal of Agronomy*.

Holliday, R. & D. Wilman, 1965. The effect of fertiliser nitrogen and frequency of defoliation on yield of grassland herbage. *Journal of the British Grassland Society* 20, 32–40.

Hoogendoorn, C.J., C.W. Holmes & A.C.P. Chu, 1992. Some effects of herbage composition, as influenced by previous grazing management, on milk production by cows grazing on ryegrass/white clover pastures. 2. Milk production in late spring/summer: effects of grazing intensity during the preceding spring period. *Grass and Forage Science* 47, 316–325.

Hume, D.E. & J.L. Brock, 1997. Morphology of tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) plants in pastures under sheep and cattle grazing. *Journal of Agricultural Science (Camb.)* 129, 19–31.

Humphreys, M.O., 1989a. Water-soluble carbohydrates in perennial ryegrass breeding. I. Genetic differences among cultivars and hybrid progeny grown as spaced plants. *Grass and Forage Science* 44, 231–236.

Humphreys, M.O., 1989b. Water-soluble carbohydrates in perennial ryegrass breeding. III. Relationships with herbage production, digestibility and crude protein content. *Grass and Forage Science* 44, 423–429.

Humphreys, M.O., 1994. Variation in the carbohydrate and protein content of ryegrasses: potential for genetic manipulation. Breeding for quality. Proceedings of the 19th EUCARPIA Fodder Crops Section Meeting held in Brugge, Belgium, 5–8 October 1994, pp. 165–172.

Hutchings, N.J. & I.J. Gordon, 2001. A dynamic model of herbivore-plant interactions on grasslands. *Ecological Modelling* 136, 209–222.

James, T., D. Meyer, E. Esparza, E.J. DePeters & H. Perez-Monti, 1999. Effects of dietary nitrogen manipulation on ammonia volatilization from manure from Holstein heifers. *Journal of Dairy Science* 82, 2430–2439.

Jarvis, S.C., D.J. Hatch & D.H. Roberts, 1989. The effects of grassland management on nitrogen losses from grazed swards through ammonia volatilization; the relationship to excretal returns from cattle. *Journal of Agricultural Science (Camb.)* 112, 205–216.

Jones, E.L. & J.E. Roberts, 1991. A note on the relationship between palatability and water soluble carbohydrates content in perennial ryegrass. *Irish Journal of Agricultural Research* 30, 163–167.

Jones, R.M. & J.N.G. Hargreaves, 1979. Improvements to the dryweight-rank method for measuring botanical composition. *Grass and Forage Science* 34, 181–189.

Jonker, J.S., R.A. Kohn & R.A. Erdman, 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *Journal of Dairy Science* 81, 2681–2692.

Kauffman, A.J. & N. St.-Pierre, 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cows. *Journal of Dairy Science* 84, 2284–2294.

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.

Kebreab, E., J. France, J.A.N. Mills, R. Allison & J. Dijkstra, 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *Journal of Animal Science* 80, 248–259.

Kennedy, E., D. Hennesy & M. O'Donovan, 2007. Achieving a 300-day grazing season. Moorepark 07. Irish Dairying - Winning on a World Stage, Moorepark Research Centre, Ireland, pp. 25–28.

Kirchgessner, M., W. Windisch & F.X. Roth, 1994. The efficiency of nitrogen conversion in animal nutrition. *Nova Acta Leopold* 70, 393–412.

Kolver, E., D. Muller, G.A. Varga & T.J. Cassidy, 1998b. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *Journal of Dairy Science* 80, 2017–2028.

Kolver, E.S., L.D. Muller, M.C. Barry & J.W. Penno, 1998a. Evaluation and application of the Cornell Net Carbohydrate and Protein System for dairy cows fed diets based on pasture. *Journal of Dairy Science* 81, 2029–2039.

Kröber, T.F., D.R. Külling, H. Menzi, F. Sutter & M. Kreuzer, 2000. Quantitative effects of feed protein reduction and methionine on nitrogen use by cows and nitrogen emission from slurry. *Journal of Dairy Science* 83, 2941–2951.

Lantinga, E.A., J.A. Keuning, J. Groenwold & P.J.A.G. Deenen, 1987. Distribution of excreted nitrogen by grazing cattle and its effects on sward quality, herbage production and utilization. In: Van der Meer, H.G., R.J. Unwin, T.A. Van Dijk & G.C. Ennik (eds.), Animal Manure on Grassland and Fodder Crops, Dordrecht: Marinus Nijhoff Publishers, pp. 103–117.

Lantinga, E.A., N. Gaborcik & B.O.M. Dirks, 1996. Ecophysiological aspects of herbage production in grazed and cut grassland. Grassland and Land use systems, Proceedings of the 16th General meeting of the European Grassland Federation, Italy, pp. 151–161.

Lantinga, E.A. & J.C.J. Groot, 1996. Optimization of grassland production and herbage feed quality in an ecological context. Utilization of local feed resources in dairy cattle. EAAP Publication No. 84, 58–66.

Lantinga, E.A., P.J.A.G. Deenen & H. van Keulen, 1999a. Herbage and animal production responses to fertilizer nitrogen in perennial ryegrass swards. II. Rotational grazing and cutting. *Netherlands Journal of Agricultural Science* 47, 243–261.

Lantinga, E.A., M. Nassiri & M.J. Kropff, 1999b. Modelling and measuring vertical light absorption within grass-clover mixtures. *Agricultural and Forest Meteorology* 96, 71–83.

Lantinga, E.A., M. Duru & J.C.J. Groot, 2002. Dynamics of plant architecture at sward level and consequences for grass digestibility: modelling approaches. Multi-function grasslands, quality forages, animal products and landscapes. Proceedings of the 19th general meeting of the European Grassland Federation, La Rochelle, France, pp. 45–55.

Le Clerc, M.H., 1976. Effects of nitrogen and rest periods on the seasonal production of a pure grass sward. 1. Dry matter production. *Irish Journal of Agricultural Research* 15, 247–255.

Ledgard, S.F., 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil* 228, 43–59.

Lee, M.R.F., E.L. Jones, J.M. Moorby, M.O. Humphreys, M.K. Theodorou, J.C. Macrae & N.D. Scollan, 2001. Production responses from lambs grazing on *Lolium perenne* selected for elevated water-soluble carbohydrate concentration. *Animal Research* 50, 441–449.

Lee, M.R.F., L.J. Harris, J.M. Moorby, M.O. Humphreys, M.K. Theodorou, J.C. Macrae & N.D. Scollan, 2002. Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. *Animal Science* 74, 587–596.

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.

Lindberg, J.E. & E. Lindgren, 1988. Influence of cutting time and nitrogen fertilization on the nutritive value of Timothy: 3. Rumen degradability and estimates energy and protein values. *Swedish Journal of Agricultural Research* 18, 91–98.

Mambrini, M. & J.L. Peyraud, 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. *Reproduction, Nutrition and Development* 34, 9–23.

Mayne, C.S., R.D. Newberry, C.F. Woodcock & R.J. Wilkins, 1987. Effect of grazing severity on grass utilization and milk production of rotationally grazed dairy cows. *Grass and Forage Science* 42, 59–72.

Mc Grath, D., 1992. A note on the influence of nitrogen application and time of cutting on water-soluble carbohydrate production by Italian ryegrass. *Irish Journal of Agricultural Research* 31, 189–192.

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.

Mead, R., R.N. Curnow & A.M. Hasted, 1998. Statistical methods in agriculture and experimental biology. Second edition. London: Chapman and Hall, 415 p.

Metcalf, J.A., D. Wray–Cahen, E.E. Chettle, J.D. Sutton, D.E. Beever, L.A. Crompton, J.C. MacRae, B.J. Bequette & F.R.C. Backwell., 1996. The effect of dietary crude protein as protected soyabean meal on mammary metabolism in the lactating dairy cow. *Journal of Dairy Science* 79, 603–611.

Miller, L.A., J.M. Moorby, D.R. Davies, M.O. Humphreys, N.D. Scollan, J.C. MacRae & M.K. Theodorou, 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* 56, 383–394.

Milne, J.A., J. Hodgson, R. Thompson, W.G. Souter & G.T. Barthram, 1982. The diet ingested by sheep grazing swards differing in white clover and perennial ryegrass content. *Grass and Forage Science* 37, 209–218.

Minson, D.J., 1990. Forage in ruminant nutrition. San Diego, CA: Academic Press, 483 p.

Mishra, S. & S.N. Rai, 1996. Influence of varying RDP:UDP ratios in diets on digestion, nitrogen utilization and milk production efficiency in goats. *Small Ruminant Research* 20, 39–45.

Moorby, J.M., R.T. Evans, N.D. Scollan, J.C. MacRae & M.K. Theodorou, 2006a. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. *Grass and Forage Science* 61, 52–59.

Moorby, J.M., W.J. Fisher, D.W.R. Davies, M.K. Theodorou, N.D. Scollan & J.C. MacRae, 2006b. Milk production from dairy cows grazing pastures of monocultures of Aberdart and Fennema ryegrass. Proceedings of the 8th Research Conference of the British Grassland Society, 4–6 September 2006, Cirenchester. British Grassland Society, Reading, pp. 25–26.

Nevens, F. & D. Reheul, 2003. Effects of cutting or grazing grass swards on herbage yield, nitrogen uptake and residual soil nitrate at different levels of N fertilization. *Grass and Forage Science* 58, 431–449.

Newbold, J.R. & S.R. Rust, 1992. Effect of asynchronous nitrogen and energy supply on growth of ruminal bacteria in batch culture. *Journal of Animal Science* 70, 538–546.

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 (Camb.)* 59, 387–392.

O'Riordan, E.G., P. O'Kiely & M.G. Keane, 1998. Efficient beef production from grazed pasture. End of project report, Grange Research Centre, Teagasc, Ireland, 48 p.

Penning de Vries, F.W.T., A.H.M. Brunsting & H.H.v. Laar, 1974. Products, requirements and efficiency of biosynthesis: a quantitative approach. *Journal of Theoretical Biology* 45, 339–377.

Peyraud, J.L., L. Astigarraga & P. Faverdin, 1997. Digestion of fresh perennial ryegrass ferilized at two levels of nitrogen by lactating dairy cows. *Animal Feed Science and Technology* 64, 155–171.

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.

Pollock, C.J. & T. Jones, 1979. Seasonal patterns of fructosan metabolism in forage grasses. *New Phytologist* 83, 9–15.

Radojevic, I., R.J. Simpson, J.A.S. John & M.O. Humphreys, 1994. Chemical composition and in vitro digestibility of lines of *Lolium perenne* selected for high concentrations of water soluble carbohydrate. *Australian Journal of Agricultural Research* 45, 901–912.

Rearte, D.H., 2005. New insights into the nutritional value of grass. Utilisation of grazed grass in temperate animal systems. Proceedings of a satellite workshop of the XXth International Grassland Congres, Cork, Ireland, pp. 49–59.

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 (Camb.)* 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.

Robson, M.J., G.J.A. Ryle & J. Woledge, 1988. The grass plant - its form and function. In: Jones, M.B. & A. Lazenby (eds.), The Grass Crop, London, New York: Chapman et Hall, pp. 25–84.

Roche, J.R., D.P. Berry & E.S. Kolver, 2006. Holstein-Friesian strain and feed effects on milk production, body weight, and body condition score profiles in grazing dairy cows. *Journal of Dairy Science* 89, 3532–3543.

Ruiz, R., L.O. Tedeschi, J.C. Marini, D.G. Fox, A.N. Pell, G. Jarvis & J.B. Russell, 2002. The effect of a ruminal nitrogen (N) deficiency in dairy cows: evaluation of the Cornell Net Carbohydrate and Protein System ruminal N deficiency adjustment. *Journal of Dairy Science* 85, 2986–2999.

Russell, J.B., J.D. O'Connor, D.G. Fox, P.J.V. Soest & C.J. Sniffen, 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science* 70, 3551–3561.

Sanderson, M.A. & W.F. Wedin, 1989. Nitrogen in the detergent fibre fractions of temperate legumes and grasses. *Grass and Forage Science* 44, 159–168.

Schulte, R.P.O. & E.A. Lantinga, 2002. Mechanistic simulation of the vertical sward structure of mixed swards. *Ecological Modelling* 149, 229–246.

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.

Shewmaker, G.E., H.F. Mayland, C.A. Roberts, P.A. Harrison, N.J. Chatterton & D.A. Sleper, 2006. Daily carbohydrate accumulation in eight tall fescue cultivars. *Grass and Forage Science* 61, 413–421.

Sinclair, L.A., P.C. Garnsworthy, J.R. Newbold & P.J. Buttery, 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science (Camb.)* 120, 251–263.

Sinclair, L.A., P.C. Garnsworthy, J.R. Newbold & P.J. Buttery, 1995. Effect of synchronizing the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science (Camb.)* 124, 463–472.

Smit, H.J., B.M. Tas, H.Z. Taweel, S. Tamminga & A. Elgersma, 2005. Effects of perennial ryegrass (*Lolium perenne* L.) cultivars on herbage production, nutritional quality and herbage intake of grazing dairy cows. *Grass and Forage Science* 60, 297–309.

Smit, H.J., S. Tamminga & A. Elgersma, 2006. Dairy cattle grazing preference among six cultivars of perennial ryegrass. *Agronomy Journal* 98, 1213–1220.

Smith, D., 1973. The nonstructural carbohydrates. In: Butler, G.W. & R.W. Bailey (eds.), Chemistry and Biochemistry of Herbage, London: Academic Press, pp. 105–155.

Smith, K.A. & J.P. Frost, 2000. Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 1: cattle and sheep. *Bioresource Technology* 71, 173–181.

Smith, K.F., R.J. Simpson, R.N. Oram, K.F. Lowe, K.B. Kelly, P.M. Evans & M.O. Humphreys, 1998. Seasonal variation in the herbage yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars with high or normal herbage water-soluble carbohydrate concentrations grown in three contrasting Australian dairy environments. *Australian Journal of Experimental Agriculture* 38, 821–830.

Smith, K.F., R.J. Simpson, R.A. Culvenor, M.O. Humphreys, M.P. Prud'homme & R.N. Oram, 2001. The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (*Lolium perenne* L.). *Journal of Agricultural Science (Camb.)* 136, 65–74.

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 (Camb.)* 138, 375–385.

Stakelum, G. & P. Dillon, 1990. Influence of sward structure and digestibility on the intake and performance of lactating and growing cattle. In: Mayne, C.S. (Ed.) Management issues for the grassland farmer in the 1990s. Occasional Publication No. 25, British Grassland Society, pp. 30–42.

Struik, P.C., 1983. Effect of temperature on development, dry matter production, dry matter distribution and quality of forage maize (*Zea mays* L.). An analysis. Mededelingen Landbouwhogeschool, Wageningen 83–3, pp. 1–41.

‘t Mannetje, L. & K.P. Haydock, 1963. The dry-weight rank method for the botanical analysis of pasture. *Journal of the British Grassland Society* 18, 268–275.

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

Tamminga, S. & M.W.A. Verstegen, 1996. Implications of nutrition of animals on environmental pollution. In: Garnsworthy, P.C., W. Haresign & D.J.A. Cole (eds.), Recent Advances in Animal Nutrition, U.K.: Nottingham University Press, pp. 121–134.

Tas, B.M., H.Z. Taweel, H.J. Smit, A. Elgersma, J. Dijkstra & S. Tamminga, 2006. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. *Journal of Dairy Science* 89, 3494–3500.

Taweel, H.Z., B.M. Tas, H.J. Smit, A. Elgersma, J. Dijkstra & S. Tamminga, 2005a. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. *Animal Feed Science and Technology* 121, 243–256.

Taweel, H.Z., B.M. Tas, B.A. Williams, A. Elgersma, J. Dijkstra & S. Tamminga, 2005b. Fibre degradation rate of perennial ryegrass varieties measured using three techniques: *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production. Proceedings of the XXth International Grassland Congress, Ireland & UK, pp. 228.

Taweel, H.Z., B.M. Tas, H.J. Smit, A. Elgersma, J. Dijkstra & S. Tamminga, 2006. Grazing behaviour, intake, rumen function and milk production of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates. *Livestock Science* 102, 33–41.

Terry, R.A. & J.M.A. Tilley, 1964. The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an *in vitro* procedure. *Journal of the British Grassland Society* 19, 363–372.

Tharmaraj, J., W.J. Wales, D.F. Chapman & A.R. Egan, 2003. Defoliation pattern, foraging behaviour and diet selection by lactating dairy cows in response to sward height and herbage allowance of a ryegrass-dominated pasture. *Grass and Forage Science* 58, 225–238.

Thompson, K.F. & D.P. Poppi, 1990. Livestock production from pasture. In: Langer, R.H.M. (ed.) Pastures, their Ecology and Management, Melbourne: Oxford University Press, pp. 263–283.

Trevaskis, L.M. & W.J. Fulkerson, 1999. The relationship between various animal and management factors and milk urea, and its association with reproductive performance of dairy cows grazing pasture. *Livestock Production Science* 57, 255–265.

Turner, L.B., M.O. Humphreys, A.J. Cairns & C.J. Pollock, 2001. Comparison of growth and carbohydrate accumulation in seedlings of two varieties of *Lolium perenne*. *Journal of Plant Physiology* 158, 891–897.

Ulyatt, M.J., D.J. Thomson, D.E. Beever, R.T. Evans & M.J. Haines, 1988. The digestion of perennial ryegrass (*Lolium perenne* cv. Melle) and white clover (*Trifolium repens* cv. Blanca) by grazing cattle. *British Journal of Nutrition* 60, 137–149.

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.

Valk, H., I.E. Leusink-Kappers & A.M. Van Vuuren, 2000. Effect of reducing nitrogen fertilizer on grassland on grass intake, digestibility and milk production of dairy cows. *Livestock Production Science* 63, 27–38.

Van Horn, H.H., G.L. Newton & W.E. Kunkle, 1996. Ruminant nutrition from an environmental perspective: Factors affecting whole-farm nutrient balance. *Journal of Animal Science* 74, 3082–3102.

Van Loo, E.N., 1993. On the relationship between tillering, leaf area dynamics, and growth of perennial ryegrass (*Lolium perenne* L.). PhD thesis, Agricultural University, Wageningen, The Netherlands, 169 p.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant: Ruminant Metabolism, Nutritional Strategies, the Cellulolytic Fermentation and the Chemistry of Forages and Plant Fibres. Corvallis, Oregon: O. & B. Books, 375 p.

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. & J.A.C. Meijs, 1987. Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows. In: Van der Meer, H.G., R.J. Unwin, T.A. van Dijk & G.C. Ennik (eds.), Animal manure on grassland and fodder crops. Fertilizer or waste?, Dordrecht, The Netherlands: Marinus Nijhoff, pp. 17–25.

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 (Camb.)* 116, 429–436.

Van Vuuren, A.M., F. Krol-Kramer, R.A. van der Lee & H. Corbijn, 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75, 2215–2225.

Waite, R., 1970. The structural carbohydrates and the in vitro digestibility of a ryegrass and a cocksfoot at two levels of nitrogenous fertilizer. *Journal of Agricultural Science (Camb.)* 74, 457–462.

Wilkins, P.W., D.K. Allen & L.R. Mytton, 2000. Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. *Grass and Forage Science* 55, 69–76.

Wilman, D., D. Droushiotis, A. Koocheki, A.B. Lwoga & J.S. Shim, 1976a. The effect of interval between harvests and nitrogen application on the proportion and yield of crop fractions in four ryegrass varieties in the first harvest year. *Journal of Agricultural Science (Camb.)* 86, 189–203.

Wilman, D., D. Droushiotis, A. Koocheki, A.B. Lwoga & J.S. Shim, 1976b. The effect of interval between harvests and nitrogen application on the digestibility and digestible yield and nitrogen content and yield of four ryegrass varieties in the first harvest year. *Journal of Agricultural Science (Camb.)* 86, 393–399.

Wilman, D., A. Koocheki & A.B. Lwoga, 1976c. The effect of interval between harvests and nitrogen application on the proportion and yield of crop fractions and on the digestibility and digestible yield and nitrogen content and yield of two perennial ryegrass varieties in the second harvest year. *Journal of Agricultural Science (Camb.)* 87, 59–74.

Wilman, D., B.M. Ojuederie & E.O. Asare, 1976d. Nitrogen and Italian ryegrass. 3. Growth up to 14 weeks: yields, proportions, digestibilities and nitrogen contents of crop fractions, and tiller populations. *Journal of the British Grassland Society* 31, 73–79.

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 (Camb.)* 89, 53–63.

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 (Camb.)* 91, 381–394.

Wilson, J.R., 1994. Cell wall characteristics in relation to forage digestion by ruminants. *Journal of Agricultural Science (Camb.)* 122, 173–182.

Woodward, S.J.R., 1998. Bite mechanics of cattle and sheep grazing grass-dominant swards. *Applied Animal Behaviour Science* 56, 203–222.

Yin, X., E.A. Lantinga, A.H.C.M. Schapendonk & X. Zhong, 2003. Some Quantitative relationships between leaf area index and canopy nitrogen content and distribution. *Annals of Botany* 91, 893–903.

Authors and affiliations

N.J. Hoekstra [1, 2, 3]
R.P.O. Schulte [1]
P.C. Struik [2]
E.A. Lantinga [3]
P. O'Kiely [4]
M.E. Van Amburgh [5]

[1] Teagasc, Johnstown Castle Research Centre, Wexford, Ireland

[2] Crop and Weed Ecology Group, Wageningen University, Haarweg 333, 6709 RZ Wageningen, The Netherlands

[3] Biological Farming Systems Group, Wageningen University, Marijkeweg 22, 6709 PG Wageningen, The Netherlands

[4] Teagasc, Grange Beef Research Centre, Dunsany, Co. Meath, Ireland

[5] Department of Animal Science, Cornell University, Ithaca, NY 14853, USA

Summary

Samenvatting

Summary

Background, problem and objectives

Livestock production has been identified as a major source of nitrogen (N) losses in agro-ecosystems. N excreted in dung and urine contributes to environmental N pollution either as ammonia and N oxides in air, or as nitrate in soil and ground water. Therefore, it is important to reduce N output through animal excretions by improving N utilisation by the animal. In general, the N utilisation of cows for milk production is often lower than 25% of N intake, although the theoretical maximum of efficiency of N utilisation may reach 40 to 45% of N intake.

Bovine N utilisation can be increased substantially through changing the composition of the diet, resulting in a significant reduction of N losses to the environment. In indoor feeding systems, this diet composition can be controlled by using protein and carbohydrate supplements. However, in many parts of Europe, a large proportion of the bovine's diet consists of grass taken up by grazing. Specifically, in Ireland and UK, increasing the proportion of grazed grass in the cow's diet is seen as an important way of improving farm profitability, resulting in increasingly longer grazing seasons. Manipulating the nutritional composition of grazed grass poses a complex challenge, since it is hard to control the diet under grazing as this depends on grassland management and environmental factors.

The objective of this research was to investigate the efficacy of grassland management tools for manipulating herbage quality and to assess the subsequent effect on the N efficiency of grazing cows. The research consisted of a combination of literature review, plot experiments, animal experiments and modelling work.

Identifying pathways for improving the N efficiency of grazing bovines

In the literature review (Chapter 2), three pathways were identified through which more efficient N utilisation by grazing bovines can be achieved by manipulation of the chemical composition of the grass forage: 1) matching protein supply to animal requirements, 2) balancing and synchronising carbohydrate and N supply in the rumen, and 3) increasing the proportion of rumen undegradable protein (RUP).

Matching the diet requirements of grazing bovines through herbage manipulation encompasses the manipulation of carbon (C) and N concentrations of growing herbage. These C and N concentrations vary both spatially within the grass sward and over time. Under grazing conditions, grassland management tools, such as the length of the regrowth period, defoliation height, fertiliser N application rate, and growing high-sugar grass cultivars, are the main pathways to manipulate herbage quality and subsequent bovine N efficiency. However, these management tools are interrelated and may show adverse effects on production. Due to the complex nature of interactions, modelling is essential in order to quantify and predict the effect of any combination of herbage management tools under specific circumstances.

The effect of grassland management tools on herbage quality

A field experiment comprising the factorial combinations of three fertiliser N application rates (0, 90 and 390 kg N / ha / year), three regrowth periods (2–3, 4–5, and 6–7 weeks), two perennial ryegrass (*Lolium perenne* L.) cultivars [Aberdart (high sugar concentration) and Respect (low sugar concentration)] 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 in 2002 and 2003.

In Chapter 3, the results of the concentrations of water-soluble carbohydrates (WSC), N, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and ash in both sheath and lamina material are reported. Expressing NDF and ADF concentrations in g / kg WSC-free dry matter (DM) increased the consistency of treatment effects. The high-sugar cultivar had generally higher WSC concentrations than the low-sugar cultivar, especially during the late season. Moreover, the relative difference in WSC concentration between the two cultivars tended to be higher for the lamina material than for the sheath material, which suggests that the high-sugar trait may be more important under grazing conditions, when lamina forms the bulk of the intake, than under mowing regimes. Longer regrowth periods and lower N application rates increased WSC concentrations and decreased N concentrations; interactions between regrowth period and N application rate were highly significant. The concentrations of NDF and ADF were much less influenced. The NDF concentration in terms of g / kg WSC-free DM tended to be higher at lower N application rates and at longer regrowth periods. The effect of cutting height on herbage chemical composition was unclear. High-sugar cultivars, N application rate and length of the regrowth period were concluded to be important tools for manipulating herbage quality.

Chapter 4 presents the results of the same experiment for N fractionation, with the hypothesis that protein degradability as measured by the Cornell Net Carbohydrate and Protein System (CNCPS) protein fractionation scheme, can be manipulated by grassland management tools. The grass 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 the length of the 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 grassland management.

The effect of grassland management tools on the quality of herbage ingested during grazing

The impact of grassland management on herbage quality has been well described, but due to asymmetric grazing of animals between individual plant organs, this information needs to be translated into the quality of the herbage ingested during grazing.

In Chapter 5, a herbage intake quality (HIQ) model is presented with the objective to assess the efficacy of individual grassland management tools for manipulating the WSC, NDF and N concentrations of the herbage intake of strip-grazing bovines throughout the growing season. There was a good correlation between predicted and observed WSC concentration in the intake (R^2 = 0.78, $p < 0.001$). The correlation between predicted and observed NDF concentrations in the intake was reasonable (R^2 = 0.49, $p < 0.05$) with a small absolute bias. Differences in the N concentration between laminae and sheaths and between clean patches and fouled patches were adequately simulated, although N concentration of the intake was over-predicted in early season and under-predicted during the late season. However, this did not affect the ability of the model to assess the efficacy of grassland management tools for manipulating the WSC and N concentration in the intake of strip-grazing bovines.

Model application showed that reduced N application rates and longer rotation lengths were effective tools for manipulating the quality of the herbage ingested during early and mid season. During late season, the large proportion of area affected by dung and urine reduced the impact of N application rate on herbage quality. In contrast, relative differences between high-sugar and low-sugar cultivars were largest during this period. This suggests that high-sugar cultivars may be an important tool to increase bovine N-efficiency during late season, when risks of N losses to water are largest. The model output showed that defoliation height affects the chemical composition of the intake of both the current and the subsequent period, but the two effects are opposite. When defoliation height is the same at the start and end of the grazing period, the chemical composition of the intake tended to be similar for the low and high defoliation height.

The effect of grassland management tools on the nitrogen efficiency of grazing bovines

In Chapter 6, we developed a tool for predicting efficacy of grassland management tools for improving the N utilisation of grazing dairy cows by linking the HIQ model to the Cornell Net Carbohydrate and Protein System (CNCPS) model, a mathematical model to evaluate diet and animal performance that was developed from basic principles of rumen function, microbial growth, feed digestion and passage, and animal physiology.

Firstly, the HIQ model was extended to predict the input required by the CNCPS model. Subsequently, the combined HIQ-CNCPS model was validated using data from a grazing experiment with two farmlets consisting of 15 dairy cows each, during three distinct periods in the grazing season. The correlations between the observed and predicted values of milk production and the N partitioning over milk, faeces, life weight change and urine were highly significant, and predictions were reasonably accurate (R^2 0.52 to 0.77 on individual cow basis; R^2 0.77 to 0.97 on herd basis) with some relative biases but only one absolute bias (5% under-prediction of faeces N). Sensitivity analyses of the CNCPS model showed that the herbage crude protein (CP) concentration was the most important herbage quality factor for improving bovine N efficiency.

In Chapter 7 we applied the HIQ-CNCPS model and the results of the previous Chapters to evaluate the effectiveness of a) the grassland management tools and b) the herbage quality pathways identified in Chapter 2 on the N utilisation of grazing dairy cows.

Within the modelled scenario the concentration of CP in the ingested dry matter (DM) was the main factor affecting N utilisation. Model predictions indicated that herbage should be managed to achieve a CP concentration of 130−150 g / kg DM in order to maximise the efficiency of N utilisation for milk production and minimise the proportion of N excreted in urine. Both N application rate and rotation length were shown to be effective tools for affecting the CP concentration of the intake and subsequent cow N utilisation. However, there was no effect of the high-sugar cultivar and defoliation height on cow N utilisation. Assessment of the effectiveness of the three herbage quality pathways for improving bovine N utilisation resulted in the following conclusions:

1) N utilisation is strongly related to the daily N intake (g / day), however, this

seems more connected to the N concentration of the ingested DM (g / kg DM), rather than the actual daily N intake. Therefore, the effect is more related to the balance between energy and N (pathway 2), than to the quantity of ingested N.

2) Because of the strong inverse correlation between the energy and N concentration in grass as affected by herbage management tools, the balance between N and energy is the most important herbage quality factor for improving bovine N utilisation. In contrast, the synchronisation between the release of energy and N seems to have little effect.

3) The proportion of protein in the form of RUP is not much affected by the herbage management tools, and is therefore not an effective pathway for improving the N utilisation of grazing cows.

It is recommended that the model will be extended to include a herbage yield and intake component. This would allow the model to be used to design herbage management systems to optimise N utilisation on a yearly basis.

Samenvatting

Achtergrond, probleem en doelstellingen

Dierlijke productie systemen zijn een belangrijke bron van stikstof (N) verliezen in agro-ecosystemen. N uitgescheiden in mest en urine draagt bij aan milieuvervuiling in de vorm van ammoniak en stikstofoxiden in de lucht of als nitraat in bodem en grondwater. Het is daarom belangrijk om de N-uitstoot in dierlijke uitwerpselen te verminderen door de N-benutting door het dier te verhogen. Normaal is de efficiëntie waarmee de N in de voeropname wordt omgezet in melk minder dan 25%, terwijl het theoretische maximum op 40 tot 45% ligt.

De N-benutting door koeien kan verbeterd worden door de samenstelling van het voer aan te passen, met een significante vermindering van N-verliezen naar het milieu als gevolg. In stalvoedersystemen kan de voersamenstelling worden geregeld door middel van krachtvoer rijk aan eiwitten of koolhydraten. In een groot deel van Europa bestaat een belangrijk deel van de voeropname uit gras opgenomen tijdens beweiding. Met name in Ierland en Groot Brittannië wordt het vergroten van het aandeel begraasd gras in het dieet gezien als een belangrijke manier om de rendabiliteit van het boerenbedrijf te vergroten, hetgeen leidt tot steeds langer wordende weideseizoenen. Het manipuleren van de nutritionele samenstelling van het rantsoen onder beweiding is een complex probleem: het is moeilijk om de samenstelling van het rantsoen te beïnvloeden omdat dit afhangt van graslandbeheer en omgevingsfactoren.

De doelstelling van dit onderzoek was om de effectiviteit van graslandbeheerstechnieken voor het beïnvloeden van de graskwaliteit en het daaruit voortvloeiende effect op de N-benutting van koeien onder beweiding te onderzoeken. Het onderzoek bestond uit een combinatie van literatuur-onderzoek, veldproeven, dierproeven en modelleerwerk.

Identificatie van manieren om de N-benutting van koeien onder beweiding te vergroten

In het literatuuronderzoek (hoofdstuk 2), zijn drie manieren geïdentificeerd door middel waarvan een betere N-benutting door koeien onder beweiding bereikt kan worden door de manipulatie van de chemische samenstelling van het gras: 1) het eiwit aanbod afstemmen op de behoefte, 2) verbeterde balans en synchronisatie van koolhydraat en eiwit levering aan de pens en 3) het vergroten van het aandeel bestendig eiwit.

Om de samenstelling van het rantsoen te manipuleren door middel van graslandbeheer moeten de koolstof- en N-gehalten van het groeiende gras worden gemanipuleerd. Deze concentraties variëren zowel ruimtelijk binnen de graszode als ook in de tijd. Graslandbeheerstechnieken, zoals de lengte van de hergroei periode, graashoogte, kunstmest-N-gift en het telen van grasrassen met een hoog suikergehalte, zijn de belangrijkste manieren om de kwaliteit van het gras en de daaruit voortvloeiende N-benutting van koeien te beïnvloeden. Deze graslandbeheers-technieken zijn echter onderling gerelateerd, en hebben mogelijk een nadelige invloed op de productie. Vanwege de complexe aard van de interacties is modelleren essentieel om de effecten van combinaties van deze graslandbeheerstechnieken te kunnen kwantificeren.

Het effect van graslandbeheerstechnieken op graskwaliteit

Een veldexperiment bestaande uit de factoriële combinatie van drie kunstmest-N-giften (0, 90 en 390 kg N / ha / jaar), drie lengtes van de hergroeiperiode (2–3, 4–5 and 6–7 weken), twee Engels raaigras (*Lolium perenne* L.) grasrassen [Aberdart (hoog suiker gehalte, HS) en Respect (laag suiker gehalte, LS)] en twee maaihoogten (*circa* 8 en 12 cm) werd uitgevoerd te Teagasc, Johnstown Castle Research Centre, Wexford, Ierland. De veldjes werden gedurende vier seizoenen in 2002 en 2003 bemonsterd.

De resultaten van de gehalten van wateroplosbare koolhydraten (WOK), N, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignine en as in zowel schede als lamina-materiaal worden in hoofdstuk 3 gepresenteerd. De effecten van de verschillende behandelingen op het gehalte van NDF en ADF waren consistenter wanneer de NDF en ADF werden uitgedrukt in g / kg WOK-vrije droge stof (DS). Het HS-ras had over het algemeen een hoger WOK-gehalte dan het LS-ras, met name tijdens het late seizoen. Bovendien was het relatieve verschil in WOK-gehalte tussen de twee rassen over het algemeen hoger voor lamina dan voor schede-materiaal. Dit suggereert dat het HS-ras van groter belang is onder beweiding, wanneer het lamina-materiaal de bulk van de opname vormt, vergeleken met maaien. Langere hergroeiperiodes en verlaagde kunstmest-N-giften verhoogden het WOK-gehalte en verlaagden het N-gehalte en de interacties waren zeer significant. Het effect van maaihoogte op de chemische samenstelling van lamina en schede was onduidelijk. De conclusie luidt dan ook: HS-grasras, kunstmest-N-gift en de lengte van de hergroei periode zijn de belangrijkste graslandbeheerstechnieken voor het beïnvloeden van graskwaliteit.

In hoofdstuk 4 worden de resultaten van hetzelfde experiment met betrekking tot N-fractionering gepresenteerd. De hypothese was dat de afbreekbaarheid van eiwit zoals gemeten in het Cornell Net Carbohydrate and Protein System (CNCPS) eiwit fractioneringssysteem, beïnvloed kan worden door graslandbeheerstechnieken. De eiwitten in gras waren zeer oplosbaar en gemiddeld 19% and 28% van de totale N was in de vorm van niet-eiwit N, 16% and 19% in de vorm van buffer-oplosbaar eiwit, 52 and 40% in de vorm van buffer-onoplosbaar eiwit en 12 and 13% in de vorm van potentieel beschikbare celwand-N voor lamina en schede. Voor zowel lamina als schede was slechts 0,9% van de totale N aanwezig in de vorm van niet-beschikbare celwand-N. Over het algemeen hadden de bestudeerde graslandbeheerstechnieken niet veel effect op de eiwitfractionering. De effecten van de lengte van de hergroeiperiode, grasras en maaihoogte waren klein en inconsistent. Hoge kunstmest-N-giften verhoogden de eiwitafbreekbaarheid, vooral gedurende het late seizoen. Dit is belangrijk aangezien onderzoek heeft aangetoond dat verhoogde afbreekbaarheid van eiwitten het potentiële verlies van N via de urine verhoogt, en deze N is vooral tegen het einde van seizoen gevoelig voor uitspoeling. Het effect was echter het sterkst voor schede-materiaal, hetgeen slechts een klein onderdeel van de totale grasopname vormt tijdens begrazing. Daarom werd geconcludeerd dat er weinig potentie is om de N-fractionering van het gras te manipuleren door middel van graslandbeheerstechnieken.

Het effect van graslandbeheerstechnieken op de kwaliteit van de grasopname onder beweiding

Er is een goede kennis van het effect van graslandbeheerstechnieken op graskwaliteit, maar omdat koeien asymmetrisch grazen tussen individuele plantorganen en de dierlijke uitwerpselen de graskwaliteit beïnvloeden, moet deze informatie worden vertaald naar de kwaliteit van het gras opgenomen tijdens beweiding.

In hoofdstuk 5 wordt een model voor de kwaliteit van grasopname (HIQ) gepresenteerd met de doelstelling om de effectiviteit van individuele graslandbeheerstechnieken voor het manipuleren van de WOK-, NDF- en N-gehaltes van de grasopname van stripgrazende koeien gedurende het groeiseizoen te bepalen. Er was een goede correlatie tussen het voorspelde en geobserveerde WOK-gehalte in de opname ($R^2 = 0,78$, $p < 0,001$). De correlatie tussen de voorspelde en geobserveerde NDF gehalten in de opname was redelijk ($R^2 = 0,49$, $p < 0,05$) met een kleine absolute afwijking. De verschillen in N-gehalten tussen lamina- en schede-materiaal en gebieden die al dan niet door excreta waren beïnvloed, werden goed gesimuleerd. Het N-gehalte in de opname werd echter overschat in het vroege seizoen en onderschat tijdens het late seizoen. Dit had evenwel geen effect op het vermogen van het model om de effectiviteit van graslandbeheerstechnieken voor het manipuleren van de WOK- en N-gehalten in de opname van stripgrazende koeien te beoordelen.

De modeltoepassing liet zien dat lagere kunstmest-N-giften en langere rotatieduur effectieve technieken waren om de kwaliteit van de grasopname te beïnvloeden tijdens het vroege en midden-seizoen. Het grote aandeel van het areaal dat beïnvloed werd door mest en urine, verminderde het effect van kunstmest-N-gift op graskwaliteit gedurende het late seizoen. Daarentegen was het relatieve verschil tussen het HS en het LS ras het grootst tijdens het late seizoen. Dit suggereert dat het HS ras een belangrijk middel zou kunnen zijn om de N-benutting van koeien te vergroten tijdens het late seizoen, wanneer het risico van N-verliezen door uitspoeling het grootst is. De modeluitvoer liet zien dat de graashoogte de kwaliteit van de opname van zowel de huidige als de volgende periode beïnvloedde, maar in tegenovergestelde richting. Als de graashoogte aan het begin en einde van de periode gelijk was, was er weinig verschil in de kwaliteit van de grasopname tussen de hoge en lage graashoogte.

Het effect van graslandbeheerstechnieken op de N-benutting van grazende koeien

In hoofdstuk 6 ontwikkelen we een model om de effectiviteit van graslandbeheerstechnieken voor het verbeteren van de N-benutting van grazende melkkoeien te verbeteren, door het HIQ model te koppelen aan het Cornell Net Carbohydrate and Protein System (CNCPS) model. Het CNCPS model is een mathematisch model om de voeding en dierlijke productie te evalueren en is ontwikkeld op basis van de grondbeginselen van pensfunctionering, microbiële groei, voedervertering en -passage en dierfysiologie.

Eerst werd het HIQ model uitgebreid om de invoervariabelen benodigd voor het CNCPS model te voorspellen. Vervolgens werd het gecombineerde HIQ-CNCPS model gevalideerd met behulp van gegevens van een beweidingsexperiment bestaande uit twee systemen met elk 15 melkkoeien, gedurende drie perioden. De correlaties tussen de geobserveerde en voorspelde waarden voor melk productie en de N-verdeling over melk, mest, gewichtsverandering en urine waren zeer significant en de voorspellingen waren voldoende nauwkeurig (R^2 0,52 tot 0,77 op basis van de individuele koe; R^2 0,77 tot 0,97 op basis van de gehele kudde) met enkele relatieve afwijkingen maar slechts één absolute afwijking (5% onderschatting van de N in mest).

In hoofdstuk 7 werd het HIQ-CNCPS model en de resultaten van de voorgaande hoofdstukken toegepast om de effectiviteit van a) de graslandbeheerstechnieken en b) de graskwaliteitsaspecten geïdentificeerd in hoofdstuk 2 voor het verbeteren van de N-benutting van grazende melkkoeien te evalueren.

Binnen het modelscenario was het CP gehalte van de DS-opname de belangrijkste factor voor het beïnvloeden van de N-benutting. Modelvoorspellingen lieten zien dat om de N-benutting voor melkproductie te maximaliseren en het aandeel van N in de urine te minimaliseren het gras zodanig beheerd moest worden dat een CP-gehalte van 130–150 g / kg DS werd bereikt. Zowel de kunstmest-N-gift als de rotatie-duur bleken effectieve middelen om het CP-gehalte in de opname en de daaruit voortvloeiende N-benutting van de koe te manipuleren. Er was echter geen effect van

HS grasras en graashoogte op de N-benutting. De beoordeling van de effectiviteit van de drie graskwaliteitsaspecten voor het verbeteren van de N-benutting van koeien leidde tot de volgende conclusies:

1) N-benutting is sterk gecorreleerd aan de dagelijkse N opname (g / dag), maar dit lijkt meer het gevolg te zijn van het N gehalte in de DS-opname (g / kg DS) dan de dagelijkse N opname zelf. Daarom is het effect voornamelijk het resultaat van een verbeterde balans tussen energie en N (aspect 2) in plaats van de hoeveelheid opgenomen N.
2) De balans tussen N en energie is het belangrijkste graskwaliteitsaspect voor het verbeteren van N-benutting door de koe, vanwege de sterke negatieve correlatie tussen het energie- en het N-gehalte in het gras. De synchronisatie tussen energie en N bleek daarentegen weinig effect te hebben.
3) Het aandeel bestendig eiwit werd niet sterk beïnvloed door graslandbeheerstechnieken en is daarom geen effectief kwaliteitsaspect om de N-benutting van grazende koeien te verbeteren.

We bevelen aan het model uit te breiden met een grasopbrengst en -opname component. Dit zou het mogelijk maken het model te gebruiken om graslandbeheerssystemen te ontwerpen die de N-benutting op een jaarlijkse basis optimaliseren.

Acknowledgements

Curriculum Vitae

Education plan

Acknowledgements

After years of reading, experimenting, modelling and writing this PhD has come to an end. Numerous people have supported me throughout this period and I would like to thank them accordingly:

Firstly, I would like to thank my supervisor Dr. Rogier Schulte for his ever stimulating input into the PhD. I am proud to be one of the few people who managed to get you to do field and lab work, and the golden tiller award (for the successful dissection of a quarter of a million *Lolium perenne* tillers) will have a permanent place in my office to prove just that!

Also, I would like to thank Rioch Fox, John Murphy and Alan Cuddihy for helping me out with the maddest of jobs (e.g. separating grass into leaf and stem; collecting freshly dropped dung pats from grazing cows at 7 o'clock in the morning). To the Johnstown crowd: a big thank you for all the friendship and support throughout my PhD. Couldn't have done it without you.

Further, I would like to acknowledge the people in the Department of Plant Sciences of Wageningen University. Thanks to my promotor Prof. Dr. Ir. Paul Struik for his support throughout the PhD and incredibly rapid response to numerous questions and manuscripts. I am equally indebted to Dr. Ir. Egbert Lantinga for his feedback and push to get that bit extra out of the data. Even though most of my contact with promoter and co-promotor was through email, their visits to Ireland ensured that they kept in touch with the actual work. There is something to be said for having supervisors all to yourself for one or two days every year, with the added bonus of sampling the local pubs, restaurants and countryside.

Hennie Halm I would like to thank for the incredible amount of work on the thousands of grass samples that have been analysed over the years. It was a pleasure working with you!

I would like to thank the 'CNCPS team' (M.E. Van Amburgh, T.P. Tylutki, D.G. Fox, L.O. Tedeschi, A.N. Pell) for hosting my visit to Cornell University in 2002 and for providing an Excel version of their model.

I am grateful to Michael O'Donovan in Teagasc, Moorepark Research Centre, Ireland, for his help with the alkane technique and to J. Nash for freeze-drying grass samples.

Many thanks to Padraig O'Kiely in Teagasc, Grange Beef Production Centre, Ireland for helping me onto the right track at the very start of the project and for the sharp comments on some of the manuscripts.

Furthermore, I would like to thank Janneke, Eveline en Elisabeth for hosting my stays in Wageningen for days, weeks or even months at a time, and providing me with a home and bicycle.

Famylje en freonen, bedankt foar de steun troch de jierren, sels safier fan hûs ha jim dochs hieltiid rjocht efter my stien. Arjan, do witst no mear fan gers dan datsto oait witte woedest. Tige by tige tank foar alles! Do bist minstens lyke bliid as ik dat de PhD no klear is: tiid foar ús nije projekt!

Nyncke

Curriculum Vitae

Nyncke Jitske Hoekstra was born on the 28th of November 1978 in Twijzelerheide, The Netherlands. She followed high school at the Lauwers College in Buitenpost and graduated from the VWO in 1997, and subsequently studied Crop Science at Wageningen University. Graduated in 2001, she specialised in organic farming systems and rural sociology, and achieved her MSc in Crop Science in September 2002. In January 2002 she was employed by Teagasc as a Walsh Fellow at the Johnstown Castle Environmental Research Centre in Wexford, Ireland, where she carried out a PhD for the Plant Sciences Group of Wageningen University in Wageningen, The Netherlands. Since July 2006, she is employed by Teagasc as a research officer at the same centre.

Nyncke Jitske Hoekstra is geboren op 28 november 1978 te Twijzelerheide. Ze behaalde haar VWO diploma op het Lauwers College te Buitenpost in 1997 en ging vervolgens plantenteeltwetenschappen studeren aan Wageningen University. Ze deed afstudeervakken in biologische bedrijfssystemen en rurale sociologie en behaalde haar MSc in september 2002. In januari 2002 werd ze aangesteld door Teagasc als een Walsh Fellow aan het Johnstown Castle Environmental Research Centre in Wexford, Ierland, waar ze een PhD deed voor de Plant Sciences Group van Wageningen Universiteit, Nederland. Ze is sinds juli 2006 werkzaam bij Teagasc als onderzoeker bij hetzelfde onderzoekscentrum.

PE&RC PhD Education Certificate

With the educational activities listed below, the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities).

Review of Literature (5.6 credits)
- N.J. Hoekstra, R.P.O. Schulte, P.C. Struik & E.A. Lantinga, 2007. Pathways for improving the nitrogen efficiency of grazing bovines. *European Journal of Agronomy* 26, 363-374

Writing of Project Proposal (7 credits)
- Improving the nitrogen efficiency of grazing bovines (2002)

Laboratory Training and Working Visits (4.2 credits)
- Methods for chemical analyses of feedstuffs and discussing options to use the Cornell Net Carbohydrate and Protein System for PhD research (3wks); Cornell University, USA (2003)

Post-Graduate Courses (2.3 credits)
- Statistical analysis & design using SAS; University College Dublin (2007)
- Making science work on the farm: workshop on decision support systems for Irish agriculture (2007)

Discussion Groups / Local Seminars and Other Scientific Meetings (8.4 credits)
- Scientific discussion seminars, Teagasc Johnstown Castle (2003-2007)
- Agricultural Research Forum, Tullamore Ireland (2003 & 2007)
- Journal Club, Teagasc Johnstown Castle (discussing scientific papers) (2006-2007)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.2 credits)
- PE&RC Weekend (2003)
- PE&RC annual meeting (2004)

International Symposia, Workshops and Conferences (5.5 credits)
- Presentation at Cornell University; USA (2003)
- International grassland conference; Ireland (2005)
- International grassland conference; China (2008)

Funding

This project was funded by a Walsh Fellowship of Teagasc, the Irish Agriculture and Food Development Authority, and was carried out at the Johnstown Castle Environmental Research Centre, Wexford, Ireland.

Cover: Arjan & Nyncke
Printing: Ponsen en Looijen, Wageningen