

CHAPTER 7

NITROGEN UTILIZATION OF PERENNIAL RYEGRASS IN DAIRY COWS

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Abstract. In intensive dairy farming, perennial ryegrass is among the main forages used because of its rapid response to nitrogen (N) and its high herbage yield. Due to a high N fertilization and cutting at a young and leafy stage, grass has a high crude-protein (CP) content that is rapidly degraded by rumen microbes. The supply of N often exceeds the supply of energy available to rumen microbes, and almost 80 % of this excess N is excreted in the urine, giving rise to a low efficiency of N utilization by grass-fed dairy cows.

Improving the efficiency of grass N utilization in dairy cattle is possible by including specific traits in grass-breeding programmes. Perennial-ryegrass cultivars differ in the water-soluble-carbohydrate (WSC) content, and WSC is a consistent and heritable trait. An increase in WSC is related with only a slight increase in total carbohydrate (CHO), with a decrease in neutral detergent fibre (NDF) and (or) the non-determined residual fraction, whereas the CP content is hardly affected. This shift in the source of energy in cultivars with an increased WSC content may better match the rapidly degradable CP in the rumen, viz. synchronization, and improve the efficiency of N utilization in the rumen. However, cultivars with an increased WSC content did not result in a significantly increased microbial protein flow to the duodenum and efficiency of microbial protein synthesis, nor in a reduced NH₃ content in rumen fluid or milk urea N (MUN), and did not result in an increased efficiency of N utilization in dairy cows. Moreover, a substantial proportion of N is recycled within the rumen and in the animal, and this may nullify the positive effect of an improved synchronization on the efficiency of microbial protein synthesis. With increased N-fertilization levels, the CP content in grass increased and this resulted in an increased N intake in dairy cows. The increased N intake was related with increased MUN, a slight increase in N excreted in milk but a strong increase in urinary N excretion, and thus a decrease in efficiency of N utilization in dairy cows. Thus there is little scope to improve the efficiency of N utilization in dairy cows by increasing the WSC content in cultivars with a high CP content, but decreasing the CP content in grass or grass-based diets to around 150 to 160 g/kg DM may result in substantial improvement.

Keywords: water-soluble carbohydrate; neutral detergent fibre; cultivar; dry-matter intake; milk urea N

INTRODUCTION

In temperate areas of the world, a high milk yield is obtained with forages that provide feed of a high nutritive value at low costs. Because of its rapid response to

nitrogen (N) fertilization and its relatively high nutritive value, perennial ryegrass (*Lolium perenne* L.) is among the main forages used in intensive dairy farming in northwestern Europe. Nitrogen fertilization leads to a high crude-protein (CP = N × 6.25) content in grass, usually at the expense of water-soluble carbohydrates (WSC), without much effect on the level of cell-wall carbohydrates as reviewed by Peyraud and Astigarraga (1998). The CP in perennial ryegrass is relatively rapidly and extensively degraded by rumen microbes. This may lead to an excess of N in the rumen, of which almost 80 % will be excreted in urine (Van Vuuren 1993). As a result, the efficiency of N use by grass-fed dairy cows is relatively low, often below 0.25 g N milk/g N intake (Van Vuuren 1993; Kolver 2003). In many regions of the world, dairy farming has become an important source of N pollution (Tamminga 1992).

Nitrogen utilization in grass-fed dairy cows can be improved by either a decrease of the N fertilization of grass (Van Vuuren 1993; Peyraud and Astigarraga 1998; Valk et al. 2000), or by partially replacing grass in the diet by a low-N roughage or high-energy concentrate feed rich in rapidly degradable non-structural carbohydrates such as starch (review Bargo et al. 2003). The main aim of such measures is to reduce N intake and (or) to balance the available N and carbohydrates (CHO) for rumen microbes, without adversely affecting dry-matter intake (DMI), digestibility and milk yield. Another way to increase the N utilization by dairy cows may be to optimize rumen degradation characteristics through forage-breeding programmes (Beever and Reynolds 1994). Humphreys (1989) showed the possibilities to select perennial-ryegrass cultivars for an increased WSC content. A higher WSC content in grass may decrease the asynchronous supply of N and CHO for ruminal microbial protein synthesis, and hence reduce rumen N losses and increase efficiency of N use. In this chapter, the effects of N fertilization and grass composition on rumen digestion, N intake and efficiency of N utilization in grass-fed dairy cows is discussed, with special emphasis on effects of perennial-ryegrass cultivars.

HERBAGE YIELD AND COMPOSITION

The herbage yield and composition are influenced by many environmental (e.g., climatic and weather conditions, soil type and nutrients in the soil, and fertilization and cutting management) and genetic factors (e.g., forage type, species and cultivar). Perennial ryegrass has a relatively late heading date (the date when 50 % of stems are with seeds). Cultivars differ in heading date and are classified as early, mid and late heading varieties (Bonthuis and Donner 2001). The herbage yield in the first cut and mean annual yield are important characteristics for cultivar evaluation tests. Smit et al. (2005a; 2005b) found that perennial-ryegrass cultivars differ in herbage yield. The N-fertilization level has a profound effect on the CP content of herbage, with an increase in CP of 50-90 g/kg DM per 100 kg/ha increase in N fertilization (Peyraud and Astigarraga 1998). The uptake rate of fertilizer N is high. After an initial increase in CP content in the first weeks after N fertilization, CP content decreases mainly due to dilution by an increased herbage mass. With an increased regrowth period, the cell-wall contents (neutral detergent fibre (NDF),

acid detergent fibre (ADF) and acid detergent lignin (ADL)) increase and the composition of the cell wall changes (Wilman and Altimimi 1982; Schäfer 1996). Moreover, the composition of CP is altered by the N-fertilization level, where an increased N-fertilization level results in a decreased proportion of true protein and increased proportion of non-protein N in grass (Wilman and Altimimi 1982; Peyraud and Astigarraga 1998).

To illustrate the relationships between CP, WSC and NDF contents of grass, a meta-analysis including year as random effect as described by St-Pierre (2001) was conducted, with data on chemical composition of diploid perennial-ryegrass cultivars in four years (Smit et al. 2005a; 2005b). The root mean square error (RMSE) and the determination coefficient (R^2) were determined by simple regression of the predicted values with the observed values. A significant quadratic relationship between CP and WSC was obtained: $WSC = 935.7 (\pm 144.7) - 7.34 (\pm 1.55) CP + 0.0158 (\pm 0.004) CP^2$; $R^2 = 0.55$, $P < 0.01$; $RMSE = 31.4$ g/kg DM (Figure 1A). This relationship indicates that the decrease in WSC content is large with an increase in CP content at low CP contents, and that a further increase in CP content above 200 g/kg DM has a minor effect on the WSC content. The relationship between WSC and CP can be attributed to a decrease in the utilization of carbon chains for protein synthesis and for production of the energy required for the nitrate reduction step before protein synthesis occurs (Peyraud and Astigarraga 1998). This inverse relationship was found to be higher in grass species with a high WSC content (Wilman and Wright 1978). However, upon comparison of cultivars differing in WSC content, this inverse relationship with CP content was not found (Tas et al. 2006). In line with results that indicate an absence of effect of N fertilization, and hence of CP content, on the cell-wall (NDF) content (Valk et al. 1996; Peyraud and Astigarraga 1998), no significant relationship ($P = 0.17$) was found between CP and NDF content (Figure 1B). The cell-wall content depends more on the growing season and maturity of the sward (Schäfer 1996; Ombabi et al. 2001). In The Netherlands, heading dates of perennial ryegrass are at the end of May and beginning of June. In June, grass in a reproductive stage had a higher proportion of stems (> 0.30 g/g), an increased NDF and ADL content and a higher ADL/NDF ratio (Tas et al. 2005). In the first two years of this study NDF content was analysed by wet chemistry, whereas in the other two years NDF content was predicted by NIRS. Although the accuracy of NDF prediction by NIRS was reasonable ($R^2 = 0.63$, bias = -40.5 g/kg DM) (Smit et al. 2005b), the intercept of the regression line differed from zero (95.7 g/kg DM), resulting in an over-prediction of NDF. The meta-analysis did not show a significant relationship ($P = 0.17$) between WSC and NDF content due to large differences among years. Similar linear relationships were found in 2000 ($WSC = 779.3 (\pm 162.7) - 1.50 (\pm 0.39) NDF$; $R^2 = 0.40$, $P < 0.001$) and in 2002 ($WSC = 874.7 (\pm 36.4) - 1.57 (\pm 0.08) NDF$; $R^2 = 0.90$, $P < 0.001$), whereas in 2001 the intercept and regression coefficient were smaller ($WSC = 255.9 (\pm 51.7) - 0.33 (\pm 0.11) NDF$; $R^2 = 0.27$, $P < 0.01$), and in 2003 no significant relationship ($P = 0.67$) was found (Figure 1C).

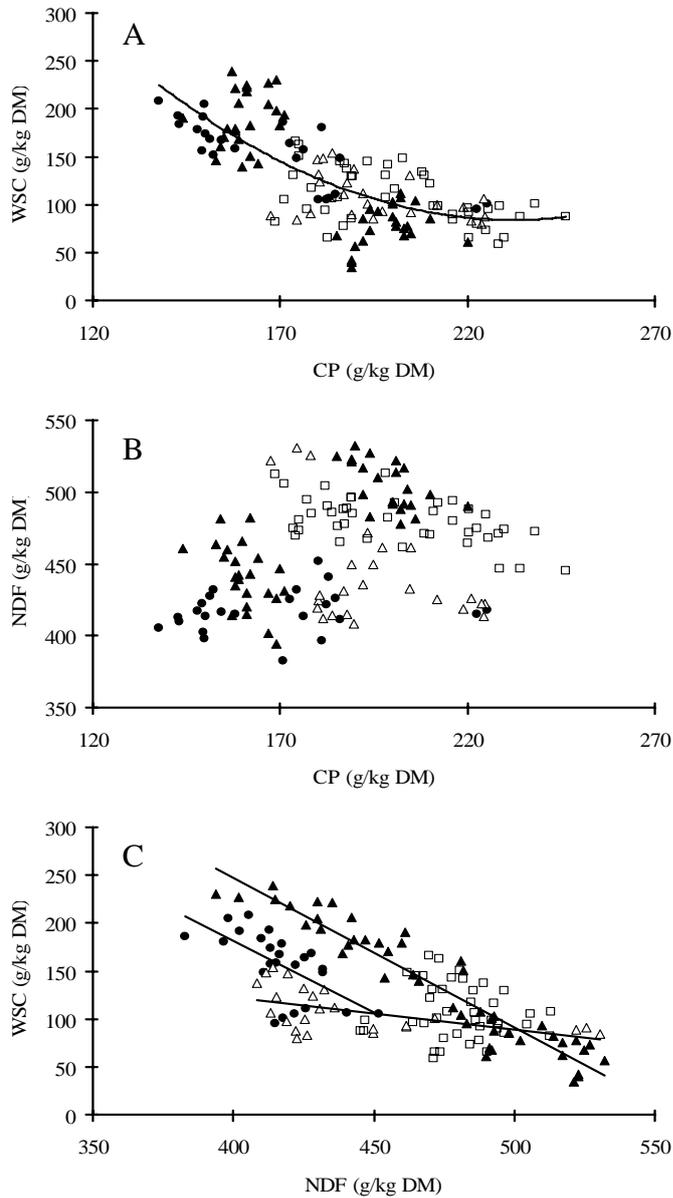


Figure 1. Relationship between A) WSC content and CP content, B) NDF content and CP content, C) WSC content and NDF content, in perennial ryegrass in 2000 (●), 2001 (Δ), 2002 (▲) and 2003 (□). Data from Smit et al. (2005a; 2005b). For details on fitted regression lines, see text

However, these relationships were determined based on the total environmental and genetic effect on the phenotypic herbage composition. Humphreys (1989) showed that perennial-ryegrass cultivars differed consistently in WSC content and that WSC is a consistent and heritable trait. At the Institute of Grassland and Environmental Research in Aberystwyth (UK), grass cultivars with a high WSC content have been developed. In comparisons among perennial-ryegrass cultivars, the higher WSC content was confirmed (Smith et al. 1998; 2002). Comparing six perennial-ryegrass cultivars in four years, the WSC content of two cultivars was consistently higher than of the other cultivars (Smit et al. 2005a; 2005b). The WSC is predominantly present as sucrose and fructans (Turner et al. 2002). When comparing two cultivars, the cultivar with a high WSC content had more large polymeric fructans, and less sucrose and small fructans, in the leaf blades and sheaths than cultivars with a 'normal' WSC content (Turner et al. 2002). The increase in WSC content was not related with a decrease in CP content when comparing cultivars (Tas et al. 2006). Therefore the inverse relationship between WSC and CP appears to be more an environmental than a genetic effect. However, a high WSC content was associated with a lower NDF content (Smith et al. 1998; 2002), or lower content of the non-determined residual fraction (OM – CP – NDF – WSC) (Smith et al. 2002). This was also observed by Tas et al. (2006). Cultivars with an increased WSC content (1 and 4) had a lower NDF content and/or a lower content of the residual fraction (Figure 2). The higher CHO and higher proportion of WSC in the CHO of the high-WSC cultivars indicate an altered carbon partitioning.

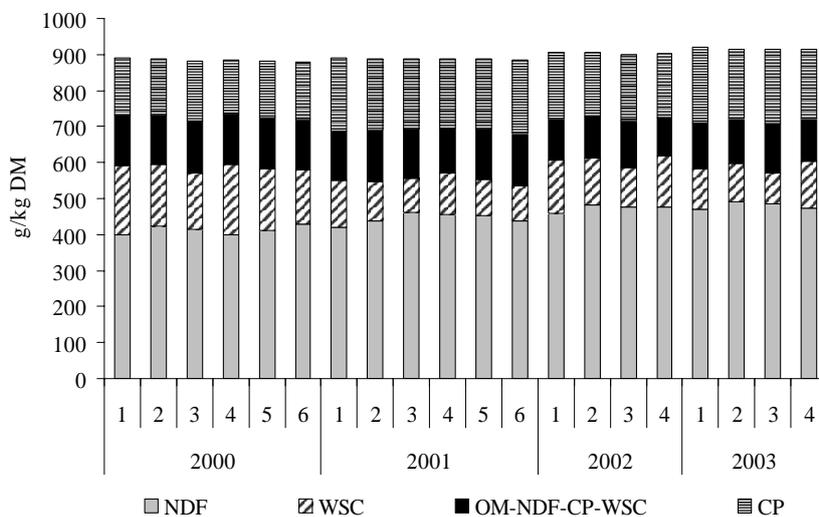


Figure 2. The neutral detergent fibre (NDF), water-soluble carbohydrate (WSC), crude protein (CP) and non-determined residual fraction (organic matter (OM) – NDF – CP – WSC) of perennial-ryegrass cultivars in four consecutive years. Cultivars 5 and 6 were not evaluated in 2002 and 2003. Data from Tas (2005) and Tas et al. (2006)

N UTILIZATION IN THE RUMEN

Rumen degradation of herbage

In the rumen of dairy cows, microbes degrade and ferment carbohydrates and protein for their maintenance and growth, and microbes produce end products including volatile fatty acids (VFA), ammonia (NH_3) and methane. The CP in fresh grass is rapidly and extensively degraded by rumen microbes and the supply of rumen-degradable protein (RDP) usually exceeds the N requirements of microorganisms (Van Vuuren et al. 1990; 1991). This excess protein is fermented to NH_3 , and the NH_3 absorbed is converted to urea in the liver, and to a large extent excreted in the urine. In fresh grass, a linear relationship was found between grass CP content and N losses in the rumen. Above a CP content of 155 g/kg DM, almost 80% of the additional CP was lost in the rumen and excreted in urine (Van Vuuren 1993). Ruminal N losses can be reduced by decreasing protein degradation and (or) increasing efficiency of N use in the rumen (Tamminga 1996).

With increased N-fertilization levels, and hence increased CP content and proportion of non-protein N in grass (Peyraud and Astigarraga 1998), both the soluble fraction and the fractional degradation rate of CP increased (Van Vuuren et al. 1991; Valk et al. 1996). Upon comparing ryegrass cultivars during the growing season, similar relationships were observed (Tas et al. in press). However, among cultivars only small differences in CP content, in fractional degradation rate and in effectively degraded CP were found (Tas et al. in press). The efficiency of microbial protein synthesis is commonly assessed as microbial N synthesis per unit of rumen available energy, expressed as apparent or true fermentable OM or CHO. A major determinant of rumen microbial protein synthesis therefore is the availability of energy-yielding substrates (Dijkstra et al. 1998). In grass, the major energy-yielding substrates are the structural (NDF) and non-structural carbohydrates (WSC). The supply of energy from structural carbohydrates depends on the cell-wall content (NDF) and the rate and extent of degradation in the rumen.

With an increased re-growth period and (or) an increase in proportion of stems, the NDF and ADL content in grass increases and this leads to a reduced fractional degradation rate and an increased undegradable fraction of NDF (Poppi et al. 1981; Schäfer 1996). Upon comparison of ryegrass cultivars, an increase in proportion of stems, NDF and ADL content resulted in a lower NDF digestibility (Tas et al. 2005). However, the rumen fill, fractional clearance rate and fractional degradation rate of NDF (estimated from the difference between fractional clearance rate from the rumen and fractional passage rate based on lignin disappearance), did not differ among these cultivars (Taweel et al. 2005b). Similarly, with *in situ* incubations, the fractional degradation rate and extent of degradation of NDF did not differ significantly among cultivars (Tas et al. in press). With both methods, the differences in fractional degradation rate of NDF among cultivars were within 0.01/h, indicating a narrow range of improvement among cultivars for these traits (Taweel et al. 2005b). The high-WSC cultivars may have supplied more CHO, and the shift from structural (NDF) to non-structural CHO (WSC) may influence (activity of) the microbial population, and consequently may reduce rumen ammonia

production and urinary urea excretion, as shown by simulations of Kebreab et al. (2002).

Synchronization of N and CHO

In addition to the amount of nutrients available for rumen microbes, the synchrony in time of availability of N and CHO for rumen microbes, depending on the rate of degradation and fermentation, may influence the efficiency of microbial protein synthesis. In fresh grass, based on *in situ* rumen incubations, the degradation rate of CP is higher than the degradation rate of OM and NDF (Van Vuuren et al. 1991; Valk et al. 1996), and this results in an asynchrony in the N and energy available for rumen microbes. Moreover, the activity of proteolytic enzymes in fresh grass after ingestion may give rise to protein degradation inside the plant and may further increase the rate of availability of N (Kingston-Smith and Theodorou 2000). The WSC are released easily from grass, with 60 % after ingestive mastication and increasing to 90 % within 1.5 h after ingestion (Boudon and Peyraud 2001). The proportions released after ingestive mastication were high for non-protein N (58 %) and free sugars (61 %), intermediate for fructans (42 %), and lower for protein-N and chlorophyll (22 % and 28 % of intake, respectively) (Boudon and Peyraud 2001). The rate and extent of gas production was lower from fructans than from sucrose and glucose (Dewhurst et al. 2000). Thus the WSC may supply energy to balance the rapid supply of N from non-protein N and rapidly degradable protein within a meal. This concept is the main interest for an increased WSC content in perennial-ryegrass cultivars.

The synchronization of N and energy can be calculated based on *in situ* degradation rates, expressed as the mean hourly ratio between N and energy (OM or CHO) (Sinclair et al. 1993). Comparing two perennial-ryegrass cultivars differing in WSC content, the cultivar with an increased WSC content had a lower hourly ratio between degraded N and energy (OM and CHO) (Tas et al. in press). The addition of WSC (inulin/sucrose, 80/20, g/g) to fresh perennial ryegrass in a RUSITEC system over 10 days showed a linear decrease in pH and NH₃ with increasing WSC levels and an increase in the efficiency of microbial protein synthesis at 1.5 times the WSC content in grass (Lee et al. 2003). However, the increased dilution rate at higher WSC concentrations in these *in vitro* systems may have contributed to this rise in microbial efficiency, since the fractional dilution rate is one of the major determinants of microbial efficiency (Dijkstra et al. 2002). In steers fed ryegrass cultivars differing in WSC concentration (83 g/kg DM), the ruminal NH₃ content was lower with the high-WSC cultivar, but the *in situ* efficiency of microbial protein synthesis and flow of N to the duodenum as proportion of N intake were not different (Lee et al. 2002). Moreover, supplementing grazing dairy cows with non-structural carbohydrates at the time of grazing or four hours after grazing, did not affect the mean ruminal NH₃, blood urea-N concentration and N excretion in milk (Kolover et al. 1998). Similarly, two cultivars (1 and 4) with a higher WSC content than four other cultivars did not result in a significantly lower mean ruminal NH₃ concentration (Taweel et al. 2005a).

Besides, the supply of soluble N (peptides, amino acids, NH_3) and (or) CHO may exceed the maximum capacity of microorganisms to use all these substrates immediately, and therefore may not be metabolized by rumen microorganisms (Dijkstra et al. 2002; Bach et al. 2005). Moreover, feed intake level, the storage of CHO in rumen microbes (Dijkstra 1993), the recycling of N within the rumen due to bacterial lysis and protozoal predation (Dijkstra et al. 1998; Koenig et al. 2000), and recycling of urea N within the body to the rumen (Lapierre and Lobley 2001) have a (large) effect on the efficiency of microbial protein synthesis. Other experiments, studying the effect of synchrony *in vitro* and *in situ* by altering feed ingredients, time of feeding, or infusing or pulse-dosing N or energy sources in the rumen, did not show consistent effects of improved synchrony on microbial protein flow and on efficiency of microbial protein synthesis (Dewhurst et al. 2000; Kaswari 2004; Bach et al. 2005).

UTILIZATION IN DAIRY COWS

Milk urea-N content

In the liver, urea is synthesized from NH_3 , which originates on the one hand from surplus NH_3 not incorporated in microbial protein in the rumen and on the other hand from losses in the amino-acid metabolism in various organs and tissues. Urea equilibrates in aqueous solutions through diffusion. Therefore, milk urea-N (MUN) content is highly correlated with blood and plasma urea-N content, as reported by many authors (reviewed by Hof et al. 1997), although slopes differed between data compilations and varied from 0.62 to 0.89 (Broderick and Clayton 1997; Kauffman and St-Pierre 2001). The best single dietary factor predicting MUN is the CP content of the diet ($R^2 > 0.77$) (Broderick and Clayton 1997; Broderick 2003; Nousiainen et al. 2004). Using data of four years (Tas 2005; Tas et al. 2006), the CP content in the diet was also well related with MUN ($\text{MUN (mg/dL)} = -7.3 (\pm 7.0) + 0.124 (\pm 0.035) \text{ CP diet (g/kg DM)}$; $R^2 = 0.74$, $\text{RMSE} = 2.0 \text{ mg/dL}$; Figure 3). This equation differed from the equations determined with fresh grass (Valk 2002, $\text{MUN (mg/dL)} = -13.4 + 0.17 \text{ CP diet (g/kg DM)}$; $R^2 = 0.71$) and with grass silage (Nousiainen et al. 2004, $\text{MUN (mg/dL)} = -14.2 + 0.17 \text{ CP diet (g/kg DM)}$; $R^2 = 0.77$). Slightly more of the variation in MUN (75 %) could be explained by the RDP balance in the diet ($\text{MUN} = 11.7 (\pm 1.7) + 0.160 (\pm 0.041) \text{ RDP balance (g/kg DM)}$, $R^2 = 0.75$, $\text{RMSE} = 2.0 \text{ mg/dL}$; Figure 3). The RDP balance was calculated according to Tamminga et al. (1994). At a RDP balance of zero, the MUN content was 10.4 mg/dL, which is similar to values reported by Hof et al. (1997) (10.3 mg/dL) and Valk (2002) (11.0 mg/dL), whereas the slope was in between the values reported by Hof et al. (1997) (0.17 RDP balance (g/6.9 MJ)) and Valk (2002) (0.25 RDP balance (g/kg DM)). The determination coefficient of the negative relationship between WSC content and MUN was slightly lower ($R^2 = 0.54$) than between CP and MUN. However, these relationships depend on the inverse (quadratic) relationship between WSC and CP in grass, as shown in Figure 1A. In the four years, only small differences among cultivars, within 2 mg/dL, were found in MUN (Tas 2005; Tas et al. 2006), and this was in agreement with the relatively small differences in CP content among

cultivars. An increased WSC content did not consistently lead to a reduced MUN content (Tas et al. 2006). Based on data from two years (2000 and 2001), Taweel (2004) found that the MUN was positively related ($r = 0.64$; $P < 0.05$) with the NH_3 concentration in rumen fluid. Moreover, the NH_3 concentration in rumen fluid was positively related ($r = 0.84$; $P < 0.001$) with the CP content in grass. These relationships indicate that the CP content in grass is well related to the ruminal NH_3 and MUN concentration. Although a substantial proportion of urea N (30-40 % of digested N) is recycled within the body (Lapierre and Lobley 2001), ultimately only a small amount is excreted in the faeces and a small amount is excreted in milk, whereas the majority is excreted in urine.

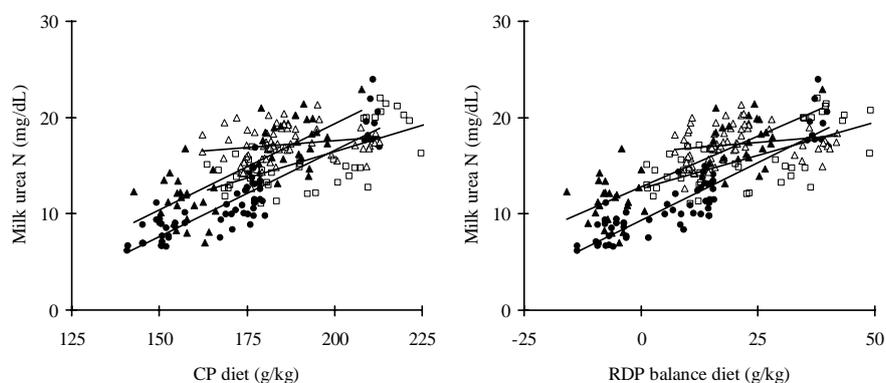


Figure 3. Relationship between MUN content with CP content and rumen degradable protein (RDP) balance of the diet in 2000 (●), 2001 (△), 2002 (▲), and 2003 (□). Data from Tas (2005) and Tas et al. (2006). For details on fitted regression lines, see text

The MUN content has not only been well correlated to the N content in the diet, but also to daily urinary N excretion (Jonker et al. 1998; Kauffman and St-Pierre 2001; Valk 2002; Broderick 2003; Nousiainen et al. 2004). According to Jonker et al. (1998), the physiological basis of this close relationship depends on a constant blood urea pool size and blood flow through the kidney, where the urea is actively and proportionally removed from the blood and excreted in urine. In 2000 a close relationship was found between MUN and urinary N excretion ($\text{N urine (g/d)} = 115.8 (\pm 14.2) + 16.1 (\pm 1.14) \text{MUN (g/d)}$, $R^2 = 0.74$, $\text{RMSE} = 39.9 \text{ g/d}$), whereas this relationship was poor and not significant in 2001 ($\text{N urine (g/d)} = 117.9 (\pm 59.2) + 9.0 (\pm 3.42) \text{MUN (g/d)}$, $R^2 = 0.09$, $\text{RMSE} = 51.2 \text{ g/d}$) (Tas 2005). More important, at a lower (30 g/d) mean urinary N excretion in 2001 than in 2000, the MUN content was on average 5.5 mg/dL higher in 2001 than in 2000 (Tas et al. 2006). The slope of the regression in 2000 was high in comparison with slopes reported by other authors (Jonker et al. 1998; Kauffman and St-Pierre 2001; Broderick 2003; Nousiainen et al. 2004; Valk 2002), and also since these slopes varied, this has a large effect on the prediction of the urinary N excretion. The efficiency of N utilization was negatively related ($R^2 = 0.41$, $P < 0.001$) with MUN

content in 2000, whereas this relationship was absent in 2001 ($P = 0.52$). Besides dietary factors, animal factors including parity, milk production, days in milk (Jonker et al. 1998) and body weight (Kauffman and St-Pierre 2001) may influence the MUN content and they may also explain part of the observed variation between years. In conclusion, the strength of the relationship between MUN and dietary components varied strongly between years and the regression equations differed substantially. Therefore, the use of MUN, given the high CP contents in our experiments, did not accurately predict the urinary N excretion. However, a high MUN is an indicator of high N losses in the protein metabolism and hence a low efficiency of N utilization.

N intake and N partitioning in dairy cows

The efficiency of N utilization by dairy cows is assessed as N excreted in milk as proportion of N intake. Therefore, efficiency of N utilization depends on the N intake and the milk protein yield ($= N \times 6.38$) by dairy cows. The N intake is determined by the N content in grass and the DMI. Based on two-year stall-feeding data, the urinary N excretion was highly related with N intake (Tas et al. 2006, Figure 4). The slope of the relationship indicated that 81.2% of the additionally ingested N will be excreted in the urine. The N excretion did not increase exponentially as observed by Castillo et al. (2000) and Kebreab et al. (2002) with N intakes above 400 g/d, although this was based on lower N intakes (< 550 g/d) compared with our data. The high N excretion with the urine implies that the response of N excretion in milk on N intake was low, with 13.3 % of additionally ingested N excreted in milk protein, although the slope did not differ significantly from zero ($P_{\text{slope}} = 0.12$). The mean proportion of N intake excreted in milk was 26.2 (range 15.2 – 33.8) and 23.7 (range 18.5 – 30.6) g/g in 2000 and 2001, respectively.

Due to relatively high CP contents in grass and high N intakes, the supply of metabolizable protein (MP) exceeds the requirements for milk protein yield (Kolver and Muller 1998), and the excess of protein is metabolized and mainly excreted in the urine. In line with these findings, the MP intake in our experiments was above the requirements for milk protein yield (19.5 to 53.2 %). The supply of amino acids for milk yield was therefore most likely not limiting milk protein synthesis. In agreement, milk protein content and yield in grass-fed or grazing dairy cows can be increased by level and type of energy supplementation with concentrates or roughages, whereas replacing RDP sources with rumen-undegradable protein sources in concentrates did not have a consistent effect on milk protein content and yield (Bargo et al. 2003). However, intake of rumen-undegradable protein was positively related with milk yield ($R^2 = 0.98$) (Bargo et al. 2003). Both the DMI and MP intake were positively correlated with milk protein yield ($r = 0.81$). Milk protein yield depends on protein supply as well as energy supply, and therefore the ratio between MP and net energy for lactation (NEL) available for milk synthesis (MP_c or $NEL_c = MP$ or NEL intake - MP or NEL maintenance requirements, respectively) is an important factor influencing the milk protein yield and the efficiency of milk protein synthesis (Hof et al. 1994). The mean ratio between MP_c and NEL_c

available for milk protein synthesis ranged in both years between 18.4 ± 0.9 and 19.8 ± 1.0 g/MJ and the mean efficiency of MPc available for milk protein synthesis was between 43.6 to 54.1 %. This mean efficiency corresponded rather well with the efficiencies determined with the regression equation (milk protein efficiency = $113.5 - 3.11 \times \text{MPc}/\text{NELc}$) reported by Hof et al. (1994) (viz. mean efficiency between 51.9 and 56.3 %). However, in the stall-feeding experiment this relationship was poor (milk protein efficiency = $91.0 - 2.0 \times \text{MPc}/\text{NELc}$, $R^2 = 0.10$, $P < 0.01$) and it was absent in the grazing experiment ($P = 0.83$). The difference in milk protein efficiency between 2000 ($54.1 (\pm 6.5)\%$) and 2001 ($52.5 (\pm 4.6)\%$) was similar to the difference in N excreted in milk as proportion of N intake (2000, $0.260 (\pm 0.039)$ g/g and 2001, $0.237 (\pm 0.027)$ g/g) (Tas 2005).

The efficiency of N utilization increases with decreasing N intakes (Figure 4). The proportion of N excreted in milk was more related with the N content of the diet ($r = -0.65$) than with N intake ($r = -0.24$). As mentioned before, MUN was strongly related with the CP content and RDP balance in the diet, and almost 80 % of the additional CP (> 155 g/kg DM) in grass was excreted in the urine (Van Vuuren 1993). Together with the relationships in Figure 4, this indicates that the CP content in the diet should not exceed 160 g/kg DM. In agreement, increasing the CP content (151, 167 and 184 g/kg DM) by replacing high-moisture corn by soybean meal in a forage-based diet, increased only slightly the milk yield, but largely increased MUN

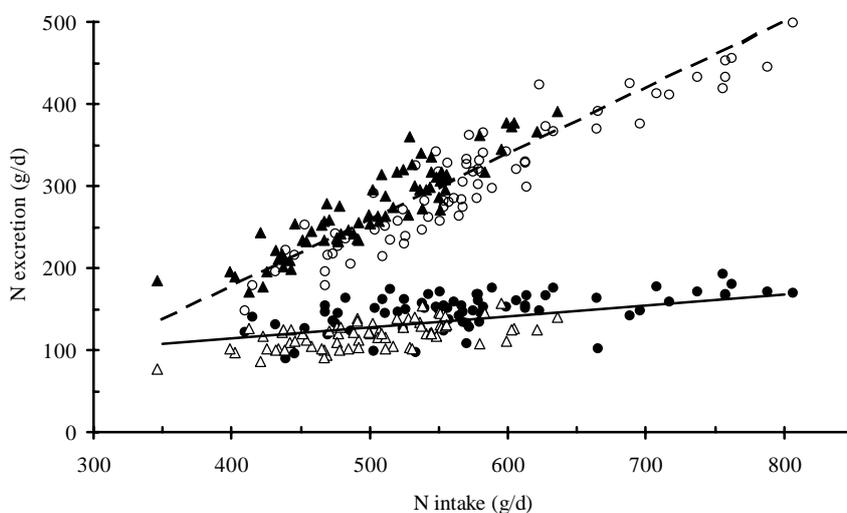


Figure 4. Relationship between N excreted in urine (dotted line; $\circ = 2000$, $\blacktriangle = 2001$) and milk (solid line; $\bullet = 2000$, $\blacktriangle = 2001$) with N intake: $N_{\text{urine}} (\text{g/d}) = -147.5 (\pm 14.6) + 0.812 (\pm 0.036) N_{\text{intake}} (\text{g/d})$; $R^2 = 0.88$, $\text{RMSE} = 24.1$ g/d; $N_{\text{milk}} (\text{g/d}) = 60.8 (\pm 9.8) + 0.133 (\pm 0.025) N_{\text{intake}} (\text{g/d})$, $R^2 = 0.55$, $P_{\text{slope}} = 0.12$, $\text{RMSE} = 16.1$ g/d. N in urine was calculated from N intake, N in faeces and N in milk assuming zero N balance. Data from Tas (2005) and Tas et al. (2006). For details on fitted regression lines, see text

and urinary N excretion and decreased the efficiency of N utilization (Broderick 2003). The increase in urinary N excretion is to a large extent an increase in urea-N excretion (Peyraud et al. 1997). The synthesis and excretion of urea requires energy, and simulations indicated that 11% of the lower milk yield in grazing dairy cows than cows fed a totally mixed ration could be attributed to these costs (Kolver and Muller 1998). The relationship between faecal N excretion and N intake was poor in 2000 ($R^2 = 0.08$, $P = 0.01$) and absent in 2001 ($P = 0.88$) (Tas et al. 2006), in agreement with the review by Peyraud and Astigarraga (1998).

The N excretion (in g/d) in milk, faeces and urine of the cultivars in the four years is presented in Figure 5. In the stall-feeding experiment (2000 and 2001), the N intake and N excreted in milk and faeces were determined (Tas et al. 2006), whereas in the grazing experiment (2002 and 2003) only the N intake and N in milk were determined. The N in urine or N in manure was calculated by subtraction assuming zero N balance. The DMI of stall-fed or grazing dairy cows was hardly affected by an increased CP content of fresh grass, due to increased N fertilization levels but at similar maturities of the sward and (or) regrowth period (Peyraud and Astigarraga 1998; Valk et al. 2000). The CP contents differed among years, as shown in Figure 2, and this largely influences the proportion of N excreted in milk (Figure 5). In 2000, the mean CP content of cultivars 1 - 6 was 158 g/kg DM with 0.28 g N milk/g N intake (Tas et al. 2006), whereas in 2003 the mean CP content was 202 g/kg DM with only 0.20 g N milk/g N intake (Tas 2005). However, differences between years in the production level and days in milk of the cows also attributed to these differences in efficiency of N utilization. Although the DMI did not differ significantly among cultivars in the stall-feeding experiment (average $P = 0.193$), the N intake differed significantly among cultivars in the stall-feeding experiment in three of the four Latin-square comparisons (mean $P = 0.057$) (Tas et al. 2006). Although N excreted in milk (in g/d) did not differ among cultivars, N excreted in milk as proportion of N intake only differed between cultivars 1, 2 and 3 in 2000. In 2002, cows grazing cultivars 1 and 4 had a higher N intake ($P < 0.05$) and N excretion in milk ($P < 0.01$) than cows grazing cultivar 3, whereas in 2003, no effects of cultivar ($P \geq 0.18$) on N intake and N excretion were found. These results indicate that there was no consistent effect of cultivars differing in WSC content (Figure 2) on efficiency of N utilization. In contrast, comparing two ryegrass cultivars differing in WSC content, Miller et al. (2001) found a similar N intake but a higher efficiency of N utilization in dairy cows fed a cultivar with an increased WSC content. Furthermore, Lee et al. (2002) found an increased DMI and rumen-digestible OM content in steers, resulting in an increase in non-ammonia N and microbial N flow to and amino-acid absorption in the small intestine for the high-WSC cultivar. However, Lee et al. (2002) did not find an increase in the efficiency of microbial protein synthesis in the rumen, as mentioned before. In both studies, the cultivar with the low WSC content had a relatively high NDF content (> 540 g/kg DM). Cultivars with an increased WSC had often a reduced NDF content (45 to 83 g/kg DM lower), a higher digestibility (0.05 - 0.07 g/g), and a lower (2.2 g/kg DM) or slightly higher (0.7 g/kg DM) N content than the low-WSC cultivar. Besides, the N content of these cultivars was low and ranged from 14.7 to 17.0 g N/kg DM (Miller et al. 2001; Lee et al. 2002). In the experiment of Miller et al. (2001),

executed after a six-week regrowth period, these differences resulted in a lower DM digestibility, and a higher digestible DMI, milk yield and milk protein yield in the high-WSC cultivar than the other cultivar. This indicates that in these comparisons not only an increased WSC content, but other differences in chemical composition of the cultivars may have had an effect on their results.

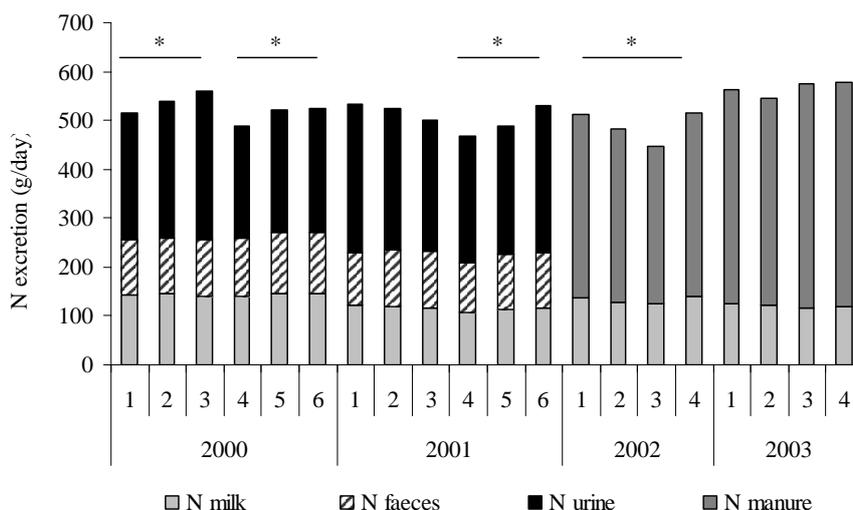


Figure 5. The N excreted in milk, faeces and urine (2000 and 2001) or in milk and manure (2002 and 2003) of perennial-ryegrass cultivars in four consecutive years. The summation is the N intake. * The N intake differs significantly among cultivars ($P < 0.05$). Data from Tas (2005) and Tas et al. (2006)

CONCLUSIONS

Perennial ryegrass that receives a high N fertilization and is cut at or grazed by dairy cows in a young and leafy stage has a high CP content that is rapidly degradable in the rumen. This leads to a low efficiency of N utilization by dairy cows. Perennial-ryegrass cultivars differ in the WSC content and WSC is a consistent and heritable trait. This increase in WSC is related with only a slight increase in total CHO, with a decrease in NDF and (or) the non-determined residual fraction, whereas the CP content is not affected. This shift in the source of energy with cultivars with an increased WSC content is thought to balance the rapidly degradable CP in the rumen, viz. synchronization, and improve the efficiency of N utilization in the rumen. However, cultivars with an increased WSC content did not result in a significantly increased microbial protein flow to the duodenum and efficiency of microbial protein synthesis, or in a reduced NH_3 content in rumen fluid and MUN, nor in an increased efficiency of N utilization in dairy cows. Moreover, the effect of an improved synchronization within a day on efficiency of microbial protein

synthesis is debatable. With increased N fertilization levels, the CP content in grass increased and this resulted in increased N intake in dairy cows. The increased N intake was related with increased MUN, a slight increase in N excreted in milk but a strong increase in urinary N excretion, and a decrease in efficiency of N utilization in dairy cows. In conclusion, there is little scope to improve the efficiency of N utilization in dairy cows by increasing the WSC content in cultivars with a high CP content, but decreasing the CP content in grass to around 150 - 160 g/kg DM may result in substantial improvement of N utilization efficiency.

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