DIGESTION AND NITROGEN METABOLISM OF GRASS FED DAIRY COWS

Promotor: **dr. ir. S. Tamminga**
buitengewoon hoogleraar op het vakgebied van de veevoeding in het bijzonder de voeding van herkauwers.

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A. M. van Vuuren

Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. C. M. Karssen
in het openbaar te verdedigen op
dinsdag 21 december 1993
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

STELLINGEN

1. De dagelijkse schommelingen in de pensfermentatie bij grazende koeien zijn meer een afspiegeling van het graasgedrag van de dieren dan van bijvoedering.
   Dit proefschrift.

2. Gezien de consequenties voor het schatten van de eiwitwaarde dient meer aandacht te worden geschonken aan de invloed van monstervoorbereiding op de oplosbare eiwitfractie van vers gras.
   Dit proefschrift.

3. Uit het oogpunt van milieubelasting dient gras niet meer dan 160 g ruw eiwit per kg droge stof te bevatten.
   Dit proefschrift.

4. De verteerbaarheid van celwanden is een beter criterium voor de selectie van grassen dan het gehalte aan water-oplosbare koolhydraten zoals bepleit door McGrath en Humphries.
   McGrath, 1988. Irish Journal of Agricultural Research 27: 131-139;
   Humphries, 1989. Grass and Forage Science 44: 231-236 en 423-430;
   dit proefschrift.

5. Verhoging van de ziekteresistentie van grassen en verbetering van de verteerbaarheid van celwanden gaan moeilijk samen.

6. De opvatting dat de introductie van klaver een belangrijke bijdrage levert aan de verbetering van de stikstofbenutting van melkveebedrijven is een misvatting.

7. De door de Gezondheidsdienst voor Dieren gehanteerde normaalwaarde voor het ureumgehalte in bloedserum van grazende koeien is niet normaal.

8. Onderzoek naar de bouw van celwanden verdient een hogere prioriteit dan onderzoek naar de relatie tussen celwandgehalte en verteerbaarheid.
   Hornstein *et al.*, 1989. Crop Science 29: 1319-1324.

9. Bij gelijke stikstofgift zijn de stikstofverliezen op grasland met hoog-produktieve grassoorten geringer dan op die met laag-produktieve grassoorten.
   Frame, 1991. Grass and Forage Science 46: 139-151.

10. Het fermentatievat in de koe is voor laboratoria voor veevoedingsonderzoek met een onregelmatige stroomvoorziening een alternatief voor in vitro systemen.
    Kabuga & Darko, 1993. Animal Feed Science and Technology 40: 191-205.

11. De haat tegen haaien en de liefde voor personenauto's die de media uitdragen, zijn verwonderlijk gezien het verschil in aantal slachtoffers dat jaarlijks aan haaien en personenauto's ten prooi valt.

12. Een kuil kan een hoop betekenen.

A. M. van Vuuren.
DIGESTION AND NITROGEN METABOLISM IN GRASS FED DAIRY COWS.
Wageningen, 21 december 1993.

Aan Marijke,
Sheila en Chantal

VOORWOORD

Het onderzoek dat in dit proefschrift is beschreven is uitgevoerd op het DLO-Instituut voor Veevoedingsonderzoek (IVVO-DLO) in Lelystad. Veel IVVO-medewerkers hebben hun steen(tje) aan dit proefschrift bijgedragen.

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In het begin van de tachtiger jaren begonnen Jac Meijs en ik met onderzoek naar de relatie tussen krachtvoergift en het melkvetgehalte bij grazende melkkoeien. De hoge ammoniakgehalten in de pens die we daarbij vonden, de produktie van melkkoeien in de weideperiode, literatuurgegevens en de discussies met Henk de Visser vormden de start van het onderzoek naar de vertering en de eiwitstofwisseling bij koeien op gras. De deskundigheid van Seerp Tamminga was daarbij onontbeerlijk. Seerp, jouw steun gedurende dit onderzoek waardeer ik bijzonder. Je was - als collega - betrokken bij de ideevorming, bij het opzetten van de verschillende experimenten, bij het vangen van fistelkoeien 's nachts in de wei en - als promotor - bij het interpreteren van de resultaten in dit proefschrift. Onze discussies vormden steeds de motivatie om verder te gaan.

Ook de gesprekken met Henk Valk brachten mij meestal weer met beide benen op de grond, in dit geval: in het gras. Daarmee bleef het onderzoek toepassingsgericht.

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De medewerkers van de DLO-Groep Landbouwwiskunde, Janneke Hoekstra, Paul Goedhart en Jan Kogut, hebben mij geholpen om de proeven zo op te zetten dat deze statistisch verantwoord waren. Hoewel ik vaak niet aan hun wens tegemoet kon komen meer dieren in de proeven op te nemen, gaven zij steeds gehoor aan mijn verzoek om begeleiding bij de statistische uitwerking van de gegevens.

Ynze van der Honing stelde mij in de gelegenheid dit proefschrift af te maken, door mij wat minder te betrekken bij andere instituutsaangelegenheden. Daardoor kwam er een nog grotere papierwinkel terecht op de schouders van Sierk Spoelstra, die mij de laatste tijd veel werk uit handen nam, vaak zelfs zonder dat ik me daarvan bewust was. Daarnaast maakte hij tijd vrij om de manuscripten te voorzien van opbouwende kritieken.

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CHAPTER 1

Introduction

Variations in protein and carbohydrates in fresh grass

Introduction

Over the last decades, dairy farming in the Netherlands has been strongly intensified. This resulted in a serious imbalance between inputs of N, P and K in purchased fertilizers, concentrates and roughage and outputs in milk and meat. On Dutch farms, an average N input of *ca.* 555 kg/ha per year has been estimated, of which only 15% is leaving the farm as milk or meat (Aarts *et al.*, 1992). A major part (*ca.* 60%; Aarts *et al.*, 1992) of the N input is from purchased anorganic fertilizers, applied to produce grass in high quantities and with a high nutritive value (Prins, 1983).

At the level of the animal, 64% of N input originates from consumed forage, which is 313 kg/ha per year, whereas N output in milk and meat has been estimated at 84 kg/ha per year (Aarts *et al.*, 1992). In the Netherlands, approximately 30 to 50% of forage consumed by dairy cows is fresh grass. Thus, improvement in the utilization of grass N by dairy cows may contribute significantly to a reduction in N losses on dairy farms.

The organs of grasses are stems, roots, and leaves. In the vegetative stage, during which stem and leaves develop, the stem of grass plants is short and consequently leaves form the major part of plants (Osbourn, 1980). Under certain conditions of temperature and light, the vegetative shoot or tiller develops into a reproductive shoot, carrying the inflorescence upwards to escape from the leaf sheaths. This coincides with stem elongation. After pollination the seed is developed.

In chloroplasts or chlorophyll-bearing cell granulas embedded in the cell content, photosynthesis takes place by which hexose and O_2 are produced from CO_2, H_2O and the energy of light. Thus, grasslands are an important sink in the global deposition of solar energy. Due to photosynthesis more than 10^{17} kcal of free energy is stored annually on earth. A part of the hexose formed are used for the synthesis of poly-saccharides: starch, hemicellulose and cellulose. Starch is stored as energy depot in the seed, for the future development of the embryo, whereas hemicellulose and cellulose are deposited in the cell wall. As plants mature, lignin is deposited in the cell walls in increasing quantities, thus adding rigidity to the cell wall. Lignin, phenolic acids, tannins and cutins protect cell walls from microbial attack and digestion.

More than 30% of dry matter (DM) in chloroplasts are lipids, mainly galactolipids (40%) and phospholipids (10%) in the thylakoid membrane. The concentration of these lipids is high when photosynthesis is high (Hawke, 1973). Another part of crude lipids can be found in waxes, surface lipids, covering the cuticular layer. This layer protects the plant from dehydration, reduces mechanical damage and inhibits microbial invasion.

Photosynthesis and polymerization require enzymes. These metabolic proteins form the major part of plant proteins. Nitrogen in the plant is transported as free amino acids (AA), ammonia-N or nitrate (Ourry *et al.*, 1990). Besides proteins, carbohydrates and lipids, grass contains other macro- and micro elements (Table 1).

The studies reported in this thesis aimed to investigate the digestive behaviour of grass differing in nutritional quality, and the effects of measures to improve the utilization of grass

N by dairy cows. In this chapter, variations in protein and carbohydrates of fresh grass as reported in literature will be discussed. In Chapter 2, the effects of herbage composition and supplement feeding on N excretion in faeces and urine, reported in literature and from preliminary experiments are presented.

In our further studies, a reduction in N intake was achieved by two different approaches. One approach was to decrease the N-content of grass by a reduction in the level of N fertilization. In Chapters 3 and 4, the results of *in situ* experiments to study the effects of variation in N fertilization and time of harvesting on chemical composition and on ruminal behaviour of N and carbohydrates of fresh grass are presented. In Chapter 5 we report an experiment in which the effects of variation in N fertilization on rumen fermentation and duodenal flow of protein and carbohydrates were studied in cannulated cows.

The second approach was to decrease the N content of the ration by "diluting" grass N with low-N feedstuffs. In Chapter 6, we investigated the effects of partial replacement of fresh grass by maize meal or sugar beet pulp on the duodenal flow of protein and carbohydrates. Results of several experiments studying the effects of partial replacement of grass by low-N feedstuffs on rumen fermentation, N excretion and milk composition are presented in Chapters 7 and 8. In Chapter 9, the results are discussed in general.

Table 1. Concentrations (g or mg per kg DM) of nutrient elements in herbage leaf (Whitehead *et al.*, 1985a)

	P g	S g	Ca g	Mg g	K g	Na g	Fe mg	Mn mg	Zn mg	Cu mg
Lolium perenne	3.5	3.1	16.6	2.6	25.7	2.6	176	84	51	10.2
Festuca arundinaceae	2.5	3.7	9.4	2.1	24.1	0.3	152	61	30	6.4
Trifolium repens	2.7	2.2	18.7	2.2	29.4	0.4	182	31	32	6.9
Medicago sativa	2.7	6.3	26.8	2.0	13.7	0.7	175	129	33	10.7

Proteins

Role and characteristics of grass protein

In plants, proteins appear mainly in the form of enzymes. Since the main enzymatic process is photosynthesis, it is not surprising that the most abundant protein in all green leaves is ribulose-1,5-biphosphate carboxylase/oxygenase (*Rubisco*; EC 4.1.1.39) (Mangan, 1982), the first enzyme in the Calvin cycle in chloroplasts. *Rubisco* is the single protein in

Fraction 1 protein. Fraction 1 protein is soluble in phosphate-buffer (pH 7.5), has a sedimentation constant of 18S and thus an estimated molecular size of 500 to 600 kDaltons (Mangan, 1982). Fraction 1 protein makes up to 50% of soluble protein in plants. In lucerne leaves, Fraction 1 protein forms 30 to 40 % of total crude protein (Mangan, 1982).

Fraction 2 protein constitutes about 25% of total leaf protein and is a mixture of soluble proteins, ranging in molecular size between 10 and 300 kDaltons, originating from chloroplasts, the cytoplasm and the cell wall.

Insoluble protein may account for up to 50% of the protein present in the leaf (Lyttleton, 1973). Part of the insoluble protein is found in the chloroplast membranes. The thylakoid membranes constitute about 40% of the chloroplast protein, present as chlorophyll-protein complexes (Mangan, 1982). Another part of insoluble protein is associated with the cellulose in primary cell walls. These proteins, *extensins*, are distinct from plant cytoplasmic proteins in being strongly basic glycoproteins, rich in arabinose, galactose, lysine, serine and tyrosine and extremely rich in hydroxyproline (Fry, 1988). These glycoproteins have been postulated to play a role in controlling cell wall development. Wilman *et al.* (1977) reported that cell wall N of perennial ryegrass accounted for approximately 10% of herbage total N. Similar results were reported by Whitehead *et al.* (1985b) and for *Bromus inermis* by Sanderson & Wedin (1989). In fresh lucerne, 18% of AA N was not solubilized in neutral detergent (Weiss *et al.*, 1986).

The free AA content of forage plants may vary considerably. Free AA N may account for 4 to 32% of total protein-N (Mangan, 1982). Free AA serve mainly for the transport of N from the source organ (root or old leaves) to the sink organ (young leaf or fruit). The main free AA are Asn, Gln, Asp, Glu, Ala, Ser and γ-amino butyric acid. After defoliation and at the onset of senescence, a substantial part of the N may be in the form of free AA. During the first days after defoliation up to 75% of the plant-N may be in the form of free AA N, of which 60-75 % being Asx (Asn + Asp) and Glx (Gln + Glu) (Bigot *et al*, 1991; Lefevre *et al.*, 1991).

The proportions of the different proteins in herbage are summarized in Table 2.

Table 2. Proportions of different proteins in herbage

Soluble proteins	Fraction 1 *Rubisco*	30 to 40%
	Fraction 2	15 to 20%
	Free AA	5 to 10%
Insoluble protein	Chlorophyll-protein complexes	*ca.* 30%
	Cell wall proteins *Extensin*	*ca.* 10%

Table 3. Amino acids composition in herbage ([1,2]A. M. van Vuuren, unpublished; [3]W. M. van Straalen, unpublished; [4]Coto and Herrera, 1989; [5]Coto *et al.*, 1990; [6]Messman *et al.*, 1992; [7]Shaposhnikov *et al.*, 1992)

	Lolium perenne				*Cynodon dactylon*			*Bromus inermis*[6]		*Festuca*[7]	
	high N[1]	low N[1]	[2]	[3]	[4]	0 N[5]	400 N[5]	- N	+ N	*pratensis*	*arundinaceae*
g AA/ kg DM	170	149	156	110	80	47	64	74	99	106	85
	% AA/total AA										
Asx	9.8	10.0	9.9	10.9	10.6[a]	11.7[a]	12.2[a]	9.3[a]	7.4[a]	11.2[a]	9.6[a]
Thr	5.2	5.2	5.2	5.3	4.5	5.2	5.2	5.5	5.8	5.7	5.0
Ser	5.4	5.2	5.4	5.6	4.5	5.0	5.1	5.4	5.3	5.0	4.4
Glx	13.1	13.3	12.0	12.6	13.3	12.7	12.5	10.9	11.1	15.9	13.1
Pro	5.2	5.1	5.4	5.9	7.6					6.2	6.1
Gly	5.9	5.9	5.7	6.1	5.6	5.7	6.1	6.1	6.3	6.8	5.6
Ala	7.0	7.0	7.1	7.5	6.0	7.4	7.6	6.8	7.0	7.1	5.6
Cys	1.4	1.5	1.6	1.4		1.2	0.9				
Val	6.4	6.3	6.4	6.6	5.1	6.2	6.7	5.8	6.2	7.4	6.7
Met	2.2	2.2	2.3	2.0	2.2	2.3	1.8				
Ile	4.9	4.8	5.0	4.8	5.1	4.9	4.9	4.3	4.7	5.5	5.2
Leu	8.8	8.9	8.9	8.7	11.6	9.1	9.2	10.0	10.6	10.2	9.0
Tyr	3.5	3.7	3.6	3.2	2.8	2.6	2.6	3.6	3.6	3.9	3.9
Phe	5.7	5.6	5.8	5.5	4.2	5.7	5.5	6.3	6.5	7.0	6.6
His	2.6	2.5	2.5	2.5	1.3			2.1	2.3	2.8	3.0
Lys	6.4	6.6	6.5	5.5	7.3	5.9	5.5	5.5	5.0	5.4	6.1
Arg	6.5	6.3	6.6	6.0	6.6			9.5	9.2	5.7	6.2

[a]Corrected for missing values

The major AA in grass are Asn, Asp, Glu, Gln and Leu, whereas the proportions of His, Cys and Met are relatively low (Table 3). From Table 3 it appears that small differences in AA composition between grass species exist. However, since data were derived from different studies, these apparent differences may also reflect differences between laboratories. The proportions of Asx and Glx may also vary due to differences in the amount of transport AA, influenced by maturity.

Effect of growing stages on grass protein

Plant composition changes with time. During early development, levels of chloroplast proteins increase markedly. Nitrogen is supplied as NO_3 and soluble reduced N (NH_4^+-N, and amino-amide N) from other tissues like roots and old leafs, and from the soil (Ourry *et al.*, 1990). Mangan (1982) stated that the concentration of free AA may vary considerably, whereas Goswami & Willcox (1969) observed no response of various growing stages on the free AA N content of ryegrass, with a mean concentration of 3.4 g/kg DM.

In leaves tissues of *Lolium temulentum*, an increase of about 67% in protein content between the juvenile and emerging phases of development was observed, whereas a similar decrease occurred from maturity to senescence (Thomas, 1990).

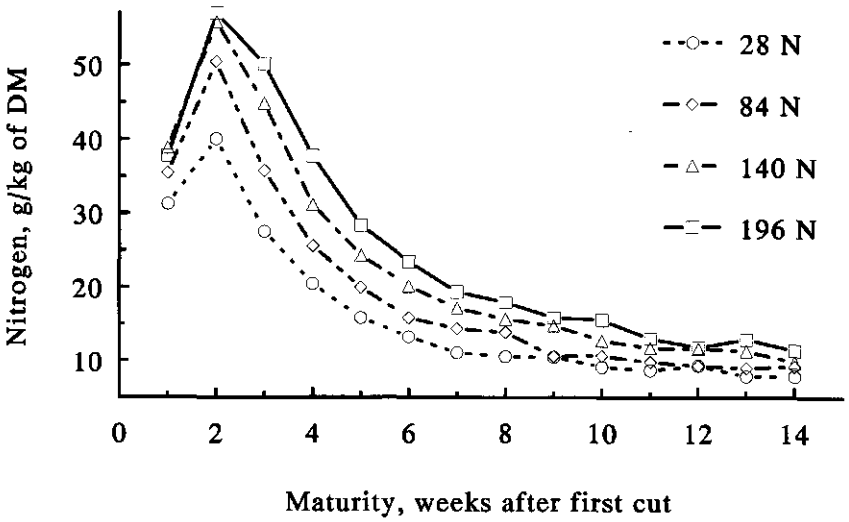

Figure 1. The influence of nitrogen fertilization (kg of N/ha) and age on the N content in *Lolium multiflorum*. From Wilman (1975)

The rate of the decline in protein content with increasing maturity depends on factors like rainfall, radiation and temperature. Givens *et al.* (1989) estimated a decline in crude protein (CP) content of spring-grown herbage of 1.43 g/kg DM with each day after April 10 (Day 100). Similar observations were reported for *Cynodon dactylon* (Prine & Burton, 1956), for *Lolium perenne* (Culleton *et al.*, 1986) and for other cool-season grasses (Collins & Cassler, 1990). Lindgren & Lindberg (1988) noticed that the rate in decline was directly related to the CP content. In their study, CP content of *Phleum pratense* daily declined by 2.3 (second cut) to 5.6% (first cut) per day. Using the data of Wilman (1975), I estimated a decline of 4.1 (± 0.3)% in *Lolium multiflorum*. In grass fertilized at high levels of N, the rate of the decline was faster than in low-fertilized herbage (Figure 1).

Maturation also changes N bound to the cell walls. The content of N per unit neutral detergent fibre (NDF) gradually decreases with maturation (Sanderson & Wedin, 1989). However, due to the increase in NDF and the decline in total CP content with increasing maturity, the proportion of NDF relative to the total N content increases (Figure 2).

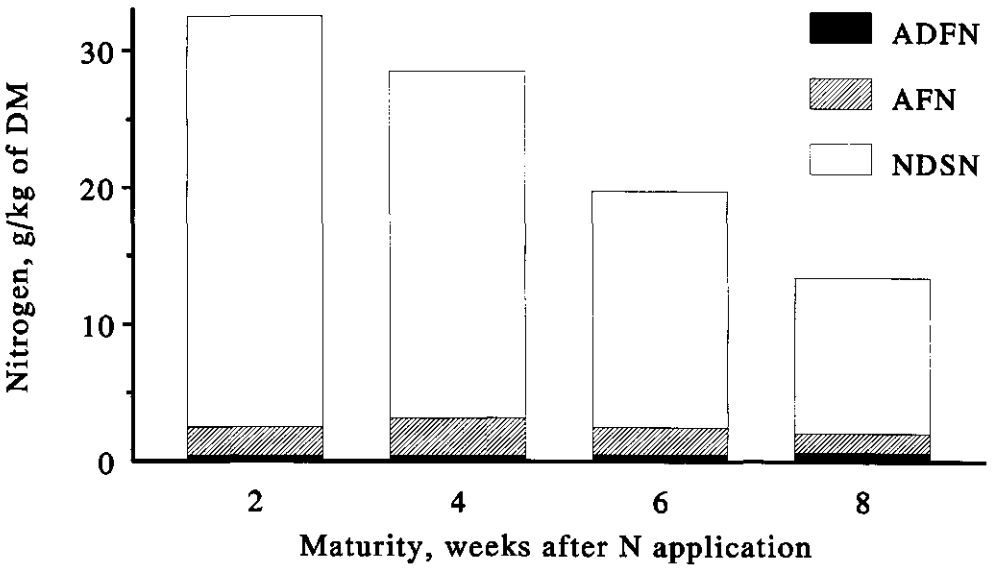

Figure 2. The effect of maturation on neutral detergent soluble N (NDSN = Total N - NDFN), acid detergent fibre N (ADFN) and available fibre N (AFN = NDFN-ADFN) in *Medicago sativa*. From Sanderson & Wedin (1989).

Effect of nitrogen fertilization on grass protein

Nitrogen fertilization is a very effective way to increase grass production (Prins, 1983; Frame, 1991) and therefore a common tool in grassland management in regions with intensive cattle husbandry. When compared to lower N fertilization levels, higher N levels result in higher DM yields at a fixed date after N application (vertical N effect) and in a reduction in growing days to reach a particular production stage (horizontal N effect) (Prins, 1983). Thus, comparing grasses harvested at a similar DM yield/ha, but fertilized at different levels of N may also imply comparing grasses at different age. Thus, the reported effects of N fertilization on grass DM composition, including CP, should be interpreted with care, since the observed differences between treatments may be amplified by differences in days of (re)growth (Table 4).

Table 4. The effects of N application rate on the CP content (g/kg DM) of *Lolium perenne* (From van Vuuren *et al.*, 1991)

N application level (kg N/ha per yr)	Vertical N effect[1]	Horizontal N effect[2]	
0	203 ± 22	94	(68)[3]
250	261 ± 31	175	(40)
400	288 ± 30	231	(30)
700	333 ± 43	288	(30)

[1]7-Day old grass (Experiment 2)
[2]Grass harvested at 2000 kg DM/ha (Experiment 3)
[3]Days of regrowth after previous cut

With increasing levels of N fertilization, the N content of grass increases (Wilman & Wright, 1983; Frame, 1991). Because the absolute decline in N with increasing maturity (g/kg DM per day) is sharper in high fertilized grass (Lindgren & Lindberg, 1988), the vertical N fertilizer effect on N content decreases with maturation (see also Figure 1). However, at a particular production stage, high N fertilized grass is younger and thus differences in DM composition are usually higher.

Goswami & Willcox (1969) studied the changes in nitrogenous fractions in ryegrass harvested at the same maturity (vertical N effect). In their study the increase in total N content was accompanied by an increase in protein N, but the proportion of protein N in total N declined from 74 to 60% (Figure 3). Up to a N application rate of 126 kg/ha, the proportions of AA N (either in peptides or free) were rather constant, *ca.* 10%. At these rates, nitrate and nitrite N was less than 3% of total N. At application rates ≥ 250 kg N/ha, the

proportion of nitrate- and nitrite-N increased to almost 10% of total N, with a decrease in the proportion of peptide N. A similar increase in the proportion of NO_3-N with increasing N fertilization was reported by Whitehead *et al.* (1985b) for *Lolium perenne*, harvested in October.

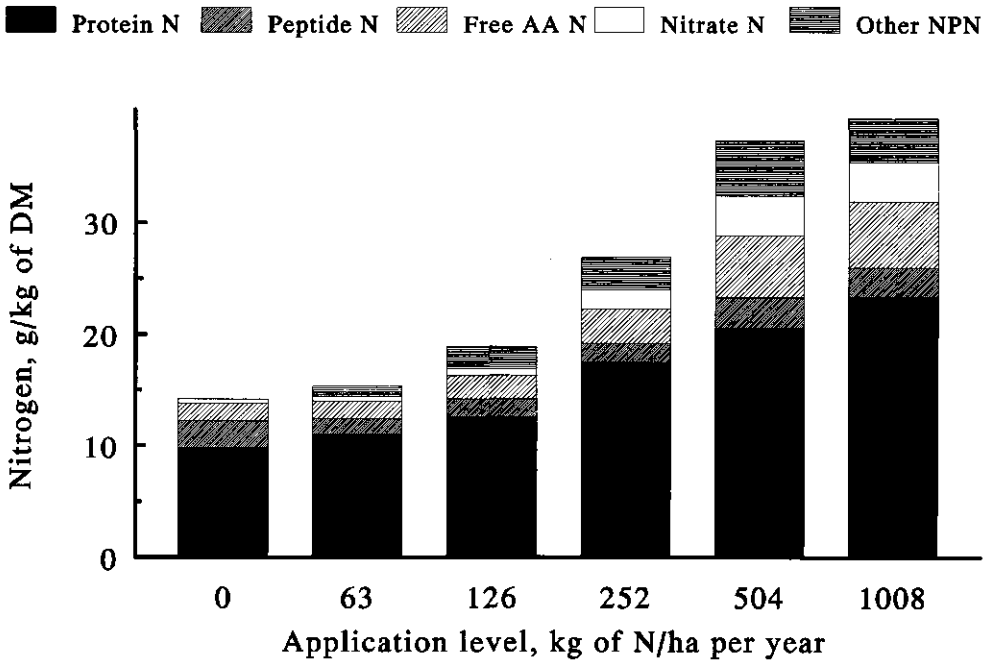

Figure 3. Effect of level of nitrogen (N) fertilization on nitrogenous components in ryegrass (AA = amino acid, NPN = no-protein N). From Goswami & Willcox (1979).

Whitehead *et al.* (1985b) also studied the effects of N application on cell wall N. At rates ≤ 100 kg N/ha, more than 10% of total herbage N was analyzed as cell wall N. At rates ≥ 250 kg N/ha, about 7% of the total N was present as cell wall N, with a minor, increasing effect of N application rate. These results were confirmed by the study of Sanderson & Wedin (1989), which showed that the increase in CP with increasing level of N fertilization was mainly an increase in neutral detergent soluble N. Consequently, the proportions of available and unavailable N in NDF declined.

Effect of rainfall, temperature and radiation on grass protein

As mentioned earlier, maturity has an important effect on herbage DM composition. Therefore, other factors than N application affecting growth also influence content and composition of CP.

Regions with a high rainfall in the growing season or with soil with a high water holding capacity or both showed high grass production yields (Morrison, 1980). The effect on the CP content is less consistent. In some experiments the CP content was not affected, whereas in other experiments the CP content decreased with increasing production (Garwood *et al.*, 1980). Intensive rainfall will have a negative effect on the CP content, due to N leaching and consequently poor N recovery from the soil (Garwood *et al.*, 1980).

Leaf growth is linearly related to degree-days based either on soil or air temperatures (Spiertz, 1982). Generally, an enhanced leaf growth will dilute herbage N and therefore the CP content will decrease at higher temperatures. However, after defoliation leaf growth will coincide with N remobilization (Ourry *et al.*, 1990), resulting in an increase in CP content.

Radiation will stimulate photosynthesis and the increase in carbohydrates will cause a dilution of CP. The relationship between radiation and plant productivity is complex, depending on light interception influenced for instance by leaf area and photosynthesis rate (Spiertz, 1982).

Carbohydrates

Carbohydrates occur as structural cell wall poly-saccharides (hemicellulose, cellulose, pectines) and as non-structural mono-, di-, oligo- and poly-saccharides (sugars, fructosans and starches) in cell contents. Non-structural carbohydrates in herbage are glucose, sucrose, fructose, fructosans and amylose-starch. Whereas free sugars are metabolic intermediates and usually occur at low concentrations, starches and fructosans are storage carbohydrates and vary widely in concentration. In grasses, starch is mainly located in the seed, and therefore starch content of grasses in a vegetative stage is low, being usually less than 10 g/kg DM (Smith, 1973). Holt & Hilst (1969) reported that 9 to 14% of nonstructural carbohydrates were poly-saccharides other than fructosans.

Non-structural carbohydrates are water-soluble. Mono- and disaccharides are extracted by 80%-ethanol. The solubility of fructosans in ethanol depends on their chain-length. The average degree of polymerization ranged between 40 (*Dactylis glomerata*) and 160 (*Phleum pratense*) (Kühbauch, 1978). In *Lolium perenne*, fructosans contained 10 to 60 fructose units (McGrath, 1988). In *Dactylis glomerata* and *Phleum pratense*, 10 to 60% of the water-soluble carbohydrates (WSC) were present as fructosans (Kühbauch, 1978). In *Lolium perenne* values of 70% have been reported (McGrath, 1988). With increasing maturity the content of

fructosans increased until week 5. After this peak fructosans were translocated or synthesized into structural carbohydrates (Blaser, 1964).

In most studies the content of WSC in grass is inversely related to the CP content (Wilman & Altimimi, 1982; Syrjälä-Qvist *et al.*, 1984; Humphreys, 1989). However, McGrath (1992) observed a poor relationship if data of different experiments were pooled, but within each experiment the relationship was evident. Probably, other environmental factors like radiation, rainfall and temperature and time of sampling overruled the effect of CP on the content of WSC. During the day, the content of WSC increases due to photosynthesis (Holt & Hilst, 1968; Van Vuuren *et al.*, 1986). During the night WSC are catabolized. Thus, concentrations of WSC are the highest at the end of the day, and low in early-morning. Soluble carbohydrates are assumed to be instantly degraded by rumen microorganisms, because the number of bacteria fermenting soluble sugars is constantly high and ruminal concentrations of soluble sugars are low (Leedle *et al.*, 1986).

Cell walls form the main mass of structural poly-saccharide. Mature plant cell walls can be divided in three main layers (Grenet & Besle, 1991). The middle lamella is situated between two adjacent cells and cement them together. The middle lamella merges with the relatively thin primary cell wall. During maturation a thicker secondary cell wall is formed, which reduces cell cavity.

The main cell wall poly-saccharide are pectic substances, hemicelluloses and celluloses. Pectines are principally found in the middle lamella and are mainly in the form of (α 1-4 linked) rhamnogalacturonans. In herbage, pectin concentrations are usually low (*ca.* 20 g/kg DM). Hemicelluloses are important for the flexibility and plasticity of the cell wall. Their content decreases from the primary to the secondary cell wall. The main hemicelluloses in grasses are xylans (ß 1-4 linked xylopyranoses) and glucans (ß 1-4 linked glucose, branched with α 1-6 linked xylose). Cellulose is continuously deposited on the inner site of the secondary cell wall and is composed of long linear chains of ß 1-4 linked glucose units.

Other important structural components in cell walls are phenolic compounds. These hydroxycinnamic acids (p-coumaric acid and ferulic acids) play a role in cell wall architecture and are linked to cell wall poly-saccharide. Lignins are formed by reduction and condensation of these acids. Lignins are the main phenolic compounds in secondary cell walls and are closely linked to the matrix of the poly-saccharide.

Different cell types in plant tissues also show considerable differences in chemical composition and physical properties. Parenchyma consist mainly of thin-walled cells; the collenchyma, providing mechanical support, consists of cells with thick walls, whereas the conducting sclerenchyma (phloem, xylem) contains lignified, inextensible walls. Lignification is the mean defence of plant cell tissues against microbial deterioration. Primary cell walls are rich in cellulose; lignified vascular tissue is high in lignified hemicelluloses.

During the developing stage cell walls are formed and cell solubles accumulate in the lamina (Deinum, 1992). In the adult stage, part of the digestible cell wall becomes

indigestible, the extent depending on the temperature. In the cells molecules are synthesized and stored or transported to other organs. At senescence, cell contents is reallocated and digestible cell walls are lost by leaching and microbial consumption.

Conclusion

The concentration, composition and rate and extent of degradation of CP and carbohydrates in fresh grass may vary significantly, influenced by growing conditions. The differences in chemical composition and physical properties will influence the rate and extent of rumen degradation, and consequently the production of volatile fatty acids, ammonia and microbial protein. Thus, differences in grassland management and growing conditions will affect animal performance and the excretion of undesired products into the environment. These aspects were investigated in the research reported in this thesis.

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CHAPTER 2

Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows.

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Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows

A M VAN VUUREN[a] and J A C MEIJS[b]
[a] *Institute for Livestock Feeding and Nutrition Research (IVVO), P.O. Box 160, 8200 AD Lelystad, The Netherlands*
[b] *CLO-Institute for Animal Nutrition 'De Schothorst', Meerkoetenweg 26, 8218 NA Lelystad, The Netherlands*

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Abstract. Theoretically a 600 kg cow producing 25 kg milk per day can utilize dietary nitrogen (N) with a maximum efficiency of 40–45%. The actual efficiency of N utilization in cows grazing intensively managed pastures, is 15 to 25%. When young leafy grass is the sole feed this figure cannot be altered substantially without reducing animal performance. Supplementing the diet of grazing dairy cows with a low-protein, high-energy feed (special concentrates or maize silage) increases the efficiency of N utilization, mainly because it lowers N intake. First results using maize silage indicate an improvement in N utilization of 25 to 30%.

Introduction

Intensification of pasture management by higher nitrogen (N) input and better grazing management has led to higher N concentrations in herbage. Van der Meer [12] estimated that on farms using abundant N fertilizer only 16% of the N input is removed in milk and liveweight. Important causes for such a low utilization of N are an inefficient uptake by herbage of N in faeces, urine and slurry and low efficiency of N utilization by ruminants.

Knowledge of the limiting factors in the utilization of N from herbage by dairy cows, both for grassland managers and nutritionists, is an important step to improve the efficiency of N utilization, thus preventing or diminishing the emission of N to the environment.

In this paper we will focus on the utilization of N by grazing dairy cows and indicate possibilities for nutritional improvement.

Nitrogen metabolism of dairy cows

Compartmentation of the proximal part of the digestive tract of ruminants slows the transit of feed, thus enabling a predigestion of slowly degradable

feed rich in fibre. The presence of cellulase producing micro-organisms in the forestomachs gives ruminants the ability to utilize energy from cellulose. Delayed passage of the feed, and microbial digestion in the forestomachs also affect the protein metabolism of ruminants.

New concepts that enable the prediction of the amount of absorbable amino acids reaching the intestines are being developed in order to take this redistribution of N into account. Such systems distinguish between two sources of amino acids entering the small intestines. One source is the non-degraded feed protein that escapes microbial digestion. The proportion of this "bypass protein" depends mainly on the rate of degradation and on the retention time of feed in the forestomachs. The second source of amino acids is microbial protein, synthesized in the forestomachs.

Microbial protein synthesis depends on the supply of (i) precursors such as peptides, amino acids, ammonia, carbon skeletons, phosphorus, and sulphur, (ii) other essential nutrients and (iii) energy [7]. Under optimal conditions the amount of protein synthesis is related to the amount of energy extracted from the feed. Demeyer and Tamminga [7] estimate a synthesis of 35 g microbial N (220 g crude protein) per kg organic matter digested in the forestomachs under ideal circumstances. However, the efficiency of microbial protein synthesis on fresh herbage seems to be somewhat higher, but reasons for this are still not clear [4]. On the other hand, we also have to bear in mind, that a significant amount of the organic matter from herbage digested in the forestomachs is protein, hence limiting the amount of energy available for the growth of the microbes [21].

Not all the protein entering the small intestines will be absorbed: intestinal digestion of different proteins may vary considerably [10]. Also, the absorbed amino acids will not be completely recovered in animal protein. Part of the N is lost during metabolism.

Minimum loss of nitrogen by dairy cows

Maintenance

In a recent review Owens [19] concluded that a 600 kg cow fed at maintenance level loses 52 to 82 g N day^{-1} (Table 1). Several factors attribute to this minimum N loss. A small proportion is lost with skin and hair. A second fraction is endogeneous urinary N (EUN), originating from N containing metabolites (e.g. amino acids, nucleic acids) expelled during metabolic processes of body tissues. A third route of N excretion is faecal output. Metabolic faecal N (MFN) consists of the residues of secreted enzymes, sloughed intestinal cells and of the gastro-intestinal biota.

Table 1. Minimum N losses (g N day^{-1}) of dairy cows [19]

A: MAINTENANCE (600 kg liveweight)			
	skin, hair	2	
	endogeneous urinary N	10–15	
	metabolic faecal N	40–65	
			67
B: MILK PRODUCTION (N content: 5.2 g kg^{-1})			
per kg:	endogeneous urinary N:		
	– absorbed nucleic acids	0.8	
	– metabolized during synthesis	0.9	
	metabolic faecal N	2.4	
		4.1	
per 25 kg:			103
TOTAL			170

Production

The production of milk protein is also coupled with inherent losses of N, originating from processes within the gastro-intestinal tract and intermediary metabolism. The production of 1 kg milk requires 460 NEL (Net Energy for Lactation, in VEM per kg dry matter (DM); 1 VEM = 6.9 kJ, [6]), under normal conditions equivalent to approximately 250 g organic matter fermented in the rumen (FOM). As stated by Owens [19] urinary N excretion increases with about 3 g per kg FOM, resulting from extra microbial nucleic acids, synthesized in the rumen and subsequently absorbed from the intestinal tract.

Another urinary N loss is due to the fact that the efficiency of N utilization is less than 100%. If the composition of the amino acids absorbed from the intestines is ideal, e.g. matches potential needs, the efficiency of amino acid utilization is considered to be approximately 85% [18]. Also extra faecal N output has to be taken into account. Various methods for estimating MFN are used. Considering a feed containing 900 g organic matter and 700 g digestible organic matter per kg DM, MFN is estimated as 4.3 (US-NRC system [20]), 4.6 (French-PDI system [22]) or 4.9 [5] g per kg DM ingested. Under normal conditions 460 NEL requires a DM intake of approximately 0.5 kg.

Thus N loss of a 600 kg cow, producing 25 kg milk day^{-1} and fed on energy and protein balance, calculated with the assumptions described above, will be at least 170 g N day^{-1} (Table 1). In this ideal situation at a production of 130 g milk N day^{-1} the efficiency of utilization of dietary N is 43%. Under practical conditions efficiencies will be lower, because of a lower N digestibility and a less ideal composition of absorbed amino acids.

Nitrogen losses by grazing dairy cows

As indicated by Van der Meer [13] intensification of grassland production and N fertilization has led to a decrease of the efficiency of N utilization. Cows weighing 550 kg and producing 20 kg milk day^{-1} utilized 21% of the ingested N on a farm where no N fertilizer was applied. On farms with an intensive N fertilization (Nitrogen Pilot Farms) only 16% of ingested N is retained in milk and liveweight (Table 2), which is far from the theoretical maximum efficiency of 40–45%.

Balance trials have been performed by Van der Honing *et al.* [9] using dairy cows fed fresh herbage indoors. The efficiency of utilization of N in these experiments was 22 to 25% (Table 3), which is higher than that calculated for the Nitrogen Pilot Farms. The reason for this difference seems mainly a different N intake.

The efficiency of N utilization in grazing dairy cows can be estimated from experiments of Meijs [14, 15, 16]. Weight gain of the spring-calving animals was small during these trials and therefore we assumed N retention to be negligible. On average about 78% of the N ingested with the herbage was excreted with faeces and urine (Table 4). This result agrees with data from indoor-experiments (74–78%), and makes it clear that the utilization of grass N on well fertilized pastures is generally less than the theoretical maximum. Efficiency of N utilization in the experiments of Meijs was

Table 2. Estimated utilization of N of unfertilized and heavily fertilized herbage by dairy cows, weighing 550 kg and producing 20 kg fat corrected milk per day [13]

N rate ($kg\,ha^{-1}\,yr^{-1}$)	N in herbage DM ($g\,kg^{-1}$)	N intake ($g\,day^{-1}$)	N excretion ($g\,day^{-1}$)		
			milk	faeces	urine
0	29.6	506 (100%)	106 (21%)	117	283
383	44.0	647 (100%)	106 (16%)	111	430

Table 3. Nitrogen balance of cows fed fresh herbage and 1 kg concentrates per day (basic data from [9])

Institute	N in herbage DM ($g\,kg^{-1}$)	N intake ($g\,day^{-1}$)	N excretion ($g\,day^{-1}$)			N retention by animal ($g\,day^{-1}$)
			milk	faeces	urine	
Anim. Physiol., Wageningen	37.2	521 (100%)	88 (17%)	126 (24%)	279 (54%)	28 (5%)
IVVO, Lelystad	38.8	460 (100%)	107 (23%)	117 (25%)	227 (49%)	9 (2%)

Table 4. Nitrogen intake and N excretion in the milk of grazing cows supplemented with a maximum of 1 kg DM day^{-1} [14, 15, 16]

Year	N in herbage DM ($g\,kg^{-1}$)	N intake ($g\,day^{-1}$)	N excretion in milk ($g\,day^{-1}$)	Efficiency of N utilization (%)
1978	36	521	122	23.4
1979	38	575	127	22.1
1981	38	560	126	22.5
1982	38	572	107	18.7
1983	35	525	123	23.4
Mean	37	551	121	22.0

somewhat higher than on the Nitrogen Pilot Farms, because of a lower herbage N content, and a higher N excretion with the milk.

Reduction of N losses by grazing animals can be pursued by two approaches:

- change the factors which determine the efficiency of N utilization without alteration of the diet (same amount of fresh herbage), or
- reduce the fraction of herbage in the diet by feeding supplements.

Reduction of nitrogen losses with herbage as sole feed

One obvious way to improve N utilization is by increasing milk protein production. However, there are indications that milk production of grazing dairy cows not receiving supplementation is limited to 20–25 kg day^{-1} by the restricted intake of energy [14] and by an insufficient supply of absorbable amino acids in the small intestine [4]. The inadequate supply of amino acids in the small intestine would arise from the excessive degradation of protein from fresh forages (Tables 5 and 6). This excessive loss of protein N in the forestomachs can not be compensated for by microbial synthesis.

In fact, improvement of N utilization can be achieved either by decreasing N intake or by increasing protein supply to the small intestine possibly resulting in a higher milk protein production. With herbage as the sole feed, the intake of N can only be decreased by lowering the crude protein (CP) content of the herbage. Crude protein levels in temperate grass species vary widely and may range from 70 to 150 g per kg DM in sub-optimal situations up to 300 g per kg DM in highly productive regions, where pastures are heavily fertilized [11]. Lowering N fertilization will decrease CP intake, mainly because of a decreased CP content in the herbage. Lower N fertilization may also decrease the rate of CP degradation in the forestomachs (Table 5), resulting in a higher proportion of feed protein escaping from

Table 5. Effect of N fertilization on the crude protein (CP) content in herbage, the rate of disappearance of CP from nylon bags incubated in the forestomachs (k_d) and estimated supply of digestible feed CP entering the small intestine (DPI). (S. Tamminga, personal communication)

N rate ($kg\,ha^{-1}\,yr^{-1}$)	CP in herbage DM ($g\,kg^{-1}$)	k_d (% hr^{-1})	DPI in herbage DM ($g\,kg^{-1}$)
0	203	8.6	44
250	261	9.7	52
400	288	10.3	57
700	332	10.9	58
maize silage	94	1.4	14

rumen degradation. However, the absolute supply of feed amino acids to the small intestine will not be altered, because of the simultaneously occurring lower CP intake.

A lower CP intake may also be achieved by feeding more mature grass. As grasses mature CP content decreases, as does the rate of protein degradation in the forestomachs (Table 6). However, digestibility and hence NEL content of more mature herbage is substantially lower. Thus energy intake with this herbage will be less, and consequently milk production will decline.

Another possibility to decrease N content is fractionation of grass into a protein-rich juice and a fibrous pressed residue. However, the NEL/CP ratio in the pressed residue is only changed moderately compared to the original material [24]. Furthermore this method is expensive and energy consuming.

Several research groups have tried to increase protein supply to the small intestine by inhibiting protein degradation in the forestomachs. One approach has been the inclusion of tannin containing legumes like sainfoin (*Onobrychis viciifolia* [4]) or *Lotus pedunculatus* [2]. Proteolysis of tannin-protein complexes is reduced or takes place more slowly. The tannin-protein

Table 6. Effect of stage of herbage maturity on crude protein (CP) content, rate of disappearance of CP from nylon bags incubated in the forestomachs (k_d) and estimated supply of digestible feed CP entering the small intestine (DPI). (S. Tamminga, personal communication)

Weeks after mowing	CP in herbage DM ($g\,kg^{-1}$)	k_d (% hr^{-1})	DPI in herbage DM ($g\,kg^{-1}$)
3	269	12.2	41
4	275	10.1	43
5	256	11.0	39
6	213	9.9	32
7	156	9.6	27
8	200	8.6	32

bonds are cleaved under the acidic conditions in the abomasum and thus do not interfere with the proteases excreted by the host-animal. However, in some cases the effects of tannins on animal performance have not been very promising [1].

Influencing proteolysis in the forestomachs has also been attempted by using formaldehyde treatment to affect the molecular structure of proteins or Monensin to affect the metabolism of microbes. However, the effects have been found variable [3].

There is little data on comparisons between different herbage species. Differences have been reported, but they may be partly overruled by variations occurring during the growing season [4].

Reduction of nitrogen losses by supplement feeding

Cows grazing on well fertilized pastures consume 50–100% more protein than they require. The easiest way to reduce N consumption is to replace part of the N-rich herbage by roughage or concentrates with a low protein content. During the summer of 1985 a stall-feeding experiment was done at Lelystad in which herbage and a combination of herbage and maize silage were compared. The aim of this experiment was to compare both rations at the same level of net energy intake, and therefore feed supply was restricted. Herbage contained 39 g N per kg DM, maize silage 17 g N per kg DM.

The results of the N balance measurements are shown in Table 7. The N in the faeces and urine was decreased by 28% when 49% of herbage DM was replaced by maize silage. This effect was mainly due to the reduced intake of N with this treatment. However, despite the lower protein intake, the protein content of the milk was increased significantly ($P < 0.05$) by maize silage. Most probably the microbial protein synthesis in the rumen was improved by the supply of energy, mainly carbohydrates, from maize silage.

Another way of reducing the N content of the diet is by supplementing herbage with concentrates. Several authors have reported a substantial

Table 7. Nitrogen balance of cows fed fresh herbage or a combination of herbage and maize silage (51/49 on DM basis)

Ration	N intake ($g\,day^{-1}$)	N excretion ($g\,day^{-1}$)			Efficiency (%)
		milk	faeces	urine	
Herbage	626 (100%)	107 (100%)	158 (100%)	361	17 (100%)
Herbage + maize silage	494 (79%)	118 (110%)	178 (72%)	198	24 (141%)

decrease of the ammonia concentration in the rumen when supplementing N-rich forages [8, 23]. The effect on N excretion will be less than with supplementation of maize silage, because of the higher N content in concentrates. Moreover, the quantities of concentrate which may be fed in addition to herbage are limited, due to possible disturbances of rumen fermentation [23]. However, when carbohydrates in the concentrate consist of highly but slowly digestible structural polysaccharides, such as soya bean hulls and palm kernel expeller, the negative effects on herbage intake and milk fat may be smaller [17].

Conclusion

Possibilities for an improvement of the efficiency of N utilization in ruminants with grass as the sole feed are limited. In most cases such measures are coupled with a decline of animal performance due to a decrease of NEL intake. A better response seems possible if grazing cows are supplemented with good quality concentrates or forages with a relatively high energy and low protein content. Preliminary experiments gave promising results. However, the consequences of such feeding systems on farm management and economics are not yet clear.

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CHAPTER 3

In sacco degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows.

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In sacco degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows

A. M. VAN VUUREN, S. TAMMINGA* AND R. S. KETELAAR†

Research Institute for Livestock Feeding and Nutrition, PO Box 160, 8200 AD Lelystad, Netherlands

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SUMMARY

In three experiments, carried out in 1985 and 1986 in the Netherlands, the effects of herbage maturation and rate of nitrogen fertilization on rumen degradability of organic matter (OM) and crude protein (CP) in fresh herbage (*Lolium perenne*) were studied using the nylon bag technique. Experimental farms at Lelystad and Swifterbant (clay soil) and Achterberg (sandy soil) provided the herbage samples. From the results, the content of digestible CP entering the small intestine (DPI) was estimated.

Crude protein content and *in sacco* degradability of OM and CP decreased with increasing grass maturity and with decreasing rate of N application. With every 100 g/kg DM decrease in CP content, the estimated content of DPI decreased by 19 g/kg DM, no matter how the CP content was manipulated.

INTRODUCTION

Fresh herbage from intensively fertilized swards contains high concentrations of crude protein (CP), of which 70–90% is present as true protein (Tamminga 1986). However, in young grass, a substantial proportion of the nitrogen may be in the form of nonprotein N (NPN) (Mangan 1982). Crude protein of highly fertilized, young grass is thus characterized by a high rate of rumen degradation (Standing Committee on Tables of Feed Composition 1990). With protein concentrations of > 200 g/kg of dry matter (DM), this will result in high ruminal ammonia concentrations (Beever & Siddons 1986; Van Vuuren *et al.* 1986) and substantial losses of N via urine (Van Vuuren & Meijs 1987).

A second component contributing to the protein value of grass is its potential as a substrate for microbial growth. Young, heavily fertilized grass contains substantial amounts of soluble protein, which may not be utilized for microbial protein synthesis very efficiently. Efficiency of microbial growth on protein as a substrate is considerably lower than on carbohydrates (Demeyer & Tamminga 1987).

Using new evaluation systems, the estimated protein supply of grazing dairy cows consuming 16 kg organic matter (OM) from herbage, meets the requirements for maintenance and a daily production of 23 kg of milk (Vérité *et al.* 1987), instead of the 30–40 kg which the system of digestible crude protein suggests (Centraal Veevoederbureau in Nederland 1977).

Protein utilization of grazing animals can be improved by changes in grassland management. As herbage matures, OM digestibility decreases (Wilman 1975) as well as the proportion of soluble N (Sanderson & Wedin 1989*b*). This may result in a decrease in the rate of CP degradation and, because of a higher escape from rumen fermentation, to an increase in the proportion of herbage protein entering the small intestine. However, since CP content also decreases with increasing maturity, the amount of unfermented plant protein reaching the small intestine per kg DM ingested may not differ very much.

Reducing the rate of application of N fertilizer reduces CP content and results in a smaller proportion of NPN (Wilman & Wright 1983). Rate of N fertilization thus influences solubility (Sanderson & Wedin 1989*a*) and probably also degradability of CP in the rumen.

Effects of grass maturation and rate of N fertilization on rumen degradability of OM and CP of fresh herbage (*Lolium perenne*) were studied using nylon

* Present address: Department of Animal Nutrition, Agricultural University, Wageningen, Netherlands.

† Present address: Experimental Farm 'Groot Kantwijk', Mengvoeder UT-Delfia bv, Vreeland, Netherlands.

Table 1. *Effect of maturation on chemical composition of* Lolium perenne *harvested in summer 1985 at Lelystad, Netherlands*

Weeks after previous (2nd) cut	Dry matter (g/kg)	Organic matter (g/kg DM)	Crude protein (g/kg DM)	Neutral detergent fibre (g/kg DM)	*In vitro* organic matter digestibility (%)
1	173	888	298	389	82·4
2	196	897	280	373	81·3
3	165	902	270	341	85·2
4	160	900	272	371	83·8
5	133	887	254	403	83·3
6	156	895	213	406	82·8
7	175	896	156	453	81·4
8	155	898	199	493	77·5

Table 2. *Effect of maturation on instantly degradable (S) and undegradable (U) fractions and rate of disappearance of the insoluble potentially degradable fraction (k_d;/h) of organic matter and crude protein of* Lolium perenne *harvested in summer 1985 at Lelystad, Netherlands (mean ± S.D.)*

Weeks after previous (2nd) cut	Organic matter			Crude protein		
	S	U	k_d	S	U	k_d
1	0·31	0·10 ± 0·007	0·067 ± 0·0063	0·37	0·04 ± 0·001	0·099 ± 0·0047
2	0·29	0·09 ± 0·016	0·081 ± 0·0056	0·25	0·04 ± 0·003	0·117 ± 0·0041
3	0·31	0·05 ± 0·009	0·091 ± 0·0047	0·22	0·02 ± 0·007	0·127 ± 0·0132
4	0·30	0·08 ± 0·007	0·075 ± 0·0122	0·28	0·04 ± 0·002	0·098 ± 0·0115
5	0·28	0·08 ± 0·017	0·075 ± 0·0010	0·26	0·04 ± 0·003	0·106 ± 0·0130
6	0·31	0·10 ± 0·014	0·065 ± 0·0017	0·31	0·06 ± 0·004	0·099 ± 0·0074
7	0·26	0·13 ± 0·015	0·062 ± 0·0031	0·21	0·09 ± 0·004	0·096 ± 0·0086
8	0·26	0·15 ± 0·027	0·051 ± 0·0074	0·30	0·09 ± 0·009	0·087 ± 0·0046

bag incubations in three experiments with grazing dairy cows in 1985 and 1986, using herbage from experimental farms at Lelystad, Swifterbant and Achterberg in the Netherlands. From the results, differences were measured in the estimated contents of digestible CP entering the small intestine of cows eating different grasses.

MATERIALS AND METHODS

Herbage

In Expt 1, herbage was sampled from one plot on clay soil at Lelystad in June and July 1985. One week before first sampling, this plot was mown (second cut) and fertilized with 83 kg N/ha. Samples were taken every 7th day for 8 consecutive weeks, by mowing an area of 10–20 m² with a cutter bar at a height of *c.* 4 cm and were then chopped with a paper guillotine to *c.* 1 cm in a climatic room at 4–7 °C. Nylon bags (polyamide 190 × 100 mm, mesh size 41 μm, porosity 20%, Nybolt, Switzerland) were filled with *c.* 30 g of fresh, chopped material, equivalent to 5 g DM, tied and stored at −18 °C until rumen incubation. A 600 g sample was lyophilysed, ground to pass through a 1 mm screen and stored for proximate and cell wall analyses.

In Expt 2, herbage from eight plots, four on sandy soil at Achterberg and four on clay soil at Swifterbant, was sampled weekly and used to evaluate the effect of N fertilization on DM yield under cutting (Lantinga *et al.* 1987). On each type of soil, N was applied at 0, 250, 400 and 700 kg N/ha per year. Proximate analyses and nylon bag incubations were carried out on twelve samples from sandy soil (mown on 14 May, 8 July and 12 September) and twelve from clay soil (mown on 21 May, 15 July and 19 September, 1985). Nylon bags and samples were prepared as in Expt 1.

In Expt 3, herbage was sampled in August or September 1986 from the plots on clay soils at Swifterbant. The same rates of N fertilization were applied, but, in contrast with Expt 2, herbage was mown to obtain *c.* 2000 kg DM/ha. Thus, the cutting interval decreased as rate of N application increased. Herbage was sampled and prepared as in Expt 1.

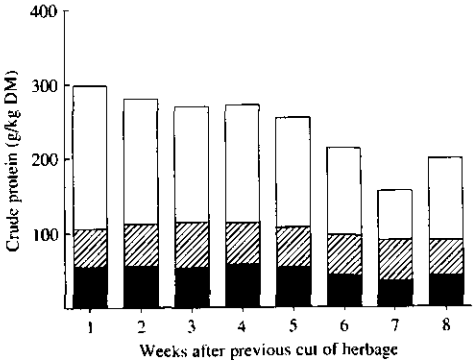

Fig. 1. Effect of maturation of *Lolium perenne* plants on (■) digestible crude protein of plant origin escaping rumen fermentation estimated by *in sacco* degradation and (▨) estimated digestible microbial protein entering the small intestine of dairy cows. Contour is total crude protein content.

Analyses and nylon bag incubations

Lyophilysed samples were analysed for DM, ash and Kjeldahl N according to the procedures of the Netherlands Normalization Institute (NEN 3332 (1974), NEN 3329 (1969) and NEN 3145 (1966), respectively). Neutral detergent fibre (NDF) was analysed by the method of Robertson & Van Soest (1981). In Expts 1 and 2, *in vitro* digestibility of OM was determined by a modified Tilley & Terry method (Van der Meer 1986). In Expt 1, crude fat was analysed by extraction with petroleum ether (boiling range 40–60 °C) (European Communities 1984).

Nylon bags were incubated as described by Van Vuuren *et al.* (1989) in three grazing cows, surgically prepared with large rumen cannulas. Incubation times were 0, 3, 6, 12, 24, 48 h and 9 (Expts 1 and 2) or 14 (Expt 3) days. After removal from the rumen, bags were cleaned with water and subsequently washed at room temperature in a domestic washing machine providing four rinses using 4 × 12 litres of water without spinning. Bags were dried at 70 °C and weighed. Residues were ground to pass through a 1 mm screen and analysed for DM, ash and N, the

Table 3. *Effect of rate of nitrogen fertilization (kg/ha per year) on chemical composition of 7-day-old* Lolium perenne *plants, grown on sandy soil at Achterberg and on clay soil at Swifterbant, Netherlands, in May, July and September 1985*

Type of soil	Days since 1 April	Applied N rate	Dry matter (g/kg)	Organic matter	Crude protein	Neutral detergent fibre	*In vitro* organic matter digestibility (%)
				(g/kg DM)			
Sand	44	0	175	905	171	415	85·8
	44	250	140	908	271	419	84·0
	44	400	120	901	328	388	84·3
	44	700	121	904	379	381	84·7
	99	0	199	907	185	419	82·7
	99	250	191	912	217	401	83·1
	99	400	183	917	247	389	84·0
	99	700	176	928	290	375	83·8
	165	0	160	848	213	403	81·5
	165	250	149	882	232	415	82·6
	165	400	144	901	261	401	82·6
	165	700	142	909	271	388	82·6
Clay	51	0	146	879	232	366	86·3
	51	250	131	891	300	375	86·1
	51	400	123	840	307	327	84·8
	51	700	121	866	357	327	85·5
	106	0	208	882	202	418	83·4
	106	250	185	889	274	395	83·7
	106	400	154	883	300	373	83·1
	106	700	149	888	359	370	83·1
	172	0	163	896	215	418	82·1
	172	250	176	898	274	409	81·6
	172	400	158	903	286	393	82·0
	172	700	153	899	339	369	84·1

Table 4. *Effect of rate of nitrogen fertilization (kg/ha per year) on instantly degradable (S) and undegradable (U) fractions and rate of disappearance of the insoluble potentially degradable fraction (k_d; /h) of organic matter and crude protein of 7-day-old* Lolium perenne *plants, grown on sandy soil at Achterberg, Netherlands, in May, July and September 1985 (mean ± S.D.)*

Days since 1 April	Days since N applied	Applied N rate	Organic matter			Crude protein		
			S	U	k_d	S	U	k_d
44		0	0·16	0·06 ± 0·004	0·046 ± 0·0062	0·06	0·04 ± 0·001	0·078 ± 0·0020
	6	250	0·14	0·05 ± 0·007	0·055 ± 0·0107	0·09	0·03 ± 0·004	0·087 ± 0·0229
		400	0·06	0·04 ± 0·007	0·064 ± 0·0036	0·07	0·02 ± 0·003	0·097 ± 0·0042
		700	0·14	0·05 ± 0·005	0·083 ± 0·0046	0·20	0·02 ± 0·004	0·110 ± 0·0067
99		0	0·13	0·08 –	0·043 ± 0·0059	0·09	0·06 –	0·078 ± 0·0101
	19	250	0·15	0·09 –	0·069 ± 0·0069	0·06	0·06 –	0·110 ± 0·0051
		400	0·16	0·08 ± 0·001	0·071 ± 0·0047	0·09	0·05 ± 0·000	0·107 ± 0·0155
		700	0·17	0·09 ± 0·034	0·084 ± 0·0176	0·13	0·03 –	0·120 ± 0·0141
165		0	0·15	0·08 ± 0·016	0·041 ± 0·0027	0·08	0·08 ± 0·009	0·084 ± 0·0130
	21	250	0·14	0·10 ± 0·020	0·047 ± 0·0054	0·07	0·07 ± 0·002	0·078 ± 0·0070
		400	0·15	0·13 ± 0·013	0·057 ± 0·0119	0·08	0·07 ± 0·040	0·084 ± 0·0118
		700	0·29	0·09 ± 0·018	0·051 ± 0·0043	0·03*	0·06 ± 0·008	0·079 ± 0·0022

* Because S was extremely low, data from this sample were omitted from further calculations.

Table 5. *Effect of rate of nitrogen fertilization (kg/ha per year) on instantly degradable (S) and undegradable (U) fractions and rate of disappearance of the insoluble potentially degradable fraction (k_d; /h) of organic matter and crude protein of 7-day-old* Lolium perenne *plants, grown on clay soil at Swifterbant, Netherlands, in May, July and September 1985 (mean ± S.D.)*

Days since 1 April	Days since N applied	Applied N rate	Organic matter			Crude protein		
			S	U	k_d	S	U	k_d
51		0	0·14	0·05 ± 0·007	0·078 ± 0·0183	0·04	0·03 ± 0·003	0·113 ± 0·0346
	13	250	0·12	0·06 ± 0·007	0·084 ± 0·0138	0·08	0·03 ± 0·004	0·120 ± 0·0429
		400	0·09	0·05 ± 0·004	0·098 ± 0·0167	0·04	0·03 ± 0·001	0·134 ± 0·0320
		700	0·12	0·04 ± 0·006	0·101 ± 0·0172	0·10	0·02 ± 0·002	0·140 ± 0·0382
106		0	0·21	0·09 ± 0·026	0·047 ± 0·0043	0·18	0·06 ± 0·006	0·092 ± 0·0175
	5	250	0·22	0·05 ± 0·006	0·056 ± 0·0108	0·16	0·05 ± 0·023	0·101 ± 0·0024
		400	0·18	0·05 –	0·070 ± 0·0074	0·15	0·03 –	0·115 ± 0·0127
		700	0·19	0·07 ± 0·021	0·078 ± 0·0047	0·25	0·03 ± 0·005	0·105 ± 0·0082
172		0	0·07	0·21 –	0·049 ± 0·0022	0·07	0·09 –	0·078 ± 0·0053
	9	250	0·19	0·13 ± 0·016	0·063 ± 0·0038	0·18	0·05 ± 0·006	0·091 ± 0·0041
		400	0·13	0·10 ± 0·014	0·050 ± 0·0044	0·07	0·04 ± 0·005	0·079 ± 0·0052
		700	0·14	0·08 ± 0·019	0·072 ± 0·0074	0·11	0·03 ± 0·001	0·101 ± 0·0087

former to be used to correct the ash and N values. Analyses were carried out as described by Van Vuuren *et al.* (1989).

Instantly degradable fractions (S) of OM and N were estimated as fractions disappearing from the bags during washing (zero incubation time). Residues in the bags after different times of incubation were fitted by a first-order degradation model, including an insoluble, potentially degradable fraction (D), degraded at a constant rate (k_d), and an undegradable fraction (U). Undegradable fractions were determined as the residues after long-term incubations (9 or 14 days) (Robinson *et al.* 1986). Residues were also fitted by including a discrete lag time. However, as lag time was not always significant and to make results comparable, all data were calculated by the model without a lag time.

Contents of fermented OM (FOM) and CP (FCP) were calculated according to Eqn (1). Digestible CP of plant origin escaping rumen fermentation (PDCP; g/kg DM) was estimated according to Eqn (2), assuming that rumen undegradable CP (U) was also

indigestible in the small intestine (W. van Straalen, unpublished).

$$\text{FOM or FCP} = \left[\left(\frac{k_d}{k_d + k_p} \times D\right) + S\right] \times (\text{OM or CP}) \quad (1)$$

where k_p is rate of passage, assumed to be 0·045/h.

$$\text{PDCP} = \left(\frac{k_p}{k_d + k_p} \times D\right) \times \text{CP} \quad (2)$$

From the amounts of FOM, FCP and PDCP the total amount of digestible CP entering the small intestine (DPI; g/kg DM) was calculated. We assumed a microbial true protein synthesis of 150 g/kg truly fermented carbohydrate (FCB = FOM − FCP − crude fat) and 75 g/kg FCP (Demeyer & Tamminga 1987; Hvelplund 1987). Digestibility of microbial protein was assumed to be 0·85 (Hvelplund 1987). In Expts 2 and 3, crude fat content was assumed to be 40 g/kg DM, being the mean fat content of the herbage in Expt 1.

The relation between chemical composition of herbage and estimated DPI was quantified using linear regression analysis (Payne *et al.* 1987).

RESULTS AND DISCUSSION

Experiment 1: grass maturation

The CP content of the herbage decreased moderately from week 1 to week 4 and more rapidly between weeks 4 and 7 (Table 1). A decrease in CP content may be due to either an increase in the proportion of stem (lower CP than leaves) or a decrease of CP in leaf and stem fractions (Lyttleton 1973) or both.

Between weeks 1 and 3, NDF decreased (Table 1), thereafter the cell wall content increased. Also the pattern of the *in vitro* digestibility suggests an improvement in the nutritive value during the first 3 weeks of regrowth, followed by a gradual decrease. These findings agreed with observations by Van Vuuren *et al.* (1989), and may be explained by an increase in the proportion of leaf to stem during the first 3 weeks, compensating for the effect of maturation on the fibre content and quality (Wilman *et al.* 1976). Increase in the concentration of structural carbohydrates with maturation was also reported by Sanderson & Wedin (1989*b*) and occurs in both leaves and stems (Bailey 1973).

Disappearance of OM and CP from nylon bags suspended in the rumen reflected the pattern in nutritive value as concluded from chemical composition and *in vitro* digestibility. Fermentable fractions (*S* and *D*) and rates of degradation of OM and CP increased until week 3 and declined between weeks 4 and 8 (Table 2). The decrease in FOM with increasing plant development may be due to increased lignification of NDF. An increase of indigestible NDF with maturation was observed by Wilman *et al.* (1977). The decrease in rates of OM and CP degradation with maturation is believed to result not only from the increase in resistance of cell walls to microbial breakdown, which additionally will lead to a slower release of cell contents, but, in the case of CP, also from the increase in proportion of N associated with cell walls (Sanderson &Wedin 1989*b*). During the first 5 weeks the undegradable CP fraction was not clearly related to growing stage; after week 5, the U fraction increased. The magnitude of the undegradable CP fraction was similar to the proportion of N in acid detergent fibre from timothy (*Phleum pratense*) and smooth bromegrass (*Bromus inermis*) reported by Sanderson & Wedin (1989*b*).

The CP fraction assumed to be effectively degraded in the rumen was high. It was estimated that 60–80% of herbage CP will be fermented, which may result in high ruminal ammonia concentrations (Beever &

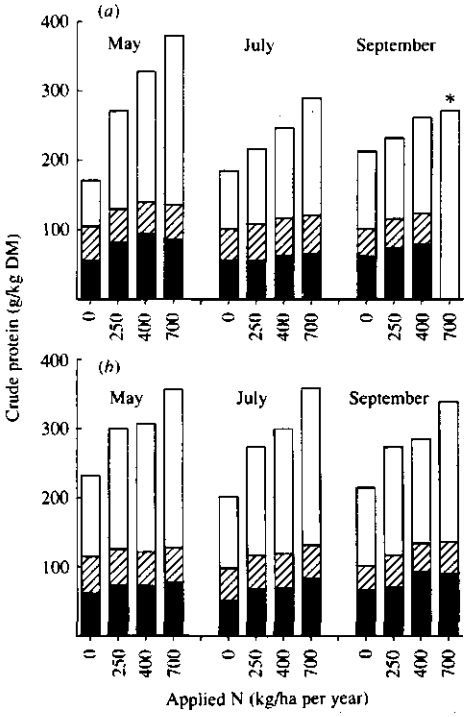

Fig. 2. Effect of rate of nitrogen fertilization on estimate (from *in sacco* degradation) of digestible crude protein of 7-day-old *Lolium perenne* plants grown on (*a*) sandy and (*b*) clay soil, entering the small intestine of dairy cows. (■) estimate of digestible crude protein of plant origin; (▨) estimate of digestible microbial protein; contour is total crude protein content; * results unreliable and omitted.

Table 6. *Effect of rate of nitrogen fertilization (kg/ha per year) on the chemical composition of* Lolium perenne *mown to obtain* c. *2000 kg DM/ha on clay soil at Swifterbant, Netherlands, in 1986*

Applied N rate	Days since previous cut	Days since 1 April	Dry matter (g/kg)	Organic matter	Crude protein	Neutral detergent fibre
				(g/kg DM)		
0	68	141	252	887	94	481
250	40	163	173	885	175	421
400	30	157	182	891	231	424
700	30	150	152	890	288	406

Table 7. *Effect of rate of nitrogen fertilization (kg/ha per year) on instantly degradable (S) and undegradable (U) fractions and rate of disappearance of the insoluble degradable fraction (k_d; /h) of organic matter and crude protein of* Lolium perenne *mown to obtain* c. *2000 kg DM/ha on clay soil at Swifterbant, Netherlands, in 1986 (mean ± S.D.)*

Applied N rate	Organic matter			Crude protein		
	S	U	k_d	S	U	k_d
0	0·10	0·12 ± 0·010	0·028 ± 0·0074	0·15	0·12 ± 0·007	0·051 ± 0·0061
250	0·15	0·07 ± 0·011	0·047 ± 0·0110	0·14	0·05 ± 0·008	0·079 ± 0·0130
400	0·13	0·06 ± 0·005	0·055 ± 0·0125	0·12	0·04 ± 0·004	0·085 ± 0·0120
700	0·13	0·05 ± 0·005	0·065 ± 0·0108	0·21	0·03 ± 0·004	0·089 ± 0·0052

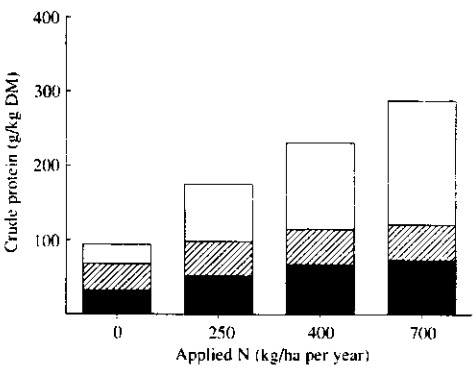

Fig. 3. Effect of rate of nitrogen fertilization on estimate (from *in sacco* degradation) of digestible crude protein of *Lolium perenne* mown to obtain *c.* 2000 kg DM/ha entering the small intestine of dairy cows. (■) estimate of digestible crude protein of plant origin; (▨) estimate of digestible microbial protein; contour is total crude protein content.

Siddons 1986; Van Vuuren *et al.* 1986). Also, Møller & Hvelplund (1988) reported high degradability of CP in perennial ryegrass, declining with plant maturity. Other data (Standing Committee on Tables of Feed Composition 1990) show that, at an assumed outflow of 0·05/h, CP degradability of fresh grass may range between 47 and 87%.

During the first 3 weeks, estimated DPI increased and then declined gradually (Fig. 1). This decline was mainly caused by the decrease in PDCP, while the estimated amount of digestible microbial protein remained reasonably constant after week 3, despite the decrease of FOM. However, as CP content decreased, carbohydrate content increased and thus a more efficient microbial synthesis was assumed, compensating for the decrease in FOM. Thus, although CP in the herbage decreased from 300 to 200 g/kg DM, DPI varied much less, from 120 to 90 g/kg DM.

Experiment 2: rate of nitrogen fertilization

The increase in N fertilizer resulted in a decrease in DM and NDF contents and an increase in CP (Table 3). Rate of N fertilization did not affect *in vitro* digestibility of OM. These results confirm those of Wilman & Wright (1983): with higher N rates, moisture and CP content increased, but OM digestibility was not changed when herbage was cut once every 7 days. Similar results were reported recently by Sanderson & Wedin (1989*a*).

There were no consistent effects of N application rate and type of soil on OM fractions (Tables 4 and 5), which may be attributed to the early growing stage

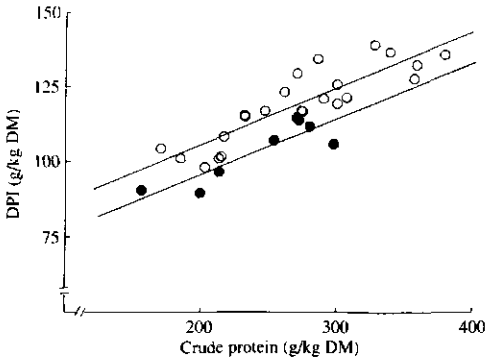

Fig. 4. Relation between crude protein content of *Lolium perenne* and estimate (from *in sacco* degradation) of digestible crude protein entering the small intestine of dairy cows (DPI). Expt 1 (●) $y = 0{\cdot}19x + 58{\cdot}7$, $r^2 = 0{\cdot}769$ (see Methods); Expt 2 (○) $y = 0{\cdot}19x + 68{\cdot}0$, $r^2 = 0{\cdot}773$.

at which the herbage was sampled. Rate of OM degradation increased with increasing N application rate, as did the soluble fraction and k_d of CP, while the undegradable CP fraction slightly decreased. Seasonal effects on the *in sacco* degradation of OM and CP were not apparent. Beever *et al.* (1987) observed seasonal effects, but the influence of season is more likely to be overruled by other environmental factors, such as rainfall and temperature, which may vary substantially in temperate climates. Mean temperatures in the week preceding sampling were 15, 17 and 13 °C in May, July and September, respectively. Time interval between N application and sampling may be another important factor, as the highest soluble CP fractions were observed at the highest N rate and at an interval between N application and sampling of less than a week.

The higher rates of OM and CP degradation at higher N rates may be attributed to increasing ratios of cell content:cell walls, indicated by the increased moisture content and the decreased NDF content. Rate of N application may also affect the rate of NDF degradation, thereby influencing the release of cell contents. However, under a 7-day cutting regime, this may be of minor importance.

As the proportions of FCP in CP and CP in OM increased with increasing N application rate, lower PDCP and lower microbial protein production is assumed, undoing, to a large extent, the positive effects of N fertilization on CP content (Fig. 2). Thus, at 0 kg N/ha per year, DPI (g/kg DM) was 50% of CP while, at 700 kg N/ha per year, this was only 40%.

Experiment 3: rate of N fertilization and maturation

Effects of N rate on chemical composition were similar to those in Expt 2 (Table 6). With increasing N rate, DM and NDF contents decreased, while CP content increased. Comparing chemical compositions at the same rate of N fertilization shows that CP content was lower and NDF content was higher than in Expt 2. Although annual variations cannot be excluded, it is more likely that the difference in plant maturity (7 days *v.* 30–68 days) is the main reason for these differences (Wilman *et al.* 1976). Undegradable OM and CP fractions were highest in herbage at the lowest N rate (Table 7). Soluble CP fraction was highest at the highest N rate. With increasing N rate, the k_d of OM and CP increased.

Estimated DPI increased with increasing N from 70 g/kg DM at 0 kg N/ha per year to 120 g/kg DM at 700 kg N/ha per year, mainly as a result of increased PDCP (Fig. 3). However, the PDI:CP ratio decreased from 78% at 0 to 44% at 700 kg N/ha per year, in agreement with the results from Expt 2.

CONCLUSION

Crude protein of fresh herbage was characterized by high rumen degradability and results in this study were similar to data reported by others (Møller & Hvelplund 1988; Standing Committee on Tables of Feed Composition 1990). Linear regression analyses showed that, when the CP content decreased by 100 g/kg DM, the DPI content decreased by 19 g/kg DM (Fig. 4).

Although the method of reducing the CP content in herbage differed in Expts 1 and 2, the relation between CP content and DPI was similar.

From these studies it can be concluded that reducing the rate of N fertilization, under practical conditions resulting in a prolonged cutting interval, will have minor effects on the supply of digestible protein entering the small intestine.

The assistance of J. J. van der Tol, P. Pijper and R. Heida (Agricultural University, Wageningen), who performed some of the nylon bag incubations, is highly appreciated.

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CHAPTER 4

Ruminal availability of nitrogen and carbohydrates from fresh and preserved herbage in dairy cows

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Ruminal availability of nitrogen and carbohydrates from fresh and preserved herbage in dairy cows

A. M. VAN VUUREN[1], S. TAMMINGA[2] & R. S. KETELAAR[3]

[1] Research Institute for Livestock Feeding and Nutrition, P.O. Box 160, NL 8200 AD Lelystad, Netherlands
[2] Present address: Department of Animal Nutrition, Wageningen Agricultural University, Haagsteeg 4, NL 6708 PM Wageningen, Netherlands
[3] Present address: Experimental Farm 'Groot Kantwijk', UT Delfia BV, Bergseweg 28, NL 3633 AK Vreelant, Netherlands

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Abstract

Ruminal degradabilities of crude protein and non-protein organic matter of fresh and preserved herbage, obtained with nylon bag studies, were compared and consequences for dairy cow rations were discussed. In Experiment 1 the effect of rate of fertilization on the in sacco degradation of fresh grass was studied. The second experiment focussed on the effect of maturation on fresh grass. In Experiments 3 and 4 the influence of maturation and dry matter content on the in sacco degradation of grass silage and hay was studied. Experiment 4 also included treatment with cell wall degrading enzymes. Fresh and preserved herbage fertilized at high rates of nitrogen, had a large surplus of fermentable nitrogen. In fresh herbage the ratio of soluble nitrogen:soluble non-protein organic matter ('carbohydrates') was lower than the ratio of insoluble, degraded nitrogen:insoluble, degraded carbohydrates. Therefore it was concluded that ingredients with a low ratio of insoluble, degraded nitrogen:insoluble, degraded carbohydrates may be appropiate supplements for grass-based diets. In preserved herbage the ratio of soluble nitrogen:soluble carbohydrates exceeded the ratio of insoluble, degraded nitrogen:insoluble, degraded carbohydrates. Wilting had no consistent effect on the ratios of nitrogen:carbohydrates. Treatment with cell wall degrading enzymes resulted in a lower ratio of soluble nitrogen:soluble carbohydrates. It was suggested that silage-based diets require supplementation with ingredients rich in soluble carbohydrates.

Keywords: rumen availability, in sacco degradation, nitrogen, carbohydrates

Introduction

Herbage, either fresh or preserved (hay, silage) features largely in the nutrient supply of ruminants. In the Netherlands, grass and grass products make up 50 to 60 % of the total energy intake of a dairy cow. The chemical composition of grass and grass products may vary considerably and depends on a wide range of genetic and environmental factors (Gill et al., 1989) such as grass species and variety, rate of fertilization, solar radiation, rainfall, maturity at time of grazing or harvesting and

method of preservation (Henderson et al., 1982). These factors not only influence composition but also the rate and extent of ruminal and intestinal degradation (Tamminga et al., 1990a; van Vuuren et al., 1989) and as a result the intake of the various components.

Highly fertilized, young herbage is characterized by a high content of crude protein (CP) and a high rate and extent of degradation of CP, causing high concentrations of ammonia-N in the rumen (van Vuuren et al., 1986) and substantial N losses via urinary excretion. In grass silages a variable proportion of CP will become solubilized and consequently becomes even more rapidly degradable in the rumen and poorly utilizable by micro-organisms (Thomas, 1982).

A better utilization of nutrients, especially N, seems possible by matching the supply of rumen-degradable N and carbohydrates. Czerkawski (1986) calculated from stoichiometric considerations an equilibrium with a capture of 22.7 to 27.7 g N per kg OM fermented, while in vivo determinations produced an average efficiency of 19.3 g N per kg OM fermented. Thus from a N economy the optimal ratio of N to OM fermented is around 25 g kg^{-1}.Under conditions of an optimal N economy, synchronization of available energy and N to the rumen biota is likely to be another important condition for an improved utilization of N.

Rumen micro-organisms use carbohydrates as their main energy source. In fresh herbage soluble sugars, fructosans and cell wall polysaccharides are the main carbohydrates. In silages the sugars and fructosans are pre-fermented to a variable degree. In fresh forages 70 to 90 % of N is present in true protein (Tamminga, 1986), mainly in soluble enzymes in chloroplasts and cytoplasm and in insoluble proteins (mainly chlorophyll) in the chloroplast membrane (Mangan, 1982). Sugars, fructosans and soluble N components are supposed to be instantly available for the rumen biota and therefore assumed to possess an infinite rate of degradation. Cell wall components and insoluble N components are however degraded at much slower but variable rates.

The objective of this study was to obtain information on the ratio of N and carbohydrates in rumen soluble, rumen insoluble effectively fermented, potentially degradable but escaping rumen fermentation and undegradable components in grass and grass silages from estimates of the disappearance of nutrients from nylon bags suspended in the rumen (Mehrez & Ørskov, 1977). Such information is required in order to understand how to optimize grass or silage-based rations.

Material and methods

Forages

In Experiments 1 and 2 the degradation of rye grass (*Lolium perenne*) was studied with nylon bag incubations in the rumen of cows. In Experiments 3 and 4 the degradation of grass silages was investigated with the same technique.

In Experiment 1, herbage originated from 8 different plots cut weekly. Mowing height was approximately 4 cm. Four plots were laid out on sandy soil, the others on clay soil. On each type of soil 4 rates of nitrogen fertilization were applied, result-

ing in 0, 250, 400 and 700 kg N ha^{-1} yr^{-1}, respectively. The herbage cut on May 14 (sandy) and 21 (clay), July 8 (sandy) and 15 (clay) and September 12 (sandy) and 19 (clay) 1985 was sampled and taken to the laboratory in cooling boxes. The herbage was chopped with a paper guillotine to a particle length of approximately 1 cm. Nylon bags (polyamide 190 × 100 mm, mesh size 41 μm, porosity 30 %; Nybolt, Switzerland) were filled with approximately 30 g of fresh material, equivalent to 5 g dry matter (DM). Bags were tied and stored at − 18 °C until rumen incubations. Sample preparations were carried out in a climatic room at 4-7 °C. A 600 g sample was lyophilized, ground to pass a 1 mm screen and stored for proximate and cell wall analyses.

Experiment 2 focussed on the effect of maturity on CP content and ruminal disappearance. In June and July 1985, herbage was sampled every seventh day, starting 9 days after previous cutting. For 8 consecutive weeks strips of one plot were mowed with a cutter bar at a height of approximately 4 cm. Samples and nylon bags were prepared as in Experiment 1.

In Experiment 3, herbage samples of week 2, 4, 6 and 8 of Experiment 2 were chopped and ensiled in 1.5 l preservation jars either directly or after wilting in the laboratory to reach DM levels of ca 300 and 450 g kg^{-1}. After 90 days, jars were opened and nylon bags were filled with the silage (approximately 5 g DM) and stored at − 18 °C until rumen incubation. Another proportion of the sample was dried to reach a DM content of ca 875 g kg^{-1} (hay) and put into nylon bags. Samples of silages and hay were oven-dried at 70 °C, ground to pass a 1 mm screen and stored for proximate and cell wall analyses.

In Experiment 4, herbage was harvested from one plot 2, 4 and 6 weeks after the previous cut and ensiled either directly or after wilting to a DM content of 300 and 450 g kg^{-1} and with or without the addition of cell wall degrading enzymes. Detailed information on experiment 4 has been published elsewhere (van Vuuren et al., 1989).

Nylon bag incubations and analyses

Dried samples of the original material were analysed for DM, N and neutral detergent fibre (NDF) as described previously (van Vuuren et al., 1989).

Nylon bags incubations were carried out as described by van Vuuren et al. (1989) in the rumen of three dairy cows, surgically prepared with a large rumen cannula (Bar Diamond, Idaho, USA). Incubation times were 0, 3, 6, 12, 24 and 48 h and 9 (Experiments 1 and 2) or 14 (Experiments 3 and 4) days. After removal from the rumen, bags were rinsed with water and subsequently washed in a domestic washing machine, without spinning. The bags were dried at 70 °C and weighed. The residues were ground to pass a 1 mm screen and analysed for DM, ash and N, the former to be used to correct the N and ash values. Analyses were carried out as described previously (van Vuuren et al., 1989).

The instantly degradable, soluble fraction (S) was estimated as the fraction disappearing from the bags during washing (zero incubation time). The residues present in the nylon bags after the various times of incubation were fitted using a first-order

model, including an insoluble, potentially degradable fraction (D), degraded at constant rate (k_d), and an undegradable fraction (U). The undegradable fraction was measured as the residue after long-term incubation: 9 or 14 days (Robinson et al., 1986).

The primary aim of these studies was to estimate the proportion of CP assumed to be fermented in the rumen. Thus, only limited numbers of bags were incubated and consequently only small quantities of feed residues per incubation time were recovered, especially from the bags incubated 9 or 14 days. After ash and N analyses the quantity of feed residues was insufficient for the determination of cell wall carbohydrates. Therefore the disappearance of non-protein, non-fat organic matter was used as an estimate of carbohydrate (CB) degradation. The OM in the silages was not corrected for volatile components.

The contents of fermented N (FN) and fermented CB (FCB) per kg DM were estimated according to the formula:

$$\text{FN or FCB} = \left[\frac{k_d}{k_d + k_p} \times D + S\right] \times \text{N or CB}$$

where:
k_d = rate of degradation of N or CB (h^{-1})
k_p = rate of passage, assumed 0.045 h^{-1}
D = insoluble, potentially degradable fraction of N or CB
S = instantly degradable fraction of N or CB

The content of soluble, instantly degradable N (SN) was calculated as $[S_n \times N]$; soluble carbohydrate (SCB) as $[S_{om} \times OM - S_{cp} \times CP]$. The contents of insoluble N (EN) and CB (ECB) assumed to be fermented in the rumen were estimated as [FN − SN] and [FCB − SCB], respectively. Undegradable N (UN) was calculated as $[U_n \times N]$ and undegradable CB (UCB) as $[U_{cb} \times CB]$.

We also estimated the contents of potentially degradable N and CB escaping from rumen fermentation according to the formula:

$$\text{PDN or PDCB} = \left[\frac{k_p}{k_d + k_p} \times D\right] \times \text{N or CB}$$

where:
k_p = rate of passage, assumed 0.045 h^{-1}
k_d = rate of degradation of N or CB (h^{-1})
D = insoluble, potentially degradable fraction of N or CB

Relationships between chemical composition and rumen availability were quantified using multiple regression analysis (Payne et al. 1987). Effects of treatments were subjected to analysis of variance.

Results

Effect of rate of N fertilization (Experiment 1)

In Experiment 1 the N content of herbage ranged between 27.3 and 60.7 g kg^{-1} DM and increased with increasing rate of N fertilization. At the higher N rates the proportion of UN was lower and that of FN higher than at low N rates. The effect of rate of N fertilization on the soluble and insoluble rumen-degradable fractions varied and depended on the time between N fertilization of the plots and sampling of the grass (van Vuuren, unpublished). Since effects of type of soil were not apparent, results from both types of soil were pooled per N rate.

The CB content of herbage decreased with increasing N fertilization (Figure 1). This decrease was mainly a decrease of PDCB. The proportion of FCB was higher at higher N rates. The content of UCB increased with the season.

The FN:FCB ratio ranged between 46.2 and 159.9 g N kg^{-1} CB and was strongly correlated with the N content (R = 0.98). Figure 2 shows that the SN:SCB ratio was lower than the EN:ECB ratio, except in May at the highest N rate, when SN:SCB exceeded the EN:ECB ratio. Also in May, the SN:SCB ratio at 0 kg N ha^{-1} was lower than 25 g kg^{-1}, the ratio at which microbial growth is assumed to be optimum (Czerkawski, 1986). The EN:ECB ratios were always higher than 25 g kg^{-1}.

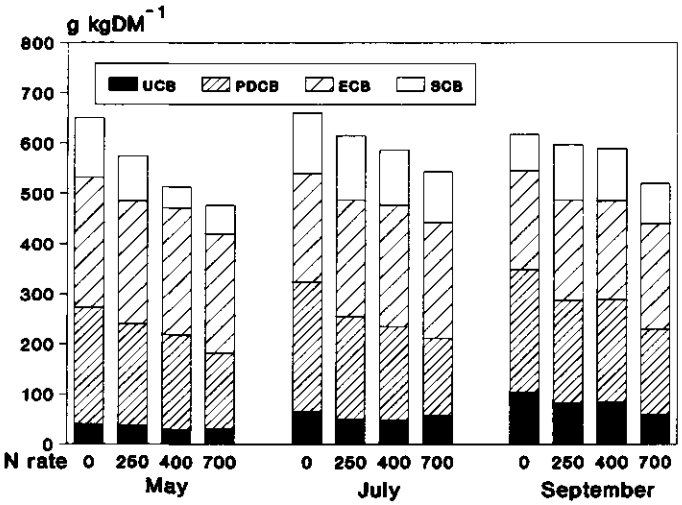

Fig. 1. Effect of rate of nitrogen fertilization (0, 250, 400 and 700 kg ha^{-1} yr^{-1}, respectively) and season on carbohydrate (CB) in fresh herbage (*Lolium perenne*). Experiment 1. (UCB = undegradable CB; PDCB = potentially degradable CB escaping from rumen fermentation; ECB = potentially degradable CB effectively fermended in the rumen; SCB = soluble CB).

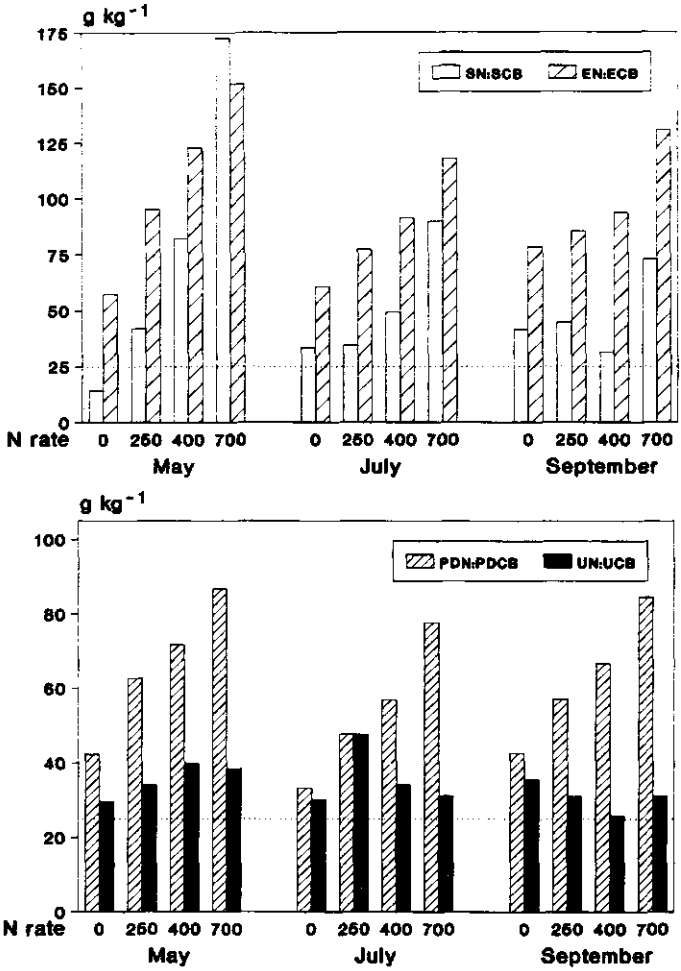

Fig. 2. Effect of rate of nitrogen fertilization (0, 250, 400 and 700 kg ha^{-1} yr^{-1}, respectively) and season on ratios between nitrogen (N) and carbohydrates (CB) in fresh herbage (*Lolium perenne*). Experiment 1. (U = undegraded; PD = potentially degradable but escaping from rumen fermentation; E = potentially degradable effectively fermented in the rumen; S = soluble).

Effect of maturity (Experiment 2)

With maturation the N content decreased from 47.7 to 31.8 g N kg^{-1} DM, mainly due to a decrease of FN (van Vuuren, unpublished). The CB content increased with aging (Figure 3). The UCB and PDCB contents decreased between weeks 1 and 3,

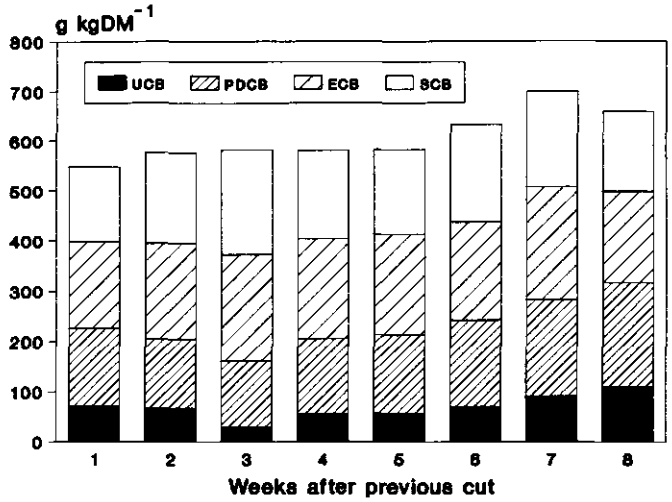

Fig. 3. Effect of maturity (weeks after previous cut) on carbohydrates (CB) in fresh herbage (*Lolium perenne*). Exp. 2. (UCB = undegradable CB; PDCB = potentially degradable CB escaping from rumen fermentation; ECB = potentially degradable CB effectively fermented in the rumen; SCB = soluble CB).

but from then onwards increased with maturation.

As in Experiment 1 the SN:SCB ratio was lower than the EN:ECB ratio, except in week 1 (Figure 4). After week 2 the EN:ECB ratio decreased with aging. The ratios of SN:SCB and EN:ECB were always higher than 25 g kg^{-1}. The ratio of PDN:PDCB increased from week 1 to 3 and subsequently decreased. The UN:UCB ratio was approximately 25 g kg^{-1} and not influenced by aging.

Effect of method of preservation (Experiment 3)

The SCB of the silages was lower than in the original herbage. The effects of maturation were similar to those found in Experiment 2: with aging SN and SCB decreased, while UN, PDCB and UCB increased (Table 1). The SN:SCB and EN:ECB ratios tended to decrease with aging. The EN:ECB ratio was not affected by method of preservation.

Ensiling of grass increased SN especially when ensiled at low DM contents and at the young stages (Tamminga et al., 1990a). Comparing data from Table 1 with those of Figure 3 it may be concluded that ensiling grass decreased the content of SCB except when ensiled without wilting (220 g DM kg^{-1}). However, the effect of method of preservation on the SN:SCB ratio was not significant.

Contrary to fresh herbage the SN:SCB ratio in the silages exceeded the EN:ECB ratio. Also in hay (87.5 % DM) the SN:SCB ratio was higher than the EN:ECB ratio. In silages the EN:ECB ratio was slightly above 25 g kg^{-1}. The PDN:PDCB ratio ranged between 13 and 27 g kg^{-1}; in hay this ratio was higher.

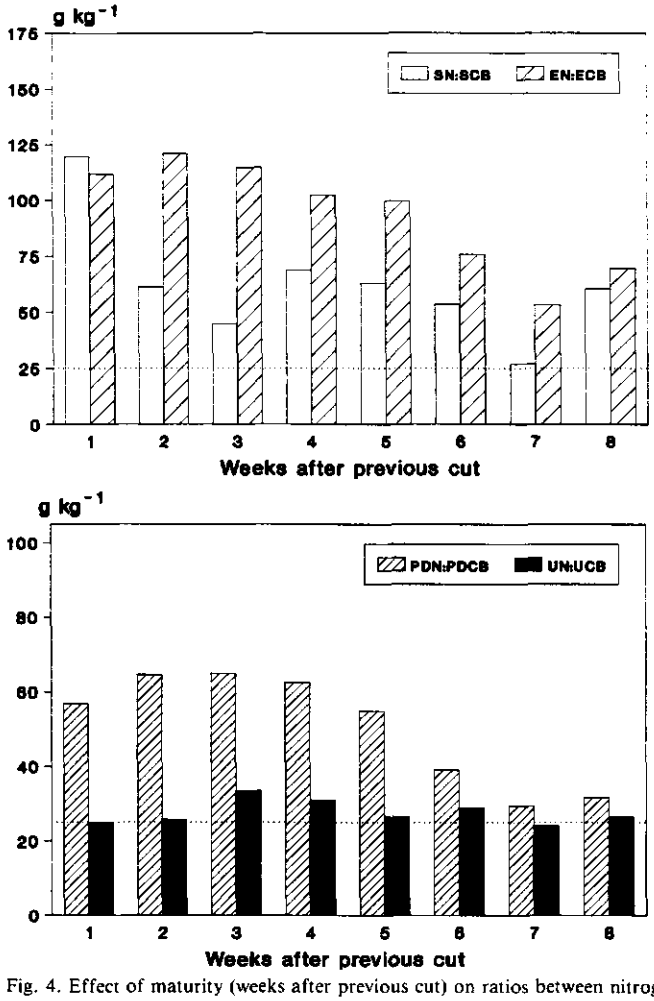

Fig. 4. Effect of maturity (weeks after previous cut) on ratios between nitrogen (N) and carbohydrates (CB) in fresh herbage (*Lolium perenne*). Experiment 2. (U = undegraded; PD = potentially degradable but escaping from rumen fermentation; E = potentially degradable effectively fermented in the rumen; S = soluble).

Effect of cell wall degrading enzymes (Experiment 4)

As observed in Experiment 3, maturation increased the CB content of the silages, mainly due to an increase of the undegraded fractions (Figure 5). Generally, treatment with cell wall degrading enzymes increased SCB (Figure 5), as stated earlier (van Vuuren et al., 1989). Also in these silages the SN:SCB ratio exceeded the

Table 1. Effect of maturity and method of preservation on the rumen availability of nitrogen and carbohydrates of grass silage (Experiment 3).

Parameter	Maturity (weeks)				Preservation (g DM kg^{-1})				LSD $P<0.05$
	2	4	6	8	220	300	450	875	
SN[1] (g kg^{-1} DM)	29.2[a2]	29.4[a]	20.6[b]	14.3[b]	28.1[x]	26.4[x]	24.8[x]	14.3[y]	3.80
EN (g kg^{-1} DM)	10.1[a]	8.1[b]	6.6[bc]	6.3[c]	5.7[xy]	5.2[x]	6.8[x]	13.4[z]	1.49
PDN (g kg^{-1} DM)	6.4	7.1	6.0	6.4	3.5[x]	4.2[x]	6.4[x]	11.6[z]	1.17
UN (g kg^{-1} DM)	1.9[a]	2.2[a]	2.3[a]	3.4[b]	1.4[x]	2.8[xy]	3.3[y]	2.4[x]	0.55
SCB (g kg^{-1} DM)	124	117	109	105	163[x]	107[y]	100[y]	86[y]	42.0
ECB (g kg^{-1} DM)	168[a]	168[a]	192[b]	186[b]	184[x]	171[xy]	165[y]	194[x]	13.8
PDCB (g kg^{-1} DM)	191[a]	210[a]	244[b]	259[b]	192[x]	226[xy]	237[y]	250[y]	36.5
UCB (g kg^{-1} DM)	67[a]	60[a]	80[b]	115[c]	56[x]	96[y]	89[y]	80[y]	18.3
SN:SCB (g kg^{-1} CB)	235	265	196	144	171	247	251	172	121.5
EN:ECB (g kg^{-1} CB)	57[a]	46[b]	36[c]	34[c]	32[x]	30[x]	39[x]	73[y]	9.6
PDN:PDCB (g kg^{-1} CB)	33[a]	32[ab]	24[b]	25[b]	19[x]	20[x]	27[x]	49[y]	8.0
UN:UCB (g kg^{-1} CB)	27[a]	34[b]	28[a]	29[a]	26[x]	29[xy]	34[yz]	30[z]	3.9

[1] SN = soluble N; EN = insoluble effectively degraded N; PDN = degradable N escaping from rumen fermentation; UN = undegradable N; SCB = soluble non-protein organic matter; ECB = insoluble effectively degradable non-protein organic matter; PDCB = degradable non-protein organic matter escaping from rumen fermentation; UCB = undegradable non-protein organic matter.

[2] Figures with different superscript differ significantly ($P<0.05$).

EN:ECB ratio (Figure 6). The SN:SCB ratio in enzyme-treated silages was usually lower than in the untreated silages.

Discussion

In this study, we have distinguished ruminal available CP and CB into a soluble fraction (S), assumed to be instantly and completely available in the rumen, and an insoluble, effectively degraded fraction (E). Our approach resulted in static figures and we realize that a more kinetic model is needed to describe the nutrient flow in ruminants more accurately. For such an approach nylon bag residues should be fitted using a model in which rate of degradation is not a constant. This method requires more data of short term incubations than we obtained in our studies.

Fresh herbage

In dairy cows fed young highly fertilized herbage, the duodenal protein supply may be relatively low and only meet the requirements of maintenance and a production of approximately 25 kg milk per day (Beever & Siddons, 1985). This is mainly caused by an extensive CP degradation in the rumen, while the intake of energy is too low to compensate for this loss by the synthesis of microbial protein. Besides, a large proportion of the energy available for microbial growth has to come from

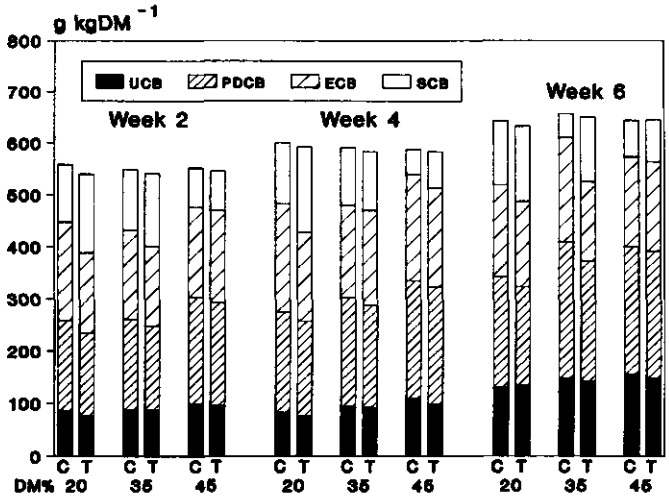

Fig. 5. Effect of maturity (weeks after previous cut), DM content (20, 30 and 45 %) and cell wall degrading enzymes (C = control; T = treated) on carbohydrates (CB) in grass silage. Experiment 4. (UCB = undegradable CB; PDCB = potentially degradable CB escaping from rumen fermentation; ECB = potentially degradable CB effectively fermented in the rumen; SCB = soluble CB).

protein and soluble sugars, which will yield less ATP per kg fermented OM than cell wall carbohydrates (Demeyer & Tamminga, 1987). An important consequence of this situation is an excessive loss of urea N via the urine (van Vuuren & Meijs, 1987).

Synchronization of the availability of N and CB in the rumen is considered to be an important condition for optimizing microbial protein synthesis. This can be achieved by optimizing both the SN:SCB and EN:ECB ratios. Usually the SN:SCB ratio of fresh herbage exceeds 25 g kg^{-1}, the value considered as an optimum for microbial protein synthesis (Czerkawski, 1986). In one occasion (Figure 2) the SN:SCB in fresh grass was lower than this value . A temporary deficit of SN can however be compensated for by SN from urea constantly secreted to the rumen in saliva and via diffusion through the rumen wall (Cheng & Costerton, 1980). Increasing the SN content in fresh grass by higher N fertilization seems therefore less appropiate. High SN:SCB ratios were observed in young herbage intensively fertilized, where a high proportion of N may be present in the form of non-protein nitrogen, presumably nitrate (Mangan, 1982).

The EN:ECB ratio in fresh herbage usually exceeds the SN:SCB ratio and the desired value of 25 g kg^{-1}. To prevent excessive N losses from the rumen a decrease of the EN:ECB ratio seems more appropiate. Theoretically a lower EN:ECB ratio in the diet can be achieved either by decreasing the ratio in the herbage by varying growing conditions or by changing the EN:ECB ratio in the diet by supplementing with appropriate concentrate ingredients. Desired growing conditions leading to an optimal EN:ECB ratio were deduced from the data of Experi-

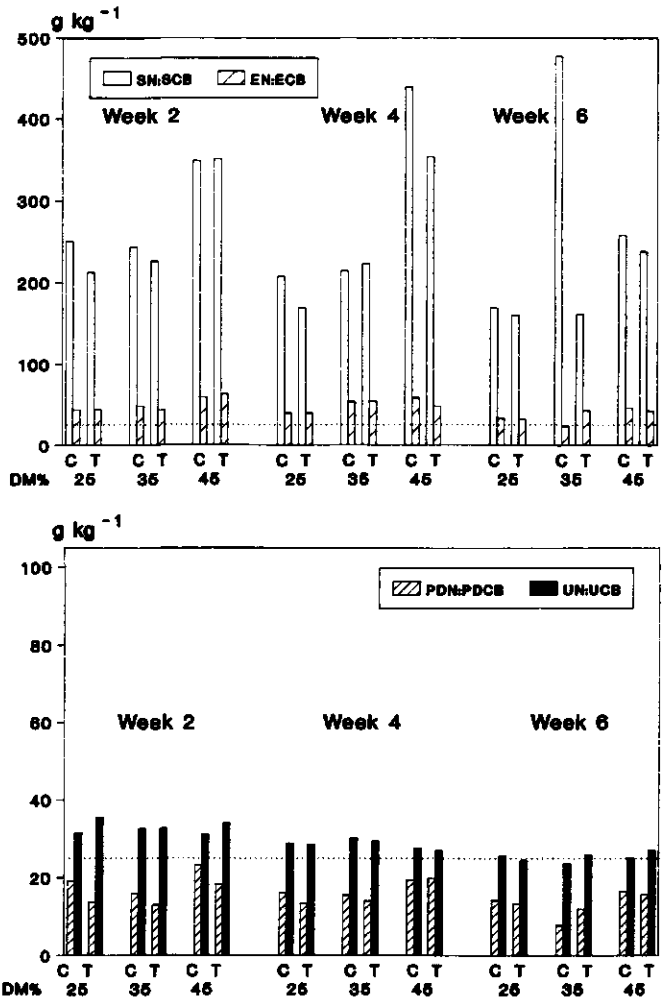

Fig. 6. Effect of maturity (weeks after previous cut), DM content (20, 30 and 45 %) and cell wall degrading enzymes (C = control; T = treated) on ratios between nitrogen (N) and carbohydrates (CB) in grass silage. Experiment 4. (U = undegraded; PD = potentially degradable but escaping from rumen fermentation; E = potentially degradable effectively fermented in the rumen; S = soluble).

ments 1 and 2. Predictions of EN:ECB in fresh herbage by multiple regression analysis were only significant with N content and time between April 1 and day of cutting (Table 2). The equations to predict EN:ECB obtained in Experiment 1 were similar to those of Experiment 2. From these equations it may be concluded that an optimum EN:ECB ratio will be reached at a N content of 11 (Experiment 1) to

Table 2. Parameters in multiple regression equations for the prediction of rumen-available nutrients of grass ($P<0.05$).

Y	Experiment	Constant	X_1[a]	X_2[b]	R^2
SN:SCB[c]	1	−126.8	4.27		0.515
	2	−29.9	2.37		0.391
EN:ECB	1	−6.3	2.74		0.878
		−22.3	2.86	0.10	0.911
	2	−24.2	3.03		0.922
FN:FCB	1	−43.5	3.20		0.948
	2	−25.8	2.68		0.882

[a] X_1 = N content (g kg^{-1} DM)
[b] X_2 = days between April 1 and cutting date
[c] SN = soluble N; SCB = soluble non-protein organic matter; EN = insoluble effectively degraded N; ECB = insoluble effectively degraded non-protein organic matter; FN = fermented N; FCB = fermented non-protein organic matter.

16 (Experiment 2) g kg^{-1} DM. Such low N contents require low rates of N fertilization (< 200 kg ha^{-1} yr^{-1}) at which the annual DM yield ha^{-1} will be substantially lower (Prins, 1983).

As the EN:ECB ratio in fresh herbage always exceeded 25 g N kg^{-1} DM and an adequate decrease of this ratio may have negative consequences for herbage yield, one may conclude that grazing dairy cows should preferable be supplemented with rumen degradable carbohydrates. The rate of CP degradation of fresh herbage is relatively high: 0.08 to 0.14 h^{-1} (van Vuuren, unpublished). Thus for optimum synchronization the supplement should have a rate of CB degradation similar to that of herbage CP. Concentrate ingredients characterized by a low EN:ECB ratio and a high rate of CB degradation are beet pulp, rice and tapioca (Tamminga et al., 1990b). Corn, milo and potato are also low in EN:ECB, but their rate of degradation is lower: around 0.05 h^{-1} (Tamminga et al., 1990b). Van Vuuren & Meijs (1987) and Valk (1990) reported a higher milk protein production by cows, when 35 to 50 % of grass DM was substituted by maize silage or by concentrates based on corn or beet pulp. This observation may indicate an improvement of the nutrient supply.

Silage

In animals given silage-based diets the efficiency of microbial protein synthesis (g microbial protein kg^{-1} OM fermented in the rumen) is usually low. This is attributed to the fact that a variable portion of OM consists of silage fermentation products, which will not contribute to the energy supply of rumen micro-organisms (Thomas, 1982). Our data suggest that asynchronous N and CB availabilities may also contribute to the low efficiency.

Different from fresh herbage, preserved herbage (silage or hay) was characterized by a high SN:SCB ratio, exceeding the EN:ECB ratio. The high N solubility of

silages results primarily from the extensive proteolysis of herbage protein during wilting and ensiling (McDonald, 1982). The high SN:SCB ratio may be a reason for the ineffective microbial protein synthesis on silage-based diets, the more since part of SCB may be fermentation products, which do not contribute to the energy supply of the micro-organisms.

The high SN:SCB ratio, in which SCB contains a certain portion of fermentation products, would suggest positive effects of feeding easily available carbohydrates when feeding grass silage. Products high in SCB and with a low SN:SCB ratio are barley, tapioca, oats, wheat and wheat flour (Tamminga et al., 1990b). To achieve full potential of such a strategy the silage and supplements should be consumed regularly over the day (complete mixture or computerized feed supply). Starch of barley, oats, tapioca, wheat and wheat flour is rapidly degradable (Tamminga et al., 1990b) which may lead to unfavourable conditions in the rumen, when consumed in a short time at large amounts (Malenstein et al., 1984). Thus also in this respect a regular consumption over the day is preferable, while the level of intake of these ingredients should be restricted.

Syrjala (1972) and Chamberlain et al. (1985) reported a reduction of the ruminal NH_3-N concentration when supplementing silage diets with sucrose and glucose. However, Chamberlain et al. (1985) indicated that starchy ingredients may stimulate the number of protozoa, thereby diminishing the positive effect of extra CB on the efficiency of microbial protein synthesis.

The EN:ECB ratio in silages with a low DM content may be relatively low (Table 1; Figure 5). Thus when feeding such type of silage the N availability may be too low for optimal protein synthesis in long feeding intervals. Indeed, Rooke et al. (1987) observed low ruminal NH_3-N levels (< 3 mmol l^{-1}) 6 hours after feeding moderately wilted silage (229 g DM kg^{-1}; 18.4 g N kg^{-1} DM) to dairy cows. In their experiments, continuous intraruminal infusions of urea or casein, expected to increase N supply to the rumen biota, had no effect on microbial protein synthesis.

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CHAPTER 5

Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents

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Protein Digestion and Intestinal Amino Acids in Dairy Cows Fed Fresh *Lolium perenne* with Different Nitrogen Contents

A. M. VAN VUUREN, F. KROL-KRAMER, R. A. VAN DER LEE, and H. CORBIJN
Dienst Landbouwkundig Onderzoek
Instituut voor Veevoedingsonderzoek
PO Box 160
NL 8200 AD Lelystad, The Netherlands

ABSTRACT

An experiment was carried out to study digestion and intestinal AA in three ruminally and duodenally cannulated, lactating cows fed freshly cut grass (*Lolium perenne*) fertilized with 500 and 275 kg of N/ha per yr, respectively. High N grass was fed in June and October, and low N grass was offered in July and September. Composite samples of the grass fed in each period also were tested for in situ degradation of OM, CP, and NDF. When low N grass was fed, the digestibilities of OM and CP were lower than when high N grass was fed. On low N grass, the duodenal N flow expressed per unit of N intake was higher, although the flow of AA N on low N grass was reduced in September, mainly because of reduced microbial protein synthesis from slower OM degradation of low N grass. Duodenal N flow per unit of N intake was related negatively to the N:OM ratio of the diet. The rate of N fertilization had no effect on ruminal OM and NDF turnover rates. Turnover and passage rates in this experiment were not different from reported data on cows on winter rations with similar DMI.

(**Key words**: grass, dairy cows, protein digestion, ruminal fermentation)

Abbreviation key: **JJ** = experimental period June and July, **Cr-NDR** = Cr-mordanted neutral detergent residue, **SO** = experimental period September and October.

INTRODUCTION

Grass, fertilized at high N rates, is characterized by high content of CP and high rate and extent of CP degradation in the reticulorumen (32), causing high ruminal concentrations of NH_3 (33) and substantial losses of N by urinary excretion (20). Excreted N results in NH_3 volatilization contributing to soil acidification and nitrate leaching, which impair ground water quality (20).

Improvement of CP utilization in the reticulorumen will reduce NH_3 absorption from the reticulorumen and, consequently, N excretion. For optimal CP utilization, the concentration of ruminally degraded N in the ration should be around 25 g/kg of OM (4). In intensively produced grass, the N content is high (32). The N:OM ratio in the diet of grazing cows can be decreased by reducing the level of N fertilization (31). This reduction may decrease not only N concentration but also OM digestibility (32). However, a reduction in CP intake may be accompanied by reduced feed protein breakdown (32), increased microbial protein synthesis, or both. Consequently, the supply of absorbable protein at the duodenum will not be altered greatly.

The aim of this experiment was to study the alterations in digestion, ruminal fermentation, and protein supply in the duodenum when dairy cows are fed grass fertilized at a lower N level.

MATERIALS AND METHODS

Design, Diets, Cows, and Statistical Analysis

The experiment consisted of four experimental periods of 8 d each. The first two periods were in June and July 1987 (**JJ**) and comprised three lactating cows, A, B, and C. The last two periods were in September and October 1987 (**SO**) and comprised cows B, C, and D. In July and September, grass was harvested from a plot fertilized with about 275 kg

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TABLE 1. Rate of N fertilization, growing stage, and mean date of harvesting of grass fed.

	Period 1	Period 2	Period 3	Period 4
Fertilization rate, kg of N/ha per yr	500	275	275	500
Cutting	3	4	7	8
Days of regrowth	24	23	30	13
Harvesting date, 1987	Jun 18	Jul 9	Sep 9	Oct 8

of N/ha per yr, whereas grass fed in June and October was fertilized at about 500 kg of N/ha per yr (Table 1).

Cows were equipped with a large ruminal cannula (i.d. 10 cm; Bar Diamond, Parma, ID) and a silicon T-type cannula (i.d. 30 mm with flanges strengthened by a steel plate) at the duodenum, 10 to 15 cm distal to the pylorus. Mean BW were 538 and 568 kg, and mean FCM yields were 24.8 and 13.5 kg/d in JJ and SO, respectively. At least 2 wk before the start of the experiment, cows were pastured on grass fertilized at about 400 kg of N/ha per yr. Four days before each measuring period, cows were tethered in a tie stall with free access to water and block salt. Cows were fed indoors and intraruminally infused with Co-EDTA and Cr-mordanted neutral detergent residue (Cr-NDR) in six portions per day. Grass (mainly *Lolium perenne*) was harvested daily at 0730, 1130, and 1600 h; in SO, harvesting at 1130 h was omitted. Grass was weighed in meals and stored at 4°C until feeding. Feeding times were 0100, 0800, 1200, 1500 (not SO), 1730, and 2100 h. At 0800 and 1730 h, .5 kg of a mineral-rich commercial compound feed (18.5% CP and 36.9% NDF in DM) was given additionally.

Using Genstat 5 (11), results were analyzed statistically within each season by ANOVA with cows as block and N fertilization rate as treatment. Treatment means were compared by Student's *t* test. Significance was determined at $P < .05$ unless otherwise indicated.

Feed Intake and Digestibility

Grass harvested in each period was sampled at each weighing. Samples were freeze-dried, ground to pass a 3-mm screen, and combined by period proportional to the DM offered. One portion of the composite sample was used for estimation of in situ disappearance of OM, CP, and NDF. Subsamples (5 g) of freeze-dried material were weighed in nylon bags and incubated in the rumen of three (other) grazing ruminally cannulated cows (30). Another portion of the composite sample was ground to pass a 1-mm screen for the analyses of the concentrations of DM, ash, N, NDF, lignin, and AA. Analytical procedures for the determination of DM, ash, and N were according to standard procedures at our institute (19). Cell-wall analyses (NDF, 72% H_2SO_4-insoluble lignin) were carried out according to Robertson and Van Soest (14). For AA analysis, freeze-dried samples were hydrolyzed in 6 *M* HCl at 110°C under reflux for 23 h and subsequently vacuum vaporized. After pH was adjusted to 2.2 with citrate buffer and norleucine was added as internal standard, AA were separated by ion-exchange column chromatography and detected by color reaction with ninhydrin. The absorption of primary AA was measured at 570 nm, and absorption of Pro was at 440 nm. Concentrations of Thr, Ser, Val, and Ile were corrected for incomplete AA recovery (18). For the analysis of Cys and Met, samples first were oxidized with performic acid (88%) for 16 h. Oxidation was stopped with HBr (48%), and samples were further processed as mentioned. Cystine and Met were determined as cysteic acid and Met sulfone, respectively. In vitro OM digestibility was determined by incubation with ruminal fluid, followed by pepsin-HCl (19).

Orts were collected at 0800 and 1730 h, weighed, and sampled proportionally. The composite samples were dried at 70°C, ground to pass a 1-mm screen, and analyzed for DM, ash, and N. The NDF content of orts OM was assumed to be similar to that of grass OM.

Feces were collected quantitatively during 144 h. Each day, the collected feces were homogenized, and proportional samples were taken for analysis. Kjeldahl N was estimated in fresh subsamples. Composite fecal samples by

cow were dried at 70°C, ground to pass a 1-mm screen, and analyzed for DM, ash, NDF, Co, and Cr. Cobalt was determined in a test solution prepared by ashing 1 g of the dried sample at 550°C and dissolving the ash in .5 *M* HNO_3. Cobalt was analyzed by atomic absorption spectrophotometry after diluting a portion of the test solution with a solution of lanthanum-(III)-chloride (10 g/L). Chromium was determined by atomic absorption spectrophotometry (34).

Duodenal Sampling

Starting on d 2 at 2000 and on d 6 at 1800 h, duodenal digesta were sampled every 4th h for 24 h. The cannula was connected with a tube, and about 4.5 kg of digesta were collected. The digesta collected immediately after removing the fistula plug were excluded from the sample. Per sampling event, a 600-ml subsample was taken from the collected digesta, and the remaining digesta were returned into the duodenum in .5-L portions at intervals of 2 to 5 min. The subsamples were pooled per cow, preserved with toluene (3 ml/L), and stored at 4°C until further processing. The 24-h sample was blended for 5 min (Ultra Turrax T45, Janke and Kunkel, Staufen, Germany), and subsamples were taken to compose one sample per period. A portion of the sample was filtered over a 41-μm screen to separate digesta into solid and liquid fractions.

Samples of total digesta and the liquid fraction were freeze-dried, ground to pass a 1-mm screen, and analyzed for DM, ash, N, NDF, Cr, and Co. Total digesta also were analyzed for AA composition. Another portion of the total digesta was centrifuged at 1500 × *g*, and the NH_3 concentration in the supernatant was determined (15). Duodenal flows of DM, OM, N, and NDF were estimated by a double marker technique (8); duodenal Cr and Co flows were assumed to be equal to their daily fecal excretion. To calculate the flow of NAN, the NH_3 concentration in the liquid fraction was assumed to be equal to that in the supernatant of total digesta; to calculate the AA N flow, the proportions of AA N in adjusted NAN were assumed to be similar to those in total digesta. The fraction of microbial protein entering the duodenum was estimated based on the AA profiles of grass, ruminal microbes, and duodenal digesta (24).

Ruminal Sampling

On d 2 to 3 and 6 to 7, samples of ruminal fluid were taken at 1715, 1900, 2045, 2245, 0045, 0415, 0745, 0945, 1145, 1315, 1515, and 1715 h for the determination of pH and concentrations of NH_3 and VFA (15). Ruminal fluid samples (1 L) were collected for isolation of bacteria at 1900 h (d 2), 0945 h (d 3), 2245 h (d 6), and 1315 h (d 7). Bacteria were isolated from each sample immediately after collection as described by Robinson et al. (16). Freeze-dried bacteria were analyzed for AA.

On d 1 to 2 and 6 to 7, ruminal evacuations were carried out at 1400, 2000, and 0600 h and at 2300, 1000, and 1700 h, respectively. Ruminal contents were removed by hand (mat) or by a plastic beaker (liquid). In contrast with the method described by Robinson et al. (16), flushing with CO_2 was omitted, and liquid (usually less than 3 L) was added to the mat material before mixing and sampling. Samples were freeze-dried, ground to pass a 1-mm screen, and analyzed for DM, ash, Co, and Cr. Composite samples by cow were created that were proportional to the ruminal DM contents at evacuation. Those samples were used for determination of NDF and lignin.

Ruminal turnover rates of OM and NDF were calculated using the model of removal from the rumen of indigestible fiber (1). In this approach, the dynamic properties of the ruminal pool are defined by linear first-order differential equations, assuming a constant rate of intake during the feeding period, zero intake during resting and ruminating periods, and a constant fractional rate of disappearance over the complete measuring period. Fractional disappearance rates and initial pool sizes were estimated by fitting the equations to data on feed intake and ruminal contents using nonlinear regression (17). Ruminal passage rates per hour of OM, NDF, and lignin were calculated as (duodenal flow)/(average ruminal contents × 24). The duodenal flow of NDF was estimated either from duodenal sampling or from fecal NDF excretion (16). Ruminal passage rates of Co and Cr were calculated as (fecal excretion)/(average ruminal contents × 24).

TABLE 2. Dry matter content and DM composition of grass fertilized at 275 and 500 kg of N/ha per yr and harvested in different seasons.

	June–July		September–October	
	500 kg of N	275 kg of N	275 kg of N	500 kg of N
DM, %	13.8	21.8	14.9	17.2
OM, % of DM	89.3	88.4	88.4	87.6
CP, % of DM	21.2	17.4	16.8	19.6
NDF, % of DM	37.3	39.8	40.9	33.1

Duodenal Supply of Ruminally Undegraded Feed Protein

The supply of undegraded feed protein in the small intestine was calculated by three different methods: 1) by regression equation (27) using the CP content (x_1) and mean date of harvest (days after April 1; x_2): undegraded fraction = $38.6 - .080x_1 + .070x_2$; 2) from the in situ data according to van Vuuren et al. (32) with a rate of passage assumed to be either fixed at 4.5%/h or variable, i.e., equal to the rate of passage of OM obtained from ruminal pool size and duodenal flow (as in method 1, the proportion of true protein was assumed to be 100%); and 3) from the duodenal flow of AA N and the estimated proportion of microbial AA N. The flow of nonmicrobial AA N was corrected for endogenous protein, which was assumed to be 3.6 g of N/kg of duodenal DM flow (3).

RESULTS AND DISCUSSION

Composition, Digestibility, and Intake

In June (high N grass) and September (low N grass), the DM content of grass was less than 15% (Table 2). There were no distinct effects of season and N fertilization rate on OM content. Reducing N fertilization decreased the CP content by 3 to 4% of DM but increased the NDF concentrations by 2.5 (JJ) and 7.8% (SO) of DM.

Also, differences in in situ degradation between treatments were smaller in JJ than in SO (Table 3). In comparison with the high N grass, low N grass was characterized by higher undegradable fractions of OM and CP and reduced rates of OM, NDF, and CP degradation except for CP in JJ.

Rates of in situ degradation of grass were similar to those reported previously (32), but soluble OM and CP fractions were much higher. This was attributed to differences in sample preparation. In the experiments of van Vuuren et al. (32), fresh grass was chopped to about 1 cm and stored at −18°C until ruminal incubation, but in the present experiment, grass was freeze-dried and ground through a 3-mm screen. This increased the fraction that disappears from the bags during washing.

The average DMI varied between 13.0 and 16.8 kg/d (Table 4) and was related positively to the DM content of the grass. Dry matter intakes in our experiment ranged between 113 (September) and 151 (July) g/kg of $BW^{.75}$, which is in agreement with intake levels reported by Dulphy et al. (6).

The apparent total tract digestibility of OM and CP of the total diet (grass + .9 kg of concentrates) was lower when low N grass was fed (Table 4) although differences were not significant for OM digestibility in JJ ($P = .15$) and CP digestibility in SO ($P = .13$). Cell-wall digestibility did not differ between treatments.

The reduction in nutritive value (higher NDF content and lower in situ degradation and total tract digestibility of OM) of low N grass in JJ was smaller than that in SO. This is mainly attributed to the smaller difference in days of regrowth in JJ in comparison with the 17-d difference in SO. The increased NDF content and decreased digestibility with increasing maturity are in agreement with previous observations (32). Posttreatment effects partially explain the smaller difference between treatments in JJ because N fertilization rates were identical (100 kg of N/ha) for both grasses until the first cut (May 11).

Digestibility also may be influenced by level of feed intake (Table 4). In that case, the difference between treatments in JJ would have

TABLE 3. Rate of in situ degradation (K_d) of the digestible, insoluble fractions (D) and the soluble (S) and undegradable (U) fractions of OM, NDF, and CP of grass fertilized at 275 and 500 kg of N/ha per yr and harvested in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
OM						
K_d, %/h	6.9	6.1	.5	4.7	6.4*	.4
S, %	40.4	40.8		34.2	46.3	
D, %	54.7	53.1*	.5	57.9	49.4**	.1
U, %	4.8	6.1*	.5	7.8	4.3**	.1
NDF						
K_d, %/h	5.6	4.9	.2	4.2	6.6**	.4
D, %	92.6	90.9	.5	89.5	93.5**	.2
U, %	7.4	9.1	.5	10.5	6.5**	.2
CP						
K_d, %/h	9.0	9.2	.2	6.4	9.1**	.5
S, %	53.1	47.2		40.3	47.5	
D, %	43.3	47.5**	.2	53.2	48.8**	.3
U, %	3.6	5.3**	.2	6.5	3.7*	.3

[1]Standard error of the difference.

*Significantly different from other N fertilization rate within the same season ($P < .05$).

**Significantly different from other N fertilization rate within the same season ($P < .01$).

been underestimated, whereas that in SO would have been overestimated. However, on grass diets, the depression in digestibility from increased feeding level may be much smaller than on diets containing preserved forages and concentrates (25).

Ruminal Fermentation and Duodenal Flow

There were no distinct effects of N fertilization rate on ruminal pH, VFA concentration, or pool size and ratios (Table 5). In SO, the proportion of acetate was lower on high N grass, but the nonglucogenic:glucogenic VFA ratio was not changed significantly ($P = .13$). Ruminal NH_3 concentration and pool size on low N grass were lower than on high N grass.

Ruminal VFA concentrations were higher than those reported by Beever et al. (2) but were similar to values reported by van Vuuren et al. (33). In the experiment of Beever et al. (2), DMI was lower than in our experiment, but van Vuuren et al. (33) estimated a DMI similar to that in the present experiment.

TABLE 4. Dry matter intake and digestibility of OM, CP, and NDF in dairy cows fed grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
DMI, kg/d	13.3	16.8	1.4	13.0	15.2*	<.1
Digestibility, %						
OM	81.0	79.2	1.1	78.1	82.0**	.9
CP	75.1	70.0*	1.1	69.8	74.7	3.4
NDF	77.0	76.7	1.4	75.6	76.8	2.0

[1]Standard error of the difference.

*Significantly different from other N fertilization rate within the same season ($P < .05$).

**Significantly different from other N fertilization rate within the same season ($P < .01$).

TABLE 5. Mean pH, concentration, pool size, proportions of VFA and concentration, and pool size of NH_3 in the rumen of dairy cows fed on grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
pH	6.6	6.1*	<.1	6.2	6.1	<.1
VFA						
Concentration, mmol/L	119	125	4	118	140*	6
Pool size, mol	6.5	8.0	.7	8.0	10.2	.2
Acetate, % of VFA	62.3	61.6	.6	64.5	62.3*	.8
Propionate	20.6	20.4	1.2	19.2	20.8	1.0
Butyrate	13.4	14.5	.6	12.9	12.6	.2
Isoacids[2]	2.4	2.1	.2	2.2	2.9**	<.1
NGGR[3]	4.2	4.4	.3	4.6	4.1	.3
NH_3						
Concentration, mmol/L	15.0	9.4**	1.1	10.9	17.0*	2.0
Pool size, mol	.82	.60	.12	.74	1.23	.12

[1]Standard error of the difference.

[2]2-Methylbutyrate + 3-methylbutyrate + isobutyrate.

[3]Nonglucogenic:glucogenic ratio = [acetate + 2 (butyrate + isobutyrate) + 2-methylbutyrate + 3-methylbutyrate + valerate]:[propionate + 2-methylbutyrate + 3-methylbutyrate + valerate].

*Significantly different from other N fertilization rate within the same season ($P < .05$).

**Significantly different from other N fertilization rate within the same season ($P < .01$).

Beever et al. (2) observed higher proportions of propionate and lower proportions of butyrate.

Generally, duodenal flows of OM and NDF were related to their intakes (Table 6). On average, 65% of the ingested OM and 80% of the ingested NDF apparently were digested in the stomach. In SO, a smaller proportion of NDF reached the small intestine ($P < .05$) when high N grass was fed. The site of digestion of OM and NDF was not affected by N fertilization rate.

The estimated duodenal NDF flow was lower than fecal NDF output. This may be explained by differences in sample preparation. Fecal samples were oven-dried at 70°C, whereas digesta samples were freeze-dried. The higher temperature may have increased NDF content (26), and, thus, fecal NDF output would have been overestimated. Fecal lignin excretion was 155, 118, 143, and 80% of duodenal lignin flow in June, July, September, and October, respectively. Another reason for the higher fecal NDF excretion may be an underestimation of the duodenal NDF flow, which also may explain the high ruminal NDF digestibility (ca. 80%). Gaillard and van't Klooster (10) also reported high ruminal NDF degradation (88% of NDF intake) in cows with T-type cannulas fed frozen grass. In their experiment (10), fecal excretions of hemicellulose and lignin were equal or higher than their duodenal flows, whereas fecal cellulose excretion was lower than duodenal flow. The proportion of digestible OM absorbed in the stomachs in our experiment (80 to 83%) was higher than values (68 to 70%) reported by others (2, 28).

In JJ, feeding low N grass increased duodenal total AA N flow relative to N intake from .47 to .57 g/g ($P = .05$) (Table 7). In SO, this ratio was not affected by N fertilization rate. In JJ (high and low N grass) and in October (high N grass), total AA N flow was about 17 g/kg of ingested OM. In September (low N grass), the total AA N flow relative to OM intake on low N grass decreased ($P = .05$), reducing efficiency of microbial protein synthesis. Although N intake was substantially reduced in this period, NH_3 concentrations were sufficient for optimal microbial growth (12). Also, ratios between fermentable N and carbohydrates, calculated as described by van Vuuren et al. (31), were not below 25 g/kg. Therefore, we conclude that the slow rate of OM degradation, as estimated in situ, led to

TABLE 6. Intake, mean duodenal flow, and ruminal digestion of OM and NDF in dairy cows fed on grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
OM						
Intake, kg/d	12.11	15.07	1.30	11.64	13.51*	.03
Duodenal flow, kg/d	4.30	5.52	.71	4.10	4.31	.20
Ruminal digestion						
% of Intake	64.4	63.6	3.0	64.8	68.1	1.4
% of TTD[2]	79.6	80.3	2.7	83.1	83.0	1.1
NDF						
Intake, kg/d	4.68	6.28*	.51	4.95	4.82	.04
Duodenal flow, kg/d	.97	1.35	.23	1.08	.89*	<.01
Ruminal digestion						
% of Intake	79.0	78.7	2.9	78.2	81.6*	.4
% of TTD	102.6	102.6	2.0	103.5	106.4	3.1

[1]Standard error of the difference.

[2]Total tract digestion.

*Significantly different from other N fertilization rate within the same season ($P < .05$).

low microbial growth rates, resulting in low efficiency of microbial protein synthesis (5) and reduced AA flow. The proportion of microbial N in total NAN was reduced by 5% (JJ; $P = .20$) to 15% (SO; $P = .01$) when low N grass was fed. The proportion of total AA N (not including Trp) in NAN ranged between 71 and 72% with an interaction between N fertilization rate and season.

The NAN flow:N intake (y) grams per gram was related to the ratio between N and OM intake (x) grams per kilogram: $y = 1.271 (\pm .0531) - .0163 (\pm .0053)x$ ($r^2 = .69$). Beever et al. (2) and Ulyatt et al. [unpublished data, cited

TABLE 7. Intake and duodenal flow of nitrogenous components in dairy cows fed on grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
N Intake, g/d	444	458	50	344	478**	2
AA N Intake,[2] g/d	339	380	36	291	391**	1
NAN Flow, g/d	307	371	39	262	309	15
AA N Flow						
g/d	207	261	29	160	224*	15
% of N Intake	46.9	56.9*	3.4	46.6	46.8	3.5
g/kg of OM Intake	17.2	17.3	1.2	13.8	16.5	1.2
Microbial N flow						
% of NAN	61.3	56.0	2.6	40.8	56.5*	2.7
g/kg of ARDOM[3]	24.1	21.8	1.8	14.2	19.1	1.9

[1]Standard error of the difference.

[2]Assuming 80% AA N in concentrate N.

[3]OM apparently digested in rumen.

*Significantly different from other N fertilization rate within the same season ($P < .05$).

**Significantly different from other N fertilization rate within the same season ($P < .01$).

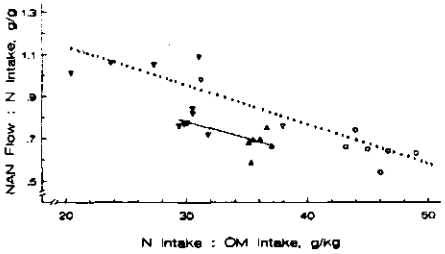

Figure 1. Relationship between N concentration in the diet (grams per kilogram of OM) and the duodenal NAN flow (grams per grams of N intake). Open symbols were derived from Beever et al. (2): ▼, ▽, ▲ = rye grass; ○ = clover.

in (2)] observed a similar relation in cattle fed perennial rye grass and white clover (Figure 1). The equations based on the results of Beever et al. (2) or on those of the present experiment were used to calculate the NAN flow for a cow consuming 15 kg of OM/d. A maximum NAN flow of .46 or .38 kg/d, respectively, was estimated at an N intake of about .57 kg/d (Figure 2). Under these circumstances, the dietary N:OM ratio is about 38 g/kg, and the NAN flow:N intake ratio is .80 and .65 g/g, respectively. Thus, a net loss of 20 to 35% of dietary N in the reticulorumen was estimated.

Rate of N fertilization had no significant effect on duodenal flows of individual AA (Table 8).

Ruminally Undegraded Feed Protein

Estimations of ruminally undegraded feed protein based on in situ data and on the in vivo results were lower than estimations based on the equation (Figure 3). In SO, the reduction in N fertilization rate decreased the estimated flow of ruminally undegraded feed protein calculated by equation but increased the flow of feed protein based on in situ data ($P < .01$). The flow of ruminally undegraded feed protein estimated in vivo was not influenced by N fertilization rate ($P = .17$).

Freeze-drying and grinding prior to in situ incubation increased the fraction that disappeared from the bags during washing. Thus, a higher fraction of CP is calculated to be fermented in the reticulorumen, and, consequently, the estimated supply of undegraded feed protein is reduced. This explains the difference between estimations based on the in situ data and those based on the equation by van Straalen and Tamminga (27); the latter was derived from results of freshly chopped grass with lower soluble CP fraction [(32); I. W. Hageman, 1990, unpublished]. The discrepancy in the effect of N fertilization between the supply of undegraded protein estimated by equations or by duodenal flow may be due to the higher cell-wall content of low N grass, resulting in an increased duodenal NDF flow (Table 6), causing a rise in endogenous protein secretion (23).

Ruminal Turnover

Rate of N fertilization did not effect ruminal pool sizes of OM and NDF (Table 9). In SO, ruminal turnover rate of OM was lower when low N grass was fed. Ruminal turnover rate of NDF and ruminal passage rates of OM, NDF, and markers (lignin, Cr-NDR, and Co-EDTA) were not influenced by N fertilization rate. Rate of passage of Cr-NDR was higher than the estimated passage rates of lignin and OM. Passage rate of NDF was lower than that for OM.

Total ruminal content and ruminal turnover of OM and NDF in SO were in agreement with

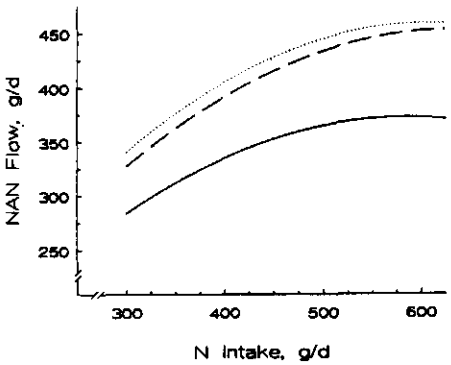

Figure 2. Estimated duodenal NAN flow in cows consuming 15 kg/d of OM of a grass-based diet at different N intake. — = Based on data from the present experiment; ..... = Beever et al. (2); - - - - = Ulyatt [cited by Beever et al. (2)].

TABLE 8. Duodenal flow of AA N in dairy cows fed on grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
	(g/d)					
Asp	17.0	21.5	2.4	14.8	21.3	2.7
Thr	9.9	12.5	1.4	8.5	12.3	1.6
Ser	10.6	13.3	1.4	9.0	13.2	1.7
Glu	18.9	24.1	2.6	15.4	15.4	2.9
Pro	7.3	9.5	1.1	7.1	9.4	1.1
Gly	19.9	24.4	2.6	15.3	23.7	3.2
Ala	16.5	20.5	2.4	14.2	20.8	2.4
Cys	2.5	3.2	.4	2.3	3.2	.4
Val	11.3	14.1	1.6	10.1	14.0	1.8
Met	2.9	3.7	.4	2.4	3.3	.4
Ile	8.7	10.8	1.2	7.7	10.5	1.4
Leu	12.9	16.3	1.8	11.4	15.4	2.2
Tyr	5.4	6.8	.8	4.1	5.5	.8
Phe	6.6	8.3	1.0	5.9	7.4	1.1
His	9.3	12.0	1.2	7.9	11.5	1.4
Lys	22.0	27.9	3.1	17.5	24.6	2.9
Arg	25.2	32.7	3.8	21.3	29.8	4.2

[1]Standard error of the difference.

values for dairy cows on rations based on hay and concentrates at similar DMI (16). The average ruminal passage rate of NDF ranged between 1.6 and 2.0%/h. Tamminga et al. (21) divided NDF into digestible and indigestible fractions and reported passage rates of 1.3 and 3.3%/h, respectively.

Subtraction of passage rates from the ruminal turnover rates calculates rates of degradation. The calculated rates of OM degradation (6.3, 6.0, 4.6, and 6.0%/h for June, July, September, and October, respectively) agreed with the degradation rate estimated by the in situ technique (Table 3). For NDF, the calculated rates of degradation differed from the in situ results (5.8, 5.7, 4.5, and 5.4%/h for June, July, September, and October, respectively).

Passage rates of lignin were similar to those of indigestible NDF in cows (21) and lignin in lambs (7, 9). Also, passage rates of Cr-NDR and Co-EDTA measured in SO were in agreement with values for cows on winter rations at similar DMI (21). In SO, passage rates of lignin, Cr-NDR, and Co-EDTA were as expected from the DMI (13). In JJ, passage rates were higher than the calculated values. In this season, Co-EDTA passage rates were more in line with values calculated with the equation reported by Tamminga et al. (22) for cows in early lactation. The difference between JJ and SO may be due to differences in stage of lactation, i.e., milk yield (29). The advanced stage of lactation also can explain the lower rate of Co-EDTA passage in period 4 when high N grass was fed.

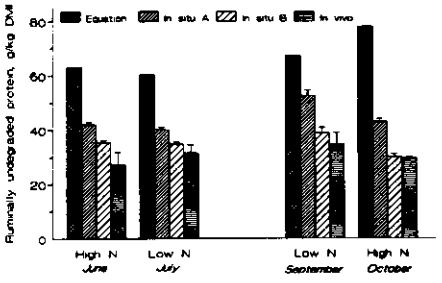

Figure 3. Effect of rate of N fertilization on the duodenal supply of ruminally undegraded feed protein estimated by different techniques. Equation = from equations of van Straalen and Tamminga (27); in situ A = from in situ results with passage rate fixed at 4.5%/h; in situ B = from in situ results with passage rate estimated from ruminal OM pool and duodenal OM flow; and in vivo = from duodenal flow measurements.

TABLE 9. Mean ruminal pool sizes, ruminal turnover rates, and passage rates (estimated from ruminal pool size and duodenal flow) in dairy cows fed on grass fertilized at 275 and 500 kg of N/ha per yr and fed in different seasons.

	June–July			September–October		
	500 kg of N	275 kg of N	SED[1]	275 kg of N	500 kg of N	SED
OM						
Pool size, kg	5.49	6.79	.47	7.09	7.49	.99
Turnover rate, %/h	9.6	9.4	.1	7.0	8.4*	.4
Passage rate, %/h	3.3	3.4	.3	2.4	2.4	<.1
NDF						
Pool size, kg	2.75	3.65	.20	3.69	3.46	.13
Turnover rate, %/h	7.4	7.3	.2	5.7	6.5	.3
Passage rate, %/h						
Measured flow	1.5	1.6	.2	1.2	1.1	<.1
Estimated flow[2]	1.9	2.0	.1	1.6	1.6	.1
Lignin passage rate, %/h	3.1	3.1	.5	2.9	2.4	.4
Cr-NDR Passage rate, %/h	6.7	6.4	.6	4.4	4.1	.3
Co-EDTA Passage rate, %/h	18.8	17.2	1.0	11.9	9.4	.5

[1]Standard error of the difference.

[2]Duodenal flow = fecal excretion/.85 (16).

*Significantly different from other N fertilization rate within the same season ($P < .05$).

CONCLUSIONS

In this experiment, treatments and periods were confounded. Therefore, results should be regarded qualitatively rather than quantitatively. Results of this experiment suggest that a maximal duodenal supply of NAN will be achieved at an N:OM ratio in grass of about 38 g/kg. At this level, 20 to 35% of dietary N will be lost in the reticulorumen. Digesta kinetics in cows fed grass in this experiment was not different from results obtained for cows on winter rations at similar DMI.

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CHAPTER 6

Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows

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Ryegrass Versus Corn Starch or Beet Pulp Fiber Diet Effects on Digestion and Intestinal Amino Acids in Dairy Cows

A. M. VAN VUUREN, C. J. VAN DER KOELEN, and J. VROONS-DE BRUIN
Dienst Landbouwkundig Onderzoek
Instituut voor Veevoedingsonderzoek
PO Box 160
NL 8200 AD Lelystad, The Netherlands

ABSTRACT

Changes in digestion and AA supply in dairy cows were studied when fresh grass was partly replaced by concentrate mixtures based either on corn starch or sugar beet pulp fiber. Treatments were tested in a Latin square utilizing three lactating cows with ruminal and intestinal cannulas. Partial replacement of grass decreased CP digestibility. When high starch concentrate was fed, NDF digestibility was lower than that of the high fiber diet, mainly because of decreased ruminal digestion of NDF. For the high starch concentrate, 39% of the ingested starch escaped ruminal fermentation. Although less OM was fermented in the forestomachs on high starch concentrate, the duodenal AA N flow was higher than for the high fiber concentrate. The proportion of microbial protein was unaffected; thus, efficiency of microbial synthesis was estimated to be higher when high starch concentrate was fed. (**Key words**: grass, nitrogen intake, digestion, protein degradation)

Abbreviation key: **DAPA** = diaminopimelic acid, **Cr-NDR** = Cr-mordanted neutral detergent residue, **HF** = high fiber concentrate mixture, **HS** = high starch concentrate mixture.

INTRODUCTION

In regions with a temperate climate, high yields of grass can be achieved by high N fertilization rates (13). Under these conditions, the CP content of grass is high, but less than 30% of the ingested grass protein reaches the duodenum (1, 23). Consequently, a relatively high proportion of ruminally degraded CP is absorbed from the reticulorumen and excreted as urea in urine. The excreted N results in NH_3 volatilization, which contributes to soil acidification, nitrate leaching, and impaired ground water quality (19).

Improvement of CP utilization in the reticulorumen reduces NH_3 absorption and, consequently, N excretion. For optimal CP utilization, the ruminally degraded N:OM ratio should be about 25 g/kg (2). A reduction in the N:OM ratio in the diet of grazing cows can be achieved by reducing N fertilization (23, 25). However, reduction in N fertilization may decrease not only CP content but also DM yield per hectare and OM digestibility (30). If the demand for high quality forage is high, reduction in N fertilization may be undesirable. Another method to reduce the N:OM ratio is dilution of grass CP by low protein feeds such as corn silage (24) or by concentrate mixtures based on low protein ingredients (9, 28). If the reduction in CP intake is assumed to be compensated by an increase in microbial protein synthesis, the intestinal supply of protein may not be reduced.

The objective of this experiment was to study the effect of partial replacement of fresh grass by low protein concentrates on the digestion and protein supply in dairy cows. Concentrate mixtures were based either on corn meal (high starch; **HS**) or sugar beet pulp (high fiber; **HF**).

MATERIALS AND METHODS

Three treatments were tested: grass (14.5 kg/d of DM); grass with HS (9.3 and 5.3 kg/d of DM, respectively), and grass with HF (9.4 and 5.4 kg/d of DM kg, respectively). Grass (about 85% *Lolium perenne*) was fertilized at 400 kg of N/ha per yr and harvested at a yield of ca. 2000 kg of DM/ha. Grass was harvested daily between 0900 and 1000 h and weighed in

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TABLE 1. Ingredient composition of experimental concentrate mixtures.

	High starch	High fiber
	(g/kg)	
Sugar beet pulp (10 to 15% sugar)	. . .	825
Soybean hulls (>31% crude fiber)	. . .	150
Corn meal	475	. . .
Hominy feed	500	. . .
Mineral-vitamin premix	25	25

five meals. Meals were stored at 4°C until feeding. Meals were offered at 1200, 1700, 2100, 0700, and 0930 h. Concentrates were fed at 1700 and 0700 h. In addition to experimental concentrates, a mineral-rich concentrate was fed at 1.7 kg/d of DM. Ingredient composition of the experimental mixtures is presented in Table 1.

Treatments were offered to three lactating cows in a 3 × 3 (treatments × periods) Latin square design. To increase the degrees of freedom, the experiment was prolonged with an extra period, in which the cows were alloted randomly to the treatments. The multiparous Dutch Friesian cows had large ruminal cannulas (i.d. 100 mm; Bar Diamond, Parma, ID) and silicon T-type cannulas (i.d. 19 mm) at the distal part of the duodenum. Cows were kept in tie stalls and had free access to water and block salt. At the start of the experiment, cows produced 23 to 36 kg/d of FCM.

Each period lasted for 28 d. The first 3 wk were used for adaptation. Starting on d 15, Co-EDTA and Cr-mordanted neutral detergent residue (Cr-NDR) were infused intraruminally in six portions per day. From d 21 to 28, feed intake was measured; feces were collected from d 22 to 28. Ruminal fermentation and duodenal flow were studied on d 21 to 22 and on d 25 to 26. Ruminal evacuations were carried out at 1600 h (d 20), at 0500 and 1400 h (d 21), and at 0000, 1100, and 1900 h (d 27). Collection and sample preparation for grass, feces, ruminal fluid, and duodenal and ruminal digesta were as described previously (23). Samples of concentrates were dried at 70°C and ground to pass a 1-mm screen. Grass and concentrates were analyzed for DM, ash, N, NDF, starch, and reducing sugars. Dry matter was determined at 103°C and ash at 550°C. Nitrogen was analyzed by the Kjeldahl method and NDF according to Robertson and Van Soest (14). Reducing sugars and starch were analyzed as described by van Vuuren et al. (27). In situ incubations were carried out with freeze-dried grass samples and concentrate samples, both ground to pass a 3-mm screen (22).

Ruminal bacteria were isolated at 1900 (d 21), 0900 (d 22), 2300 (d 25), and 1545 h (d 26) as described by Robinson et al. (16) and analyzed for diaminopimelic acid (DAPA). The DAPA was analyzed by ion-exchange column chromatography after preoxidation in performic acid (88%) and hydrolysis in 6 *M* HCl at 110°C under reflux. Duodenal digesta and feces were analyzed for DM, ash, N, NDF, starch, NH_3, AA, DAPA, Co, and Cr. A portion of the freeze-dried ground sample of duodenal digesta was dissolved in water and filtered over a 41-μm screen to separate the digesta into solid and liquid fractions. Both fractions were freeze-dried and analyzed for DM, ash, Cr, and Co. In addition, N and DAPA were determined in the liquid fraction, and NDF was determined in the solid fraction. Cobalt (23) and Cr (29) were determined by atomic absorption spectrophotometry. Duodenal flows of OM, N, and DAPA were calculated by the double-marker technique based on concentrations in total digesta and the liquid fraction; flows of OM and NDF were based on concentrations in total digesta and the solid fraction (5). Flow of OM was expressed as the average of flows estimated either on the basis of the solid or the liquid fraction. Flows of NAN and AA N were calculated as described previously (23). Differences in DMI interfered with the interpretation of duodenal AA flows. Therefore, the duodenal AA N flows were expressed per kilogram of DMI. The flow of microbial protein entering the duodenum was estimated based on the DAPA:N ratio in ruminal bacteria and duodenal DAPA flow.

Ruminal turnover rates of OM and NDF were calculated as described by van Vuuren et al. (23). Ruminal passage rates per hour of OM, NDF, and lignin were calculated as (duodenal flow)/(average ruminal contents × 24). Duodenal NDF flow was estimated either from duodenal sampling or from fecal NDF excretion (16). Fractional passage rates of Co and Cr were calculated as (fecal excretion)/(average ruminal contents × 24).

Data were analyzed by ANOVA for a Latin square design using GENSTAT (6) with cows × periods as the block structure. Differences between treatments were compared by Student's *t* test. Significance was determined at $P < .05$.

RESULTS AND DISCUSSION

Composition and Digestibility

Chemical composition and in situ degradation parameters of grass and concentrate mixtures are presented in Table 2. Chemical composition and in situ degradation of the grass samples differed between periods. Concentrations of CP and NDF of grass fed in period 3 were 40 to 50 and 30 to 40 g/kg of DM lower, respectively, than in the other periods. The lower CP and NDF contents were compensated by higher sugar concentrations. Rate of in situ CP degradation of grass sampled in period 3 was lower than that of the other periods, but rate of in situ degradation of OM and NDF was significantly higher in period 4 than in the other periods. Rates of in situ degradation of grass were similar to those reported by van Vuuren et al. (23). Soluble OM and CP fractions were higher than those reported by van Vuuren et al. (26) because of differences in sample preparation before ruminal incubation (23).

Differences in CP concentrations between grass and concentrates, especially HS, were relatively small. Concentrations of OM and CP in HF were lower than in HS; only small differences occurred with in situ OM degradation. In situ CP degradation of HF was lower than that of HS. Rate of starch degradation of HS was 11%/h, which was much higher than

TABLE 2. Harvest dates (1988), chemical composition, and rate of in situ degradation (K_d) of OM, CP, NDF, and starch and soluble (S), insoluble, potentially degradable (D), and undegradable (U) fractions of ryegrass and concentrate mixtures.

	Ryegrass				Concentrates		
	Period 1	Period 2	Period 3	Period 4	High starch	High fiber	Mineral mixture
Mean date of harvesting	Jun 12	Jul 10	Aug 7	Sep 4	. . .	. . .	. . .
DM, %	17.2	18.8	21.6	16.3	87.8	90.8	87.4
OM, % of DM	90.2	89.8	90.4	86.8	94.1	90.2	90.6
K_d, %/h	5.1^{b}	5.0^{b}	5.2^{b}	5.9^{a}	9.9	9.4	6.1
S, %	34.6	35.8	43.1	33.9	23.6	21.3	46.5
D, %	60.3^{b}	57.7^{c}	52.9^{d}	61.5^{a}	72.4	72.8	51.2
U, %	5.1^{b}	6.5^{a}	4.1^{d}	4.6^{c}	4.0	3.9	2.3
CP, % of DM	19.7	18.4	14.2	19.9	14.6	12.4	17.8
K_d, %/h	9.1^{a}	8.9^{a}	7.2^{b}	8.6^{a}	10.1	6.9	6.1
S, %	45.1	42.3	45.3	42.1	55.9	47.3	58.9
D, %	50.9^{b}	52.8^{a}	50.4^{b}	53.5^{a}	41.1	49.0	39.3
U, %	3.9^{b}	4.9^{a}	4.3^{b}	4.4^{b}	2.6	3.7	1.8
NDF, % of DM	42.5	42.5	38.7	41.6	23.3	40.7	37.5
K_d, %/h	4.3^{b}	4.4^{b}	4.8^{ab}	5.4^{a}	. . .[1]	9.6	4.7
D, %	90.7^{ab}	89.7^{b}	91.7^{ab}	93.5^{a}	. . .	93.0	90.0
U, %	9.3^{a}	10.3^{a}	8.3^{ab}	6.5^{b}	. . .	7.0	10.0
Lag time, h	0	0	0	0	. . .	.86	0
Starch, % of DM	. . .	. . .	. . .	. . .	46.1	4.7	7.1
K_d, %/h	. . .	. . .	. . .	. . .	11.2	. . .	. . .
S, %	. . .	. . .	. . .	. . .	23.4	. . .	. . .
D, %	. . .	. . .	. . .	. . .	76.4	. . .	. . .
U, %	. . .	. . .	. . .	. . .	.2	. . .	. . .
Sugar, % of DM	12.8	15.2	23.4	10.2	2.6	4.7	7.4

^{a,b,c}Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Not determined.

that reported for starch in corn meal and hominy feed [4.0 and 5.3%/h, respectively (20)]. The relatively high rate of starch degradation in HS (Table 2) may be partly due to pelleting (11) but also indicates contamination of hominy feed by corn gluten feed, as was also concluded from the relatively high CP concentrations of HS (21). In HF, concentration of NDF was lower, and rate of in situ degradation of NDF was higher, than that reported for beet pulp (20).

Partial replacement of grass by concentrate mixtures had no effect on OM digestibility but decreased CP digestibility from 70 to 68% (Table 3). When HS was fed, cell-wall digestibility was reduced from 79 to 74%. With concentrate mixtures, starch digestibility was high (≥95%). Digestibility of OM was higher than that reported by Valk et al. (21) for similar diets. The higher proportion of concentrates in the production trial (21) may have reduced ruminal digestion. In the production trial, CP digestibility on HF was significantly lower than that on HS, which was attributed to a higher excretion of metabolic fecal N (21). In our study, differences in CP digestibility between HS and HF were not significant. The decrease in NDF digestibility is in agreement with the results of Valk et al. (21). Sutton et al. (17) also reported a decrease in NDF digestibility when HF was replaced by HS in a hay and concentrate diet. Robinson et al. (15) observed decreased NDF digestibility when the proportion of starch in the diet was increased from 8 to 14%, but a further increase in starch had no further negative effect on NDF digestibility.

TABLE 3. The effect of partial replacement of ryegrass by high starch and high fiber concentrates on DMI and coefficients of digestibility of OM, CP, NDF, and starch in dairy cows.

	Supplement			
	None	High starch	High fiber	SED[1]
DMI, kg/d				
Ryegrass	14.5[a]	9.3[b]	9.3[c]	.1
Concentrates	1.7[c]	7.0[b]	7.2[a]	<.1
Total	16.3	16.3	16.5	.1
Digestibility, %				
OM	78.6	78.0	78.9	1.0
CP	70.0[a]	67.5[b]	67.8[b]	.6
NDF	78.7[ab]	74.5[b]	79.2[a]	1.4
Starch	86.3[b]	97.7[a]	95.5[a]	1.5

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

Ruminal Fermentation and Duodenal Flow

Ruminal pH (not tabulated) was not different among treatments. Concentrations of VFA were lower when HS was fed than for HF (27). Partial replacement of grass significantly decreased ruminal NH_3 concentrations.

Partial replacement of grass by HS changed the site of OM and NDF digestion from the rumen to the intestine (Table 4). When HS was fed, more than 1 kg/d of starch reached the small intestine. Nocek and Tamminga (11) estimated that 32 to 35% of corn starch and 22 to 29% of hominy feed starch escaped ruminal fermentation. Using the in situ data, we estimated a ruminal escape of 22%, corrected for 10% escape of soluble starch and 25% decrease of ruminal escape that was due to pelleting (11). Correction of duodenal starch flow for microbial starch, assuming that microbial OM contains 65% CP and 5% starch (11), indicated that –2, 994, and 123 g/d of feed starch reached the small intestine for herbage, HS, and HF, respectively, suggesting that, for HS, 39% of feed starch escaped ruminal fermentation.

Rate of NDF degradation of HS was not determined in our experiment, but de Visser et al. (4) reported a degradation rate for corn bran NDF of <2%/h. When we assumed a similar degradation rate for HS NDF and a passage rate of 3%/h, we estimated a reduction in ruminal degradation of 5 percentage units. A depression in ruminal cell-wall digestion may be due to low pH (10). However, in the present experiment, pH was not different among treatments (6.1, 6.2, and 6.1 for the unsupplemented diet, HS, and HF, respectively) (27). A depression in cell-wall digestion also may be caused by the addition of readily fermentable carbohydrates per se (7).

Intestinal NDF digestibilities agreed with those assumed by Robinson et al. (16), which were based on data obtained from experiments with reentrant-cannulated cows (18).

Although N intake was reduced by partial replacement of grass (Table 5), duodenal NAN

TABLE 4. The effect of partial replacement of ryegrass by high starch and high fiber concentrates on intake, duodenal flow, and ruminal digestion of OM, NDF, and starch in dairy cows.

	Supplement			
	None	High starch	High fiber	SED[1]
OM				
Intake, kg/d	14.57	14.83	14.80	.14
Duodenal flow, kg/d	5.92[b]	7.24[a]	5.84[b]	.24
Ruminal digestion[2]				
% of Intake	59.4[a]	51.2[b]	60.6[a]	1.7
% of TTD[3]	75.6[a]	65.7[b]	76.7[a]	1.8
NDF				
Intake, kg/d	6.61[b]	5.73[c]	6.72[a]	.03
Duodenal flow, kg/d	1.68[b]	2.08[a]	1.73[b]	.08
Ruminal digestion				
% of Intake	74.7[a]	63.8[b]	74.3[a]	.9
% of TTD	95.0[a]	85.6[b]	93.8[a]	2.0
Starch				
Intake, kg/d	.12[c]	2.54[a]	.38[b]	.02
Duodenal flow, kg/d	.12[b]	1.12[a]	.24[b]	.05
Ruminal digestion				
% of Intake	−29.6[b]	56.4[a]	34.7[a]	7.2
% of TTD	−33.6[b]	57.8[a]	36.5[a]	8.3

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Uncorrected for microbial OM.

[3]Total tract digestion.

TABLE 5. The effect of partial replacement of ryegrass by high starch and high fiber concentrates on protein digestion in dairy cows.

	Supplement			
	None	High starch	High fiber	SED[1]
N Intake				
g/d	459[a]	441[b]	428[c]	5
g/kg of OM	31.6[a]	29.8[ab]	29.0[b]	.7
NAN Flow, g/d	409	424	396	19
AA N Flow				
g/d	252[ab]	273[a]	241[b]	8
% of N intake	56.1[b]	62.5[a]	56.6[b]	1.7
g/kg of OM intake	17.3[ab]	18.4[a]	16.2[b]	.6
Microbial N flow				
% of NAN	62.1	61.5	61.3	3.1
g/kg of OMARD[2]	29.4[b]	34.3[a]	27.2[b]	1.1

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]The OM apparently digested in rumen.

TABLE 6. The effect of partial replacement of ryegrass by low protein concentrates on the duodenal AA N flow in dairy cows.

AA	Supplement			SED[1]
	None	High starch	High fiber	
	(g/kg of DMI)			
Asx[2]	1.28[ab]	1.35[a]	1.21[b]	.05
Thr	.74[ab]	.79[a]	.69[b]	.03
Ser	.77[b]	.86[a]	.75[c]	.02
Glx[3]	1.31[b]	1.54[a]	1.26[c]	.03
Pro	.60[b]	.70[a]	.56[b]	.03
Gly	1.30	1.34	1.24	.07
Ala	1.25[ab]	1.33[a]	1.13[b]	.05
Cys	.20[b]	.24[a]	.21[b]	.01
Val	.90[ab]	.96[a]	.83[b]	.04
Met	.23[ab]	.23[a]	.20[b]	.01
Ile	.70[ab]	.74[a]	.65[b]	.03
Leu	1.03[b]	1.15[a]	.95[b]	.03
Tyr	.38	.41	.37	.02
Phe	.53[ab]	.56[a]	.49[b]	.02
His	.68[b]	.77[a]	.66[b]	.02
Lys	1.62	1.66	1.52	.07
Arg	1.94[ab]	2.12[a]	1.84[b]	.07
DAPA[4]	.11	.13	.11	.01

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Asp and Asn.

[3]Glu and Gln.

[4]Diaminopimelic acid.

flow did not differ ($P = .44$) among treatments. Partial replacement of grass by concentrates had no significant effect on duodenal AA N flow. With HS, duodenal AA N flow was higher than when HF was fed, both absolutely (32 g/d) and relative to OM and N intakes. The estimated efficiency of microbial protein synthesis for HS was higher than for grass alone or for grass with HF. For most AA, duodenal flows were significantly higher when HS was fed (Table 6). Addition of high amounts (>30% of ration DM) of nonstructural carbohydrates often resulted in a depressed efficiency of microbial protein synthesis (8), which often is attributed to ruminal acidification accompanied by inhibited microbial growth (3, 10), uncoupled fermentation, or a decrease in ruminal turnover rates (8). In cows fed a hay-based diet containing 60 or 90% concentrates of ground corn, efficiencies were 30% lower than for concentrates based on rolled barley (12), but information on ruminal pH was not presented. In our study, ruminal pH and propionate concentrations were not different among treatments (27). Also, ruminal OM turnover rate was not depressed when HS was fed (Table 7).

In continuous cultures, the decrease in microbial efficiency was curvilinear when the ratio between nonstructural carbohydrates and ruminal degradable CP widened (8). In our experiment, these ratios were 1.6, 2.3, and 1.3 g/g for the unsupplemented diet, HS, and HF, respectively. For the in vitro experiments, no ratios below 2.4 were studied, but the efficiency of microbial protein synthesis was expected to be depressed by narrow ratios (e.g., <1.1) mainly representing all-forage diets (8). Our data support this concept, which can be explained by the higher rate of carbohydrate fermentation when HS was fed (Table 2), resulting in faster microbial growth, thereby increasing the efficiency of protein synthesis (3).

When the unsupplemented diet or grass with HF was fed, NAN flows were 20 g/d

TABLE 7. The effect of partial replacement of ryegrass by high starch and high fiber concentrates on the mean ruminal pool sizes, ruminal turnover rates, and passage and degradation rates of OM and NDF in dairy cows.

	Supplement			
	None	High starch	High fiber	SED[1]
OM				
Pool size, kg	7.20[a]	6.01[b]	6.30[b]	.31
Turnover rate, %/h	8.7[c]	11.4[a]	10.7[b]	.2
Passage rate, %/h	3.2[c]	5.1[a]	3.9[b]	.2
Degradation rate, %/h	5.3[b]	6.3[a]	6.8[a]	.2
NDF				
Pool size				
kg	3.69[a]	2.80[b]	3.12[b]	.18
% of OM pool	51.1[a]	46.6[b]	49.4[ab]	.6
Turnover rate, %/h	7.7[c]	9.3[b]	9.8[a]	.1
Passage rate, %/h				
Measured flow	1.8[c]	3.2[a]	2.3[b]	.1
Estimated flow[2]	1.7[b]	2.6[a]	2.2[a]	.1
Degradation rate, %/h	5.7[b]	6.2[a]	7.5[a]	.2

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Duodenal flow = fecal excretion/.85 (16).

lower than values estimated from the relationship between NAN flow expressed per unit of N intake and the N:OM ratio in the diet (1). The increase in nonstructural carbohydrates in the diet from 15 and 13% of DM for grass and HF, respectively, to 26% for HS, considered in combination with the estimated ratios between nonstructural carbohydrates and rumen degradable CP, indicates that more microbial N flow and a greater efficiency for HS would be likely (8). These increases were confirmed by our observations. The higher duodenal protein supply with HS in our study agrees with the increase in milk protein yield in a production trial using similar diets (21).

Ruminal Turnover

Partial replacement of grass significantly reduced ruminal pool sizes of OM and NDF (Table 7). Inclusion of concentrates in the diet increased ruminal turnover of OM and NDF by >2 and 1.6%/h, respectively. Concentrates also increased passage rates, which were more extreme when HS was fed. However, increased passage rates could not completely account for the increase in turnover rates. Thus, an increase in degradation rates had to be assumed as well. Passage rate of lignin was increased with the concentrates, but passage rates of Cr and Co were not affected (Table 8).

When only grass was fed, turnover and passage rates were comparable with those observed in other cows fed grass (23). When grass was replaced partially by concentrates, an increase in ruminal turnover was expected from the higher rate of OM degradation of the HS and HF diets (Table 2). Based on the proportions of grass and concentrate OM, degradation rates of OM of 5.4, 7.3, and 7.1%/h were expected for the unsupplemented diet, HS, and HF respectively. Especially for HS, the degradation rate estimated from ruminal evacuations and duodenal flow was lower than that calculated from in situ incubations, which was attributed to the decrease in ruminal fiber degradation.

The decreased ruminal turnover of NDF on HS compared with HF is in agreement with results of Robinson et al. (16), who observed a linear decrease in turnover of NDF when the starch content in the diet increased. Passage rate of NDF, estimated from fecal NDF excretion, did not differ from that estimated from duodenal NDF flow except when HS was fed. This latter result is due to the compensatory increase in intestinal NDF degradation. When

TABLE 8. The effect of partial replacement of ryegrass by high starch and high fiber concentrates on the mean ruminal pool sizes and passage rates of indigestible markers in dairy cows.

	Supplement			
	None	High starch	High fiber	SED[1]
Lignin				
Pool size, kg	.24[a]	.17[b]	.20[ab]	.02
Passage rate, %/h	3.5[a]	4.7[b]	4.6[b]	.3
Passage rate, %/h				
Cr-NDR[2]	5.1	5.7	5.5	.6
Co-EDTA	15.5	16.2	16.2	1.3

[1]Standard error of the difference.

[2]Cr-mordanted neutral detergent residue.

the unsupplemented diet, HS, and HF were fed, 84, 72, and 82%, respectively, of duodenal NDF were excreted in the feces. Thus, for HS, fecal NDF excretion was significantly less than 85% of duodenal NDF flow, which was assumed (16).

CONCLUSIONS

Because of the relatively low N content of grass used in this experiment, no large differences in N intake were established. Consequently, differences in duodenal protein supply were small when grass was replaced partially by concentrate mixtures. When grass was replaced by high starch concentrates, the AA N supply to the small intestine was slightly higher, despite a depression of ruminal OM degradation. Grass of different periods varied in chemical composition and in in situ degradation. Thus, absence of interactions between period and treatment, a prerequisite for Latin square designs, was not ensured.

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CHAPTER 7

Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows

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Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows

A. M. van Vuuren, C. J. van der Koelen and J. Vroons-de Bruin

Institute for Livestock Feeding and Nutrition Research (IVVO), P.O. Box 160, 8200 AD Lelystad, Netherlands

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Abstract

Six grazing dairy cows, fitted with rumen cannula, were supplemented with either high-starch concentrate, containing 258 g starch per kg dry matter (DM), or low-starch concentrate (15 g starch per kg DM) in a latin-square arrangement of treatments. Animals received 1 kg of high-starch concentrate (1HS), 7 kg of high-starch concentrate (7HS), or 7 kg of low-starch concentrate (7LS) in two equal meals per day fed at 06h00 and 16h00. After a three-week adaptation, rumen samples were taken at 4-hour intervals in two 24-hour periods. In the first 24-hour period samples from the swards were taken immediately before rumen sampling.

Total sugar content of herbage increased during daytime with the highest concentration directly before sunset.

Patterns of ruminal pH values did not differ ($P > 0.05$) between treatments, and values were minimal at 24h00. Volatile fatty acids (VFA) and ammonia peaked at 24h00. Extra high- or low-starch concentrate decreased ruminal concentrations of ammonia and branched-chain VFA (ammonia concentrations were 19, 13 and 12 mmol/litre for treatments 1HS, 7HS and 7LS respectively). Acetate/propionate and non-glucogenic/glucogenic ratios within VFA, and percentage of milk fat tended ($P > 0.05$) to be lowest for treatment 7HS.

Introduction

In the Netherlands, fertilization of grassland for dairy production has increased from an average of 150 to more than 300 kg nitrogen (N) per hectare during the last twenty years (van Dijk et al., 1983). Such fertilization reduces the period required for swards to reach the grazing stage (1700 kg organic matter (OM) per hectare). Over the past two decades grasslands have been renewed and nearly become monocultures, consisting primarily of perennial ryegrass. These factors have altered the

botanical and chemical composition of the swards, resulting in a change in the composition of herbage consumed by grazing dairy cows (van Vuuren, 1985). Highly digestible grasses, such as *Lolium perenne*, at an early stage of maturity with a low proportion of stems have less lignified cell walls, a relatively high rate of digestion (Cammell et al., 1983) and probably a decreased 'structural index'.

To meet energy requirements, and to overcome variation in herbage allowance, it became common practice to supply concentrates to dairy cows during the grazing period. The surplus of nitrogen in herbage is balanced to some degree by using concentrate mixtures low in protein but rich in energy (approximately 7.0 MJ net energy (E_N) per kg), mainly composed of ingredients with a high proportion of easily fermentable carbohydrates. However this greater quantity of rapidly fermentable carbohydrates together with a low proportion of 'structural fibre' may enhance the risk of decreased fibre digestion, resulting in lower feed intake and low milk fat (Keuning, 1980).

Information about rumen fermentation in grazing dairy cows is limited (van Adrichem, 1962; Beever & Siddons, 1986). The aim of the present experiment was to study the influence of 1 kg versus 7 kg of concentrate on rumen parameters in grazing dairy cows.

However, because de Visser & de Groot (1981) observed smaller variations in diurnal patterns of some rumen parameters of cows fed winter rations if the proportion of ingredients rich in starch in the concentrate mixture was decreased, composition of the concentrate mixture was included as a second variable.

Material and methods

Animals, feed and treatments

The experiment was carried out with six rumen-cannulated, pluriparae, Dutch-Friesian dairy cows, 6 to 18 weeks into lactation at the start. Animals grazed in swards at 80 to 90 % *Lolium perenne* (Meijs, 1981) at a daily herbage allowance of 29 kg OM above 4 cm cutting height. Cows were split up into two blocks. Within each block treatments were allocated in a latin square arrangement. Treatments were: daily supplementation with either 1 kg of a ground and pelleted concentrate mixture, containing 258 g starch per kg dry matter (treatment 1HS), 7 kg of this high-starch concentrate (7HS), or 7 kg of a mixture with a low concentration of starch (15 g/kg dry matter: treatment 7LS). Table 1 presents the ingredients and their proportion in both mixtures. Concentrates were given in two equal portions at milking (06h00 and 16h00).

Grazing periods were alternately three or four days. After one week for changing over to a new diet, treatments continued for four weeks. The three-day grazing period of the fourth week was used for measurements. Due to weather conditions herbage had to be stall-fed in the first three weeks of the experiment.

Mean herbage intake was assumed to be the same as that measured with intact cows receiving the same treatments and grazing in another part of the same pasture in a grazing experiment conducted simultaneously (Meijs, 1984). Yields of OM of the swards were similar in the three measuring periods (approximately 1600 kg

Table 1. Ingredients (g/kg) and chemical composition of concentrate mixtures and chemical composition of herbage. From Meijs (1984).

Ingredient	Concentrate mixture		Herbage[1]
	high-starch	low-starch	
maize	119	–	
sugar-beet pulp	208	349	
tapioca meal	228	–	
linseed expeller	166	74	
coconut expeller	139	50	
palm kernel expeller	–	150	
soya bean hulls	–	300	
cane molasses	99	30	
tallow	5	9	
MgO	5	5	
vitamin/mineral premix	32	33	
dry matter (g/kg)	876	904	235 (51)
nitrogen (g/kg DM)	23	22	39 (7)
crude fibre (g/kg DM)	92	223	242 (14)
sugars (g/kg DM)	104	78	123 (58)
starch (g/kg DM)	258	15	–

[1] Data of herbage based on 78 estimates; standard deviation in parenthesis.

OM/ha). Growth periods were 12, 23 and 27 days for period 1, 2 and 3 respectively (Meijs, 1984).

Sampling and analysis

During the first 24-hour period of the measuring period the diurnal variation in chemical composition of herbage was studied by sampling (hand-cutting) a diagonal in the sward immediately before each rumen sampling. Grass samples were transported in a cooling box (10 °C). In a climate chamber, at 7 °C, grass was chopped at about 5 cm with a paper-cutter, mixed by hand and divided into two subsamples. One subsample was stored at 4 °C for 36 hours (maximum) for determination of N solubility by the method of Tagari et al. (1962). The other subsample was stored at −80 °C, freeze-dried, ground to pass a 1-mm screen, and stored for analysis. Total sugar content was determined by extracting 5 g of sample in 40% (v/v) ethanol at room temperature for 1 hour. After purification and weak hydrolysis the concentration of reducing sugars was determined with Luff-Schoorl reagent (Netherlands Normalization Institute, 1974). Other feed assays were completed as described by Tamminga (1981).

Immediately before the first and third 24-hour period 1.5 litres of a Cr-EDTA solution were introduced into the rumen at the afternoon milking (16h00). From this point rumen samples were taken every four hours (20h00, 24h00, 04h00, 08h00, 12h00, and 16h00). A stainless steel tube (length 50 cm; inner diameter 40 mm)

closed at one end with a steel disc and perforated with approximately 120 holes, 2.5 mm in diameter, was inserted through the cannula and positioned such that the perforated end reached the liquor phase in the ventral sac of the rumen. Samples (approximately 200 ml) were taken by using a 100-ml Janet syringe with a plastic tube (length 70 cm; inner diameter 2.5 mm).

The pH value of rumen fluid was measured immediately after sampling with a portable pH meter (C18, WPA Scientific Instruments). Samples were stored in melting ice and after 30 to 45 minutes aliquots of 5 ml were taken for analysis of volatile fatty acids (VFA), DL-lactic acid and ammonia. Another aliquot of approximately 100 ml was stored at −20 °C for chromium analysis as described by Tamminga et al. (1983). Analysis of VFA, DL-lactate and ammonia were completed as described by Robinson et al. (1986).

During the week of rumen sampling milk yield was measured on 5 days and samples from each milking were taken and analysed for fat and protein as described by Meijs (1981).

Average values of rumen parameters were calculated using the results of both 24-hour periods for each time of sampling for each animal for each period. Mean pH values were calculated as the negative logarithm of the average hydrogen concentration. Average values were used for comparing patterns of rumen fermentation for different treatments.

Daily means of rumen parameters, and milk production for each animal for each period were subjected to analysis of variance techniques from the statistical package GENSTAT (Alvey et al., 1982) with the model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + \varepsilon_{ijk}$$

where μ is the mean, Y_{ijk} is the estimate of the observation in cow i (C_i), in period j (P_j) for treatment k (T_k), and ε_{ijk} is the residual term of that observation.

Results

Herbage composition

Average concentrations (± standard deviation) of DM in period I, II, and III were respectively 203 ± 4.1, 239 ± 8.4, and 190 ± 13.1 g/kg. Average concentrations of total sugars in these periods were respectively 149 ± 6.5, 160 ± 5.2, and 101 ± 5.1 g/kg DM, and average concentrations of nitrogen 38.2 ± 0.98, 32.3 ± 0.31, and 42.9 ± 0.47 g/kg DM.

Concentration of total sugars in herbage increased during daytime, reached highest values at 20h00 and decreased during the night (Fig. 1). Concentrations of total sugars and N differed significantly between periods ($P < 0.001$). Diurnal variation in N content was smaller than in sugar content.

The soluble N fraction was 0.16 in the first period. This was significantly ($P < 0.001$) lower than for the last two periods, when the soluble N fraction was 0.24. Apart from a peak at 04h00 a distinct diurnal pattern in N solubility could not be detected.

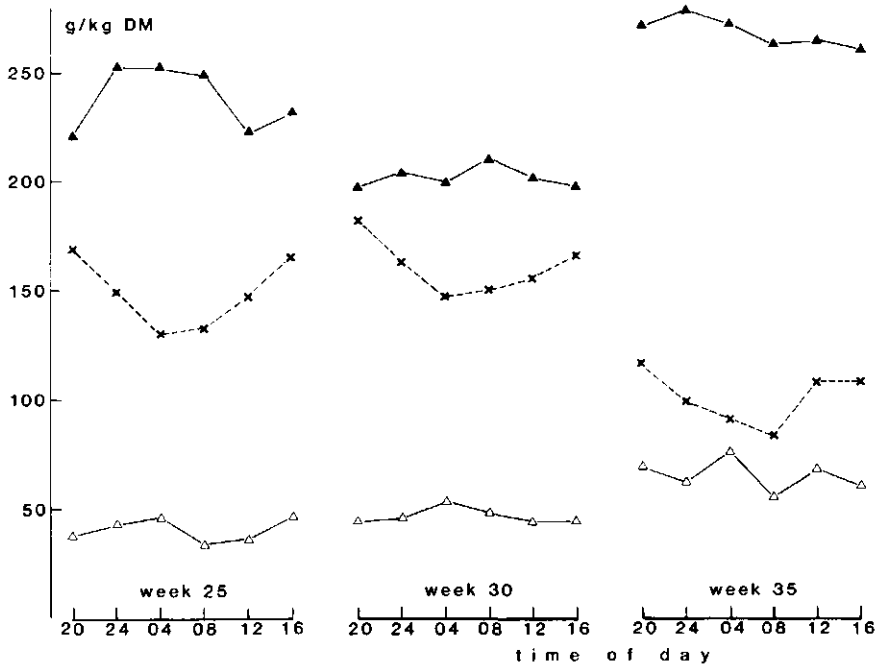

Fig. 1. Daily variation in concentrations of crude protein (▲), soluble crude protein (△), and total sugars (X) in herbage, sampled in period I (week 25), period II (week 30) and in period III (week 35).

Rumen parameters and milk production

Daily patterns of ruminal pH values and concentrations of total VFA, and ammonia are in Fig. 2.

Treatments did not significantly affect ruminal pH values, except for the 08h00 samples ($P < 0.05$). At that time pH values were inversely related to the intake of easily fermentable carbohydrates. Lowest pH values were at 24h00, when measurements varied between 5.2 and 6.2. The diurnal variation of total VFA was the inverse of the pH pattern, with maximum concentrations at 24h00. Concentrations of total VFA were not affected by treatments ($P > 0.05$). Ammonia concentrations peaked at 24h00. Ammonia concentrations were highest on 1HS, but varied considerably among periods: average ruminal ammonia concentrations were 11, 13 and 21 mmol/l in the three consecutive experimental periods. Therefore the concentration of ammonia was significantly higher ($P < 0.05$) for treatment 1HS only at 16h00. Proportions of the iso-acids (iso-butyrate, and 2-methyl- and 3-methyl-butyrate) followed patterns of ammonia.

Table 2 presents daily means of rumen parameters and milk production. Average

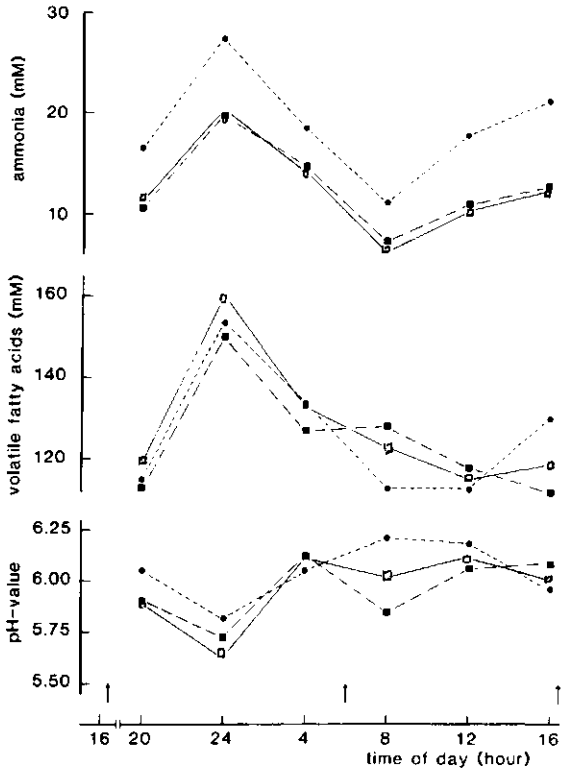

Fig. 2. Pattern of pH value, and concentrations of volatile fatty acids and ammonia in rumen fluid of grazing dairy cows, daily supplemented with 1 kg (●), and 7 kg (■) of a high-starch concentrate, and 7 kg (□) of a low-starch concentrate. Arrows indicate time of feeding concentrate.

production of 4 % fat corrected milk (FCM) in the three consecutive measuring periods was 24.1, 17.2, and 16.3 kg/day respectively.

When feeding 1 kg of concentrate the proportion of iso-acids and concentration of ammonia were higher than when feeding 7 kg of concentrate ($P < 0.05$). Other rumen parameters were not significantly influenced by the amount of concentrate.

Composition of the concentrate affected the outflow rate of rumen fluid ($P < 0.05$), being higher on the low-starch concentrate. Other rumen parameters and milk composition were not affected by composition of concentrate ($P > 0.05$).

Discussion

Herbage composition

Diurnal variations in total sugar content were similar to observations reported by

Table 2. The effect of amount and composition of concentrate on rumen fermentation and milk production of grazing dairy cows. Per treatment $n = 6$. Values in one row with different superscript vary significantly ($P < 0.05$).

	Treatment			Least significant difference
	1HS	7HS	7LS	
Intake (kg OM/day)[1]: herbage →	13.4	11.3	12.8	
concentrate →	0.8	5.4	5.2	
Rumen fermentation[2]				
pH value	6.0	5.9	5.9	0.13
total VFA (mmol/l)	127	127	130	16.7
acetate/propionate	3.2	2.8	3.2	0.61
non-glucogenic/glucogenic	4.4	3.9	4.2	0.58
iso-VFA/total VFA (%)	2.7[a]	2.2[b]	2.1[b]	0.37
ammonia (mmol/l)	19[a]	13[b]	12[b]	1.7
DL-lactic acid (mmol/l)	1.0	1.0	0.8	0.76
rumen fluid outflow rate (/d)	5.0[ab]	4.8[a]	5.3[b]	0.37
Milk production				
yield (kg/day)	19.3	20.0	18.9	3.56
fat content (g/kg)	41	38	41	3.4
protein content (g/kg)	33	35	33	2.3

[1] From different cows in a grazing experiment (Meijs, 1984).
[2] Based on two 24-hour periods; 6 samples per 24 h.

Holt & Hilst (1969) and Smith (1973). Due to photosynthesis the concentration of total sugars increases during daylight and decreases during the night. As shown by Kühbauch (1973) total sugars extracted in a 40 % (v/v) ethanol solution consist mainly of mono- and disaccharides and low-polymere fructosans. However most diurnal variation can be attributed to fluctuations in concentration of sucrose (Smith, 1973).

Total sugars are rapidly fermented by rumen microbes. If rate and extent of fermentation exceeds the absorption of produced VFA, high concentrations of VFA and low pH values may develop.

Daily fluctuations in total N existed, but were less pronounced as compared with patterns of total sugars. Towards the end of the summer period nitrogen concentrations increased, a tendency also reported by Cammell et al. (1983) and Losada et al. (1982).

N-solubility varied slightly during the day.

Rumen parameters and milk production

Although rumen samples were taken not too frequently, the results indicate that patterns of rumen fermentation (VFA, NH_3, pH value) of grazing dairy cows differ from patterns on winter rations. On winter rations patterns of rumen fermentation are mainly affected by frequency and time of feeding concentrates (Robinson et al., 1986). In our experiment the effect of feeding concentrates was less pronounced and thus amount and composition of concentrate mixtures apparently did not influ-

ence patterns of VFA concentration and pH value (Fig. 2).

Concentrations of ammonia peaked at 24h00, probably as a result of a considerable ingestion of herbage in early evening. Behaviour studies with a Kienzle vibrarecorder visualized a 'bulking period' at late-afternoon/early-evening, when animals grazed for three hours or longer, with minor or no resting periods (Meijs, unpublished data). We assume that this eating period, in addition to the higher DM content of the herbage at that time, results in a relatively high rate of DM consumption between 18h00 and 21h00, succeeding in high concentrations of fermentation products. During night-time grazing activities are scarce and concentrations of fermentation products decrease. Between 08h00 and 16h00 ammonia concentrations gradually raise, probably as a result of a moderate herbage intake.

The relatively high concentrations of ammonia indicate a substantial degradation of protein in the rumen, which agrees with results reported by Beever & Siddons (1986). Concentrations of ammonia decreased when feeding 7 kg of concentrate irrespective of its composition. An explanation may be found in the grazing experiment where herbage intake was lower when feeding 7 kg of concentrate. A lower herbage intake, combined with a higher intake of concentrate not only changes the ration between digestible organic matter (DOM) and digestible crude protein (DXP), but also may result in differences in rumen-degradable crude protein (RDXP). Crude protein (XP) in herbage is highly degradably in the rumen: 75 to 85 % of herbage XP disappeared in incubations in sacco (Veen, 1984; van der Tol & van Vuuren, 1986). Calculations based on incubations in sacco of concentrate ingredients (S. Tamminga, personal communication) showed that rate and extent of XP degradation in the rumen may have been less when feeding more concentrate, thus resulting in lower ammonia concentrations.

DOM/DXP ratio increased from 3.8 on diet 1HS to 4.4 when feeding 7 kg of concentrate. A higher DOM/DXP ratio may have been benificial for microbial growth, resulting in relatively higher incorporation of ammonia-N into microbial protein. Lower XP degradation and/or more efficient microbial protein synthesis may also explain the lower proportions of branched-chain iso-acids observed when feeding 7 kg of concentrate (Papas et al., 1984). Another result could have been a higher duodenal protein supply, possibly explaining the increase of milk protein observed in the grazing experiment (Meijs, 1984).

Concentrations of VFA peaked at 24h00, an observation also reported by Mpatwa et al. (1978). The 'bulking period', the relatively high DM content of herbage eaten in early-evening, and the higher sugar concentration in the DM may have been responsible for this phenomenon. Accounting for the change in DM content the difference in sugar content between morning and evening was 15 g per kg fresh herbage. This difference was rather constant, despite significant variations between periods (Fig. 1). VFA concentrations decreased during night, probably due to low herbage consumption. Between 04h00 and 20h00 fluctuations in VFA concentrations were small, which might indicate a moderate and gradual ingestion of herbage with a relatively lower DM content.

Low concentrations of DL-lactate suggest low production of lactate and/or adequate fermentation of lactate. However with the four hours interval of sampling

the peaks (generally short-lasting) in the concentration of lactate (Counotte, 1981; Robinson et al., 1986) could have been overlooked.

From the amount or composition of the concentrate mixture no significant effect on pH values and concentrations of total VFA or lactic acid in the rumen have been observed. From data of his grazing experiment Meijs (1984) calculated average daily consumptions of 2.0, 3.6, and 2.1 kg of starch plus sugars for treatments 1HS, 7HS, and 7LS respectively. The main starch component in the high-starch concentrate was tapioca. In incubations in vitro of tapioca in rumen liquor Malestein et al. (1982) observed a moderate decrease of pH value and a minor increase in concentration of L-lactate. On the other hand, in studies in vivo feeding wilted grass-silage de Visser & de Groot (1981) found a negative effect on rumen fermentation when supplementing high amounts of concentrate containing 300 g/kg tapioca. Our experiment does not confirm the latter results. However observations from the grazing experiment may explain this contradiction: Meijs (1984) observed a lower herbage intake for treatment 7HS, resulting in a decreased ingestion of neutral detergent (ND) fibre of approximately 1 kg per day. ND fibre of abundantly fertilized young herbage is rapidly fermentable (S. Tamminga, unpublished results). Therefore it seems that rate and extent of carbohydrate degradation was not extremely different among treatments, and changes in VFA concentrations and pH values were not apparent. Changing some ND fibre into tapioca starch could have resulted in a change in the composition of fermentation products, indicated by the tendency for a shift in acetate/propionate ratio for treatment 7HS. This is also reflected by a slightly lower milk fat content in treatment 7HS.

Outflow rates of rumen fluid were higher than those observed by Cammell et al. (see Beever & Siddons, 1986) in steers. In contradiction to their results our data did not show a seasonal influence on outflow rates. However a possible effect of season may have been counteracted by the influence of milk yield on the fluid outflow rate (van Vuuren, 1984). The higher outflow rate for treatment 7LS probably results from the higher intake of herbage.

Conclusions

Our study indicates that rumen fermentation in dairy cows grazing on highly fertilized, relatively young perennial ryegrass is not a steady process, and low pH values, and high concentrations of VFA and ammonia in rumen fluid occur, even with moderate supplementation of concentrate. As in other experiments (Beever & Siddons, 1986) our data demonstrate a substantial excess of nitrogen in dairy cows consuming heavily fertilized, young herbage.

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CHAPTER 8

Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows

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TABLE 4. Nitrogen content of diets and proportional N excretion in milk, feces, and urine in dairy cows on grass-based diets supplement with concentrate mixtures high in starch or fiber (Experiment 1).

	Supplement		
	High starch	High fiber	SED[1]
N, g/kg of DM	26.1[a]	23.8[b]	.1
N Excretion, % of N intake			
Milk	29	29	1
Feces	36[b]	42[a]	2
Urine[2]	35[a]	29[b]	2

[a,b]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Estimated as (N intake) – (N excretion in milk and feces).

TABLE 5. Chemical composition of grass and supplements used in Experiment 2.

		Concentrates			
	Grass[1]	Corn silage	Cr_2O_3 Mixture	High starch	Fiber and starch	High fiber
DM, %	17.4	36.0	89.6	87.6	88.9	89.9
Ash, % of DM	11.0	6.0	9.5	6.5	8.6	10.0
N, % of DM	3.8	1.4	2.4	1.8	1.6	1.6
NDF, % of DM	48.1	43.1	38.1	10.3	23.3	37.2
Sugars, % of DM	8.6	1.4	9.6	4.2	8.0	11.9
Starch, % of DM	. . .	26.8	7.5	56.7	29.5	.9

[1]Grass harvested between May 22 and July 13, 1989.

TABLE 6. The effect of replacement of corn silage in grass-based diets by concentrate mixtures high in starch or fiber or both on intake of OM, N, sugars, starch, and NDF and on rumen measurements in rumen-cannulated dairy cows (Experiment 2).

	Supplement				
	Corn silage	High starch	Fiber and starch	High fiber	SED[1]
Intake, kg/d					
OM					
Grass	10.5	9.1	9.5	10.2	.8
Total	17.1	15.8	16.6	17.5	.8
N	.55	.50	.52	.57	.03
NDF	8.4[a]	5.8[c]	7.0[b]	8.4[a]	.5
Sugars	1.6[bc]	1.4[c]	1.7[b]	2.1[a]	.1
Starch	1.1[c]	3.6[a]	2.2[b]	.1[d]	.1
Rumen fermentation					
pH	6.3	6.2	6.2	6.2	.1
VFA, mmol/L	114	112	111	114	4
NGGR[2]	4.6	4.8	4.2	4.4	.3
NH_3 N, mmol/L	11.9[a]	11.8[a]	7.9[b]	9.8[ab]	1.1

[a,b,c,d]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Nonglucogenic:glucogenic ratio of VFA.

TABLE 7. Nitrogen content of diets and proportional N excretion in milk, feces, and urine in dairy cows on grass-based diets supplement with corn silage or concentrate mixtures high in starch or fiber or both (Experiment 2).

	Supplement				
	Corn silage	High starch	Fiber and starch	High fiber	SED[1]
N, g/kg of DM	27.3[c]	28.8[a]	27.7[b]	27.7[b]	.1
N Excretion, % of N intake					
Milk	26	27	28	27	.8
Feces	30[c]	35[b]	39[a]	40[a]	1
Urine[2]	44[a]	38[b]	34[c]	33[c]	1

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]Standard error of the difference.

[2]Estimated as (N intake) − (N excretion in milk and feces).

Total OM intake was higher when grass was partly replaced by beet pulp and corn by-products (Table 9). Differences were only significant in Experiment 3A, in which beet pulp was tested, mainly because of the large variations in Experiment 3B, in which corn products were tested. Because of the higher OM intake on supplemented diets, differences in N intake were not significant. On beet pulp, cows consumed more NDF than on grass, and sugar intake also was increased on DP. Corn by-products significantly increased starch intake.

When DP and EP were fed, mean rumen pH and VFA did not differ from grass without supplementation. The NGGR and mean NH_3 N concentration decreased when grass was partly replaced by DP or EP. Partial replacement of grass by ECS and HCS gave a small decrease in mean rumen pH but did not significantly influenced mean VFA, NGGR, or mean NH_3 N. The differences between beet pulp and corn by-products also were reflected in the diurnal variation in rumen NH_3 N concentration (Figure 2).

In the feeding trial, N intakes were .63, .55, .55, .54, and .55 kg/d for grass without supplementation or supplemented with DP, EP, ECS, and HCS, respectively. Excretion of N in milk was significantly higher when supplements were fed (Table 10). Supplementation reduced urinary N excretion. With EP, the estimated urinary N excretion was significantly higher than for the other supplements.

DISCUSSION

Composition of Supplements

The beneficial effect of supplementing carbohydrates to ruminants fed grass silage on N excretion has been reported (3, 8). The rumen behavior of CP of fresh grass may vary sub-

TABLE 8. Chemical composition of grass and supplements used in Experiment 3.

		Concentrates						
			Minerals		Supplements[1]			
	Grass[2]	Cr_2O_3	$\bar{X}$[3]	SE	DP	EP	ECS	HCS
DM, %	15.5	89.3	89.5	.2	89.7	20.5	57.1	47.7
Ash, % of DM	12.2	10.5	9.9	.8	7.1	7.8	1.8	2.1
N, % of DM	3.5	2.6	2.5	.2	1.6	1.6	1.5	1.4
NDF, % of DM	50.2	37.8	39.8	.6	41.5	50.9	9.4	22.8
Sugars, % of DM	6.1	7.1	8.2	.6	9.9	.6	.2	.3
Starch, % of DM	. . .	11.6	9.3	1.5	.9	. . .	66.5	54.4

[1]DP = Dried sugar beet pulp; EP = ensiled pressed sugar beet pulp; ECS = high moisture ear corn silage; HCS = high moisture ear corn silage with husks.

[2]Grass harvested between August 14 and October 5, 1989.

[3]Mean of three mixtures.

TABLE 9. Effects of partial replacement of ryegrass by low protein supplements on daily intake of OM, sugars, starch, NDF, and N and on rumen measurements in rumen-cannulated dairy cows (Experiment 3).

	Experiment 3A				Experiment 3B			
	Supplements[1]				Supplements[2]			
	None	DP	EP	SED[3]	None	ECS	HCS	SED
Intake, kg/d								
OM								
Grass	11.4[a]	9.3[b]	9.5[b]	.3	11.6[a]	9.3[b]	9.9[b]	.3
Total	13.0[b]	15.4[a]	14.9[a]	.4	13.2	14.9	15.8	.8
N	.54	.53	.53	.01	.55	.52	.54	.02
NDF	7.2[b]	8.0[a]	8.2[a]	.2	7.3	6.5	7.3	.2
Sugars	.8[b]	1.2[a]	.7[c]	<.1	.8[a]	.7[b]	.7[ab]	<.1
Starch	.2	.2	.2	. . .	.2[b]	2.7[a]	2.6[a]	.4
Rumen fermentation								
pH	6.4	6.2	6.2	<.1	6.4[a]	6.3[b]	6.3[ab]	<.1
VFA, mmol/L	106	112	115	4	104	111	106	3
NGGR[4]	4.8[a]	4.6[b]	4.6[b]	<.1	5.0	5.0	5.3	.4
NH_3 N, mmol/L	19.1[a]	12.5[b]	12.7[b]	1.3	19.6	16.6	15.9	1.4

[a,b,c]Within a row and experiment subcolumn, means not sharing common superscript differ ($P < .05$).

[1]DP = Dried sugar beet pulp; EP = ensiled pressed sugar beet pulp.

[2]ECS = High moisture ear corn silage; HCS = high moisture ear corn silage with husks.

[3]Standard error of the difference.

[4]Nonglucogenic:glucogenic ratio of VFA.

stantially from that of CP in silage. The CP of fresh grass is characterized by a relatively low solubility (27), but a large proportion of CP in silage, especially at a low DM content, is solubilized as a result of fermentation (23). Comparing in situ degradation of CP and carbohydrates between forages (23, 28) and concentrate ingredients (24), van Vuuren et al. (27) suggested that feedstuffs with a high proportion of soluble carbohydrates, such as barley, tapioca, oats, and wheat, would be suitable supplements for silage-based diets but that feedstuffs with a high fraction of insoluble, fermentable carbohydrates, such as beet pulp, rice, and, to a lesser extent, corn, milo, and potato, would be suitable supplements for

TABLE 10. Effect of partial replacement of grass by low protein supplements on N content of diets and proportional N excretion in milk, feces, and urine in dairy cows (Experiment 3).

	Supplement[1]					
	None	DP	EP	ECS	HCS	SED[2]
N, g/kg of DM	35.9[a]	30.9[b]	31.4[b]	31.4[b]	30.2[c]	.2
N Excretion, % of N intake						
Milk	16[b]	22[a]	20[a]	21[a]	21[a]	1
Feces	26[b]	29[a]	27[b]	30[a]	29[a]	1
Urine[3]	58[a]	49[c]	53[b]	49[c]	50[c]	1

[a,b,c]Within a row, means not sharing common superscripts differ ($P < .05$).

[1]DP = Dried sugar beet pulp; EP = ensiled pressed sugar beet pulp; ECS = high moisture ear corn silage; HCS = high moisture ear corn silage with husks.

[2]Standard error of the difference.

[3]Estimated as (N intake) – (N excretion in milk and feces).

cows fed fresh grass. In our experiments, supplements based on corn and beet pulp were tested.

Total carbohydrates (sugars, starch, and NDF) in concentrate mixtures based on sugar beet pulp always were lower than in mixtures based on corn (Tables 2 and 5). Also, in DP and EP, carbohydrate concentrations were lower than in the ensiled corn by-products (Table 8). This difference is attributed to pectins, which form an important portion of carbohydrates in beet pulp; however, pectins were not determined by our sugar, starch, or NDF analyses. The deficit of 22% (Experiment 1) to 26% (Experiment 3) of DM is mainly in agreement with reported pectin concentrations in beet pulp of ca. 19.5% of DM (17). The higher starch and lower NDF content of S2 versus S1 is attributed to the exclusion of hominy feed.

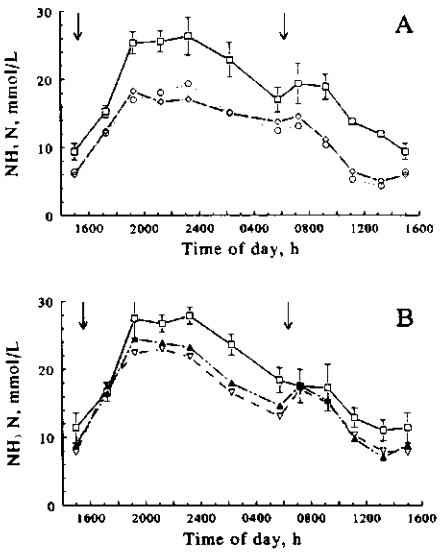

Figure 2. The effect of partial replacement of grass (□) by dried (○) or ensiled (◊) pressed sugar beet pulp (A) or by high moisture ear corn silage without (▲) and with husks (▽) (B) on diurnal patterns of rumen NH_3 N concentrations in dairy cows. Arrows indicate time of supplement feeding. Horizontal bars present standard error of the difference.

Utilization of N in the Rumen and N Excretion

Partial replacement of fresh grass by concentrate mixtures increased OM intake in Experiments 1 and 3, as observed by Meijs and Hoekstra (14). Consequently, differences in N intake between treatments were small (Experiment 1) or insignificant (Experiment 3). Despite similar N intake, partial replacement of grass by concentrate mixtures lowered rumen NH_3 N concentrations (Tables 3 and 6).

Data were used to relate rumen NH_3 N concentration to diet composition. The relation between rumen NH_3 N (Y, millimoles per liter) and the ratio between ingested N and digestible OM (X, grams per kilogram) was $Y = .59 (\pm .07)X - 11.6 (\pm 3.0)$; $r^2 = .83$; residual mean square = 2.12 (Figure 3).

In general, at a similar ratio of N to digestible OM, rumen NH_3 N concentrations were higher when corn products were fed than when diets included beet pulp. This difference is explained by rumen escape of corn OM, thus providing less energy for microbial growth and NH_3 N uptake. To account for the variation in rumen escape of OM and CP, the relationship between rumen NH_3 N and the ratio between rumen-fermented CP and OM also was estimated. Fermented CP and OM of grass were estimated from equations (1); fermented CP and OM of supplements were calculated from the Dutch feed tables (2). The relationship was inferior to that just mentioned, which was at-

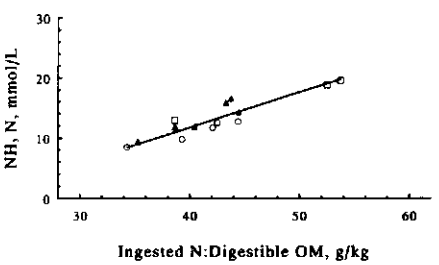

Figure 3. Relationship between rumen NH_3 N (Y) in dairy cows and the ratio between ingested N and digestible OM (X) in grass-based diets unsupplemented (□) or supplemented with feedstuffs based on either corn starch (▲) or beet pulp fiber (○). $Y = .59 (\pm .07) X - 11.6 (\pm 3.0)$; $r^2 = .83$.

tributed to errors introduced in the estimation of fermented CP and OM.

Differences in rumen behavior between corn and beet pulp cannot be explained from the results of nylon bag studies (24). The ratio between fermented N and fermented carbohydrates of corn did not differ substantially from that of beet pulp (15.2 vs. 19.3 g of N/kg of carbohydrate, respectively). Tamminga et al. (24) observed no differences between corn and beet pulp in fermented carbohydrates. But, similar to the present studies, carbohydrate was defined as NDF plus starch plus sugars, which underestimates total carbohydrates content in beet pulp because pectins (ca. 20% of DM) are not taken into account. Thus, on beet pulp mixtures, more OM is available for microbial growth; consequently, more NH_3 N is incorporated into microbial protein. In in vitro experiments (20), a narrower ratio between rumen-degradable CP and nonstructural carbohydrates resulted in higher production of VFA and microbial protein.

Partial replacement of grass CP by supplemental carbohydrates also may lead to a higher ATP yield per kilogram of fermented OM (4). Combined with the higher OM intake, this higher ATP yield may result in higher microbial protein synthesis. Rumen NH_3 N concentrations in addition to an increased fluid outflow rate were lower when intake increased in dairy cows (6). Liquid dilution rate and microbial protein synthesis also were increased when sucrose was infused in cattle fed grass silage (10). Another explanation for the higher NH_3 N when corn products were included in the diet is an increase in rumen protozoa, resulting in enhanced microbial protein degradation (3).

The higher fecal N excretion with concentrate mixtures compared with corn silage supplementation, as observed in Experiment 2 (Table 7), indicates a higher supply of indigestible CP in the small intestine. Also, Huhtanen (8) observed a higher excretion in fecal N when sugars were infused in cows fed silage, and he suggested that this increase was caused by a larger amount of indigestible residue of microbial protein produced in the reticulorumen. Another explanation for higher fecal N output may be an increase in hindgut fermentation. In 3 cows in Experiment 1, equipped with a T-type cannula in the duodenum, 1.1 kg of starch escaped rumen fermentation when S1 was fed (A. M. van Vuuren, 1993, unpublished data). When S1 was fed, rumen NDF degradation was reduced, which was partly compensated by an increased intestinal degradation. Because of the higher supply of fermentable carbohydrates in the hindgut, microbial growth increased, resulting in an extra output of fecal N.

In Experiment 3, milk N output increased significantly when grass was partly replaced by by-products; changes in fecal N excretion were small, but changes in urinary N output were larger. Our data are in agreement with those of Jarvis et al. (9), who reported no large changes in fecal N excretion, but large effects in urinary N excretion, when heifers were changed from high to low fertilized grass. A decrease in urinary N excretion decreases undesired N losses to the environment (9, 22).

Loss of N in urine originates from various sources, such as surplus of NH_3 N in the rumen, microbial nucleic acids, and conversion losses during turnover (maintenance) and protein synthesis for milk and meat (22). In the feeding trials, N excretion in urine was significantly related to the ratio between N and digestible OM in the diet (Figure 4). From Figures 3 and 4, we concluded that, in grass-

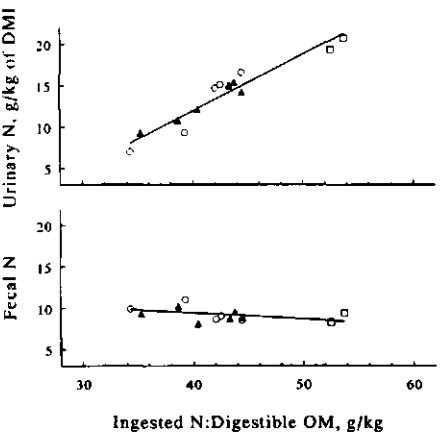

Figure 4. Relationship between N excretion in urine and feces by dairy cows and the ratio between N and digestible OM in grass-based diets unsupplemented (□) or supplemented with feedstuffs based either on corn starch (▲) or sugar beet pulp fiber (○).

based diets, rumen NH_3 N that is not incorporated into microbial N was the main source of N loss in urine. A lower ratio of N to digestible OM also decreased the ratio between absorbable protein and net energy in the diet, which leads to higher efficiency of utilization of absorbed proteins for milk production (22), thereby reducing N losses during milk protein synthesis.

Composition of Rumen VFA

Variations in NGGR were high; thus, differences in NGGR among treatments were not significant except in Experiment 3A, in which supplementation with DP or EP decreased NGGR (Table 9). When grass was partly replaced by mixtures containing corn starch, NGGR was higher than when mixtures containing tapioca starch were used (29).

Sutton et al. (21) demonstrated the relationship between rumen VFA composition and milk fat content. In the present experiments, the relationship between milk fat content and NGGR depended on the type of supplement (Figure 5). At similar NGGR, milk fat content on corn-based supplements was lower than on diets based on beet pulp. In the feeding trials, milk fat concentrations were lower on treatments with the highest starch intake (H. Valk, 1990, unpublished data). Only in Experiment 1 did the lowest milk fat content coincide with the lowest rumen NGGR. Because 20 to 35% of the ingested starch is absorbed postruminally (12, 15), plasma glucose concentrations could be elevated, and concentration of plasma insulin could increase (11), causing low milk fat content, even at higher rumen NGGR.

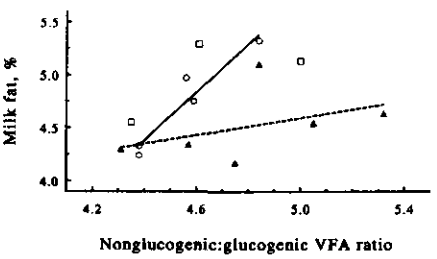

Figure 5. Relationship between milk fat content of cows in feeding trial and nonglucogenic:glucogenic VFA ratio in cannulated cows fed grass-based diets unsupplemented (□) or supplemented with feedstuffs based either on corn starch (▲) or sugar beet pulp fiber (○). R^2 = .43 and .96 for corn starch and pulp fiber, respectively.

CONCLUSIONS

Partial replacement of grass by low protein feeds decreased N excretion in urine; effects on N output in feces were minor. Milk N output may be slightly improved. The improved utilization of N was caused mainly by a reduction in NH_3 N losses in the reticulorumen. Supplements based on beet pulp yielded more fermented OM and gave lower NH_3 N concentrations than corn-based supplements. Corn-based supplements decreased milk fat content, presumably because of the higher postruminal absorption of glucogenic nutrients.

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CHAPTER 9

General Discussion

Introduction

In North-west Europe, pasture feeding is the main method of feed supply in summer. Pastures in temperate regions consist of different grass species or varieties or both. Within a sward, tillers appear in different sizes and different stages of development. Thus, as presented in Chapter 1, the relative proportions of the different plant constituents (protein, sugar, cell walls) can vary substantially and are also affected by various environmental factors, like radiation, rainfall, temperature and the supply of nutrients (N, P, K etcetera). Due to these variations, the nutritive value of grass for ruminants varies significantly. This is illustrated in Table 1, presenting the analytical results of samples of perennial ryegrass from various experiments in the Netherlands and of commercial swards from a survey by Givens *et al.* (1989). The higher content of crude protein (CP) and lower content of neutral-detergent fibre (NDF) in the Dutch grass samples indicate that these grasses grew under more intensive circumstances and were harvested at a less mature stage than the grasses of the survey of Givens *et al.* (1989). The lower dry matter (DM) and higher organic matter (OM) content of the younger, more heavily fertilized grass, indicate a higher ratio between cell contents and cell wall contents in grass grown under more intensive circumstances. This is in agreement with the review by Wilman & Wright (1983).

Grazing dairy cows must extract a major part of nutrients required for their maintenance and milk production from grass. The organic nutrients absorbed from the gastro-intestinal tract, can be divided into ketogenic, glucogenic and aminogenic nutrients, used as precursors for the synthesis of lipids (body and milk), lactose and proteins (meat and milk), respectively (Tamminga & van Vuuren, 1988).

Acetic and butyric acid are the main ketogenic nutrients. These short-chain volatile fatty acids (VFA) are end-products of microbial fermentation of carbohydrates (CB) and proteins in the forestomachs. Other ketogenic nutrients are long-chain fatty acids, which are either absorbed from the gastro-intestinal tract or provided by lipolysis of body fat. Most of the required glucose is derived from gluconeogenesis from lactate and glucogenic VFA (propionic and valeric acid) and amino acids (AA). Also the glucogenic VFA are end-products of rumen fermentation; lactate may either originate from fermentation or be produced by active skeletal muscle. Essential and non-essential AA are absorbed from the intestinal tract and originate either from unfermented feed protein or from micro-organisms leaving the forestomachs with the digesta.

Until recently, young, highly-digestible herbage was considered an ideal feed for dairy cows. However, research during the last decade has shown that the nutrient supply of grazing dairy cows is insufficient for milk productions higher than 28-30 kg/day. This is due to the limited OM intake from fresh grass (130-145 g OM per kg metabolic weight) (Meijs, 1981; Dulphy *et al.*, 1989) and to the chemical composition of grass, resulting in an unbalanced nutrient supply (Chapter 4).

Table 1. Variations in the chemical composition of perennial ryegrass sampled in various experiments in The Netherlands and of samples of commercial swards sampled in England and Wales

Parameter	The Netherlands[1]			England and Wales[2]		
	Mean	Range	*n* values	Mean	Range	*n* values
Dry Matter (g/kg)	172	118-241	46	206	126-431	173
Organic matter (g/kg DM)	890	861-911	46	924	800-957	173
Crude protein (g/kg DM)	225	144-328	46	174	45-285	130
Neutral-detergent fibre (g/kg DM)	472	341-568	46	575	415-687	171

[1]Chapter 4; Steg *et al.*, 1994; I. Hageman, unpublished.; C. Salaün,unpublished
[2]Givens *et al.*, 1989

In this General Discussion, the factors that affect the supply of aminogenic, glucogenic and ketogenic nutrients from grass will be considered. Because our research studied particularly on nitrogenous compounds in grass, the supply of aminogenic nutrients will be emphasized. Supply of nutrients depends on total DM intake and DM composition. Therefore, aspects of DM intake from grass will be considered firstly. Finally, some concepts to improve the nutrient supply of grazing animals and the effects on the excretion of N will be discussed.

Feed intake

The major factor influencing the supply of nutrients is determined by the total DM intake. Farmers strive to a maximum proportion of herbage in the diet of grazing animals. However, the intake of herbage, even at high allowances is limited to 130-145 g OM/kg metabolic liveweight (W^{kg}) per day (Meijs, 1982; Dulphy *et al., 1989).* Thus, for cows from 550 to 700 kg liveweight, maximum daily herbage intake varies between 15 and 20 kg OM, which on a net energy basis, will be insufficient to meet the requirements of maintenance and a milk production of 25 to 35 kg. Factors that limit roughage intake are not yet elucidated and may

comprise external factors like grassland management and weather conditions, and feed-animal interrelationships (de Jong, 1986).

As shown by Van Soest (1982) several studies support the hypothesis that NDF content of forage is the primary restrictive determinant of voluntary feed intake. This hypothesis implies that voluntary NDF intake is a constant. But, as indicated by Van Soest (1982), with good quality forage (NDF content < *ca.* 550 g/kg DM) NDF intake increased with increasing NDF content. The NDF content of the grasses fed in the experiments described in Chapters 5 and 6 was lower than of the grass silages fed by Bosch (1991) (Table 2). The lower NDF content resulted in a lower NDF intake, which supports the hypothesis that for high quality forages other factors than rumen NDF fill limit voluntary feed intake. Therefore, we concluded that the NDF content is not a limiting factor for grass intake.

Table 2. Comparison of NDF characteristics between grass silage and fresh grass and NDF intake and rumen pool size in dairy cows fed either mainly grass silage or fresh grass.

Parameter		Grass silage[1]	Fresh grass[2]
NDF content, g/kg DM		446 - 641	331 - 425
Rate of NDF degradation, %/h		3.8 - 5.9	4.2 - 6.6
Undegradable fraction, %		11 - 24	6 - 11
NDF intake	kg/d	5.6 - 8.0	4.7 - 6.6
	g/kg MBW[3]	50 - 70	41 - 56
NDF rumen pool size	kg	4.7 - 6.9	2.8 - 3.7
	g/kg MBW	42 - 60	25 - 33

[1]Bosch, 1991
[2]Chapters 5 and 6
[3]MBW = (Body Weight)$^{3/4}$

Also the degradation characteristics of NDF may play a role in feed intake regulation. Undegradable NDF may be an important mass in the rumen, because it will leave the rumen only by passage. The relationship between undegradable NDF and rumen DM pool size is presented in Figure 1. With the increase in undegradable NDF from 20 to 100 g/kg of forage DM the rumen DM pool increased. At a higher NDF content the DM pool tended to decrease, but the evidence for this is based on only one observation. This suggests that dairy cows respond to a decrease in forage quality by increasing rumen content. If quality further

decreases rumen fill reaches a limit. Contrary to more matured grass silage, the undegradable NDF content of grass fed in our experiments was low and consequently rumen DM pool sizes were less than the maximum fill observed by Bosch (1991). Thus, it is clear that the intake of fresh grass or good quality grass silage is not limited by rumen NDF fill.

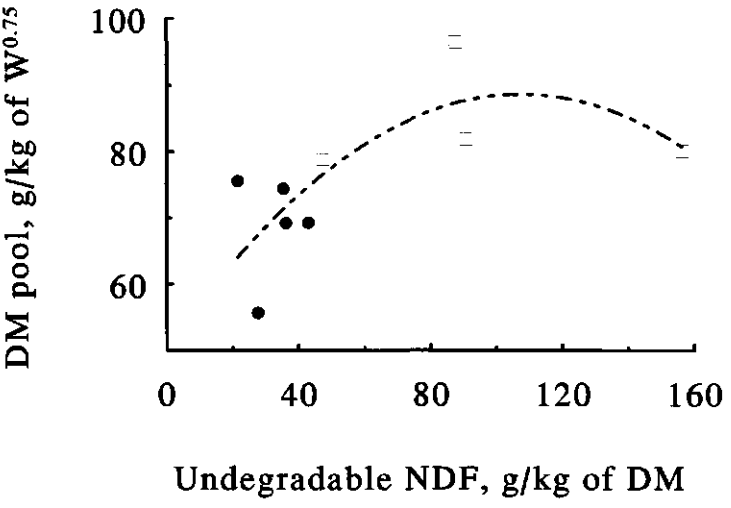

Figure 1. Relationship between undegradable NDF and rumen DM pool size. Data of grass from Chapters 5 and 6 (●); data of grass silage from Bosch (1991; □).

Bosch *et al.* (1991) suggested that the increase in rumen fill, depended on the energy requirement. However, other factors may be involved as well. With a decrease in roughage quality, concomitant with the increase in undegradable NDF content, the rate of degradation will be reduced and hence the rate of increase in functional specific gravity and rate of passage (Sutherland, 1988). The animal, to some extent, will compensate for this by an increase in rumen fill, and thus, the daily flow (fill x passage rate) will be less changed.

Madsen *et al.* (1993) combined *in situ* degradation characteristics and passage rates, to calculate the physical fill factor of feeds. From the data of Chapters 5 and 6 we calculated fill factors of NDF of 0.71 to 1.06 days. The observed NDF intake was 60 to 90% of the intake calculated as (rumen NDF pool size)/(NDF fill), assuming a maximum NDF pool size of 6 kg (Bosch, 1991). Thus also the NDF fill factor cannot explain the limited DM intake from fresh grass.

Another factor to be considered is rumen distension. The daily consumption of 16 kg OM from fresh grass with a DM content of 15%, implies a simultaneous intake of intracellular

water of 120 kg. Consequently, rumen distension when fresh grass is eaten, will be higher than when the animal consumes preserved, dried forages. Good quality grass is rapidly fermented, resulting in high rumen concentrations of VFA and NH_3. This leads to a high osmolarity of rumen fluid and consequently to an influx of water into the rumen, which also will add to rumen distension. Data of Chapters 5 and 6 were compared with data obtained in cows fed mainly grass silage *ad lib* (Bosch, 1991). No direct relationship was observed between rumen volume and DM intake (Figure 2). Generally, total rumen volumes on grass were lower than on grass silage. We therefore concluded that rumen distension is not a physical limitation for grass intake. Further analyses showed, that the relationship between rumen volume and intake depended on the stage of lactation (milk yield), in agreement with the conclusion by Bosch (1991). Within a specified stage of lactation a positive relationship existed between rumen volume and DM intake.

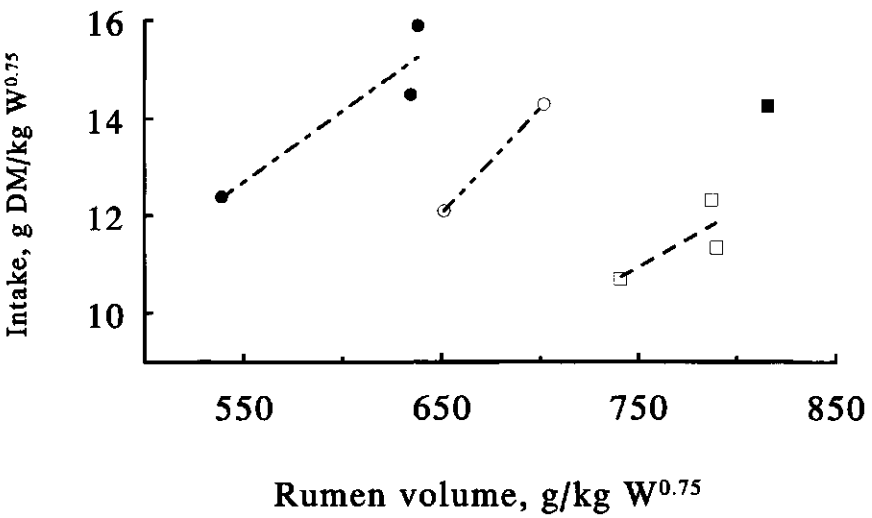

Figure 2. Relationship between total rumen volume (g wet weight/kg $W^{0.75}$), dry matter intake (kg/d per kg $W^{0.75}$) and milk yield in dairy cows fed mainly forage. ●: Grass, 25 kg FCM; ○: grass, 13 kg FCM; □: grass silage, 11 kg FCM and ■: grass silage, 19 kg FCM. Data of grass silage from Bosch (1991)

The high rate of rumen fermentation of high quality grass will result in a rapid increase of ruminal VFA and NH_3 concentrations, and consequently in a rapid increase in osmolarity and fermentation heat. Increasing rumen temperature reduced feed intake of cows offered a 50% lucerne, 50% concentrate diet (Gengler *et al.*, 1970). Due to the high rate of

fermentation, rumen temperature may increase when cows consume high quality grass. This effect may be enhanced when ambient temperature is high. Increases in VFA concentrations, osmolarity and rumen temperature may act together to control feed intake (Anil *et al.*, 1993). Also ruminal NH_3 concentrations are high in grass fed dairy cows (Chapter 7) and may well play a role in feed intake regulation. High concentrations of NH_3 in grass silage often coincided with a limited intake of that silage (Gill *et al.*, 1986).

Another factor that may influence grass intake. is a limitation of the time spent chewing. Studies on eating and ruminating activities in cattle, show that 12 to 17 hours per day is spent on chewing, with no significant relation to voluntary intake (Figure 3). A positive relationship between DM intake and chewing activity was observed only by Metz (1975), feeding hay wafers, and by Teller *et al.* (1989) comparing direct-cut and wilted grass silage. In the experiments of Bosch (1991) and DeBoever (1991), who fed grass silage of different qualities, this relationship tended to be negative. Thus, it seems that with grass silage low in quality or DM content or both, the low intake is related to the maximum time that the animal can chew. This has also been suggested by Kamatali (1991).

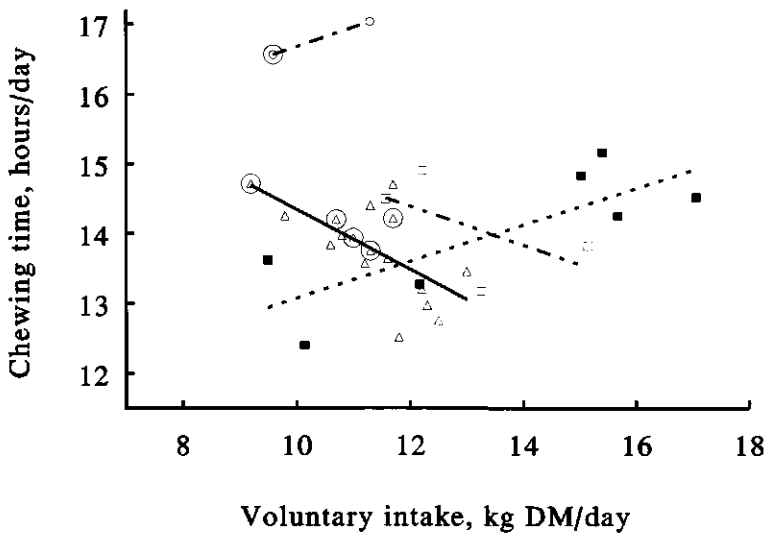

Figure 3. Relationship between *ad libitum* forage intake and total chewing time (eating and ruminating) of dairy cows. Data from Metz (1975; ■) feeding hay wafers, Teller *et al.* (1989, ○), Bosch (1991, □) and DeBoever (1991, △) feeding grass silage either wilted or direct-cut (encircled symbols).

Recently reported grazing times in dairy cows were between 5.1 and 9.3 h/day (Rogalski *et al.*, 1989; Stakelum & Dillon, 1989). Also earlier studies reported that cows spent 6 to 14 h/d grazing (Church, 1973). Factors that inversely affect grazing time are daily herbage allowance (Rogalski *et al.*, 1989) and herbage height (Stakelum & Dillon, 1989). If we also consider that grazing cows spend 5 to 6 h/d ruminating (Castle *et al.*, 1950), the total time that cows in pasture spend chewing approximates the maximum chewing capacity of dairy cows. Our experiments were carried out under a zero grazing regimen. Thus, time spent eating (grazing) could be less than in pastured animals, and consequently DM intake could be somewhat higher. However, under a zero grazing regimen, feed intake is usually less than when animal are pastured, which is attributed to the higher nutritional quality of the grass selected during grazing.

Supply of aminogenic nutrients

Aminogenic nutrients are supplied as AA in digestible protein entering the small intestine. These proteins originate from digestible feed protein that escaped from rumen fermentation and from microbial protein, synthesized in the rumen and leaving the rumen with the digesta. Modern protein evaluation systems estimate the duodenal supply of both sources (van Straalen *et al.* 1994).

Rumen escape of grass protein

The proportion of digestible protein escaping from rumen fermentation is estimated from the degradation characteristics obtained by *in situ* incubations (Chapter 3). From Table 2 in Chapter 1, it can be concluded that 50 to 60% of grass protein is soluble. However, in nylon bags studies the soluble CP fraction varied between 5 (Chapter 3) and 50% (Chapters 5 and 6), significantly influenced by the method of sample preparation. Protein solubility of 40 to 50% was observed when grass samples were freeze-dried and ground, prior to *in situ* incubation, whereas chopping and freezing of grass resulted in soluble fractions of 5 to 30%. Thus, differences in sample preparation may lead to variation between experiments and research groups.

The fate of the CP fraction disappearing from the nylon bags is also not clear. Freeze-drying and grinding ruptures cell walls, and thus free protein and intact chloroplast are washed out of the bags. Fraction I leaf protein is assumed to be instantly degradable in the rumen (Nugent & Mangan, 1981). However, it is doubtful, if the protein fraction washed out from nylon bags but still enclosed by the chloroplast membranes, is instantly available for microbial proteolysis.

Van Straalen & Tamminga (1989) presented equations to estimate escape grass protein from the CP content and the day of harvesting, expressed as days elapsed since April 1. These equations were based on 28 data, obtained from Experiments 1 and 3 of Chapter 3 and from one experiment carried out by I. Hageman (unpublished data). Since then, results from other experiments became available (I. Hageman [unpublished data], H. Valk [unpublished data], C. Salaün [unpublished data] and Steg *et al.* [1994]), increasing the number of observations up to 79. This expanded database was used to re-examine the relationships between chemical composition and *in situ* degradation parameters. Five observations were excluded: three because we did not regard them as fed under practical circumstances and two because results were too diverged. Relationships for diploid species (n=46) differed from those for tetraploid species (n=28). Moreover, the relationships for tetraploid grasses were less significant than for diploid grass.

Table 3 presents the means and ranges of the CP degradation characteristics. No significant relationships were found between chemical composition and the extent of the soluble fraction (R^2 = 0,39) or the rate of degradation (R^2 = 0.32). For the undegradable (U) and rumen escape (E) fractions, the following relationships were obtained:

$$U = -44.6\ (7.6) + 0.21\ (0.03) \times [NDF] + 10^{-3} \times \{0.65\ (0.12) \times [CP]^2 - 0.75\ (0.11) \times [CP] \times [NDF]\} \qquad R^2 = 0.78 \quad \text{Eqn (1)}$$

$$U = 50.6\ (10.8) - 0.43\ (0.10) \times [CP] + 0.19\ (0.07) \times D + 10^{-3} \times \{0.97\ (0.25) \times [CP]^2 - 0.76\ (0.28) \times [CP] \times D \qquad R^2 = 0.66 \quad \text{Eqn (2)}$$

$$E = 39.6\ (3.0) - 1.31\ (0.17) \times Gd + 10^{-3} \times \{3.22\ (0.34) \times [NDF] \times Gd - 0.11\ (0.02) \times [CP] \times [NDF]\} \qquad R^2 = 0.73 \quad \text{Eqn (3)}$$

$$E = 48.5\ (2.5) - 0.10\ (0.01) \times [CP] + 0.07\ (\pm 0.01) \times D \qquad R^2 = 0.64 \quad \text{Eqn (4)}$$

where: U and E are expressed as % of CP,
standard error of coefficients is presented in parenthesis,
[CP] and [NDF] are concentrations in g/kg DM,
D = day of harvesting expressed as days after April 1 and
Gd = growth stage in days elapsed since previous cut

Table 3. Mean and variation of date of harvesting, growing stage and *in situ* degradation characteristic of crude protein of 46 samples of diploid grass. Data from Chapter 3, Steg *et al.* (1994), I. Hageman (unpublished), H. Valk (unpublished) and C. Salaün (unpublished)

Parameter	Mean	Minimum	Maximum
Date of harvesting, days after April 1	113	47	206
Growing stage, days after previous cut	25	15	44
Soluble fraction, % of CP	15.0	4.1	30.7
Undegradable fraction, % of CP	7.6	2.3	25.4
Rate of degradation, %/h	9.6	5.9	14.9
Rumen escape,% of CP	32.5	22.2	48.6

Variations in CP and NDF content explained almost 80% of the variation in the undegradable CP fraction (Equation 1) and in combination with growth stage 73% of the variation in ruminal protein escape (Equation 3). These parameters were also explained by variations in CP content and date of harvesting (Equations 2 and 4).

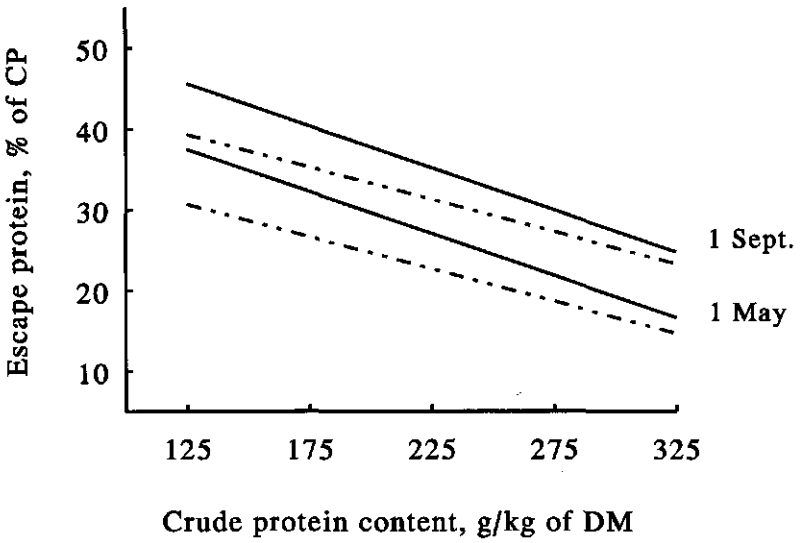

Figure 4. Estimated relationship between CP content and escape CP at different harvesting dates. - - - - = according to van Straalen & Tamminga (1990); ------ = according to new equation.

The relationship between CP and the proportion of escaped protein estimated by the Equation 4, in comparison to the equation by van Straalen & Tamminga (1990) is illustrated in Figure 4. The new equation resulted in a higher proportion of escape protein, particularly at CP concentrations < 200 g/kg of DM. Thus, the quantitative reduction in escape protein with a decrease in CP content is less abrupt than predicted earlier in Chapter 3. The equation of van Straalen & Tamminga (1990) frequently underestimated protein escape (Figure 5a). The new equation overestimated *in situ* protein escape for tetraploid grasses (Figure 5b).

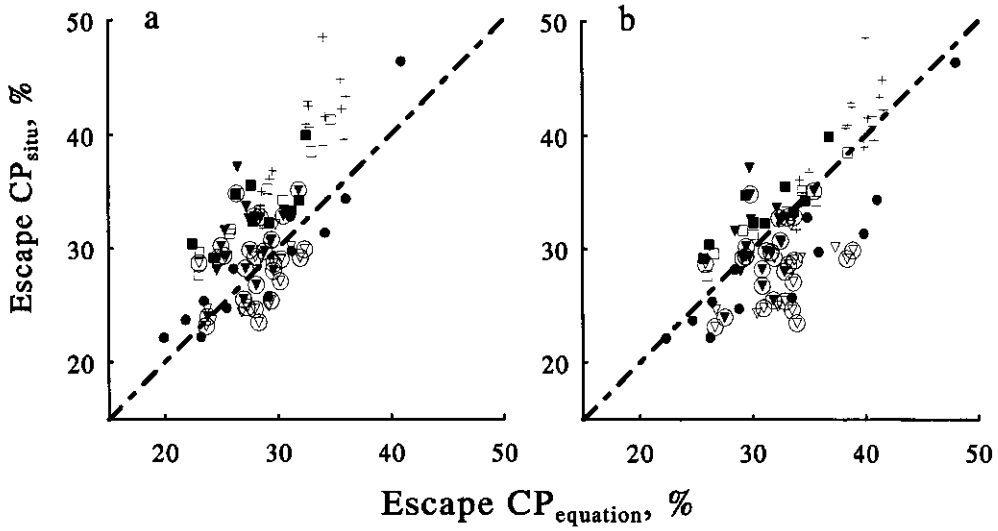

Figure 5. Prediction of *in situ* estimated escape crude protein (CP) based on (a) the equation of van Straalen & Tamminga (1990) and (b) Equation 4. ●: Data from Chapter 3; +: Steg *et al.* (1994); ∇ & ▾: I. Hageman (unpublished); □: H. Valk (unpublished); ■: C. Salaün (unpublished). Encircled symbols: tetraploid species.

Microbial protein synthesis

The duodenal supply of microbial protein depends on the building blocks and energy available for microbial growth and the efficiency of microbial protein synthesis. Available energy is usually expressed as the amount of OM fermented in the rumen; the efficiency of microbial synthesis as microbial protein produced per kg fermented OM (FOM).

Fermentable organic matter

Microbial growth is directly related to the quantity of substrate available to the micro-organisms. A proportion of the OM degraded in the rumen, is used by the micro-organisms as "building-blocks", e.g. peptides, AA, NH_4^+ and (branched-chain) VFA, which are incorporated in own macro-molecules. As shown in Chapter 3, the fermentation of heavily-fertilized grass results in an abundant supply of AA and NH_4^+. If we also assume that other essential nutrients, like minerals, are not limiting, microbial growth in the rumen of dairy cows grazing heavily-fertilized grass, will mainly depend on the energy, released from the ingested OM, i.e. CB and CP.

In Chapter 4, ruminal CB degradation was estimated indirectly as the degradation of non-protein, non-fat OM. To estimate the relationship between chemical composition and the supply of fermented FOM, data from various *in situ* incubations (Chapter 3; Steg *et al.*, 1994; I. Hageman, unpublished; H. Valk, unpublished and C. Salaün, unpublished) were used. Fermented OM was calculated either from the results of *in situ* incubations ($FOM_{in\ situ}$) or on the basis of digestible OM, fat content and the amount of escaped protein calculated by Equation 4 (FOM_{DVE}).

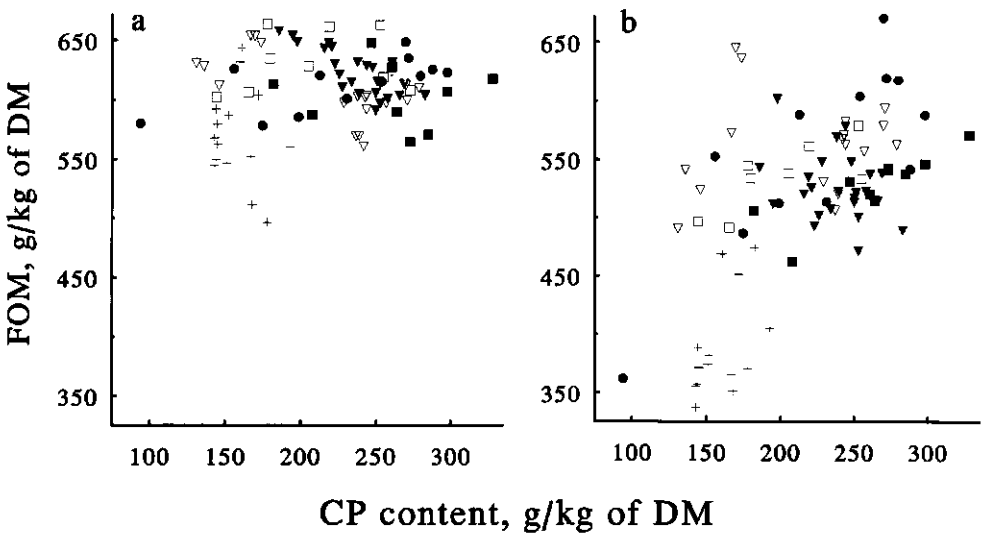

Figure 6. The relationship between the crude protein (CP) content of grass and fermentable organic matter estimated either on the basis of digestible OM, fat content and the amount of escaped protein (Centraal Veevoederbureau, 1991a) (a) or from *in situ* data (b). Data from Chapter 3 (●); Steg *et al.* (1994; +); I. Hageman (unpublished; ∇ and ▾); H. Valk (unpublished; □) and C. Salaün (unpublished; ■)

Values based on the *in situ* OM degradation were significantly lower than the estimates based on digestible OM, with the difference between both estimates ranging from 14 to 234 g FOM/kg DM. The content of $FOM_{in\ situ}$ decreased with decreasing CP content, whereas FOM_{DVE} was rather constant with a tendency to decline at CP concentrations > 200 g/kg of DM (Figure 6). Thus, at a high CP content the difference between both estimates was lower then at lower CP concentrations (Figure 7).

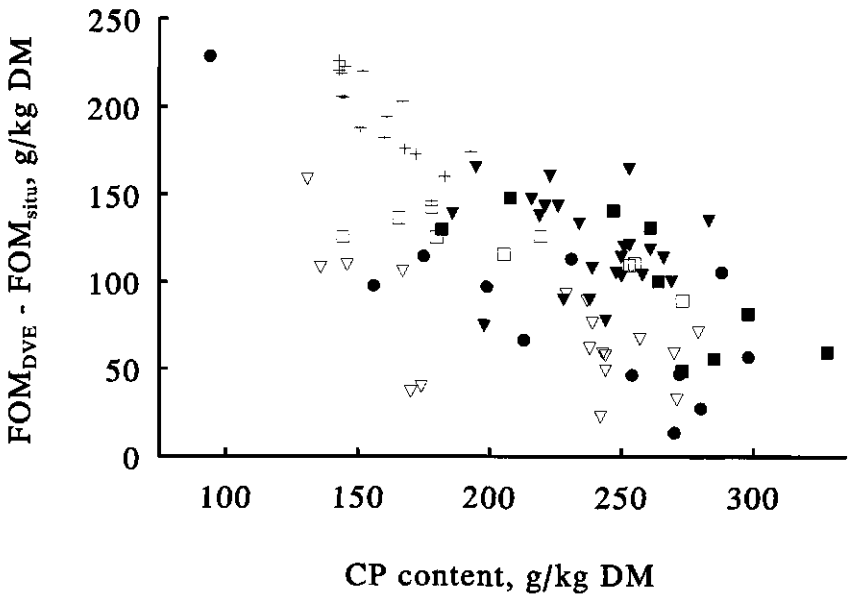

Figure 7. The relationship between crude protein (CP) content of grass and the difference in fermentable organic matter estimated by the DVE-equations (FOM_{DVE}) and from *in situ* data (FOM_{situ}). Data from Chapter 3 (●); Steg *et al.* (1994; +); I. Hageman (unpublished; ▽ and ▼); H. Valk (unpublished; □) and C. Salaün (unpublished; ■).

The difference between both methods of estimation may be explained by various factors. Fermented OM based on *in situ* degradation was estimated with a rate of passage fixed at 4.5%/h. In our *in vivo* trials (Chapters 5 and 6), the passage rate of OM in grass-fed dairy cows ranged between 2.4 and 3.4%/h. If a passage rate of 3%/h, instead of 4.5%/h, was used, the difference in estimated FOM was less, ranging between -34 and 171 g/kg DM. However, this correction had no influence on the range width and the tendency that the difference decreases with increasing CP content.

Differences between both methods also reduced when we assumed compensatory hind gut fermentation or an increased ruminal retention of low quality grass. Both explanations may

be possible. In sheep fed chopped forages, a decrease in overall cell wall digestibility coincided with an increase in the proportion of digestible cell wall digested in the large intestine (Ulyatt *et al.*, 1975). Ruminal retention is influenced by several factors, like structure of the rumen filter-bed and size and functional specific gravity of feed particles (Lechner-Doll, *et al.*, 1991). The rate of increase in functional specific gravity is related to the rate of degradation of feed particles (Sutherland, 1988). In roughage with a lower quality and a slower rate of degradation the increase in functional specific gravity will be more gradual, and thus, the slower rate of degradation is compensated by a slower rate of passage. This compensation is not taken into account when estimating FOM from the *in situ* OM degradation. Using FOM_{DVE} we estimated passage rates as a function of the degradation rate. Passage rates ranged between 0.21 and 0.60 times degradation rate (mean 0.41). When we assumed a K_p of 0.41 x K_d, the difference between $FOM_{in\ situ}$ and FOM_{DVE} was reduced to values between -29 and 92 g/kg of DM.

Based on *in situ* and *in vivo* experiments (Bosch, 1991), Tamminga (1993) estimated that 85 to 90% of the potentially degradable NDF fraction was digested, independent from the rate of NDF degradation, which is also explained by assuming compensatory hind gut fermentation or a positive relationship between rate of passage and rate of degradation or both. In our *in vivo* experiment (Chapter 5), no significant differences in the proportion of OM digested in the rumen were observed when grass fertilized at a lower N level was fed, although overall digestibility decreased by 2 to 4 %-units. In experiments using the mobile bag technique to estimate intestinal digestion in lactating dairy cows, the mean transit time of the bags was 14 h (van Straalen *et al.*, 1993). These observations suggest that a compensatory hind gut fermentation in lactating cows will be of minor importance.

Efficiency

The second factor that determines the duodenal supply of microbial protein is the efficiency by which microbial protein is synthesized. In Chapter 3, we assumed a microbial protein synthesis of 150 g per kg fermented CB (FCB) and 75 g per kg fermented protein. From the *in vivo* studies (Chapters 5 and 6) we calculated that on the diets containing > 80% grass, 55 to 102 g (average 79 ± 15) microbial protein was synthesized per kg OM degraded in the rumen to VFA or incorporated in microbial OM, assuming that microbial DM consists of 8.4% N (Robinson *et al.*, 1987), 13% ash (Czerkawski, 1986) and that 75% of microbial CP is amino acid protein (Centraal Veevoederbureau, 1991a). In the DVE-protein-system, an efficiency of 112 g per kg FOM_{DVE} is assumed.

The low efficiencies observed in our experiments, can be explained by an underestimated duodenal OM and non-ammonia N (NAN) flow. In Chapter 5, we already discussed the underestimated duodenal NDF flow, which may be due to unrepresentative sampling from the

T-type cannulas. In Chapter 6, the observed efficiencies were higher. The differences between both experiments could be caused by differences in experimental surgery. The animals used for the experiment in Chapter 5 had T-type cannulas with a large internal diameter (30 mm) directly after the pylorus, whereas in the experiment in Chapter 6, smaller cannulas (i.d. 19 mm) were placed at the distal part of the duodenum. Although we expected more representative sampling from the wider cannulas, generally, reconstitution factors (Faichney, 1980) in Chapter 5 were higher than those in Chapter 6 (Table 4), indicating less representative sampling (Ortigues *et al.*, 1990). One explanation could be that opening of the cannula directly after the pylorus influenced abomasal motility, resulting in less representative sampling.

Table 4. Reconstitution factor (RF) calculated from the concentrations of markers in total duodenal digesta and in the liquid phase as described by Faichney (1980) and the estimated efficiency of microbial protein synthesis (g/kg FOM).

	Chapter 5				Chapter 6		
	500	275	275	500	No supplement	High starch	High fibre
RF	0.361	0.165	0.202	0.395	0.159	0.200	0.305
Eff.	87	80	55	72	102	113	96

The lower efficiency in our experiments may also be attributed to the relatively high CP content of the five diets, ranging between 185 and 229 g/kg OM (Demeyer & Tamminga, 1987). However, from our few data no relationship was found between CP content and the efficiency of microbial protein synthesis. If we omit the low value observed in the experiment of Chapter 5 when feeding low N grass in September, the mean efficiency increases from 79 to 85 g/kg FOM. The efficiency observed in our experiments was lower than those calculated from experimental data of Beever *et al.* (1985, 1986a and 1986b) and Ulyatt *et al.* (1988). Integrating their 32 data from cannulated grass-fed steers, an average of 105 $\pm$ 16 g microbial protein per kg degraded OM was calculated. This difference can be mainly attributed to the relatively high proportion of microbial protein in their experiments. Whereas in our experiments a maximum 62% of duodenal NAN was estimated as microbial protein, Beever *et al.* observed (1986b) or assumed (1985, 1986a) a microbial proportion of 76%. This higher proportion of microbial NAN is probably due to the low intake level in their experiments: 75 g *versus* 118 g OM/d per kg $W^{0.75}$ in our experiments. So, with the lower feed intake rumen retention time increases, resulting in a more extended feed protein degradation. However, the lower efficiency observed in our experiments cannot be explained by the difference in feed intake, because generally an increase in feed intake coincides with an increase in the efficiency of microbial synthesis (Demeyer & Tamminga, 1987). Differences in chemical

composition between the grasses fed in our experiment and in those of Beever *et al.* (1985, 1986a, 1986b) and Ulyatt *et al.* (1988) can also explain the difference in efficiencies. Generally, the NDF content in our grasses was lower than in grasses used in the experiments reported from the UK. Consequently, our grasses contained more soluble cell contents, although average CP concentrations differed only 20 g/kg DM. The higher concentrations of cell contents, may also explain the low efficiency of microbial protein synthesis (Demeyer & Tamminga, 1987).

Also van 't Klooster & Rogers (1969) observed a higher efficiency (142 g/kg FOM; n=1) on grass (CP: 188 g/kg DM) than we observed.

Duodenal protein supply

Using Equation 4 and FOM_{DVE} to calculate escape protein and available energy, respectively, the duodenal NAN flow was estimated from the OM intake and the chemical composition of herbage in various experiments (Chapter 5 and 6, n=5; van 't Klooster & Rogers, 1969, n=1; Beever *et al.*, 1985, 1986a, 1986b, n=28; Ulyatt *et al.*, 1988, n=3). As mentioned above, the experiments of Beever, Ulyatt and colleagues, all involved steers of *ca.* 180 kg liveweight. Crude fat content was fixed at 40 g/kg DM. Efficiency of microbial CP synthesis was set at 150 g/kg FOM_{DVE} (Centraal Veevoederbureau, 1991a). An endogenous protein flow of 30 g per kg duodenal OM was assumed (Brandt *et al.*, 1980; van 't Klooster & Rogers, 1969). Predictions were compared to the reported NAN.

The relationship between predicted (X) and observed (Y) duodenal NAN flow is shown in Figure 8. Although the relationship was significant (R^2=0.92), the prediction overestimated the flow ($Y = 0.85 \times X$) especially with respect to the flows reported in Chapter 5. Possible explanations for this discrepancy have been described in the previous paragraph.

Available protein in the small intestine (g DVE/kg DM) of the grass studied *in situ* was calculated using the assumptions presented in Table 5.

A curvilinear relationship was found between CP and predicted DVE content (Figure 9a). Extrapolation suggests that at a CP level > 270 g/kg DM, the DVE-value of grass is at a plateau level of *ca.* 125 g DVE/kg DM. Similar relationships were obtained by Minson (1990) reviewing published data and in Chapter 5 where we combined our own data and those of Beever *et al.* (1986b). However, those studies showed a plateau level at CP concentrations of 215 to 240 g/kg DM. The difference between *in situ* and *in vivo* calculations may be due to the decrease in the efficiency of microbial protein synthesis, caused by the increase in the proportion of protein in FOM and the increase in the ratio between cell contents and cell walls (Demeyer & Tamminga, 1989). The average CP content in our samples was 225 g/kg DM (Table 1). According to Figure 9a, the DVE-value of grass at this CP content is 113 g/kg

DM. At this concentration and a herbage-OM intake of 16 kg/day, the supply of available protein (aminogenic nutrients) is *ca.* 2 kg DVE/day.

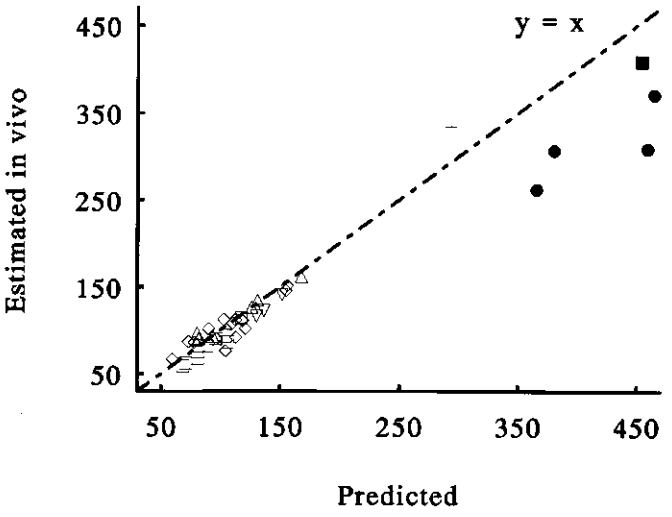

Figure 8. The relationship between the duodenal non-ammonia nitrogen flow predicted from the chemical composition of herbage and estimated in cannulated grass-fed cattle. Data from Chapter 5 (●), Chapter 6 (■), Beever *et al.*, 1985 (□), Ulyatt *et al.*, 1988 (▽); Beever *et al.*, 1986a (◊) and 1986b (△) and van 't Klooster & Rogers, 1969 (+). Y = 0.853 (±0.022) x X; R^2=0.92.

Table 5. Assumptions and calculations used to predict available protein in the small intestine (DVE) in cattle fed grass.

Parameter	Abbreviation (unit)	Calculation[1]
Protein escape	%B	Eqn. (4) [p. 100]
Undegradable CP fraction	%U	Eqn. (2) [p. 100]
Digestible escape CP	DVBE (g/kg DM)	(%B x 1.1 - %U)/100 x CP
Microbial CP	MCP (g/kg DM)	150 x (DOM - %B/100 x CP - 40)
Digestible microbial protein	DVME (g/kg DM)	0.75 x 0.85 x MCP
Indigestible DM	IDM (g/kg DM)	OM - DOM + 0.5 x ash
Digestible protein to compensate metabolic faecal N loss	DVMFE (g/kg DM))	0.075 x IDM
Available protein	DVE (g/kg DM)	DVBE + DVME - DVMFE

[1]All concentrations in g/kg DM. CP = crude protein, OM = organic matter, DOM = digestible OM, D = day of harvesting expressed as days after April 1.

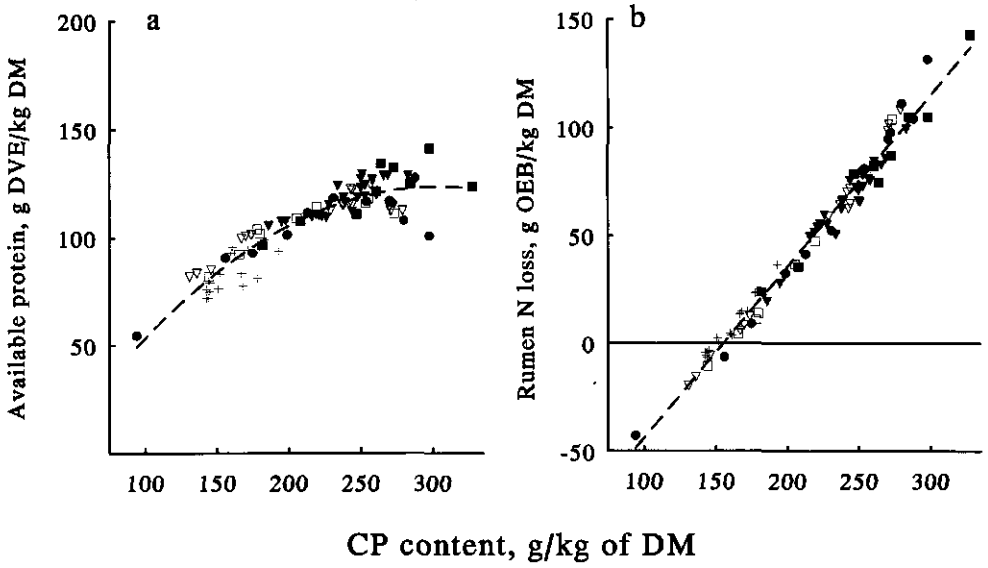

Figure 9.The relationship between the crude protein (CP) content of grass and available protein in the small intestine (a) and between CP and rumen N losses (b), based on *in situ* data from Chapter 3 (●); Steg *et al.* (1994; +); I. Hageman (unpublished; ∇ and ▾); H. Valk (unpublished; □) and C. Salaün (unpublished; ■).

Protein losses

The estimated protein losses in the rumen was linearly related to the CP content (Figure 9b). This relationship suggests that at a CP content > 155 g/kg DM, 79% of the extra CP in grass is lost in the rumen. This proportion will be excreted via urine. The relatively high losses are due to the high proportion of protein fermented in the rumen, which is not completely captured in microbial protein.

Supply of glucogenic nutrients

Starch and sugars absorbed from the small intestine contribute directly to the glucogenic nutrient supply of ruminants. As mentioned earlier, the starch content of grass in a vegetative stage, is usually less than 10 g/kg DM (Smith, 1973), whereas soluble CB are almost completely fermented in the forestomachs. Therefore, the direct supply of glucogenic nutrients in unsupplemented grazing cows is low. Thus, for her glucogenic requirements the grazing

cow depends on gluconeogenesis, for which the animal uses propionic, valeric and lactic acid and glucogenic AA, especially glycine, alanine and glutamine (Madsen, 1983). In our experiments, but more significantly in those of Beever *et al.*, (1986b) and Dillon *et al.* (1989), the proportion of propionic acid in grazing animals was relatively high. The latter authors also reported average lactate concentrations in grass-fed cows of 5 to 10 mmoles/L. Thus, although the direct supply of glucogenic nutrients is low, the supply of glucogenic precursors per kg ingested feed by grass-fed animals may be relatively high.

Glucose supply and requirements of a 600 kg cow consuming 16 kg herbage OM were calculated. The production of propionic and valeric acid were estimated from the OM apparently degraded in the rumen according to Czerkawski (1986). From the observed proportions of propionic and valeric acid in rumen fluid (Chapters 5, 6 and 8) we estimated a production of 23.4 Moles of propionic and valeric acid per day at this level of intake. Assuming that the synthesis of 1 Mol of glucose requires 2 Mol of propionic or valeric acid, the glucose supply from these VFA is 2100 g/d.

Two other sources for glucose synthesis were taken into account: microbial poly-saccharides and excessive AA. Rumen microbial DM contains 17% poly-saccharides (Czerkawski, 1986). Although starch intake in grazing animals is low, fructosans may be used to synthesize these poly-saccharides. Therefore, we assumed that 10% of duodenal microbial DM flow added to the supply of glucose. The excess of AA was calculated as the difference between the supply and the requirements of available protein (DVE). Requirements of DVE were calculated assuming that 1920 g DVE and 12.9 kVEM (NEL) were available for milk production. We also assumed a minimum amino acid excess of 364 g, originating from the surplus of non-essential AA (Tamminga, 1981). Total glucose supply thus also depended on milk production, i.e. on the surplus of available protein, and ranged between 2570 and 3065 g per day at milk yields of 30 and 10 kg/d, respectively.

Glucose requirement for maintenance was fixed at 250 g/d (Oldham & Emmans, 1988). Requirements for milk production were calculated by two approaches. The first method was according to MacRae *et al.* (1988), who assumed a constant requirement of 59.1 g per L of milk (40 g fat and 47 g lactose). The other method was based on the results of Bickerstaff *et al.* (1974). From their experiment we concluded that the efficiency of lactose production from glucose is related to milk yield: 70% at a milk yield of 10 kg/d increasing to 87% at a yield of 30 kg/d. Requirements for the production of 10 to 30 kg of milk/d ranged between 840 and 2020 g/d (MacRae *et al.*, 1988) or between 920 and 1870 (Bickerstaff *et al.*, 1974), respectively (Figure 10). Thus at a production of 27 kg of milk, which can be produced according to the energy intake, glucose is in surplus of 725 (MacRae *et al.*, 1988) or 815 (Bickerstaff *et al.*, 1974) g/d.

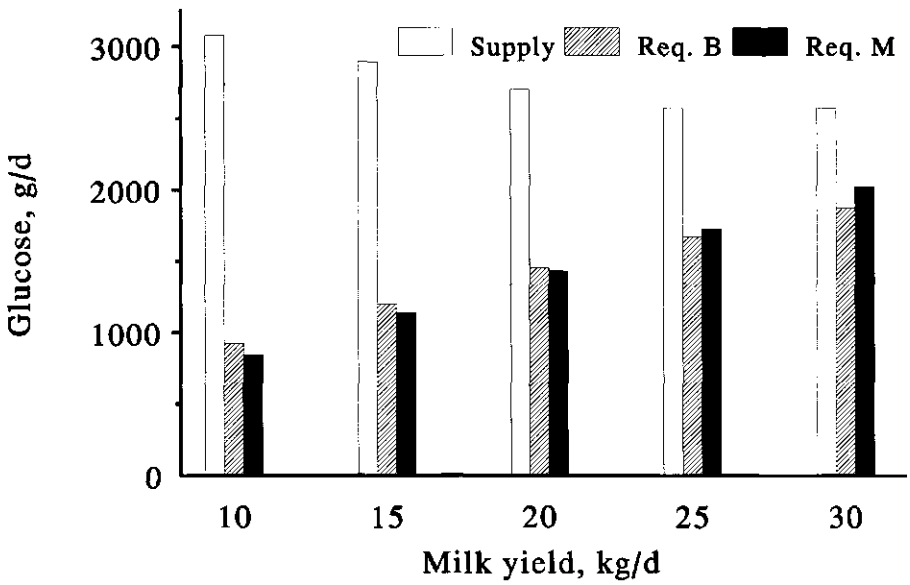

Figure 10. Glucose supply and requirements of a 600 kg dairy cow consuming 16 kg herbage OM and producing 10 to 30 kg of milk/day. Req. B = Calculated with varying efficiency from data of Bickerstaff *et al.*, 1974; Req. M = Calculated with fixed efficiency according to MacRae *et al.* 1988.

The presented calculations of the glucose supply should be regarded with some caution. It was assumed that the production of propionic and valeric acid were produced in the proportions similar to the observed concentrations. However, Dijkstra *et al.* (1993) have demonstrated that the absorption of the individual VFA may differ and is influenced by rumen volume, VFA concentrations and ruminal pH. Using the mean parameter values, observed in our experiments, we estimated that the proportions in which acetic acid, propionic acid and butyric acid are absorbed, were 43 : 34 : 23. This implies that the production of propionic acid estimated with the proportions in rumen fluid is underestimated.

MacRae *et al.* (1988) assumed a glucose requirement of 59.1 g per kg milk, but regarded this as the absolute minimum. According to the results of Bickerstaff *et al.* (1974) the requirement varied between 67 and 54 g/kg milk. Finally it should be realized that the supply of glucose for milk production depends on the requirements of other tissues. The partitioning of glucose is influenced by the endocrinological status of the animal. Therefore, in growing animals an important portion of glucose may not be available for milk synthesis.

Supply of ketogenic nutrients

Dietary fat, but particularly acetic and butyric acid are the main ketogenic nutrients for ruminants. Feeding or infusing sucrose, glucose or lactose in the rumen at levels of 10 to 20 % of total DM intake, often resulted in an small increase of the proportion of butyric acid by 2 to 4 %-units (Chamberlain *et al.*, 1985; Huhtanen, 1987; Miettinen & Setälä, 1989; Casper *et al.*, 1989 and 1990), in most cases concomitant with a similar decrease in the proportion of acetic acid. An increase in butyric acid production has also been observed in sheep fed molasses (Demeyer, 1991).

The positive relationship between dietary sugar and the proportion of butyric acid was also apparent in our experiments (Figure 11). The relationship was influenced by supplementation as well as the type of supplement. At a similar sugar intake, including high-starch supplements, especially maize meal, resulted in a higher proportion of butyric acid than including pulp or without supplementation. Butyric acid is the main fermentation product of rumen protozoa (Prins & Durand, 1991). Thus, an increase in butyric acid may indicate an increase in the number of protozoa, as observed in starch-supplemented diets (Chamberlain *et al.*, 1985). On the other hand, the high butyric acid production observed with molasses fed animals has been attributed to bacterial activity (Demeyer, 1991).

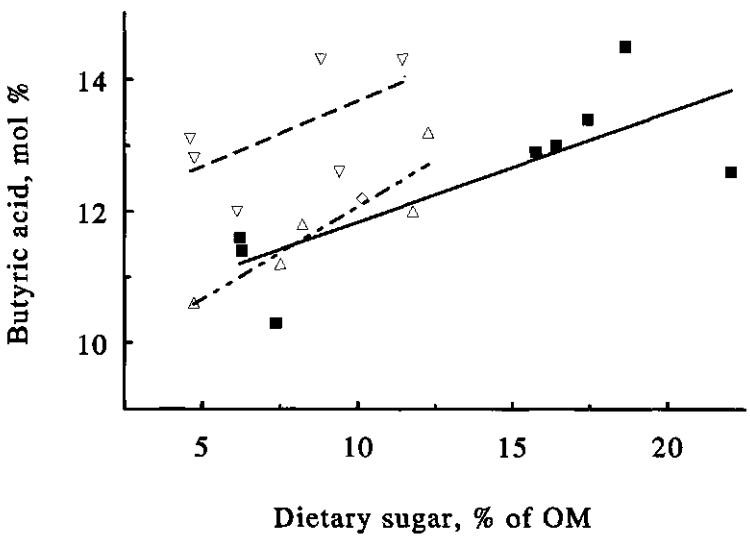

Figure 11. Effect of sugar content in grass on the proportion of butyric acid in rumen volatile fatty acids. Grass (■): $Y = 0.17 \times X + 10.2$, $r^2 = 0.62$; grass + maize (▽): $Y = 0.20 \times X + 11.7$, $r^2 = 0.35$; grass + pulp (△): $Y = 0.28 \times X + 9.2$, $r^2 = 0.83$.

Stoichiometric calculations (Czerkawski, 1986) using the data from Chapters 5, 6 and 8, showed that per kg FOM (hexose) from grass, on average 6.4 Mol acetic and 1.5 Mol butyric acid are produced, but, as emphasized in the previous paragraph, the production of acetate may be overestimated due to differences in absorption rates (Dijkstra *et al.*, 1993). Baldwin & Smith (1983) estimated a total body oxidation of acetate of 31.8 Mol/d. Based on the estimations of MacRae *et al.* (1988), 30 kg of milk require 11.5 Mol of acetate for ATP production. This suggests that 20 Mol acetate is required for maintenance. Thus, at an intake of 16 kg OM, approximately 45 (65 - 20) Mol acetate would be available for milk production. MacRae *et al.* (1988) estimated that for the synthesis of 1 kg of milk, at least 0.85 Mol acetate is needed. According to these calculations, the ketogenic VFA supply in grazing cows is sufficient for a production of 53 kg of milk.

In ryegrass, linoleic and linolenic acid comprise more than 70% of the fatty acids (Hawke, 1973). In grass samples used in our study on the effect of maturity on *in situ* degradation (Experiment 1 in Chapter 3), these unsaturated fatty acids were found in concentrations between 10 and 16 g/kg DM (Figure 12). Thus, at an intake of 16 kg OM/d approximately 225 g of unsaturated fatty acids are ingested. Long-chain fatty acids influence rumen fermentation and milk fat synthesis (Storry, 1981). Bakker & Veen (1985) reported a decrease in milk fat production at a daily intake of 200 g soya bean oil, containing 56% linoleic and 8% linolenic acid.

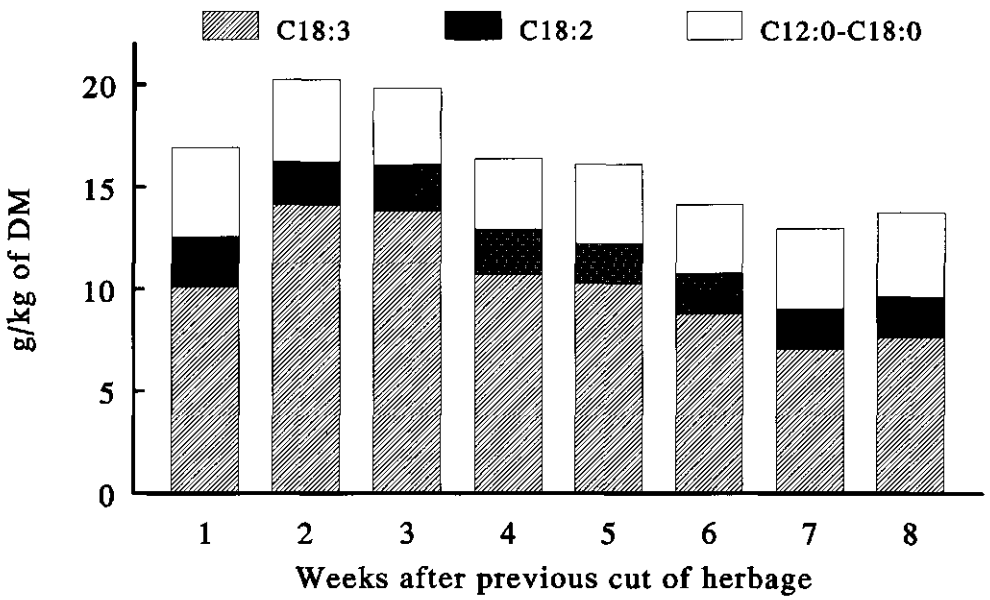

Figure 12. Effect of maturity on the content of the main fatty acids in ryegrass.

In grass-fed animals (Chapters 5 and 6) the mean duodenal flow of crude fat was 48 g per kg OM intake (not published). Assuming a crude fat digestion of 95% and an OM intake of 16 kg per day, approximately 730 g crude fat/day is absorbed.

We therefore conclude, that the decrease in milk fat content, often observed in the first months of the grazing season, is not caused by a deficit in ketogenic nutrients, but is probably due to the high intake of unsaturated fatty acids, possibly in combination with the high proportion of propionic acid (Sutton, 1989).

Table 6. Estimated energy supply from nutrients required for 1 kg milk

Nutrient	Requirement	Energy content kJ/kg or kJ/Mol	Energy supplied MJ
Aminogenic	55 g	22.6[1]	1.24
Glucogenic	60 g	16.7[1]	1.00
Ketogenic VFA	0.93 Moles	482.3[2]	0.45
Total. MJ			2.70
Total, kVEM			0.39

[1]Bondi, 1987
[2]Centraal Veevoederbureau, 1991b

Energy

According to the calculations in the previous paragraphs, the intake of 16 kg of grass OM provides a dairy cow with sufficient aminogenic, glucogenic and ketogenic nutrients to produce *ca.* 35, 40 and > 53 kg of milk, respectively. However, based on the energy intake, a maximum production of 27 kg of milk can be expected. This discrepancy, emphasizes the significant bias in the assumptions made. The assumed requirements for aminogenic, glucogenic and ketogenic nutrients to produce 1 kg of milk were compared with the energy requirement. As presented in Table 6, the required nutrients provided only 85% of the required energy (0.46 kVEM/kg milk). Similarly for maintenance requirements, the nutrient requirements yielded only 50% of the required energy. Thus, the nutrient requirements for maintenance and milk production were underestimated significantly. In contrast, the assumed supply of available protein, glucose, fat and ketogenic VFA, correlates with an energy intake of 21 kVEM, which is approximately 4 kVEM higher than that calculated from the energy content of grass (Centraal Veevoederbureau, 1992). Therefore, the surplus of nutrients is probably much less than estimated in the previous paragraphs. This suggests that the

efficiencies for the absorption and conversions are much less than assumed. The efficiency of lactose production from propionate was assumed at 72% (90% for the conversion of propionate to glucose and 80% for that of glucose to lactose). This is in agreement with estimates of van Es (1980) who calculated an efficiency of 75% on energy basis. However, provisional data of de Visser (unpublished) obtained in an experiment with grass fed dairy cows cannulated in portal and hepatic veins, suggest that approximately 15 to 20% of glucose is synthezised from AA, which indicates a deficit of other glucogenic precursors.

Improvement

Grass quality

The effect of an increase in N content of grass on N excretion by a 600-kg dairy cow was estimated, assuming a DM intake of 17.5 kg and maintenance requirements of 5.0 kVEM and 110 g DVE/d. The assumptions for the expected changes in chemical composition and nutritional quality of the herbage were based on the observations reported in Chapters 3, 5 and 6. With increasing N content in the grass we assumed a decrease in OM and NDF content and in the undegradable NDF fraction, and an increase in OM digestibility (Table 7). Rumen escape protein and undegradable protein were calculated according to equations 2 and 4 presented on p. 100, with July 1 as the date of harvesting. The digestibility of the degradable NDF fraction was fixed at 85% (Tamminga, 1993) of which 85% was assumed to be degraded in the rumen (Robinson *et al.*, 1987). Faecal OM output was estimated firstly with the assumed overall OM digestibility and secondly, with the assumed parameter values for fermentation and digestion of the model. To obtain similar values it was necessary to vary the intestinal digestibility of non-NDF non-CP OM. The energy value (VEM) was estimated from the obtained DOM and digestible CP contents (Centraal Veevoederbureau, 1992); available microbial protein and required available protein for metabolic faecal losses were calculated according to the Centraal Veevoederbureau (1991a).

The NDF digestibilities obtained by this model were slightly higher if compared to the data reported in Chapter 5, whereas CP digestibility seemed overestimated for the highest N content if compared to values reported in Chapter 5.

Milk production to be achieved from the estimated energy intake was calculated by assuming a requirement of 0.46 kVEM/kg of milk. The estimated milk production and the ratio between DVE and kVEM available for production was used to calculate the efficiency of milk protein synthesis (van Straalen *et al.*, 1994), which resulted in the amount of milk protein to be secreted.

Table 7. Assumed and calculated effects of nitrogen content on the chemical composition and nutritional qualities of ryegrass. Assumptions based on results reported in Chapters 3, 5 and 6.

	Nitrogen content			
	25	30	35	40
Assumed values				
Organic matter (OM), g/kg DM	910	900	890	880
Neutral detergent fibre (NDF), g/kg DM	450	415	380	345
OM digestibility, %	70	75	80	85
Digestibility of duodenal non-NDF, non-CP OM in small intestine, %[1]	0	25	50	75
Fermented non-NDF non-CP OM in large intestine, g	0.5 x FOM/DOM x duodenal non-NDF, non-CP OM output			
Undegradable NDF fraction, %	10	8	6	4
Digestibility of degradable NDF, %	85			
Fraction of digested NDF degraded in rumen	85			
Faecal CP, g	Undegradable feed protein + duodenal non-CP OM output x 0.015 + {Fermented non-CP OM in large intestine)}x 0.15			
Calculated values				
NDF digestibility, %	76.5	78.2	79.9	81.6
CP digestibility, %	60.1	72.1	80.1	84.9
Energy, VEM	801	879	960	1039
DVE, g	72	90	104	113

[1]See text on p. 115

Due to the assumed increase in energy content with increasing herbage N content, the predicted milk yield increased and consequently milk N excretion increased by 40% (Table 8). With an increase in herbage N content, the model predicted a 60% decrease in faecal N output, but an almost threefold increase in urinary N excretion. However, as mentioned above, the excretion of faecal N seemed underestimated. In our model urinary metabolic N losses were calculated as the difference between N intake and N-excretion by milk, faeces and urinary rumen loss and thus, the underestimation of faecal N loss, overestimated urinary metabolic N loss. The increase in urinary excretion was due to an almost fivefold increase in predicted rumen loss and an almost twofold increase in urinary metabolic loss. Thus with an increase in herbage N content the excretion of N shifts from

faeces to urine. This prediction agrees with the observations *in vivo* reported in Table 7 of Chapter 2 and Table 10 in Chapter 8. With increasing N content the estimated efficiency of N utilization (milk N excretion/N intake) decreased.

Table 8. Estimated excretion of nitrogen (g/day) of a 600 kg cow consuming 17.5 kg of herbage DM with different nitrogen content (g/kg DM). Estimates are based on the assumptions presented in Table 7.

		Nitrogen content			
Excreta		25	30	35	40
Milk		105	118	130	143
Faeces		174	147	122	105
Urine	Rumen loss	44	102	165	233
	Metabolic loss	114	159	196	218
	Total	158	261	361	451
Urine + Faeces		333	407	483	557
Per kg milk		17	18	19	19
Proportional (% of intake)					
	Milk	24.0	22.4	21.2	20.5
	Faeces	39.9	27.9	19.9	15.1
	Urine	36.1	49.7	58.9	64.5

Thus, a reduction in N loss by grass-fed dairy cows, can be achieved by reducing the N content of herbage offered. However, such a reduction will also decrease the energy content of herbage and consequently milk production. The possibility to decrease the CP content of herbage and subsequently N excretion, thus depends on the production level of the animals. This implies that farmers should obtain plots with herbage of different CP contents, available to herds of cattle of different production levels. Under practical grassland management this will be difficult to achieve.

A reduction in N content can be achieved by decreasing the level of N fertilization and feeding more mature grass (Chapter 3). Under practical conditions both measures are combined. Such management results in a decrease of herbage quality: higher concentrations of (undegradable) NDF and lower OM digestibility. From the relationship shown in Figure 1 we can expect that the animal can compensate for this reduction to some extent:

Undegradable NDF content and rumen fill in grass fed animals were low in comparison to silage fed animals, and an increase in undegradable NDF will result in an increase in rumen fill. With a decrease in quality, the rate of passage will decrease and consequently, the proportion of FOM in DOM may hardly change. Due to the increase of rumen fill a decrease of the rate of passage will not alter daily rumen output (g) and feed intake.

If chewing activity in grazing cows is a limiting factor in grass intake, changes in the DM content of grass may lead to a higher DM intake within the same time of grazing. With a reduction in the level of N fertilization, the DM content of grasses increases (Wilman & Wright, 1982), which can lead to an increase in DM intake (Valk, personal communication). This will also compensate for the reduction in nutritional quality.

With a reduction in N content, the content of water-soluble CB usually increases (Wilman & Wright, 1983). The increase in rapidly fermentable CB will lead to an increase in the rate of VFA production and consequently to a decrease in rumen pH. As reported in Chapter 7, and confirmed by observations by Mpatwa (1978) and Dillon *et al.* (1989), rumen pH in grazing cows reaches low values, especially in late-evening. At low pH, the activity of cellulolytic bacteria may be impaired (Mould & Ørskov, 1983), which may be amplified by the increase in water-soluble CB. Contrary to the DM effect, this may result in a reduction in feed intake.

We therefore conclude, that a reduction in N content mainly reduces urinary N losses. If the reduction in N content coincides with a reduction in energy value, the efficiency of N utilization (milk N/consumed N) will be unaltered. However, the effects on feed intake and consequently animal performance are not yet clear an requires further research.

Supplement Feeding

The maximum milk N output at an intake of 17.5 kg grass DM/d, as estimated in Table 8, was 143 g/d, the equivalent of 28 kg of milk. According to the model this can be achieved if the herbage contains 40 g N/kg DM, a N content that led to a predicted urinary N loss of more than 400 g/d. To reduce urinary N losses and to improve the efficiency of milk N synthesis in high yielding dairy cows, N intake should be reduced without decreasing the energy or DVE intake. This can be achieved by partial replacement of grass by low protein feeds. The main purpose of such measures is to reduce rumen N loss by decreasing the supply of fermentable CP without a decrease in rumen microbial protein synthesis, achieved by maintaining an adequate supply of FOM. Synchronization of available N and available OM seems advantageous for an optimum N economy. With the *in situ* results and feed intake reported in Chapter 6, we estimated on a hourly basis, the degradation of CP and CB, assuming that concentrates were consumed in two and herbage in four equal meals per day. Synchronization is illustrated in Figure 13 by the ratio between fermented N (FN) and FCB.

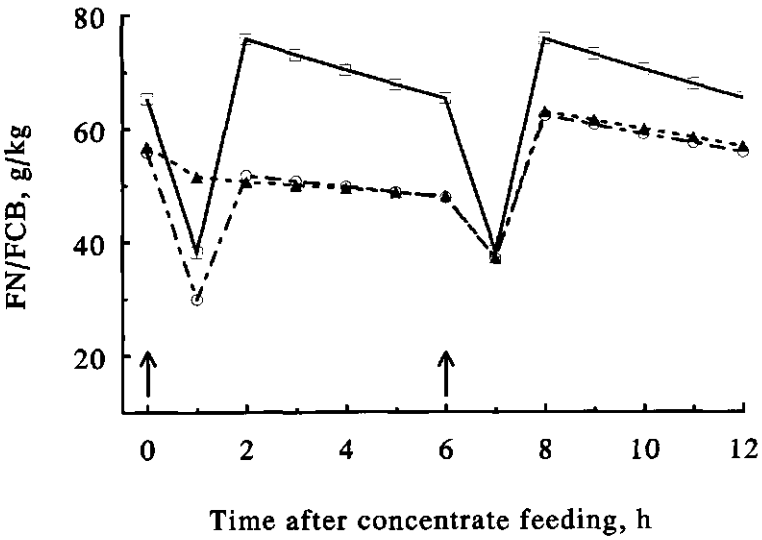

Figure 13. The pattern of the ratio between fermented nitrogen (FN) and fermented carbohydrates (FCB) when feeding herbage as a sole feed (□) or with maize meal (▲) or beetpulp (○). Concentrates consumed in one (t=0), herbage in two (arrows) equal meals per 12 h. Data from Table 2 of Chapter 6. Neutral detergent fibre was estimated as OM - (crude protein + starch + sugar + fat). Assumed passage rates were 4.5 and 6.0% for grass and supplements, respectively.

During the first hour after the assumed meal, the ratio dropped especially for grass and grass with beetpulp. This is caused by the high supply of soluble CB (sugars) assumed to be degraded within the first hour. On all three diets, CP fermentation always delivered more than 30 g N per kg FCB. Partial replacement of grass decreased the FN/FCB ratio, but type of CB (maize starch *versus* beetpulp fibre) had no effect on this ratio. However, because approximate 40% of the maize starch escaped from rumen fermentation, the total amounts of FN and FCB on maize starch will be lower than on beetpulp.

Results *in vivo* may differ, due to grass-concentrate interactions. As reported in Chapter 6, ruminal degradation of NDF was reduced when the diet contained maize meal. This will increase the FN/FCB ratio, which explains the somewhat higher rumen NH_3 N concentration when feeding the concentrate mixture based on maize.

Supplementation will also affect the intake of energy and protein, the ratio between protein and energy available for milk production and consequently the efficiency of milk protein synthesis (van Straalen *et al.*, 1994). Using the model presented in Table 7 and the data for the high starch and high fibre concentrates reported in Chapter 6, replacement of 5 kg DM of 30-N herbage by these concentrates resulted in a decrease in N excretion by faeces + urine

of 40 to 55 g/d, without a reduction in milk N output (Table 9). Thus, the efficiency in N utilization (milk N output/N intake) increased to 26%. This agreed with the *in vivo* results reported in Table 7 of Chapter 8. However, as noticed previously, the model underestimated faecal N output.

Feeding maize starch will also affect the supply of glucogenic nutrients. We observed that *ca.* 40% of the consumed starch escaped from rumen fermentation, accounting for approximately 1 kg/d (Chapter 6) If 60% is digested in the small intestine (Nocek & Tamminga, 1991) and the remaining fermented in the large intestine, the supply of glucogenic nutrients will be increased, which will reduce the ratio between protein and energy available for milk production and consequently increase the efficiency of milk protein synthesis (van Straalen *et al.*, 1994). Thus, although ruminal N losses may be higher when including maize starch in the diet due to the decrease in FOM, the metabolic N loss may be lower than when including high fibre mixtures. However, as reported in Chapter 8, urinary N excretion (expressed as % of N intake) on diets containing maize starch was similar or higher than on diets containing beet pulp fibre. This suggests that compared to high fibre concentrate, the N gain in the intermediary metabolism when feeding high starch concentrate did not completely compensate the extra rumen N loss, as is also suggested by our model (Table 9).

Table 9. Estimated excretion of nitrogen (g/day) of a 600 kg cow consuming 17.5 kg of herbage DM with 30 g N/kg DM or 12,5 kg herbage DM and 5.0 kg concentrate DM based on starch or NDF (Chapter 5).

Excreta		Concentrate		
		None	High starch	High Fibre
Milk		118	128	121
Faeces		147	151	149
Urine	Rumen loss	102	111	69
	Protein metabolism	159	101	135
	Total	261	212	204
Urine + Faeces		407	363	353
Per kg milk		18	14	15
Proportional (% of intake)				
	Milk	22.4	26.1	25.6
	Faeces	27.9	30.7	31.4
	Urine	49.7	43.1	43.1

Extra glucose from maize starch also affects the partitioning of nutrients (Sutton, 1989), which lead to a decrease in milk fat synthesis accompanied by an increase in body fat. This will also reduce glucose available for milk synthesis, which is also influenced by the physiological status (stage of lactation) of the animal. Thus, a prediction of the final outcome of such feed measures is complex and still remains uncertain.

Conclusions

In heavily-fertilized grass in the grazing stage the ratio between FN and FCB is high, which results in high N losses in the rumen, which is almost quantitatively excreted in the urine. With a reduction in N-fertilization, the N content of grass in the grazing stage will decrease, which has a direct effect on ruminal N loss. This reduction also affects the supply of nutrients to the animal. Due to the law of diminishing returns, the magnitude of this effect depends on the starting-point. From the *in vivo* data reported in Chapter 5 it was concluded that a maximum supply of available protein is reached at a CP content in herbage of *ca.* 225 g/kg DM. Although the *in situ* data suggested that the maximum content of available protein is reached at a CP concentration of 270 g/kg DM, the estimated gain in DVE correlated with a high ruminal N loss (Figure 9).

To obtain an optimum efficiency of milk protein synthesis from available protein (≈ 64%) and a minimum rumen N loss, the CP content should be around 135 to 150 g/kg DM. At this moment, such low concentrations can only be obtained by low levels of N fertilization or by harvesting at an old growing stage. Such measures have a negative effect on herbage yield (kg DM/ha) and herbage quality in terms of OM digestibility. However, to some extent the animal can compensate this by an increase in rumen fill and hind gut fermentation. Besides, chemical composition and nutritional quality also depend on other growing factors (Chapter 1). This is illustrated in Table 4 of Chapter 5, which shows only minor differences in OM digestibility with a reduction in the level of N fertilization.

A further reduction in rumen N loss can be achieved by replacing part of the grass by low protein feeds. These feeds may be either rich in fibre or starch. The effect of supplementation on the availability of nutrients for milk synthesis and subsequently metabolic N loss, are complex and must be elucidated before a more accurate prediction can be made.

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Summary

Summary

Until recently, young, highly digestible grass was considered an ideal feed for dairy cows. However, research during the last decades has shown that the nutrient supply of grazing animals is insufficient for milk productions above *c.* 29 kg per day. Experiments in England and New Zealand have shown that the efficiency of protein utilization is relatively low and consequently, a high proportion of ingested nitrogen is excreted in urine and faeces. This reports the effects of grassland management and feeding strategies on the digestion and availability of nutrients from perennial rye-grass (*Lolium perenne*) in dairy cows.

Chapter 1 is a literature review of the various factors affecting the composition and nutritional quality of grass. To facilitate high yields of dry matter and a high feeding value, grass is fertilized with high levels of nitrogen. Level of nitrogen fertilization, weather conditions and the duration between nitrogen application and harvesting date affect the content and quality of grass protein. Level of nitrogen fertilization and maturity also influence other components of grass, like carbohydrates and lipids.

In Chapter 2 it is emphasized that high levels of nitrogen fertilization result in high concentrations of crude protein, which are easily fermented in the forestomachs. Thus, in grazing dairy cows, an important part of grass protein is fermented in the rumen and subsequently excreted in the urine. The actual efficiency of nitrogen utilization in cows grazing intensively managed pastures, is 15 to 25 %. We estimated that theoretically a 600 kg cow producing 25 kg milk per day can utilize dietary nitrogen with a maximum efficiency of 40 to 45%. This chapter discusses changes in grassland management and nutrition aiming at improvements of nitrogen utilization. When grass is the sole feed, efficiency of nitrogen utilization cannot be improved substantially without have a detrimental effect on animal performance. Supplementing the diet of grazing dairy cows with low protein, high energy feeds increases the efficiency of nitrogen utilization, mainly because it reduces nitrogen intake.

Chapters 3 and 4 contain details of experiments using the nylon bag technique. Chapter 3 discusses the effects of grass maturation and rate of nitrogen fertilization on rumen degradability of organic matter and crude protein in fresh grass. These results were used to estimate the content of digestible protein entering the small intestine. Crude protein content and *in situ* degradability of organic matter and crude protein decreased with increasing grass maturity and with decreasing nitrogen application. With every 100 g per kg dry matter decrease in crude protein content, the estimated content of digestible protein entering the small intestine decreased by 19 g per kg dry matter, irrespective of how the crude protein content was manipulated.

Chapter 4 discusses rumen degradability of crude protein and non-protein organic matter (~ carbohydrates) of fresh and preserved grass, obtained in four nylon bag studies, and consequences for dairy cow rations. In Experiment 1, the effect of level of fertilization on the in situ degradation of fresh grass was studied. The second experiment focused on the effect

of maturation on degradation of fresh grass. Experiments 3 and 4 dealt with the influence of maturation and dry matter content of grass silage and hay. Experiment 4 also included treatment with cell wall degrading enzymes. Fresh and preserved grass fertilized at high levels of nitrogen, contained large surpluses of fermentable nitrogen. In fresh grass the ratio of [soluble nitrogen]:[soluble carbohydrates] was lower than the ratio of [insoluble, degraded nitrogen]:[insoluble, degraded carbohydrates]. Therefore, it was concluded that ingredients with a low ratio of [insoluble, degraded nitrogen]:[insoluble, degraded carbohydrates] may be considered appropriate supplements to grass-based diets. In preserved grass the ratio of [soluble nitrogen]:[soluble carbohydrates] exceeded the ratio of [insoluble, degraded nitrogen]:[insoluble, degraded carbohydrates]. Wilting had no consistent effect on the [nitrogen]:[carbohydrates] ratio. Treatment with cell wall degrading enzymes resulted in a lower [soluble nitrogen]:[soluble carbohydrates] ratio. From these results it was concluded that silage-based diets require supplementation with ingredients rich in soluble carbohydrates.

Chapter 5 reports an experiment in which the digestion and intestinal amino acid supply were studied in three rumen and duodenal cannulated lactating cows fed freshly cut grass. Grass was fertilized at levels of 275 or 500 kg of nitrogen/ha per year. High-nitrogen grass was fed in June and October; low-nitrogen grass in July and September. When low-nitrogen grass was fed, the digestibilities of organic matter and crude protein were lower than found with high-nitrogen grass. On low-nitrogen grass, the duodenal nitrogen flow expressed per unit of nitrogen intake was higher. The flow of amino acid nitrogen on low-nitrogen grass was slower in September, mainly because of reduced microbial protein synthesis attributed to slower organic matter degradation of low-nitrogen grass. Duodenal nitrogen flow per unit of nitrogen intake was inversely related to the nitrogen:organic matter ratio of the diet. Rate of nitrogen fertilization did not affect ruminal turnover of organic matter and neutral detergent fibre. Turnover and passage rates recorded in this experiment did not differ from reported data on cows fed winter rations at similar levels of dry matter intake.

Changes observed in vivo in digestion and amino acid supply when fresh grass was partly replaced by concentrate mixtures (either maize starch or sugar beet pulp fibre) are presented in Chapter 6. Partial replacement of grass decreased crude protein digestibility. When high starch concentrate was fed, overall digestibility of neutral detergent fibre was lower than on the high fibre diet, mainly because of decreased ruminal digestion of neutral detergent fibre. With the high starch concentrate, 39% of the ingested starch escaped ruminal fermentation. Although less organic matter was fermented in the forestomachs on high starch concentrate, the duodenal amino acid nitrogen flow was higher than on the high fibre concentrate. The proportion of microbial protein was unaffected; thus, efficiency of microbial synthesis was estimated to be higher when high starch concentrate was fed.

In the experiment reported in Chapter 7, six grazing dairy cows each fitted with a rumen cannula, were supplemented with high or low starch concentrates. The cows received 1 kg

or 7 kg of high or 7 kg of low starch concentrate in two equal meals per day fed after milking. After a three-week adaptation period, samples were taken of grass and rumen fluid. Total sugar content of grass increased during daytime with the highest concentration directly before sunset. Patterns of ruminal pH values did not differ between treatments and were minimal at midnight. Volatile fatty acids and ammonia peaked at midnight. Supplementation with 7 kg of concentrate decreased rumen concentrations of ammonia and branched-chain volatile fatty acids. Acetate:propionate and non-glucogenic:glucogenic ratios of volatile fatty acids and percentage of milk fat tended to be lowest when the diet included 7 kg of high starch concentrate.

Chapter 8 describes three ruminal fermentation studies carried out in combination with three feeding trials. These experiments were carried out to investigate the effect of partial replacement of grass by low protein feedstuffs on pH and concentrations of volatile fatty acids and ammonia in the rumen and on nitrogen excretion in milk, urine, and faeces by dairy cows. Feedstuffs tested were the high-starch and high-fibre concentrates used in the experiment reported in Chapter 6, maize silage, dried and ensiled pressed sugar beet pulp and high-moisture ear maize silage with or without husks. Partial replacement often increased dry matter intake, resulting in minor effects on nitrogen intake. Urinary nitrogen excretion ranged between 30 and 58% of nitrogen intake and decreased by 30 to 40% when grass was partially replaced. The reduction in urinary nitrogen excretion corresponded to a decrease of rumen ammonia. Faecal nitrogen output ranged between 25 and 30% of nitrogen intake and tended to increase with inclusion of low protein feed. Replacement by concentrate mixtures based on maize reduced milk fat content without changing rumen volatile fatty acid composition; for mixtures based on beet pulp, milk fat content remained unaffected.

In the General Discussion (Chapter 9), the supply of aminogenic, glucogenic and ketogenic nutrients from grass is estimated from the obtained data. Supply of nutrients depends on total dry matter intake and on the composition of the dry matter.

From the rumen evacuation data it was concluded that rumen fill is not a factor limiting grass intake. Possible limiting factors are discussed, like fermentation products (ammonia, volatile fatty acids) and the maximum capacity in chewing activity (grazing and ruminating).

Relationships between crude protein content and the proportion of protein escaping from rumen fermentation were used to predict the supply of available protein in the small intestine. A curvilinear relationship was found between crude protein and predicted amount of available protein. Extrapolation suggested that a plateau level of *c.* 125 g available protein (DVE) per kg dry matter is reached at crude protein concentrations above 270 g per kg dry matter. This was higher than concluded in Chapter 5 from the *in vivo* results, where a maximum duodenal non-ammonia nitrogen flow was predicted when grass contains 225 g crude protein per kg dry matter. The nitrogen losses in the rumen increased linearly with crude protein content.

The predicted supply of available protein based on *in situ* data agreed with *in vivo* data

for grazing steers reported in literature, but was higher than the duodenal protein flow in dairy cows observed in our experiments. This discrepancy was mainly attributed to the low efficiency of microbial protein synthesis estimated in our experiments. Possible explanations discussed included the higher intake level and higher proportion of soluble carbohydrates and proteins in grass observed in our experiments compared to those reported in steers. Differences may also result from methodological errors. It was estimated that cows consuming 16 kg of grass organic matter and producing 27 kg of milk per day are in a large positive balance of glucogenic and ketogenic nutrients. However, comparing nutrient and energy requirements showed that the assumed nutrient requirements for maintenance and milk production are too low, whereas the nutrient supply was overestimated.

An estimate was made of the effect of a decrease in nitrogen content in grass on the flow of nitrogen in grazing dairy cows, using a simplified model. Decreasing the nitrogen content in grass from 40 to 25 g per kg dry matter improved nitrogen utilization and sharply reduced nitrogen excretion in urine and faeces concomitant with an decrease in the proportion excreted in urine.

The model was also used to predict the effects of partial replacement of grass by low protein concentrates. This resulted in a reduction in urinary nitrogen excretion with only minor changes in faecal nitrogen output. The efficiency of nitrogen utilization increased.

It was concluded that the crude protein content in grass can be reduced to concentrations of c. 225 g per kg dry matter without significant negative effects on the supply of aminogenic nutrients. Yet even at that level, urinary ruminal and metabolic nitrogen losses will be significant. Since further decrease in protein content is correlated to a reduction in dry matter yield and in the nutritive value of grass, a further reduction of nitrogen losses should be established by partial replacement of grass by low protein feeds.

Samenvatting

Samenvatting

Jong, goed verteerbaar gras werd tot voor kort beschouwd als een ideaal voedermiddel voor melkkoeien. Onderzoek in de laatste decennia heeft echter aangetoond dat melkkoeien met een melkproduktie van meer dan ca. 29 kg per dag onvoldoende voedingsstoffen uit gras kunnen opnemen. Bovendien bleek uit proeven, uitgevoerd in Engeland en Nieuw-Zeeland, dat de efficiëntie waarmee melkkoeien graseiwit omzetten in melk en vlees relatief laag is. Hierdoor wordt een groot deel van de opgenomen stikstof uitgescheiden in urine en mest. In het onderzoek dat in dit proefschrift gerapporteerd wordt, is nagegaan welke effecten graslandbeheer en voedingsmaatregelen hebben op de vertering van Engels raaigras (*Lolium perenne*) en op de beschikbaarheid van voedingsstoffen uit gras bij melkkoeien.

Hoofdstuk 1 is een literatuuroverzicht van de verschillende factoren die de chemische samenstelling en de voederkwaliteit van gras beïnvloeden. Veehouders streven naar hoge droge-stofopbrengsten en een goede voederkwaliteit van gras, hetgeen zij onder meer trachten te bereiken door hoge kunstmestgiften. Stikstofgift, weersomstandigheden en de tijd tussen stikstofgift en het oogsten van het gras bepalen in belangrijke mate eiwitgehalte en eiwitkwaliteit. Stikstofgift en groeistadium beïnvloeden ook de overige componenten, zoals koolhydraten en vetten.

In Hoofdstuk 2 wordt uiteengezet dat de hoge stikstofbemesting leidt tot hoge gehalten aan eiwit, dat in de voormagen snel fermenteert. De vrijkomende ammoniak wordt voor een belangrijk deel via de urine uitgescheiden. De efficiëntie waarmee koeien eiwit uit goedbemest gras omzetten in melkeiwit bedraagt hierdoor slechts 15 tot 25%. Geschat wordt dat een melkkoe van 600 kg met een melkproduktie van 25 kg per dag in een ideale situatie het voereiwit benut met een efficiëntie van 40 tot 45%. Maatregelen in graslandbeheer en in de voeding die in diverse studies onderzocht zijn om de eiwitbenutting van grazende dieren te verbeteren, worden besproken. Indien uitsluitend gras gevoerd wordt, zijn de mogelijkheden voor een verbetering van de eiwitbenutting gering, omdat deze gepaard gaan met een daling van de melkproduktie. Aanvulling van het rantsoen met voedermiddelen met een laag eiwitgehalte verbetert de eiwitbenutting zonder een daling van de melkproduktie.

In Hoofdstuk 3 en 4 worden proeven beschreven waarin de nylon-zakjes-techniek is gebruikt. In Hoofdstuk 3 onderzochten we de effecten van groeistadium en stikstofgift op de verteerbaarheid van organische stof en ruw eiwit van gras. Met de resultaten is het aanbod aan beschikbaar eiwit in de dunne darm geschat. Het ruw-eiwitgehalte en de *in situ* verteerbaarheid van organische stof en ruw eiwit namen af naarmate het gras ouder werd en de stikstofgift lager. Voorspeld werd dat een daling van het ruw-eiwitgehalte met 100 g per kg droge stof leidt tot een afname van de hoeveelheid beschikbaar eiwit in de dunne darm met 19 g per kg droge stof, ongeacht de wijze waarop het eiwitgehalte verlaagd werd (veroudering of lagere stikstofbemesting).

In Hoofdstuk 4 is een vergelijking gemaakt tussen de *in situ* verteerbaarheid van eiwit en die van niet-eiwit organische stof (~koolhydraten) van gras en grasprodukten. In Experiment

1 is het effect van de stikstofgift op de vertering van vers gras onderzocht. In een tweede experiment stond het effect van het groeistadium op de verteerbaarheid van vers gras centraal. In de Experimenten 3 en 4 werden de effecten van maaistadium en droge-stofgehalte van graskuil en hooi bestudeerd. In Experiment 4 is bovendien het effect van celwand-afbrekende enzymen nagegaan. Vers en geconserveerd gras van percelen met een hoge stikstofbemesting bevatten een overschot aan fermenteerbare stikstof. In vers gras was de verhouding tussen oplosbare stikstof en oplosbare koolhydraten lager dan de verhouding [onoplosbare, gefermenteerde stikstof]:[onoplosbare, gefermenteerde koolhydraten]. Dit leidt tot de conclusie, dat aan grazende koeien bijvoedering van voedermiddelen met een lage verhouding [onoplosbare, gefermenteerde stikstof]:[onoplosbare, gefermenteerde koolhydra-ten] de voorkeur verdient. In graskuil was de verhouding tussen oplosbare stikstof en oplosbare koolhydraten veel hoger dan die tussen onoplosbare, gefermenteerde stikstof en onoplosbare, gefermenteerde koolhydraten. Voordrogen had geen effect op de stikstof:kool-hydraten verhoudingen. Inkuilen met celwandafbrekende enzymen leidde tot een lagere verhouding [oplosbare stikstof]:[oplosbare koolhydraten]. Geconcludeerd werd dat in rantsoenen met graskuil, voedermiddelen met een hoog gehalte aan oplosbare suikers zullen leiden tot een verbetering van de eiwitbenutting.

Hoofdstuk 5 beschrijft een proef, waarin de vertering en het aanbod aan aminozuren in de dunne darm is nagegaan bij gecanuleerde melkkoeien gevoerd met raaigras bemest met stikstofgiften van 275 en 500 kg/ha per jaar. "Hoog-bemest" gras werd gevoerd in juni en oktober en "laag-bemest" gras in juli en september. De verteerbaarheid van organische stof en ruw eiwit van het laag-bemeste gras was lager dan die van het hoog-bemeste gras. Op het laag-bemest gras was het aanbod van niet-ammoniak-stikstof in de dunne darm, uitgedrukt per opgenomen kg droge stof, hoger dan op hoog-bemest gras. In september was de darm-doorstroming van aminozuurstikstof op laag-bemest gras echter lager. Dit laatste werd toe-geschreven aan een verlaagde microbiële eiwitsynthese in de pens als gevolg van een langzame organische-stofvertering. De stikstofdoorstroming in de dunne darm uitgedrukt per eenheid opgenomen stikstof, was omgekeerd evenredig met de stikstof:organische stof verhouding in het rantsoen. De stikstofgift had geen effect op de penscapaciteit voor organische stof en celwanden. De gemeten penscapaciteit was in deze proef niet verschillend van de resultaten die eerder zijn beschreven voor winterrantsoenen met een gelijk voer-opnameniveau.

In de proef beschreven in Hoofdstuk 6, is onderzocht of de dunne-darmdoorstroming verandert indien een deel van het gras vervangen wordt door krachtvoeder gebaseerd op maïszetmeel of op celwanden uit bietenpulp. Door gedeeltelijke vervanging van gras door deze krachtvoeders daalde de verteerbaarheid van ruw eiwit. Met het zetmeelrijke kracht-voeder was de verteerbaarheid van totale celwandfractie lager dan met het celwandrijke krachtvoeder, hetgeen voornamelijk berustte op een verminderde celwandafbraak in de pens.

Met het zetmeelrijke krachtvoeder ontsnapte naar schatting 39% van het voerzetmeel aan pensfermentatie. Hoewel bij gras met zetmeelrijk krachtvoeder minder organische stof in de pens werd afgebroken, was de darmdoorstroming van aminozuurstikstof hoger dan die bij gras met celwandrijk krachtvoeder. Omdat het aandeel microbieel eiwit niet wijzigde, werd een hogere efficiëntie van de microbiële eiwitsynthese berekend.

In de proef beschreven in Hoofdstuk 7 kregen zes grazende pensfistelkoeien naast gras, 1 of 7 kg zetmeelrijk of 7 kg celwandrijk krachtvoer. Na een aanpassingsperiode van drie weken werd het verloop van de pH en van de gehalten aan vluchtige vetzuren en ammoniak in de pens gemeten. Tevens werd het verloop in chemische samenstelling van het gras gemeten. Het suikergehalte in gras steeg gedurende de dag, waarbij de hoogste gehalten vlak voor zonsondergang werden gemeten. De behandelingen hadden geen effect op het verloop van de pH van de pensvloeistof. De laagste pH-waarden en de hoogste gehalten aan vluchtige vetzuren en ammoniak werden gemeten om middernacht. Bijvoedering met 7 kg krachtvoer leidde tot een daling van de gehalten aan ammoniak en vluchtige vetzuren met een vertakte koolstofketen. Bij 7 kg zetmeelrijk krachtvoer waren de verhoudingen tussen azijnzuur en propionzuur en tussen glucogene en niet-glucogene vluchtige vetzuren in de pens en het melkvetgehalte iets verlaagd.

In Hoofdstuk 8 zijn drie studies beschreven waarin pensfermentatiemetingen en voederproeven gelijktijdig zijn uitgevoerd. Deze studie had tot doel om het effect van gedeeltelijke vervanging van gras door eiwitarme voedermiddelen op pensfermentatie en stikstofuitscheiding na te gaan. De geteste voedermiddelen waren, naast het zetmeelrijke en het celwandrijke krachtvoer uit Hoofdstuk 6, snijmaïskuil, gedroogde bietenpulp, perspulp, corncob-mix en maiskolvensilage. Omdat gedeeltelijke vervanging van gras vaak leidde tot een hogere voeropname, waren de verschillen in stikstofopname soms gering. Via de urine werd 30 tot 58% van de opgenomen stikstof uitgescheiden; gedeeltelijke vervanging van gras reduceerde dit aandeel met 30 tot 40%. Deze verlaging ging gepaard met een daling van het ammoniakgehalte in de pens. Met de mest werd 25 tot 30% van de opgenomen stikstof uitgescheiden. Dit uitscheidingspercentage nam iets toe bij gedeeltelijke vervanging van gras door eiwitarme voedermiddelen. Bij gebruik van zetmeelrijke voedermiddelen daalde het melkvetgehalte; bij rantsoenen met celwandrijke componenten bleef het melkvetgehalte ongewijzigd.

In de Algemene Discussie (Hoofdstuk 9) zijn op basis van de proefuitkomsten schattingen gemaakt van het aanbod aan aminogene, glucogene en ketogene voedingsstoffen uit gras. Dit aanbod wordt bepaald door de totale voeropname en door het gehalte aan voedingsstoffen in het voer.

De penscapaciteit bleek geen fysische belemmering voor de opname van gras. Andere oorzaken voor de beperkte grasopname worden besproken, zoals de vorming van fermentatieprodukten en een maximale kauwcapaciteit.

Er werden relaties vastgesteld tussen het ruw-eiwitgehalte en het percentage eiwit dat aan pensfermentatie ontsnapt. Deze relaties zijn gebruikt om het aanbod aan verteerbaar eiwit in de dunne darm te schatten. Er bestond een kromlijnig verband tussen het ruw-eiwitgehalte in gras en het voorspelde gehalte aan darmverteerbaar eiwit. Bij extrapolatie bleek dat rond een ruw-eiwitgehalte van 270 g per kg droge stof een plateau-waarde wordt bereikt van darmverteerbaar eiwit van 125 g per kg droge stof. Dit ruw-eiwitgehalte, gebaseerd op *in situ* resultaten, was veel hoger dan berekend in Hoofdstuk 5, waar we op basis van *in vivo* gegevens een maximaal eiwitaanbod bereikten bij een ruw-eiwitgehalte van 225 g per kg droge stof. De veronderstelde stikstofverliezen in de pens bleken recht-evenredig met het ruw-eiwitgehalte.

De darmdoorstroming van niet-ammoniak-stikstof voorspeld uit de chemische samenstelling van het gras kwam wat literatuurgegevens van vleesvee betreft goed overeen met de vastgestelde waarden. Ten opzichte van de resultaten uit de proeven met melkkoeien gaf de voorspelling een overschatting van de flux. Dit verschil werd vooral toegeschreven aan de lage efficiëntie van microbiële eiwitsynthese in onze proeven. Mogelijke verklaringen zijn het hogere opnameniveau in onze proeven en het hogere gehalte aan oplosbare koolhydraten en eiwit in ons gras ten opzichte van die in de proeven met vleesvee. De verschillen kunnen ook het gevolg zijn van meetfouten.

Geschat werd dat koeien die per dag 16 kg organische stof opnemen uit gras en 27 kg melk produceren ruim voorzien worden van glucogene en ketogene voedingsstoffen. Bij een vergelijking van de behoeften aan energie en aan voedingsstoffen op basis van het energiegehalte, bleken de schattingen van de behoeften aan voedingsstoffen voor onderhoud en melkproduktie te zijn onderschat, terwijl het aanbod aan voedingsstoffen volgens deze vergelijking juist werd overschat.

Met een vereenvoudigd model is het effect van een daling van het stikstofgehalte in gras op de stikstofstromen in grazende melkkoeien voorspeld. Verlaging van het stikstofgehalte in gras van 40 naar 25 g per kg droge stof verbeterde de stikstofbenutting en leidde tot een scherpe daling van de stikstofuitscheiding in mest en vooral urine.

Het model is ook gebruikt om de gevolgen van gedeeltelijke vervanging van gras door eiwitarme krachtvoeders te berekenen. Deze maatregel leidt slechts tot geringe veranderingen in de stikstofuitscheiding in mest, maar wel tot een verlaging van de stikstofuitscheiding in urine en tot een verbetering van de stikstofbenutting.

Op grond van de proefresultaten werd geconcludeerd dat een verlaging van het ruw-eiwitgehalte in gras tot ca. 225 g per kg droge stof geen grote nadelige gevolgen heeft voor het aanbod aan beschikbaar eiwit. Bij dit niveau blijft de stikstofuitscheiding via de urine echter aanzienlijk, afkomstig van verliezen in de pens en in de intermediaire stofwisseling. Omdat een verdere verlaging van het eiwitgehalte in gras gepaard gaat met een daling van de drogestofopbrengst en de voederwaarde, zullen stikstofverliezen verder verminderd kunnen worden door gedeeltelijke vervanging van gras door eiwitarme, koolhydraatrijke voedermiddelen.

CURRICULUM VITAE

Adrianus Marinus van Vuuren werd op 27 september 1950 geboren te Alphen aan de Rijn. Hij behaalde in 1967 het HBS-B diploma aan de Rijks Hogere Burgerschool te Roermond. In september van datzelfde jaar begon hij met zijn studie Diergeneeskunde aan de Rijksuniversiteit Utrecht. In september 1972 behaalde hij het doctoraal examen, gevolgd door het diergeneeskundig examen in juni 1974. Na gedurende één jaar als waarnemend dierenarts te hebben gewerkt trad hij op 1 juni 1975 in dienst bij het Instituut voor Veevoedingsonderzoek te Lelystad. Zijn onderzoek betrof de verteringsfysiologie bij melkkoeien en stond aanvankelijk in het teken van het "laag-melkvet syndroom" gedurende de stalperiode. Later werden deze experimenten ook in de weideperiode uitgevoerd. De resultaten van die proeven vormden de aanleiding tot nader onderzoek naar de eiwitstofwisseling bij grazende melkkoeien. De bevindingen hiervan zijn in dit proefschrift vastgelegd.