Feeding and Grazing Management for Dairy Cattle:

Opportunities for Improved Production

Sander Abrahamse

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Thesis

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Abstract

An adequate feed intake is an important prerequisite to realize high milk production in dairy cows, especially during grazing. The analysis of feed intake behaviour can assist in understanding variation in daily intake and in improving its prediction. Indeed, our results indicated that differences in feed intake behaviour were more pronounced when varying the type of roughage than when varying the type of concentrate. Dry matter intake (DMI) was reduced when a ration high in grass silage was fed, but milk production was only numericaly affected. Grazing management might result in increased herbage intake and higher intake of nutrients from herbage by improved nutritional composition of herbage. In the first grazing experiment, herbage DMI was indeed increased when allocating cows daily (1Da) to a new grazing plot compared to every four days (4D) allocation, but only when pasture mass on offer and sward surface height (SSH) were high. Grazing time increased numerically and ruminating time decreased between days in the 4D treatment, coinciding with differences in rumen fermentation characteristics and milk composition. Milk yield was greater in 1Da than in 4D, but milk fatty acid (FA) composition, potentially influencing human health, showed hardly any difference. In a subsequent experiment, herbage DMI was again greater when allocating twice daily (2D) compared to once daily (1Db), especially when SSH was high. Grazing behaviour was more equally distributed in 1Db than in 2D and milk yield was increased in 2D compared to 1Db at high SSH, but as before milk FA composition hardly differed between treatment. The last grazing experiment aimed to take advantage of the higher sugar contents of grass in the afternoon than in the morning. It showed that grazing behaviour and herbage intake were similar between morning (MA) and afternoon allocation (AA), but cows receiving a fresh plot in the afternoon had a longer evening meal than cows receiving a fresh plot in the morning. This, in combination with differences in diurnal chemical composition of the grass between treatments probably caused higher intake of sugars in AA, resulting in a higher milk fat content. However, milk production remained unaffected. In conclusion, the results of this thesis indicate that short-term feed intake behaviour is related to DMI and therefore may be a helpful tool in optimizing DMI and milk production in high-production dairy cows. Increased pasture allocation frequency improves intake and milk yield in grazing dairy cows, especially when offered SSH is high enough. In intensive stripgrazing systems, reallocation of dairy cows following afternoon milking instead of morning milking has no added value.

Voorwoord

Na een heel aantal jaren met veel plezier aan mijn proefschrift te hebben gewerkt, is het ook best een groot plezier om het nu af te hebben. Een drukke, leerzame maar vooral hartstikke leuke tijd is daarmee afgerond. Het werk kwam zeker niet alleen van mijzelf, maar ook van iedereen die er aan meegeholpen heeft. Aan jullie, maar ook aan iedereen die de broodnodige mentale ondersteuning gaven, is nogal wat dank verschuldigd. Als eerste wil ik mijn promotor en co-promotor, Seerp en Jan, van harte bedanken voor hun begeleiding tijdens dit project. Seerp, zonder jou was dit project niet eens van start gegaan, en het feit dat ik bij jou kon promoveren heeft me mede doen besluiten met dit promotieonderzoek te starten. Je overzicht, logica en betrokkenheid, vooral in de jaren voor je emeritaat en tijdens de 'schrijf-fase' hebben me erg geholpen. Jan, jouw overdonderende kennis van zaken, altijd aanwezige motivatie en (werkelijk) niet-aflatende kritische blik op al mijn werk hebben de kwaliteit nogal in positieve zin beïnvloed, super bedankt daarvoor! Ik ga er wel vanuit dat je vanaf komende zomer op Molenperk(.nl) gaat kamperen. Bruno, het was bijzonder prettig iemand te hebben waar ik mee samen kon werken. Ondanks je afkeer van vroeg opstaan hoop ik dat het toch een positieve invloed heeft gehad: niet alleen heb je koeien van dichtbij leren kennen, ook heeft het je voorbereid op de tijd met jullie fantastische kids (ook vroeg uit bed…)! Het werk tijdens alle proeven was onderdeel van het afstudeervak of de stage van een record aantal studenten, die hopelijk wat van mij geleerd hebben, maar die er zeker van kunnen zijn dat ik een hoop van hen geleerd heb. Jullie maakten de uitvoering en uitwerking van de proeven een stuk plezieriger door samen te werken. Hopelijk heb ik na afloop van jullie afstudeervakken en stages al mijn waardering daarvoor uitgesproken, want de volgende opsomming en 'dank-je-wel' is onvoldoende om mijn dank uit te spreken: Alemayehu, Angela, Ard, Arjan, Arne, Bart, Benno, Denis, Dennis, Gert-Jan, Giacomo, Gijs, Iwan, Likawent, Miguel, Miriam, Mosé, Olivia, Rianne, Vronie, Wibe en Wilco, bedankt/thanks/grazie/merci/obrigado/msgana! De proefuitvoering komt ook deels van de hand van de medewerkers van de Ossekampen, waar vooral Leen, Arie, Ronald en Ilona veel geholpen hebben. Bedankt voor al die keren vroeg-uit-bed om te melken, ook dank aan Antoinette en Frans voor het aansturen en plannen van de proeven. Mijn werkomgeving bij Diervoeding was super, met leuke collega's waarmee ik talloze gezellige koffiemomentjes 'onder de trap', BBQ's, playbackshows (helaas heeft de beste act niet gewonnen), ANU-brunches, nieuwjaarsborrels, kleiduivenschiet-competities en een heleboel andere activiteiten beleefd heb. De ondersteuning van het werk kwam bij ANU van alle kanten: uit het lab door Saskia, Jane-Martine, Truus, Meike en niet te vergeten Dick met zijn duizenden vetzuuranalyses en de lastige alkaanbepalingen. Bart, bedankt voor het opstarten van het praktische werk aan het einde van jouw, en het begin van mijn project. Anja, mede-Zeeuw, bedankt voor jouw hulp bij de schatting van de maaltijd-criteria, éé! De meeste tijd bij Diervoeding heb ik doorgebracht samen

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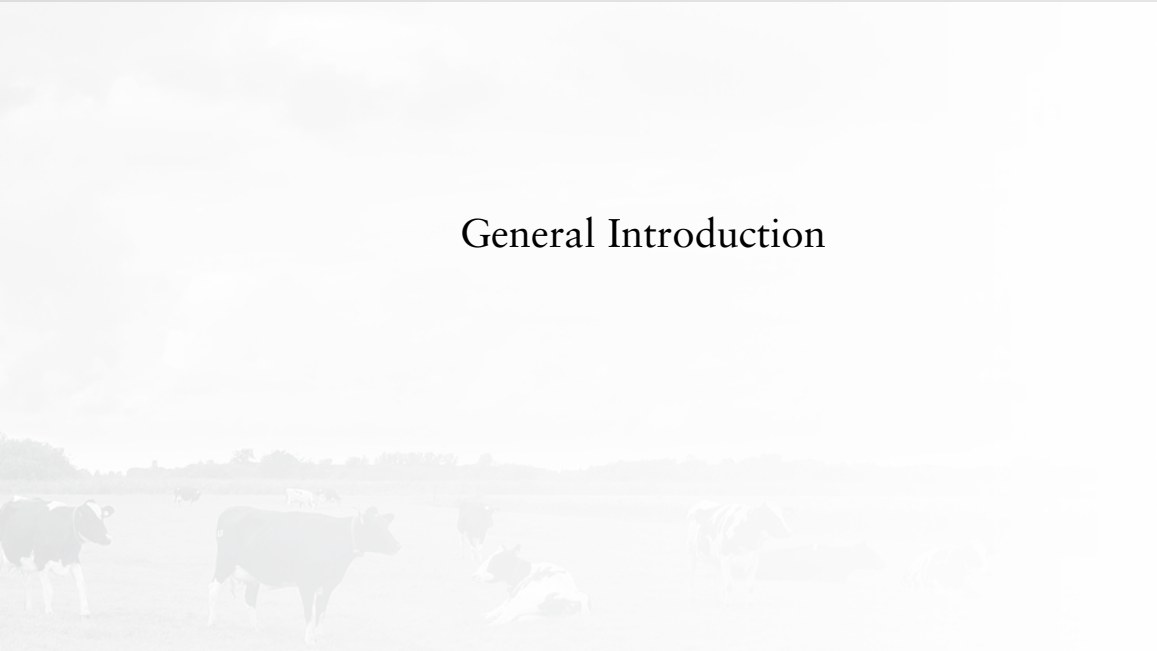
CHAPTER 1

General Introduction



An adequate feed intake is an important prerequisite to realize high milk production in dairy cows (Kolver and Muller 1998; Gibb et al., 1999). Feed intake is commonly expressed as kg of dry matter (DM) per unit of time (usually 24 h) and depends on a large number of factors either linked to the animal or to the feed (Zom et al., 2002). Regardless the way feed is offered to the animal, cows ingest their feed within a day in a number of discrete meals, which are alternated with periods of rumination and periods of "idling". Daily intake depends on the number of meals and intake per meal. It has therefore been suggested that analysis of feed intake behavior can assist in understanding variation in daily intake and in improving its prediction (Forbes, 1995).

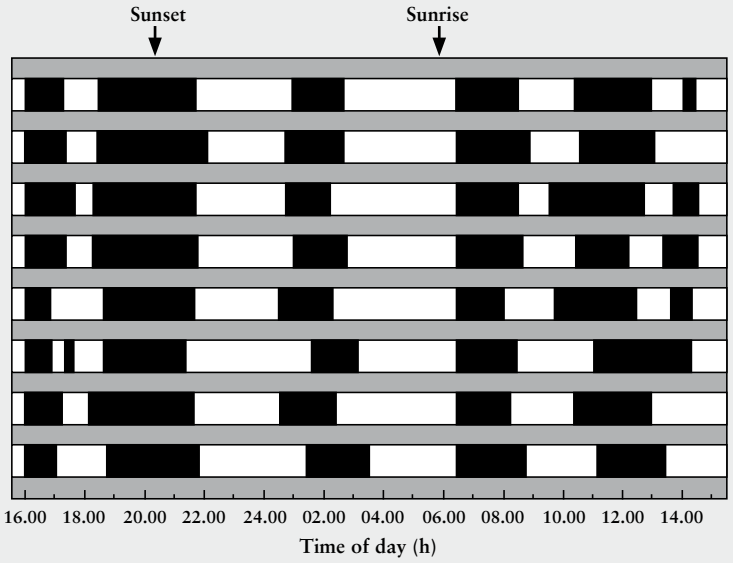


Figure 1.1

Distribution of grazing time of eight grazing cows. Dark bars represent grazing behavior (Gibb et al., 1998).

With winter diets as well as under grazing a large meal is normally taken in the morning and in the early evening, with the longest meal usually around sunset (Taweel et al., 2004). This is illustrated in Figure 1.1, showing the typical eating pattern of eight grazing dairy cows (Gibb et al., 1998). Figure 1.2 shows that, likewise, eating time is increased when cows are milked (and offered fresh feed) when fed *ad libitum* with a Total Mixed Ration (TMR) and housed in a tie-stall (Dado and Allen, 1994).

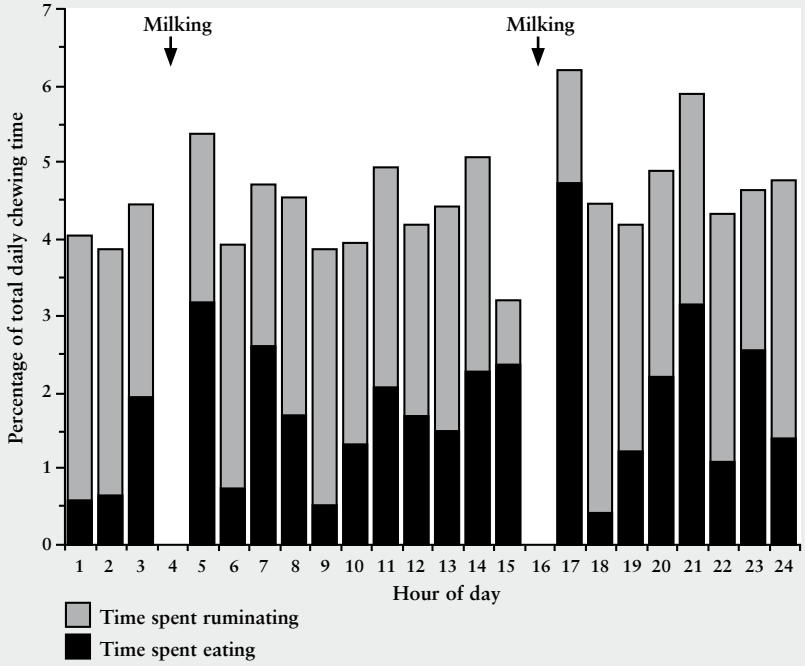


Figure 1.2

Distribution of eating and ruminating time of cows in a tie-stall fed total mixed ration (TMR; Dado and Allen, 1994).

Of the different ways to supply nutrients to dairy cows, grazing is amongst the cheapest (Coleno and Duru, 1999) and it can therefore help to increase profitability of dairy farmers. Recent research in The Netherlands has shown (Figure 1.3) that even under widely varying situations like grazing in combination with an automatic milking system, grazing can be more cost-effective than stall-feeding (Evers et al., 2009).

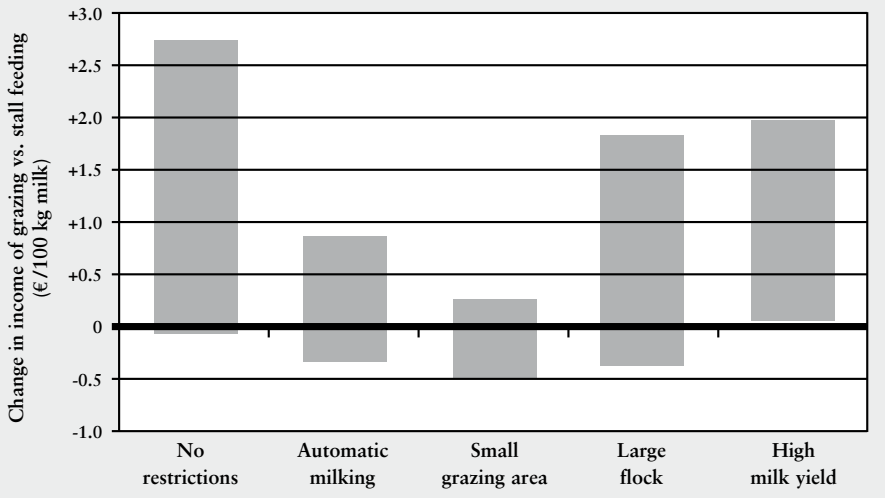


Figure 1.3

Range of the financial benefit of grazing over stall-feeding under different circumstances in The Netherlands (Evers et al., 2009).

Besides, the grazing management system plays a role in other aspects like the image society has of dairy farming, animal health and labor costs. In an evaluation of the effect of grazing management on different factors, it appeared that each of the described management systems showed clear advantages and disadvantages (Table 1.1) (Van Vuuren and Van den Pol-Van Dasselaar, 2006).

Over the last years a decrease in the fraction of dairy cows on pasture is observed, as shown for the Netherlands in Figure 1.4. The number of zero-grazed animals increased between 1997 and 2008 from 8 to21 percent of all dairy cows (CBS, 2009). It is however unclear why, during the last years, large fluctuations existed in the fraction of dairy cows with unrestricted and restricted grazing. The increase in cows not grazing at all is probably influenced by the almost linear increase in annual milk yield in The Netherlands over the last decades from 3800 kg/year in 1950 to 7926 kg/year in 2008 (CBS, 2009).

Table 1.1

The effect of grazing on various aspects. The scores indicate the relative value across systems (i.e., rows) for each characteristic, ranging from -- to ++, with ++ signifying a positive score for the point in question. Adapted from (Van Vuuren and Van den Pol-Van Dasselaar, 2006).

	Grazing management system			
Viewpoint	Unrestricted	Restricted	Zero-fresh	Zero-ensiled
Acceptance by society	++	+	-	-
Natural behavior	++	++	+	+
Animal health	++	+	+/-	+/-
Grass yield and use	-	+	++	+
Adequate nutrient supply	-	+/-	+	++
Nitrogen losses	-	+	++	++
Phosphorus losses	-	+/-	+	+
Ammonia volatilization	++	+	-	+/-
Energy use, methane emission	+	-	--	--
Labour	++	+	-	+
Economics	+	+	+/-	-

The most important reason for the decrease in the fraction of cows grazing unrestricted is probably the restrictions of this management system on dry matter intake (DMI) (Bargo et al., 2002). Dairy cows with a high proportion of forages in their diet and grazing dairy cows had a lower milk production than potentially possible (Kolver and Muller, 1998; Peyraud et al., 2004). Grazing dairy cows can reach a maximal milk production of 28 to 30 kg/d (Kolver and Muller, 1998; Van Vuuren and Van den Pol-Van Dasselaar, 2006). Grazing management, resulting in variation in grassland characteristics including herbage mass, SSH, regrowth duration and time of allocation of cows, can result in higher intake of nutrients from herbage, both by increased herbage DMI and by improved nutritional composition of herbage (Chilibroste, 2005; Rearte, 2005; Wales et al., 2005).

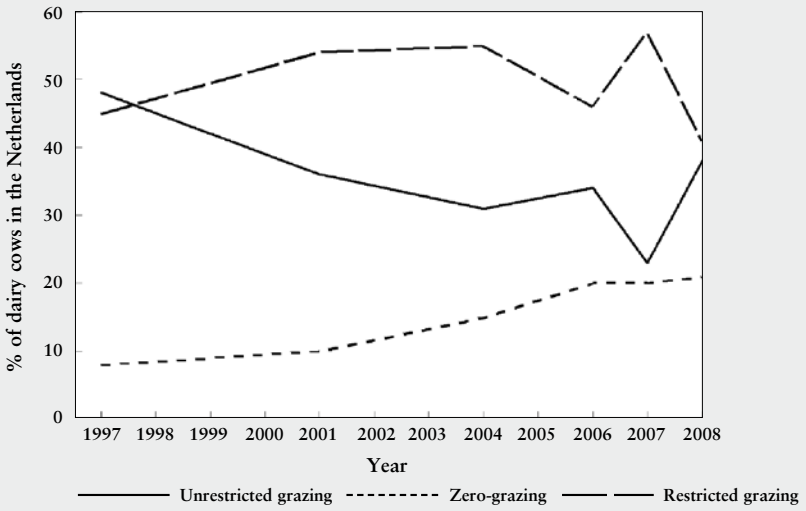


Figure 1.4

The distribution of grazing management of dairy cows in the Netherlands between 1997 and 2008 (CBS, 2009).

Under grazing, feed intake is limited by animal and dietary factors (Wales et al., 2005) and the daily herbage DMI can be represented as (Rook, 2000):

Herbage DMI (kg/d) = grazing time (min/d)*bite mass (kg DM/bite)*bite rate (bites/min)

The grazing behavior variables grazing time (GT), bite mass (BM) and bite rate (BR) can be measured using grazing recorders. Bite mass is the product of bite volume, plant density and dry matter content of the grass, in which in turn bite volume is the resultant of bite area and bite depth. As an example of the influence of grassland characteristics on grazing behavior, Wade et al. (1989), found that dairy cows always graze around 34% of sward surface height (SSH), regardless of SSH. Under Dutch conditions a lower percentage (27%) was found, provided SSH was 20 cm at minimum (Chilibroste, 1999). Bite size is predominantly limited by grass height (Gibb, 2006) but obviously also by sward density. The latter is mainly determined by sprout density and leaf to stem ratio (Agnew et al., 2004) and by DM content of herbage. The DM content of grass in turn varies in the course of the day reaching its maximum in late afternoon and evening, and varies within the vertical distribution of the herbage, decreasing towards the lower parts of the sward (Delagarde et al., 2000).

In dairy cows, nutrients become available through absorption from the reticulo-rumen and from the lower gut. Before nutrients can become available in the reticulo-rumen, the feed has to be ingested and subjected to microbial fermentation. The availability of different nutrients and their synchronization is therefore dependent on the pattern of feed intake (Chilibroste et

al., 2003). During microbial fermentation, the organic matter (OM) in the feed is converted to volatile fatty acids (VFA), fermentation gases, microbial biomass and fermentation heat. The major part of the VFA is acetate (HAc), propionate (HPr) and butyrate (HBu) and in addition small quantities of VFA with branched chains, iso-butyrate (iHBu), valerate (Val) and iso-valerate (iVal), occur. Between these VFA, HAc and HBu are nutrients of lipogenic nature, i.e. they are primarily used in the formation of milk fat. Propionic acid (HPr) is glucogenic and, after conversion into glucose in the liver, is primarily used as precursor in the synthesis of lactose and glycerol, a small (ca. 10%) component of milk fat. The amounts of lipogenic and glucogenic nutrients that become available for the animal is affected by nutrient intake, and affects milk composition and energy balance (van Knegsel et al., 2005). About 50% of the OM in microbial mass is true protein (i.e. amino acids) of which about 85% is absorbed. Amino acids are aminogenic nutrients that contribute to meet the amino acid requirements of the cow, notable requirements for the synthesis of milk protein. Absorbed nucleic acids are almost completely excreted in the urine (Tamminga and Chen, 2000).

The VFA pattern can be represented as the HAc/HPr (C2/C3) ratio or as the ratio between lipogenic and glucogenic nutrients, the so-called non-glucogenic to glucogenic ratio (NGR), which is calculated as:

$$NGR = (HAc + 2*HBu + 2*iHBu + HVal + iHVal)/(HPr + HVal + iHVal)$$

The proportions in which VFA are formed in the rumen varies, and depend on the diet composition and the conditions in the rumen. Notably the forage to concentrate ratio is important, as is the chemical composition of the diet as shown in Table 1.2 (Bannink et al., 2006). These figures were obtained from estimates of average VFA patterns over a day and originate from a large number of data on lactating cows fed indoor diets, reported in literature.

Table 1.2

The partial conversion of feed components to VFA in the rumen of lactating cattle fed on concentrate or roughage rich diets (Bannink et al., 2006).

	Relative conversion to VFA production							
	Concentrate diets				Roughage diets			
	HAc	HPr	HBu	BCVFA	HAc	HPr	HBu	BCVFA
Soluble sugars	0.53	0.16	0.26	0.06	0.64	0.08	0.24	0.04
Starch	0.49	0.31	0.15	0.05	0.49	0.22	0.21	0.08
Hemicellulose	0.51	0.12	0.32	0.05	0.44	0.19	0.32	0.06
Cellulose	0.68	0.12	0.20	<0.01	0.56	0.20	0.17	0.07
Protein	0.44	0.18	0.17	0.21	0.56	0.29	0.08	0.06

Table 1.2 shows that HAc is always the principal end product of rumen fermentation and that especially cellulose results in a high proportion of HAc. Sugars and hemicellulose result

in a relatively high proportion of HBu, whereas a significant part of starch is converted to HPr. Stage of lactation and the related feed intake level appears to have a marked influence on the VFA proportions, independently of the dietary composition. Robinson et al. (1986) extensively studied the effect of level of feed intake on the VFA pattern at 6 stages in the lactation. Although DMI level influenced the results since DMI decreased during the lactation, this experiment showed that NGR as well as its variation within a day (Robinson et al., unpublished results) increased with the progression of the lactation period (Figure 1.5). This increase in variation is probably influenced by changes in the feed intake pattern.

Information on VFA patterns in the rumen of grazing cattle is scarce, but it is expected that diurnal variation is larger than under stall feeding conditions when fed on (mixed) diets. Grazing animals have more opportunity to select and the chemical composition of grass shows substantial diurnal variation (Delagarde et al., 2000). Van Vuuren et al. (1986) found the content of sugars to vary between 130 and 175 g per kg DM in summer and between 80 and 120 g per kg DM in autumn. The highest values were measured in the late afternoon and evening and the highest VFA concentrations were observed around midnight.

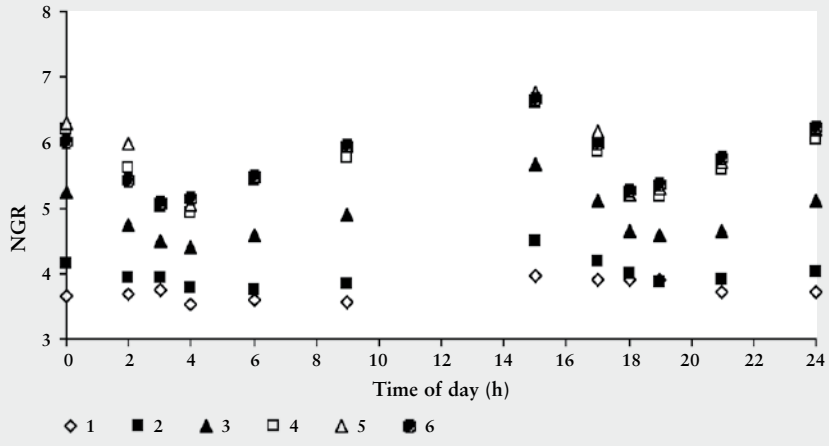


Figure 1.5

Non-glucogenic to glucogenic volatile fatty acid ratio (NGR) in the rumen of dairy cattle in different stages of lactation (1 = early lactation, 6 = dry period; Robinson et al., unpublished results).

Chilibroste et al. (1998) followed the course of the VFA pattern in the rumen of dairy cattle that were allowed to graze during different lengths of time after a fasting period of 16.5 h. The results in Figure 1.6 show that the NGR in the total pool of VFA decreased, but that the pool of freshly added VFA started very low (<3.0) and gradually increased during the grazing period and at the end approximated the level reported by Van Vuuren (1993). The pool of freshly added VFA in Figure 1.6 is calculated from the concentrations of the different VFA in

the total VFA at each time minus the concentration of these respective VFA at the preceding observation.

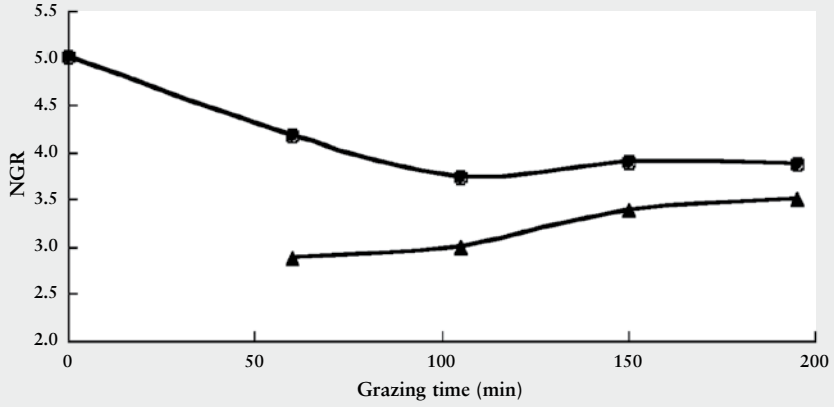


Figure 1.6

Variation in the non-glucogenic to glucogenic volatile fatty acid ratio (NGR) in the rumen pool of total (●) and freshly added (▲) volatile fatty acids (Chilibroste et al., 1998).

In the nutritional characteristics of grass, the concentration of sugars is an important aspect. Not only is sugar expected to improve herbage DMI, it also increases the amount of energy available for rumen microbes to increase rumen fermentation (Miller et al., 2001; Lee et al., 2002; Taweel et al., 2005). Several attempts have been made to improve productivity of dairy cows when feeding high-sugar grass varieties (Miller et al., 2001; Taweel et al., 2005; Moorby et al., 2006; Tas et al., 2006) but only Miller et al. (2001) found elevated milk yield on the variety with high sugar content. Sugar in grass is produced in the leafs while they are being stored in the stem and pseudostem (Fulkerson and Donaghy, 2001). The sugar concentration in grass is strongly influenced by physiological conditions of growth. Higher light intensity and rate of photosynthesis increase sugar content in grass and cause sugar content to increase during daylight (Delagarde et al., 2000). This offers opportunities to improve productivity by increased sugar intake by means of altering grazing management.

Rotational grazing management systems, especially stripgrazing, are assumed to be efficient systems due to constant grass quality and quantity between days (Kuusela and Khalili, 2002; Chilibroste, 2005). Scientific evidence on the benefits of stripgrazing systems is however scarce, and there have even been authors that doubt whether effect of allocation frequency on productivity are imagined (Parsons and Chapman, 2000).

The hypotheses of this thesis were:

1. In addition to diet composition, feed intake level strongly determines the type of nutrients that are supplied to the intermediary metabolism of a dairy cow. Feed intake level is in turn strongly influenced by the feed intake pattern.
2. The feed intake pattern changes in dependence of diet composition and also due to stage of lactation.
3. Under grazing the feed intake pattern depends on grazing management, with grass supply, plant density and grass height as determining factors.

This thesis describes the results of various experiments to determine the effects of quantitative and qualitative changes in nutrient supply on feed intake, feed intake behavior, the resulting rumen fermentation parameters and milk yield and composition. In Chapter 2 the effects of type and concentration of roughages and concentrates at two stages of lactation were studied. Chapters 3, 5 and 6 present the effects of different grazing management systems. In Chapter 3, the allocation of a new grazing plot once every 4 days was compared with daily allocation to a new plot. Chapter 5 presents the results of a comparison between a once daily and twice daily allocation of a new plot and Chapter 6 compares morning allocation with afternoon allocation of a new plot. An increased interest in milk quality, notably the fatty acid composition in milk fat as this might influence human health (Lock and Bauman, 2004), has developed in recent years. This aspect, based on the results of samples of milk from the experiment in Chapter 3, is discussed separately in Chapter 4. The results of all Chapters are discussed and general conclusions are formulated in the General Discussion in Chapter 6.

REFERENCES

Agnew, R. E., R. S. Park, C. S. Mayne, and A. S. Laidlaw. 2004. Potential of near infrared spectroscopy to predict the voluntary intake of grazed grass. Animal Feed Science and Technology 115(1-2):169-178.

Bannink, A., J. Kogut, A. M. Van Vuuren, J. Dijkstra, S. Tamminga, J. France, and E. Kebreab. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. Journal of Theoretical Biology 238(1):36-51.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Milk Response to Concentrate Supplementation of High Producing Dairy Cows Grazing at Two Pasture Allowances. Journal of Dairy Science 85(7):1777-1792.

CBS. 2009. StatLine (database). Centraal Bureau voor de Statistiek, Voorburg [http://statline.cbs.nl].

Chilibroste, P. 1999. Grazing time: the missing link? A study on the plant-animal interface by integration of experimental and modelling approaches. PhD Thesis, Wageningen University, Wageningen, The Netherlands.

Chilibroste, P. 2005. Pasture characteristics and animal performance. In Quantitative Aspects of Ruminant Digestion and Metabolism. J. France, M. Forbes and J. Dijkstra, ed. CAB International, Wallingford, UK.

Chilibroste, P., M. J. Gibb, and S. Tamminga. 2003. Pasture characteristics and animal performance In Quantitative Aspects of Ruminant Digestion and Metabolism. J. Dijkstra, J. France and M. Forbes, ed. CAB International, Wallingford, UK.

Chilibroste, P., S. Tamminga, P. L. Van der Togt, and J. Van Bruchem. 1998. Effect of allowed grazing time, inert rumen bulk and length of starvation before grazing on the weight, composition and fermentative end-products of the rumen contents of lactating dairy cows. Grass and Forage Science 53(2):146-156.

Coleno, F. C., and M. Duru. 1999. A model to find and test decision rules for turnout date and grazing area allocation for a dairy cow system in spring. Agricultural Systems 61(3):151-164.

Dado, R. G., and M. S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. Journal of Dairy Science 77(1):132-144.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1-2):49-68.

Evers, A., M. de Haan, A. Van den Pol-Van Dasselaar, and B. Philipsen. 2009. Weiden onder moeilijke omstandigheden. Animal Sciences Group, Wageningen, The Netherlands.

Forbes, J. M. 1995. Voluntary feed intake and diet selection in farm animals. CAB International, Wallingford, UK.

Fulkerson, W. J. and D. J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Australian Journal of Experimental Agriculture 41(2):261-275.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. Pages 141-157 in Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Gibb, M. J., C. A. Huckle, and R. Nuthall. 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science 53(1):41-46.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science 63(4):269-287.

Kolver, E. S., and L. D. Muller. 1998. Performance and Nutrient Intake of High Producing Holstein Cows Consuming Pasture or a Total Mixed Ration. Journal of Dairy Science 81(5):1403-1411.

Kuusela, E., and H. Khalili. 2002. Effect of grazing method and herbage allowance on the grazing efficiency of milk production in organic farming. Animal Feed Science and Technology 98(1-2):87-101.

Lee, M. R. F., L. J. Harris, J. M. Moorby, M. O. Humphreys, M. K. Theodorou, J. C. MacRae, and N. D. Scollan. 2002. Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. Animal Science 74(3):587-596.

Lock, A. L., and D. E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39(12):1197-1206.

Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): Milk production from late-lactation dairy cows. Grass and Forage Science 56(4):383-394.

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

Parsons, A. J., and D. F. Chapman. 2000. The principles of pasture growth and utilisation. Pages 31-89 in Grass, its production and utilisation. A. Hopkins, ed. Blackwell Science, Oxford, UK.

Peyraud, J. L., R. Mosquera-Losado, and L. Delaby. 2004. Challenges and tools to develop efficient dairy systems based on grazing: how to meet animal performance and grazing management. Pages 373-384 in Proceedings of XXth European Grassland Federation: Land use systems in grassland dominated regions. Luzern, Switzerland. VDF, Zürich, Switzerland.

Rearte, D. H. 2005. New insights in the nutritive value of grass. In: Utilization of grazed grass in temperate animal systems. Pages 49-60 in Proceedings of a satellite workshop of the XXth International Grassland Congress. J. J. Murphy, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

Robinson, P. H., S. Tamminga, and A. M. Van Vuuren. 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livestock Production Science 15:173-189.

Rook, A. J. 2000. Principles of foraging and grazing behaviour. Pages 229-246 in Grass, its production and utilization. A. Hopkins, ed. Blackwell Science, Oxford, UK.

Tamminga, S., and X. B. Chen. 2000. Animal-based techniques for the estimation of protein value of forages. Pages 215-231 in Forage evaluation in ruminant nutrition. D.I. Givens, E. Owen, R. F. E. Axford, and H.M. Omned, ed. CAB International, Wallingford, UK.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Effects of Perennial Ryegrass Cultivars on Milk Yield and Nitrogen Utilization in Grazing Dairy Cows. Journal of Dairy Science 89(9):3494-3500.

Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake Regulation and Grazing Behavior of Dairy Cows Under Continuous Stocking. Journal of Dairy Science 87(10):3417-3427.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121(3-4):243-256.

van Knegsel, A. T. M., H. van den Brand, B. Kemp, J. Dijkstra, and S. Tamminga. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reproduction Nutrition Development 45(6):665-688.

Van Vuuren, A. M., and A. Van den Pol-Van Dasselaar. 2006. Grazing systems and feed supplementation. Pages 85-101 in Fresh herbage for dairy cattle. A. Elgersma,, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Van Vuuren, A. M., C. J. Van Der Koelen, H. Valk, and H. De Visser. 1993. Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. Journal of Dairy Science 76(10):2982-2993.

Van Vuuren, A. M., C. J. van Der Koelen, and J. Vroons- de Bruin. 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. Netherlands Journal of Agricultural Science 34:457-467.

Wade, M. H., J. L. Peyraud, G. Lemaire, and E. A. Comeron. 1989. The dynamics of daily area and depth of grazing and herbage intake of cows in a five day paddock system. Pages 111-1112 in Proceedings of XVIth International Grassland Congress, Nice, France.

Wales, W. J., C. R. Stockdale, and P. T. Doyle. 2005. Plant and sward characteristics to achieve high intake in ruminants. In: Utilization of grazed grass in temperate animal systems. Pages 37-47 in Proceedings of a satellite workshop of the XXth International Grassland Congress. J. J. Murphy, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

Zom, R. L. G., J. W. Van Riel, G. André, and G. Van Duinkerken. 2002. Voorspelling voeropname met koemodel 2002. Praktijkrapport 11:1-50. Praktijkonderzoek Veehouderij, Lelystad.

CHAPTER 2

The Effect of Silage and Concentrate Type on Intake Behavior, Rumen Function, and Milk Production in Dairy Cows in Early and Late Lactation

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ABSTRACT

The objective of this experiment was to evaluate the effect of feeding total mixed rations (TMR) that differ in structural and non-structural carbohydrates to dairy cows in early and late lactation on short-term feed intake, dry matter intake (DMI), rumen fermentation variables and milk yield. A 5 × 5 Latin square experiment with 15 dairy cows was repeated during early and late lactation. The five treatments were a TMR with (all on dry matter basis) 55% roughage (a 50/50 mixture of corn silage and grass silage) and 45% concentrate (a 50/50 mixture of concentrate rich in structural carbohydrates and concentrate rich in non-structural carbohydrates; treatment CON), a TMR with the concentrate mixture and 55% grass silage (RGS) or 55% corn silage (RCS), and a TMR with the roughage mixture and 45% of the concentrate rich in structural carbohydrates (CSC) or the concentrate rich in non-structural carbohydrates (CNS). Meal criteria, determined using the Gaussian-Gaussian-Weibull method per animal per treatment, showed an interaction between lactation stage and treatment. Feed intake behavior variables were therefore calculated with meal criteria per treatment-lactation stage combination. Differences in feed intake behavior were more pronounced between treatments differing in roughage composition than between treatments differing in concentrate composition, probably related to larger differences in chemical composition and particle size between corn silage and grass silage than between the two concentrates. The number of meals was similar between treatments but eating time was greater in RGS (227 min/d), and lesser in RCS (177 min/d) than the other treatments. Intake rate increased when the amount of grass silage decreased, whereas meal duration decreased simultaneously. These effects were in line with a decreased DMI of the RGS diet vs. the other treatments, probably related to the high neutral detergent fiber (NDF) content. However, this effect was not found in CSC, although NDF content of the TMR, fractional clearance rate of NDF, and fractional degradation rate of NDF was similar between CSC and RGS. Rumen fluid pH was lesser, and molar proportions of acetic acid and of propionic acid lesser and greater, respectively, in RCS compared with all other diets. Milk production did not differ between treatments. There was no effect of type of concentrate on milk composition, but diet RCS resulted in a lesser milk fat content and greater milk protein content than diet RGS. Lactation stage did affect short-term feed intake behavior and DMI, although different grass silages were fed during early and late lactation. The results indicate that short-term feed intake behavior is related to DMI and therefore may be a helpful tool in optimizing DMI and milk production in high production dairy cows.

INTRODUCTION

In dairy cows, DMI is critical to achieve high milk production. In general, DMI does not meet the energy requirements for maintenance and production in high productive early lactating animals. This results in a negative energy balance accompanied by an increase in the incidence of metabolic diseases and a reduction in reproductive performance (van Knegsel et al., 2005). A better understanding of factors affecting DMI provides opportunities to increase DMI and thereby milk production (Grant and Albright, 1995), potentially increasing profitability of dairy farming. The study of short-term feeding behavior may assist in understanding variation in daily DMI (Tolkamp et al., 2002). In particular, understanding of the factors limiting DMI due to short-term constraints (e.g., rumen fill) and rumen pH dynamics (resulting in subacute rumen acidosis, for example) are of relevance.

Cows eat in discrete meals alternated with periods of ruminating and idling (non-eating behavior). These meals are separated by the meal criterion, which is the length of the longest interval still considered an interval within meals (Tolkamp et al., 1998). Hence, daily DMI can be described in terms of the number of meals per day, the length of meals, and the intake rate (IR) during meals (Dado and Allen, 1994). Manipulation of any of these variables may result in a change in DMI. This might be caused by several management factors including feeding space (De Vries et al., 2004), animal factors like lameness, and potentially also by the ration fed. A direct effect of the intake pattern on DMI could occur if, for example, a few large meals reduced rumen pH more severely than many small meals. Due to interference with rumen fermentation, this might affect the profile of supplied nutrients.

Much research was carried out on dietary factors affecting DMI. One of these factors is the type of silage fed to dairy cows. Deswysen et al. (1993) evaluated the effect of corn silage vs. grass silage on eating behavior in heifers, and found that intake was greater with corn silage than with grass silage, associated with a shorter eating time and fewer meals, implicating a greater IR within meals with corn silage. Similar results on eating behavior were found by Dulphy et al. (1980) in sheep, although DMI in this experiment was lesser on corn silage than on grass silage. The short-term feed intake behavior results in these experiments are potentially related to the greater NDF and lesser starch content in grass silage in comparison to corn silage (Dulphy et al., 1980). Not only is fermentation rate of NDF in the rumen generally slower than fermentation rate of starch, also, particle size distribution in grass silage differs from that in corn silage, with a greater proportion of large particles in grass silage (Bruining et al., 1998). This results in lesser clearance of OM (through degradation and passage) of grass silage than of corn silage. In early to midlactating dairy cows, increasing NDF content in the diet corresponded to increased time spent eating at the expense of time spent ruminating (Beauchemin, 1991), although in this experiment, forage: concentrate ratio was not kept constant between treatments.

High-producing dairy cows are often fed concentrates to supply sufficient energy and nutrients to meet their requirement. Differences in concentrate chemical composition, similar to differences in roughage chemical composition, resulted in altered feed intake behavior, although the effects may be limited, because only small differences in particle size occur in pelleted concentrates. When feeding diets containing 75% basic TMR plus an additional 25% pelleted concentrate high in starch or high in NDF to 24 lactating dairy cows, Miron et al. (2004) found that the number of meals and total time spent eating per day was greater on the NDF rich concentrate. The intake per meal and the rate at which cows consumed feed were greater in the treatment with high starch concentrate.

In most of the experiments evaluating the effect of lactation stage on DMI, the effect of lactation stage was confounded by changes in the diet (Kertz et al., 1991). When feeding the same diet throughout the lactation, dairy cows were found not to regulate feed intake accurately according their requirements for maintenance and milk production (Coppock et al., 1974; Oldenbroek, 1984). Effects of lactation stage on short-term feed intake behavior are hardly studied. From early to peak lactation, De Vries et al. (2003) found an increase in meal duration and frequency, although the effects seemed to stabilize at the end of their study period (between 35 and 94 DIM). In contrast, Friggens et al. (1998) did not find an effect of lactation stage on short-term feed intake behavior except for the time spent eating per day.

The objective of this experiment was therefore to evaluate the effect of different silages (grass and corn silage) and different concentrates (high in NDF or high in starch content) in TMR fed to dairy cows in early and late lactation on short-term feed intake, daily intake,

rumen fermentation variables and milk yield. We hypothesized that increasing the amount of structural carbohydrates (NDF) in the TMR at the expense of non-structural carbohydrates would result in longer and larger meals, slower rate of fermentation of the feed in the rumen, thus resulting in a reduction of rumen fermentation of the TMR. We also hypothesized that inducing this difference through the composition of the concentrate, rather than through the silage fed, would result in smaller differences between treatments. No effect of lactation stage on intake behavior was expected.

MATERIALS AND METHODS

Animals and Experimental Design

Two 5 × 5 Latin square experiments were conducted to evaluate the effect of 5 treatments using 15 dairy cows during early (experiment 1: between February 20th and June 11th 2004), and late lactation (experiment 2: between August 27th and December 14th 2004). Both experiments were approved by the Institutional Animal Care and Use Committee of Wageningen University. Each period in the Latin square design lasted 3 wk, with the first 2 weeks for adaptation. The treatments were as follows: a basic TMR with (all on DM basis) 55% roughage (27.5% corn silage and 27.5% grass silage) and 45% concentrate (a 50/50 mixture of a concentrate rich in structural carbohydrates and a concentrate rich in non-structural carbohydrates; treatment CON), ii) a TMR with 55% corn silage and 45% of the concentrate mixture (treatment RCS), iii) a TMR with 55% grass silage and 45% of the concentrate mixture (treatment RGS), iv) a TMR with 55% of the roughage mixture and 45% of the concentrate rich in non-structural carbohydrates (treatment CNS), and v) a TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates (treatment CSC). The proportion of feed ingredients in each of the diets was based on DM content of silages obtained before the start of the experiment. The ingredient composition of concentrates and the proportion of feed ingredients in the different treatments based on actual DM content during the experiments are presented in Tables 2.1 and 2.2. The chemical composition of the concentrate and silages is presented in Table 2.3.

The 15 Holstein Friesian cows, of which 5 were previously fitted with a rumen cannula (10 cm i.d.; Bar Diamond Inc., Parma, ID) in the dorsal sac, were grouped according to parity, DIM, milk yield during the previous lactation, and presence of a rumen cannula and were randomly assigned to the treatments. At the start of experiment 1 and 2, cows (5 primiparous, 10 multiparous) were 61 ± 5.9 and 245 ± 6.2 DIM (values expressed as means ± SE), respectively. The same cows were used during both experiments, with one exception, because one cow was culled between experiments. This cow was replaced by a cow of equal parity and DIM in experiment 2. At the end of the second period of experiment 2, data from one cow were excluded after repeated stealing feed from an incorrect treatment.

Treatments and Feeding

The cows were housed in a free-stall barn with an allowance of more than one cubicle per cow. The cubicles were bedded daily with sawdust on top of rubber matrasses. Individual eating behavior was continuously monitored throughout the study using feed bins (Nedap-Agri, Groenlo, the Netherlands) equipped with automated intake registration. Cows in each treatment had access to 2 feed bins. Weight changes of the bins (± 0.1 kg) were recorded, and time of start and end per visit were used to calculate intake and IR per visit. The bins were

calibrated at the start of each period. Feed intake registration failed for 1 group during period 3 in experiment 1 (treatment RGS) and all groups during 2 d in period 2 in experiment 2 due to a registration problem with the computer, without affecting feed intake behavior. Water was available from 3 water troughs *ad libitum*.

The different TMR were mixed 3 times a week or more frequently if necessary to prevent storage problems caused by warm weather. After mixing, TMR were stored in a cooling unit at 4°C and fed daily *ad libitum* (± 10% orts as-fed) during morning milking. Cows were milked twice daily at 0730 and 1930 h. During each milking, all cows received 1 kg as fed of a protein-rich concentrate (Table 2.1) to prevent protein deficiency of cows fed the RCS treatment. The chemical composition of the TMR fed is presented in Table 2.4.

Table 2.1

Ingredient composition of the concentrates used in the total mixed rations, and of the concentrate fed during milking (concentrate MP).

Ingredient (% as fed)	Concentrate rich in non-structural carbohydrates (g/kg)	Concentrate rich in structural carbohydrates (g/kg)	Concentrate MP (g/kg)
Corn	25.0	-	-
Barley	11.8	-	-
Wheat	10.0	-	-
Soy hulls	-	15.0	-
Lupins < 33.5%CP	-	15.0	-
Palm kernel expeller <22% crude fiber	-	12.0	-
Corn glutenfeed	-	-	19.3
Coconut expeller	-	-	2.5
Sunflower seed, extracted	-	-	2.8
Rapeseedmeal	17.8	7.0	3.0
Soybeanmeal (Mervobest)	6.2	4.5	17.8
Soybeanmeal solvent extracted	5.3	5.1	38.0
Molasses	7.5	7.5	5.0
Citruspulp	10.6	12.9	-
Beetpulp	2.5	17.0	-
Vinasses	-	-	7.0
Palm oil	0.1	1.0	-
Fat, animal origin	-	-	1.0
Limestone	1.0	0.3	1.8
Sodium bicarbonate	1.0	1.0	-
Mineral-vitamin mixture[1]	0.8	0.8	1.0
Sodium chloride	0.2	0.2	0.6
Magnesium oxide	0.2	0.2	-
Monocalcium phosphate	0.1	0.7	0.2

[1] Contained per kilogram of mix: 93 g of Ca, 400 g of Mg, 5 mg of S, 4 g of Cu, 3.3 g of Mg, 322 mg of I, 97 mg of Co, 80 mg of Se, 2600000 IU of vitamin A, 580000 IU of vitamin E (Premix 2033, PreMervo, Utrecht, the Netherlands).

Sampling and Analyses

Feed Samples and Chemical Analyses. A representative sample of individual feedstuffs and of TMR was taken twice every period. The feedstuff samples were pooled per feedstuff per experiment, whereas the TMR samples were pooled per treatment per period. All samples were oven-dried for 24 h at 70°C. Feed samples were ground to pass through a 1-mm sieve and analyzed for DM, inorganic matter (ash), CP, crude fat (CF), NDF, ADF, acid detergent lignin (ADL) and sugars as described by Abrahamse et al (2008), and starch was analyzed using enzymatic hydrolysis (ISO 15914; ISO, 2004). Net energy for lactation (NE_L) was calculated using the feed unit lactation (VEM) system (Van Es, 1975) and intestinal digestible protein (DVE) and degraded protein balance were calculated according to (Tamminga et al., 1994). Data used for these calculations were obtained from the concentrate supplier (concentrates) and near-infrared reflectance spectroscopy carried out by BLGG in Oosterbeek, The Netherlands (silages).

Table 2.2

Proportion of feed ingredients in each of the treatments during early lactation (experiment 1) and late lactation (experiment 2).

	Early lactation[1]					Late lactation				
Variable	RGS	CSC	CON	CNS	RCS	RGS	CSC	CON	CNS	RCS
Grass silage (%)	58.7	28.8	30.3	31.8	0.0	54.8	26.8	26.8	26.9	0.0
NDF concentrate (%)	20.5	45.5	21.2	0.0	22.0	22.8	44.6	22.4	0.0	22.0
Starch concentrate (%)	20.8	0.0	21.5	39.9	22.2	22.4	0.0	22.0	44.4	21.6
Corn silage (%)	0.0	25.7	27.0	28.3	55.9	0.0	28.6	28.8	28.7	56.4

[1] RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

DMI and Feed Intake Behavior. After manually screening the DMI data to detect registration errors, visits to the feed bins were grouped into meals on the basis of the estimated meal criteria (i.e., the longest interval not separating two meals). Meal criteria were estimated for each individual cow per dietary treatment during early and late lactation using the frequency distribution of log-transformed intervals between visits. A three-population model was used, with two Gaussian distributions to describe within-meal intervals and a Weibull distribution to describe between-meal intervals (Tolkamp et al., 1998; Yeates et al., 2001). Eating time was calculated as meal duration minus intervals within meals. Some variables were analyzed per day as well as per meal (DMI, number of visits, eating time), whereas others were only analyzed per meal (IR, meal duration) or per day (number of meals).

Rumen Fluid. During the last day of each period, rumen fluid samples from the rumen-cannulated animals were taken after every visit to the feed bins between morning and evening milking. During the first 2 h after each visit, rumen fluid was sampled every 15 min, thereafter

every 30 min. Sampling was performed by taking equal amounts from the front and middle of the ventral sac and from the cranial sac, using a solid, perforated plastic tube (85 cm long; 2.5 cm in diameter). Rumen pH was measured immediately after sampling using an electronic pH meter (pH electrode HI 1230, Hanna Instruments B.V., IJsselstein, the Netherlands). Time below pH 5.8, indicative of rumen acidosis (Kolver and De Veth, 2002), was calculated as the sum of the time pH was below 5.8 per cow per day (min), assuming a linear increase or decrease between two consecutive samplings per cow. After rumen pH measurement, two subsamples of rumen fluid were taken, acidified with phosphoric acid or TCA, and stored at -20°C pending VFA and NH_3-N analyses, respectively, as described by Taweel et al. (2005).

Table 2.3

Chemical composition of the silages and concentrates fed during early lactation (experiment 1) and late lactation (experiment 2; g/kg DM unless otherwise stated).

	Early lactation					Late lactation				
Variable	Grass Silage	Corn silage	NDF Concentrate	Starch Concentrate	Concentrate MP[1]	Grass Silage	Corn Silage	NDF Concentrate	Starch Concentrate	Concentrate MP[1]
DM (g/kg)	**437.9**	**340.9**	**891.8**	**890.4**	**901.7**	**326.0**	**337.9**	**908.1**	**892.6**	**900.1**
OM	**899.5**	**965.4**	**920.8**	**924.5**	**895.8**	**896.0**	**946.3**	**916.9**	**924.3**	**897.1**
CP	**137.0**	**69.9**	**193.5**	**201.0**	**368.0**	**195.0**	**79.4**	**194.4**	**191.1**	**359.3**
Crude fat	**28.6**	**27.5**	**40.0**	**24.8**	**41.1**	**41.5**	**29.9**	**41.4**	**22.2**	**41.3**
NDF	**543.9**	**420.8**	**315.8**	**145.8**	**188.6**	**472.4**	**375.4**	**322.1**	**145.1**	**183.3**
ADF	**344.9**	**259.4**	**253.5**	**114.1**	**150.1**	**275.8**	**215.6**	**251.3**	**104.9**	**122.1**
ADL[2]	**38.3**	**21.0**	**25.1**	**21.0**	**30.7**	**21.5**	**17.6**	**30.0**	**20.3**	**23.4**
Sugars	**46.9**	**7.4**	**113.9**	**112.0**	**113.2**	**24.4**	**5.8**	**102.5**	**97.9**	**109.0**
Starch	**-[3]**	**300.5**	**17.0**	**303.5**	**28.0**	**-[3]**	**326.0**	**10.2**	**300.8**	**38.2**
NE_L, MJ/kg DM[4]	**5.4**	**6.6**	**7.3**	**7.4**	**7.4**	**6.0**	**6.4**	**7.4**	**7.3**	**7.4**
DVE[5]	**59.0**	**50.0**	**125.5**	**126.3**	**210.4**	**67.0**	**48.0**	**128.3**	**126.0**	**210.4**
OEB[6]	**0.0**	**-31.0**	**15.3**	**18.5**	**115.8**	**31.0**	**-27.0**	**14.0**	**12.1**	**115.8**

[1] Concentrate fed in the milking parlor.
[2] Acid detergent lignin.
[3] Not determined.
[4] Net energy for lactation calculated with VEM (feed unit lactation) system (Van Es, 1975).
[5] Intestinal digestible protein (Tamminga et al., 1994).
[6] Degraded protein balance (Tamminga et al., 1994).

Rumen Evacuations. The fistulated cows were deprived from feed for 12 h after the evening milking on the last day of each period. Rumen evacuations were carried out before and after fasting to determine rumen pool sizes and to calculate the fractional clearance rate (Kcl). The cows were brought to a tie-stall where rumen evacuations were carried out using the methodology described by Taweel et al. (2004), with one modification. The contents of the 10% sample fraction were squeezed through cheesecloth, and from the remaining solid material, a representative sample was taken. The original rumen contents were reconstituted by adding rumen fluid proportional to the weight of the squeezed solid and fluid. Rumen pool sizes of

the different components (DM, OM, NDF, ADL and CP) were calculated as the product of the total DM weight of the rumen content, and the content of each of the components at both evacuations. Rumen pool sizes before fasting were used for statistical analyses on rumen pool sizes, whereas values of both evacuations were used to calculate Kcl based on the assumption of first-order kinetics using the equation RP(t) = RP(0) × e-Kcl × t, where RP(0) and RP(t) the rumen pool sizes (kg) immediately before and after overnight fasting, respectively, and t the time (h) between both evacuations.

Table 2.4

Chemical composition of the different total mixed rations fed during early lactation (experiment 1) and late lactation (experiment 2; g/kg DM unless otherwise stated).

Variable	Treatment[1]					Lactation stage	
	RGS	CSC	CON	CNS	RCS	early	late
Determined							
DM (g/kg)	514.9	475.6	482.2	480.8	446.0	487.6	472.2
OM	904.5	916.5	920.9	921.8	940.3	921.3	920.3
CP	175.4	151.1	150.7	153.1	128.4	143.2	160.3
Crude fat	34.1	34.7	31.9	30.1	29.6	29.9	34.3
NDF	402.9	399.1	368.3	335.9	327.1	390.3	343.0
ADF	259.3	263.5	235.9	212.0	206.9	253.3	217.7
ADL[2]	26.6	26.3	24.7	23.3	21.4	28.0	20.9
Sugars	60.0	53.9	55.4	54.4	47.1	60.2	48.1
Starch	62.0	97.1	150.9	194.5	251.7	138.5	163.9
Calculated[3]							
RNSP	170.1	180.6	163.7	153.8	156.3	159.2	170.7
NE_L (MJ/kg DM)[4]	6.4	6.6	6.6	6.7	6.9	6.6	6.7
DVE	91.6	87.4	87.7	88.1	83.9	86.6	88.8
OEB	15.3	2.5	3.0	3.6	-9.2	-0.9	7.0

[1] RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.
[2] Acid detergent lignin.
[3] Calculated values based on the ratio of different roughage and concentrate ingredients in the total mixed rations and the data in table 3. RNSP (residual non-starch polysaccharides) is calculated as OM – CP – Crude fat – NDF – Sugars – Starch; NE_L (in MJ/kg DM) is calculated with the VEM system (Van Es, 1975); DVE is intestinal digestible protein (Tamminga et al., 1994); OEB is degraded protein balance (Tamminga et al., 1994).
[4] Net energy for lactation calculated with VEM (feed unit lactation) system (Van Es, 1975).

In Situ Incubations. To evaluate rumen degradation of OM, N, NDF and starch, *in situ* rumen incubations were carried out in a separate experiment between February 10th and March 12th, 2005, using five non-lactating Holstein-Friesian cows and one cow in early lactation.

One of the non-lactating cows delivered 4 d before the end of the incubations. Before to the start of the *in situ* rumen incubations, cows were adapted during two wk to the CON treatment. Incubations were performed according to the all-out method described by Tas et al. (2006) with some modifications. Only concentrate and silage samples of experiment 2 were incubated in the rumen. Pelleted concentrates were ground to pass a 3 mm screen (type ZM 100 Retsch, Haan, Germany), whereas grass and corn silage samples were cut with a paper cutter at a length of 0.5 to 1 cm before incubations. Polyamide bags (8.5 × 16.5 cm; pore size 40 µm; PA 40/30, Nybolt, Zurich, Switzerland) were filled with approximately 5 g of DM and incubated for 2, 4, 8, 12, 48, 72, and 336 h. Bags for the short-term incubations (2 to 48 h) were randomly distributed over three cows, whereas bags for the long-term incubations (336 h) were randomly distributed over the other three cows (including both lactating cows). After incubations, bags were immediately placed in ice water to stop fermentation and later rinsed with tap water. All bags were washed in a washing machine during 45 min with 55 L cold water without centrifuging. After freeze-drying, residues of silages were ground to pass a 1 mm screen (type ZM 100 Retsch, Haan, Germany), and all residues were pooled per feed per incubation time and analyzed for DM, ash, N, NDF, and starch as described above. Data were fitted to the non-linear first-order model of Robinson et al. (1986) using the PROC NLIN procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) and the effective degradability (ED) was calculated according to Tas et al. (2006) assuming a fractional passage rate of 0.045 /h for silages and 0.060 /h for concentrates (Tamminga et al., 1994). Effective degradability of each TMR was calculated from the ED of the individual feeds, corrected for the contents of each of the chemical components.

Milk Yield and Composition. Individual milk yield was recorded throughout the experiment. During the last two d of each measurement period, milk samples were taken during four consecutive milkings per cow and stored and analyzed for fat, protein, lactose, and urea content as described by Abrahamse et al. (2008). The fat- and protein-corrected milk (FPCM, kg/d) was calculated as [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] × milk yield (kg/d).

Statistical Analyses

The effect of lactation stage was confounded with the effect of different grass and corn silages used in experiment 1 and 2. In the description of statistical analyses, this is taken together as the effect of lactation stage. Results are presented as means with their SEM. Amount of FPCM, content of milk components and rumen variables were averaged per cow per period. Consequently, data were analyzed as a replicated 5 × 5 Latin square using the MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) with model effects for treatment, lactation stage, and period and the random effect of cow. A similar model was run for meal criterion data, with the exception that cow was a fixed effect. The interaction between treatment and lactation stage was included in the model for meal criteria and milk components, but it was excluded from the model for all other variables due to lack of significance ($P > 0.05$). The number of meals, eating time, DMI, and milk yield of each cow were calculated per day. Consequently, data was analyzed as a replicated 5 × 5 Latin square with model effects for treatment, lactation stage, period, day, and the interaction between lactation stage and treatment as fixed effects and cow as random effect, using the MIXED procedure of SAS. Day was used as a REPEATED model statement (Littell et al., 1998) with a first-order autoregressive covariance structure [AR(1)] (Tempelman, 2004). Differences were considered significant at a probability of $P < 0.05$, and posthoc analyses were carried out using the Tukey test to test pairwise comparisons.

RESULTS

Chemical Composition of TMR

Formulation of the different TMR resulted in an expected decrease in NDF and increase in starch and sugars content between treatments that differed in silage component (order RGS – CON – RCS) and in concentrate component (order CSC – CON – CNS; Table 2.4). In addition, due to the lesser CP and CFAT content of the corn silage than the grass silage, CP and CFAT decreased in the order RGS – CON – RCS. The decrease in CP resulted in a decrease in DVE and degraded protein balance between treatments differing in silage components.

DMI and Feed Intake Behavior

Meal criteria differed between lactation stages, but there was no effect of cow, treatment, and period ($P > 0.05$; Table 2.5). A significant interaction between treatment and lactation stage ($P = 0.024$) was found, caused by a greater meal criterion in early lactation than in late lactation for CSC and CNS. Due to the interaction between treatment and lactation stage, individual meal criteria per treatment for both experiments were used to calculate meals.

Table 2.5

Meal criteria, dry matter intake (DMI) and feed intake behavior per day and per meal of dairy cows fed different carbohydrate sources during early (experiment 1) and late (experiment 2) lactation.

	Treatment[1]						Lactation stage			P-value		
Variable	RGS	CSC	CON	CNS	RCS	SEM	early	late	SEM	Treatment	Lactation stage	T × L[2]
Meal criterion (min)	18.5	16.4	16.5	18.3	18.4	1.46	20.5	14.9	0.96	0.835	<0.001	0.024
Per day												
DMI (kg)	17.3^{a}	20.1^{b}	19.5^{b}	19.5^{b}	19.7^{b}	0.30	19.5	19.0	0.24	<0.001	0.017	0.065
Meals (no)	7.4	7.5	7.3	7.5	7.7	0.16	7.2	7.7	0.12	0.225	<0.001	<0.001
Eating time (min)	227^{d}	210^{c}	214^{c}	199^{b}	177^{a}	3.2	231	178	2.5	<0.001	<0.001	<0.001
Per meal												
Visits (no)	2.8^{ab}	2.7^{ab}	2.8^{b}	2.8^{ab}	2.5^{a}	0.08	2.9	2.5	0.07	0.010	<0.001	0.002
Eating time (min)	33^{d}	30b^{c}	31^{c}	28^{b}	24^{a}	0.7	34	24	0.6	<0.001	<0.001	<0.001
Meal duration (min)	37^{d}	33^{bc}	34^{c}	32^{b}	28^{a}	0.9	38	28	0.7	<0.001	<0.001	<0.001
Intake rate (g DM/min)	75^{a}	92^{b}	89^{b}	97^{c}	105^{c}	2.1	81	102	1.6	<0.001	<0.001	0.005

^{a,b,c} Means in rows with difference superscripts differ ($P < 0.05$).

1 RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

2 Treatment × Lactation stage interaction.

The DMI was lesser ($P < 0.001$) in RGS compared with the other treatments, and DMI was slightly lesser ($P = 0.017$) in late lactation than in early lactation (Table 2.5). The calculated energy balance (using the Dutch feed evaluation system for energy; Van Es, 1975) was positive for each treatment in both lactation stages. There were differences between treatments with a more positive energy balance in RCS than in RGS (23.7 vs. 4.9 NE_L/d) and a more positive

energy balance in late lactation than in early lactation (30.8 vs. 1.6 NE_L/d; results not shown). The number of meals was similar between treatments, whereas in late lactation the number of meals was greater ($P < 0.001$) than in early lactation (7.7 and 7.2 meals per day, respectively; Table 2.5). Eating time in RGS (227 min/d) was greater, and that in RCS (177 min/d) was lesser ($P < 0.05$), than in all other treatments. Moreover, eating time in early lactation (231 min/d) was greater ($P < 0.001$) than in late lactation (178 min/d). However, an interaction between treatment and lactation stage was observed ($P < 0.001$) in the number of meals and in eating time per day (Figure 2.1). In early lactation, the number of meals in RGS (6.6/d) was lesser ($P < 0.001$) than in RCS (7.8/d) whereas in late lactation, diet did not affect the number of meals. Dietary effects on eating time per day were also more pronounced in early lactation than in late lactation. In early lactation, eating time per day in RGS was greater ($P = 0.002$), and in CNS and RCS lesser ($P = 0.002$ and $P < 0.001$, respectively) than in CON, whereas in late lactation, only eating time in CON was greater ($P < 0.020$) from that in CNS.

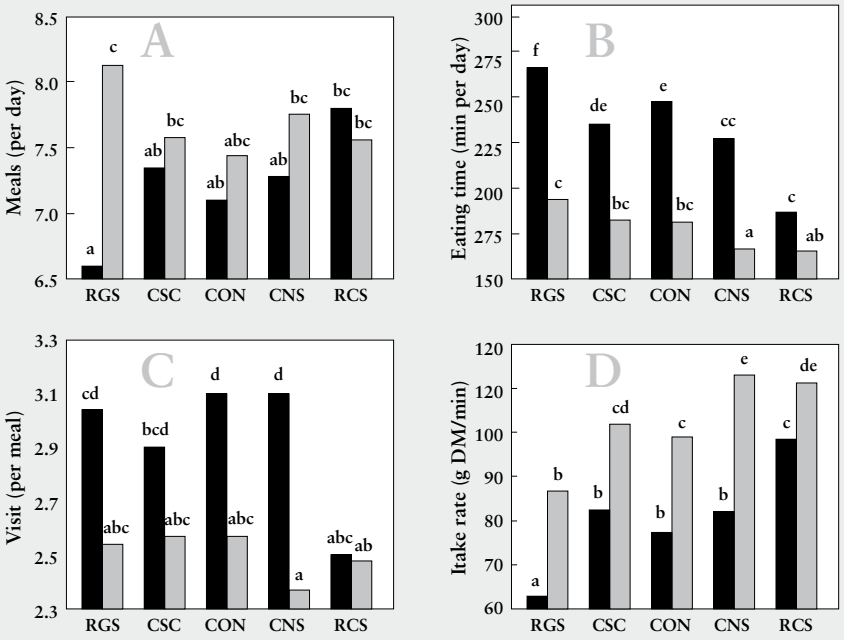

Figure 2.1

Number of meals (A) and eating time (B) per day, and number of visits (C) and intake rate (D) per meal of dairy cows fed different carbohydrate sources during early (■) and late (□) lactation.

Means with unlike letters differ within the variable tested (P < 0.05).
RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

IR was greater ($P < 0.001$) in late lactation than in early lactation. The number of visits per meal, eating time per meal (the net time spent eating during meals) and meal duration (the total time spent on eating behavior, including within-meal intervals) differed between treatments ($P < 0.05$). Intake rate per meal showed the largest differences between treatments with a greater IR in RCS and CNS ($P < 0.01$) than in the other diets, and a greater IR in CON and CSC ($P < 0.001$) than in RGS. The interaction between treatment and lactation stage was significant for all intake variables per meal. The number of visits per meal was lesser in RCS in early lactation than in CNS and CON ($P < 0.005$), whereas in late lactation it was similar between treatments. Eating time and meal duration per meal were longer in RGS in early lactation than in CNS, CSC and CON ($P < 0.001$), whereas in RCS, it was shortest ($P < 0.001$), although in late lactation, it only differed between CON and CNS ($P = 0.047$; not shown in Figure 2.1). The differences in IR between diets were rather similar in early compared with late lactation. However, in late lactation, IR per meal in CNS was greater ($P < 0.05$) than in CON, CSC and RGS, whereas in early lactation, IR per meal in CNS was only greater than in RGS ($P < 0.001$) and lesser than in RCS ($P < 0.001$; Figure 2.1).

Table 2.6

Rumen pH, VFA and ammonia-N (NH_3-N) of dairy cows fed different carbohydrate sources during early (experiment 1) and late (experiment 2) lactation.

	Treatment[1]						Lactation stage			P-value	
Variable	RGS	CSC	CON	CNS	RCS	SEM	early	late	SEM	Treatment	Lactation stage
Ruminal pH	6.12^{b}	6.17^{b}	6.06^{b}	6.11^{b}	5.84^{a}	0.070	6.11	6.00	0.059	<0.001	0.022
Time below pH 5.8 (h/d)	1.3^{a}	0.9^{a}	1.9^{a}	2.5^{a}	5.9^{b}	0.54	1.0	1.6	0.46	<0.001	0.061
Total VFA (mM)	124.1	118.0	119.7	117.7	119.8	5.24	109.6	130.1	3.44	0.907	<0.001
Acetate (mol/100 mol)	61.5^{b}	61.7^{b}	60.1^{b}	60.2^{b}	56.3^{a}	0.80	60.2	59.7	0.57	<0.001	0.415
Propionate (mol/100 mol)	21.2^{a}	21.1^{a}	21.5^{a}	22.3^{a}	25.7^{b}	0.69	22.1	22.6	0.43	<0.001	0.421
Butyrate (mol/100 mol)	13.5	13.5	14.3	13.7	13.3	0.31	13.6	13.7	0.22	0.162	0.688
Isobutyrate (mol/100 mol)	0.8	0.7	0.9	0.7	0.7	0.05	0.9	0.7	0.03	0.661	0.029
Valerate (mol/100 mol)	1.6^{a}	1.7^{a}	1.8^{ab}	1.7^{a}	2.6^{b}	0.21	1.8	1.9	0.15	0.004	0.595
Isovalerate (mol/100 mol)	1.4	1.4	1.4	1.4	1.4	0.13	1.4	1.4	0.11	0.969	0.271
NGR[2]	3.88^{b}	3.89^{b}	3.85^{b}	3.69^{b}	3.11^{a}	0.137	3.75	3.62	0.093	<0.001	0.295
NH_3-N (mg/L)	178.3^{c}	121.7^{b}	106.6^{b}	99.0^{b}	50.0^{a}	9.31	93.9	128.4	7.82	<0.001	<0.001

^{a,b,c} Means in rows with different superscripts differ ($P < 0.05$).

[1] RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

[2] The non-glucogenic:glucogenic VFA ratio (NGR) was calculated as [acetate + 2 (butyrate + isobutyrate) + valerate + isovalerate]/[propionate + valerate + isovalerate].

Although investigation of the effects of parity on DMI and milk production was not part of the objectives of this article, short-term feed intake behavior differed ($P < 0.05$) between primiparous and multiparous cows. In comparison to primiparous cows, multiparous cows had

a greater DMI and number of meals per day, and eating time per meal was lesser, although IR was not increased as compared to multiparous cows. This resulted in a greater milk production per day in multiparous than in primiparous cows, although other milk variables were similar between different parities (data not shown).

Table 2.7

Rumen pool sizes immediately before fasting and their fractional clearance rates of dairy cows fed different carbohydrate sources during early (experiment 1) and late (experiment 2) lactation.

	Treatment[1]						Lactation stage			P-value	
Variable[2]	RGS	CSC	CON	CNS	RCS	SEM	early	late	SEM	Treatment	Lactation stage
RDM (kg)	17.0^{ab}	17.9^{b}	16.7^{ab}	16.1^{ab}	14.5^{a}	1.42	18.5	14.7	1.31	0.035	<0.001
ROM (kg)	15.3	16.3	15.2	14.8	13.4	1.32	16.9	13.3	1.21	0.054	<0.001
RCP (kg)	3.7^{c}	3.4^{bc}	3.2^{bc}	3.0^{b}	2.3^{a}	0.28	3.4	2.9	0.26	<0.001	0.001
RNDF (kg)	8.0	9.2	8.6	8.4	8.3	0.75	9.9	7.2	0.70	0.163	<0.001
RADL (kg)	0.8^{ab}	0.9^{b}	0.8^{ab}	0.8^{ab}	0.7^{a}	0.08	0.9	0.7	0.07	0.025	<0.001
Kcl$_{DM}$ (h^{-1})	5.4	5.7	5.4	5.2	4.9	0.42	4.7	5.9	0.39	0.089	<0.001
Kcl$_{OM}$ (h^{-1})	5.5	5.7	5.4	5.3	5.0	0.43	4.7	6.0	0.41	0.098	<0.001
Kcl$_{CP}$ (h^{-1})	5.2	5.8	5.2	5.4	5.9	0.42	5.0	5.9	0.35	0.387	0.003
Kcl$_{NDF}$ (h^{-1})	5.0^{b}	5.1^{b}	4.7^{ab}	4.4^{ab}	3.9^{a}	0.45	3.9	5.3	0.42	0.002	<0.001
Kcl$_{ADL}$ (h^{-1})	3.6	4.1	3.6	3.3	3.5	0.42	3.2	4.0	0.38	0.093	<0.001

^{a,b,c} Means in rows with different superscripts differ ($P < 0.05$).

1 RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

2 RDM = rumen DM content; ROM = rumen OM content; RCP = rumen CP content; RNDF = rumen NDF content; RADL = rumen acid detergent lignin (ADL) content; KclDM = fractional clearance rate of DM; KclOM = fractional clearance rate of OM; KclCP = fractional clearance rate of CP; KclNDF = fractional clearance rate of NDF; KclADL = fractional clearance rate of ADL.

Rumen Variables

The effect of treatment and lactation stage on rumen pH, VFA concentration, NH_3-N and molar proportions of different VFA is presented in Table 2.6. In general, rumen variables from cows fed the RCS diet differed from the other treatments, with small differences between the latter diets. Rumen pH was lesser ($P < 0.05$), and the duration of ruminal pH < 5.8 was longer ($P < 0.01$) for RCS than the other diets. The non-glucogenic:glucogenic VFA ratio was lesser for RCS ($P < 0.05$), mainly due to the lesser molar proportion of acetate ($P < 0.01$), and greater molar proportions of propionate ($P < 0.05$) and valerate ($P < 0.05$). Rumen NH_3-N concentrations decreased when grass silage was replaced with corn silage with no effect of concentrate type. Late-lactating animals had a lesser pH ($P = 0.022$) than early lactating animals, but the duration of ruminal pH < 5.8 did not differ between early and late lactation. Total VFA ($P < 0.001$) and NH_3-N concentration ($P < 0.001$) were greater in late lactation, but VFA molar proportions did not differ, except for lesser isobutyrate proportions in late lactation than in early lactation ($P = 0.029$). Pre-fasting rumen pool sizes differed ($P < 0.05$) between

treatments, except the pool size of NDF and OM (Table 2.7). Rumen fractional clearance rates were similar between treatments, except for the rumen Kcl NDF that was lesser in RCS than in RGS and CSC. Rumen pool sizes were lesser ($P < 0.01$), and fractional clearance rates were greater ($P < 0.05$) in late lactation as compared with early lactation.

Table 2.8

Estimates of organic matter (OM), nitrogen (N), neutral detergent fiber (NDF) and starch degradation characteristics of the different silages and concentrates used during late lactation.

	Individual feedstuffs				Total Mixed Rations (TMR)[1]				
Variable[2]	Grass silage	Corn silage	NDF concentrate	Starch concentrate	RGS	CSC	CON	CNS	RCS
U_{OM} (%)	13.0	17.3	4.6	5.4					
W_{OM} (%)	36.4	45.7	39.1	46.2					
D_{OM} (%)	50.6	37.0	56.3	48.4					
kd_{OM} (h^{-1})	0.032	0.017	0.041	0.043					
ED_{OM} (%)	57.4	55.9	61.9	66.6	60.5	59.0	60.0	61.1	59.6
U_N (%)	9.4	18.0	5.1	3.8					
W_N (%)	68.4	70.4	46.7	34.3					
D_N (%)	22.2	11.6	48.3	61.9					
kd_N (h^{-1})	0.048	0.009	0.035	0.029					
ED_N (%)	79.8	72.3	64.3	54.5	71.2	70.8	68.3	65.8	63.6
U_{NDF} (%)	13.6	32.3	10.4	26.8					
D_{NDF} (%)	86.4	67.7	89.6	73.2					
kd_{NDF} (h^{-1})	0.028	0.010	0.040	0.036					
ED_{NDF} (%)	33.0	12.7	36.0	27.6	33.1	28.7	26.8	24.5	20.0
W_{Starch} (%)	nd[3]	79.3	20.6	46.8					
D_{Starch} (%)	nd[3]	20.7	79.4	53.2					
kd_{Starch} (h^{-1})	nd[3]	0.047	0.023	0.063					
ED_{Starch} (%)	nd[3]	89.9	42.4	74.1	73.3[4]	88.2[4]	82.9[4]	80.6[4]	85.5[4]

[a,b,c] Means in rows with unlike superscripts differ ($P < 0.05$).

[1] RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

[2] U = Undegradable fraction; W = washable fraction; D = potential degradable fraction; kd = fractional degradation rate; ED = effective degradability [ED = W + (kd/(kd + kp) * D)], where kp was assumed 0.045/h for silages and 0.060/h for concentrates (Tamminga, 1994). Effective degradability of TMR was calculated from the ED of individual feedstuffs, corrected for the concentration of each chemical component.

[3] Not determined.

[4] Assuming starch content of grass silage is zero.

Estimates of degradation characteristics of silages and concentrates fed during experiment 2 are presented in Table 2.8. Effective degradability of N and NDF of the different treatments decreased, whereas the ED of starch increased and ED of OM (EDOM) was similar when concentrations of corn silage in the TMR increased. Similar results were found with increasing concentrations of starch concentrate, although the ED of starch decreased and EDOM increased slightly. Also, the differences were smaller than when silage components were changed in the TMR.

Milk Production and Composition

Milk and FPCM yield were similar between treatments (Table 2.9). Fat content was lesser in RCS than in RGS, CSC, and CON ($P < 0.05$). The effect of dietary treatment on milk protein content was dependent of lactation stage (treatment × lactation stage interaction, $P = 0.012$). In early lactation, protein content in RCS was greater than in RGS ($P < 0.001$), whereas type of carbohydrate in the concentrate had no effect. In late lactation, milk protein content was similar between treatments. Milk- and FPCM production was greater in early lactation than in late lactation ($P < 0.001$). In early lactation, milk fat ($P < 0.001$) and milk protein content ($P < 0.001$) was lesser and milk lactose content greater ($P < 0.001$) than in late lactation. Milk urea was also lesser ($P < 0.001$) in early than in late lactation.

Table 2.9

Milk yield and composition of dairy cows fed different carbohydrate sources during early (experiment 1) and late (experiment 2) lactation.

	Treatment[1]						Lactation stage			P-value		
Variable	RGS	CSC	CON	CNS	RCS	SEM	early	late	SEM	Treatment	Lactation stage	T × L[2]
Milk yield												
Milk (kg/d)	24.1	25.1	25.6	25.5	26.7	1.41	31.5	19.1	1.27	0.391	<0.001	0.760
FPCM[3] (kg/d)	24.1	25.2	25.3	25.3	24.7	1.41	30.1	19.6	1.21	0.891	<0.001	0.803
Milk composition												
Fat (%)	4.10^{b}	4.08^{b}	3.89^{b}	3.87^{ab}	3.55^{a}	0.165	3.63	4.18	0.152	<0.001	<0.001	0.525
Protein (%)	3.45^{a}	3.50^{ab}	3.50^{ab}	3.58^{b}	3.53^{b}	0.067	3.29	3.74	0.064	0.004	<0.001	0.012
Lactose (%)	4.49^{a}	4.54^{ab}	4.58^{b}	4.57^{b}	4.56^{b}	0.029	4.64	4.46	0.027	<0.001	<0.001	0.135
Urea (mg/dL)	33.2^{d}	28.2^{c}	26.7^{bc}	26.2^{b}	21.9^{a}	0.61	25.1	29.1	0.51	<0.001	<0.001	0.307
Amount												
Fat (g/d)	966	998	981	966	896	61.8	1139	780	52.4	0.565	<0.001	0.801
Protein (g/d)	807	854	877	900	906	46.4	1034	699	39.8	0.163	<0.001	0.443
Lactose (g/d)	1080	1152	1179	1183	1209	66.3	1468	844	57.9	0.280	<0.001	0.651

[a,b,c] Means in rows with unlike superscripts differ ($P < 0.05$).

[1] RGS = TMR with 55% grass silage and 45% of the concentrate mixture; CSC = TMR with 55% of the roughage mixture and 45% of the concentrate rich in structural carbohydrates; CON = TMR with 55% of the roughage mixture and 45% of the concentrate mixture; CNS = TMR with 55% of the roughage mixture and 45% of the concentrate rich in nonstructural carbohydrates; RCS = TMR with 45 % of the concentrate mixture and 55% corn silage.

[2] Treatment × Lactation stage interaction.

[3] FPCM = fat- and protein-corrected milk.

DISCUSSION

Meal Criteria Estimation

In recent literature on feed intake behavior in dairy cows, the intake pattern is separated into bouts or meals, based on frequency distributions of interval lengths between feeding events. From such distributions, the meal criterion is estimated using log-transformations (Tolkamp et al., 1998; Yeates et al., 2001). The log-transformed intervals between visits to feeding bins used in these models are in better agreement with the satiety concept, implying that the initiation of a meal is not independent of the time since the last meal (Tolkamp and Kyriazakis, 1999).

When estimating the meal criteria, several factors need to be considered. The first choice is that of the model to fit the data. Estimation of meal criteria is done both with two population models [Gaussian-Gaussian (DeVries et al., 2003; Huzzey et al., 2005) and Gaussian-Weibull (Melin et al., (2005)] and three population models [Gaussian-Gaussian-Weibull, GGW; Yeates et al., 2001; Melin et al., 2005]. The choice between the different models in the current experiment was carried out according to Melin et al. (2005), using the minimum function value described by Yeates et al. (2001). In agreement with results reported by Yeates et al. (2001) and Melin et al. (2005), the GGW model was found to fit the data best. Indeed, Weibull distributions are believed to be in better agreement with the satiety concept, because the probability of cows initiating a new meal is expected to increase with time since the last meal (Yeates et al., 2001).

The second choice is to decide which meal criteria to use for grouping visits to the feeding bins into meals. Because in the present study, significant effects of lactation stage and treatment x lactation stage interactions occurred, meal criteria per treatment-lactation stage combination were used to pool intake data. No consistent effect of treatment on meal criteria estimation was found, similar to findings of Tolkamp et al. (2002), who tested if the ratio of concentrate to grass silage influenced meal patterns in dairy cows. Also Melin et al. (2005) found no differences in estimated meal criteria between cows milked 3 times daily vs. 6 times daily, when using the GGW model.

In our study, the effect of cow on meal criterion was not significant. De Vries et al. (2003) found no differences in intake behavior variables when a meal criterion was used estimated from intake data from all animals within periods or estimated from intake data per animal per period, and hence used one meal criterion based on pooled data. However, Huzzey et al. (2005) found differences between these two methods and used meal criteria for individual cows. To test with a similar approach if the statistical method applied in this experiment resulted in satisfactory results, the meal criterion estimated using data of all animals per treatment-lactation stage combination (pooled data, Mp) or using individual animal data (Mi) was used to calculate the number of meals per treatment. Both meal criteria resulted in similar numbers of meals per treatment [Mi = 0.95 (± 0.02) × Mp + 0.27 (± 0.13); r2 = 0.85], although in the current experiment, in line with Huzzey et al. (2005), a significant relationship between meals calculated based on individual or based on combined meal criteria was observed; Huzzey et al. (2005) found a consistent deviation between both meal frequencies in the postcalving period. Thus significant relationship does not guarantee that the various short intake variables do not differ between both meal criteria estimation methods. This is another reason to rather use a statistical test to determine effects on the meal criteria estimation and decide which meal criteria to use to calculate feed intake variables. The present statistical test enables the estimation of meal criteria based on the contribution of various sources of variation (animal, treatment, lactation stage).

Lactation Stage and Different Silages

In contrast to our hypotheses, lactation stage significantly affected several feed intake behavior variables. When comparing both experiments described in the present study, the effect of lactation stage is confounded with the use of different silages and concentrate batches in early and late lactation. Such confounding effects occur regularly when testing lactation stage effects (e.g., Kertz et al., 1991). Confounding effects of the animals used were limited, because all cows except one were used in early and in late lactation. Because the composition of the grass silage differed to a much larger extent than that of concentrates or corn silage (Table 2.3), potential confounding effects between lactation stage and feeds between experiments are expected to be caused mainly by grass silage. Storage of silage used in early lactation to be used also in later lactation was not feasible due to practical constraints at the research farm. However, even within silages over time, during storage of corn silage, the degradability of starch increases (Newbold et al., 2006), indicating that storage of silages between experiments would not have ensured similar availabilities of nutrients to the animals. Treatment RGS (with grass silage as the largest component of the diet) indeed showed the greatest differences between early and late lactation in eating time and number of meals (Figure 2.1). Moreover, the interaction between treatment and lactation stage in visits per meal and IR per meal indicate a disturbing effect of grass silage between early and late lactation.

There was a tendency for an interaction between treatment and lactation stage in DMI per day. Intake was lesser in RGS than in all other treatments in early and late lactation, but the numerical difference between RGS and the other treatments was smaller in late lactation than in early lactation (2.0 vs. 2.7 kg/d, respectively). This larger difference in intake between RGS and the other treatments in early lactation coincided with greater DM, NDF, and ADL contents of grass silage in early compared to late lactation. Indeed, DMI is expected to be lesser when silages are greater in NDF (Allen, 2000). In addition, a greater ADL content of silage decreases the digestibility of the diet, decreasing fractional passage rate and DMI through effects on rumen fill (Jung and Allen, 1995).

Even under the hypothesis that grass silage did influence the experimental results, from Figure 2.1 it is clear that there were also differences in feed intake behavior due to lactation stage. Lactation stage significantly affected all intake parameters studied (Table 2.5), even for the diet without any grass silage (diet RCS; Figure 2.1). Such effects of lactation stage on feed intake behavior have only been investigated to a limited extent. Friggens et al. (1998), when studying feed intake on a complete lactation in 20 cows, found a gradual decrease in DMI as the lactation progressed, after a steep increase at the start of the lactation. In line with the present results, the time spent eating was decreased with lactation stage. In contrast, De Vries et al. (2003) found an increase in time spent eating, only between period 1 (35 DIM) and period 2 (57 DIM) but not in periods after peak lactation. Moreover, in line with our results, De Vries et al. (2003) observed a greater number of meals per day in late lactating cattle than in early lactating cattle.

To investigate net effect of feed intake behavior without confounding effects of nutrient composition, digestibility, and particle size of the diet, it would be necessary to impose a specific feed intake behavior regime on animals fed similar diets. An attempt to do so could be carried out using automatic feed bins allowing cows to eat only during certain times combined with registration of the actual intake and time.

Feed Intake Behavior in Different Treatments

Effects of NDF and starch in the diet on short term feed intake behavior in ruminants have been studied before (Abijaoudé et al., 2000; Allen, 2000; Miron et al., 2004). However, a comparison of such effects upon changing the type of silage or the type of concentrate using the same concentrate:silage ratio within a single experiment has to our knowledge not been reported in line with our hypothesis, the present results indicate that changing the composition of silage in the TMR has a larger effect on short-term feed intake behavior than changing the concentrate composition (Table 2.5). This may be related to the larger differences in NDF and starch content of the TMR between RGS and RCS than between CSC and CNS (Table 2.4), as well as to the differences in particle size between both silages (Allen, 2000) that are not apparent in the pelleted concentrates.

Although effects of forage cell-wall constituents on voluntary DMI have been frequently studied (e.g. Allen, 2000), experimental evidence on their effects on short term feed intake behavior is limited. Dado and Allen (1995) studied the effects of a low-fiber diet (NDF 25% of DM) vs. a high-fiber diet (NDF 35% of DM) and found that eating time and ruminating time were increased in the latter treatment. However, in their experiment, the concentrate:forage ratio differed between treatments. Dulphy et al. (1980) reviewed effects of fresh cut forage cell-wall constituents on feeding behavior of wethers. Greater amounts of cell-wall constituents decreased IR and eating time at the expense of ruminating time, and also decreased the number of meals. Although in the present experiment diet did not affect the number of meals, the decreased IR at greater NDF and lesser starch contents between RGS, CON, and RCS, as well as the resulting longer meal duration, were in line with the results Deswysen et al. (1993) found with heifers fed grass and corn silage, even though the NDF content of grass and corn silage was lesser in the current experiment (grass silage 54.4 and 47.2% and corn silage 42.1 and 37.5% in early and late lactation, respectively) than in the experiment by Deswysen et al. (1993; 63 and 50%, respectively). The ratio between NDF content in grass silage vs. corn silage was similar between both experiments, varying between 26 and 29% greater NDF contents in grass silage than in corn silage.

The effects on short-term feed intake behavior variables were in line with a decreased DMI of the RGS diet vs. the other treatments, probably related to the greater NDF content. Nevertheless, in spite of the similar NDF content of RGS and CSC, DMI was not decreased in CSC compared to CON and CNS. The reason for this is not clear, beacause both the relative amounts of ADL and ADF in NDF, rumen Kcl of NDF and ED of NDF degradation characteristics were similar. Apparently, the way of distribution of NDF (through silage or concentrates) affects the effects on short-term feed intake behavior. This could be due to effects on particle size, as shown with chopped alfalfa silage by Beauchemin et al. (1994), although Allen (2000) only found significant effects of chop length on DMI in 3 out of 20 comparisons.

Miron et al. (2004) studied the effects of replacing a concentrate high in starch by a concentrate high in NDF on short-term feed intake behavior. Concentrates were fed separately from a TMR in 3 meals per day. Meal duration was not affected by concentrate type, but eating time and number of meals per day were lesser whereas meal size was greater with the high-starch concentrate. Dry matter intake was lesser on the high-starch diet than on the high-NDF diet, which is mainly attributed to greater NDF *in vitro* digestibility in the high NDF-concentrate and potential larger in vivo effects related to a lesser pH with the high-starch diet (Miron et al., 2004). In the present experiment, eating time (per day) and IR (per meal) differed between CSC and CNS, but DMI did not differ. This indicates that manipulating intake behavior does not necessarily lead to changes in DMI.

Rumen Fermentation

The TMR high in starch (RCS and CNS) were expected to have a lesser rumen pH than TMR high in NDF (RGS and CSC). However, effective OM degradability was hardly different between these treatments (Table 2.8). The DMI on the RGS treatment was lesser than on the RCS treatment (Table 2.5). Thus, although ED did not differ, the total supply of fermentable nutrients with RGS was lesser than with RCS, explaining the differences in pH and in the time pH below 5.8 (Table 2.6). Moreover, IR per meal was greater in RCS than in RGS, also contributing to the difference in the time that pH was below 5.8. In contrast, there were no differences in DMI or IR between CSC and CNS, and given the similar effective OM degradability values, no differences in pH between CSC and CNS were observed.

Between TMR differing in concentrate component (CNS vs. CSC), only numerical differences were found in time pH of rumen fluid was below 5.8 and in average pH of rumen fluid (Table 2.6). This was in line with the absence of a difference in DMI (Table 2.5) and OM content of the two TMR (Table 2.4) and only very small numerical effects on EDOM (Table 2.8) between CNS and CSC.

Although pool size ROM in late lactation was more than 20% less than in early lactation (Table 2.7), rumen fluid VFA concentration was almost 20% greater in late lactation (Table 2.6). This was not due to a smaller rumen fluid volume during late lactation, because DM content of rumen contents was 18.6% in early lactation and 17.2% in late lactation (data not shown). These results indicate that OM of the TMR in late lactation was fermented faster than in early lactation. From Table 2.7, it is clear that the Kcl of OM in late lactation is both the result of greater passage rate of material from the rumen, indicated by the Kcl of ADL, and from a greater fractional degradation rate of OM. Although no data are available on fractional degradation rate of OM in early lactation, faster fermentation of OM in the rumen may have increased VFA production rate to a greater extent than VFA absorption and passage rates. The absorption of VFA depends on the concentration of VFA in the rumen fluid, with in general an increase in the fractional absorption rate when pH decreases as VFA concentration in rumen fluid increases (Lopez et al., 2003), although an interaction between pH and VFA concentration has been observed (Dijkstra et al., 1993). Indicative of the build-up of the concentration of VFA in rumen fluid was the concentration of VFA in the rumen fluid at the start of the rumen evacuations: there was a difference between late lactation (140 m*M*) and early lactation (125 m*M*, $P = 0.006$).

Milk Production

The replacement of NDF with starch in feed is expected to decrease the molar proportion of acetate and increase that of propionate (Bannink et al., 2006). The increase in NDF when the amount of grass silage in the TMR was increased coincided with an increase in the concentration of acetate in rumen fluid. The decreased milk fat content in RCS (Table 2.9) is directly related to the lesser acetate:propionate ratio and lesser pH in the rumen (Table 2.6), and both have been related to a depression of milk fat content, albeit through different mechanisms (Bauman and Griinari, 2003).

In early lactation, milk protein content was greater in RCS than in RGS, whereas intake of CP (calculated from DMI and CP content of the TMR) was lesser in RCS than in RGS. In both treatments the DVE requirements were greater than DVE intake in early lactation (DVE intake/ DVE requirements were less than 1 in both treatments, data not shown). The energetically more efficient microbial protein synthesis from corn silage than from grass silage (Givens and Rulquin, 2004) might explain the increase in milk protein with RCS. In late lactation,

RCS did not result in an increase in milk protein, because intake of DVE was greater than the requirements (DVE intake/DVE requirements were 1.51 in RGS and 1.48 in RCS) in both treatments. In late lactation, milk protein was therefore more likely limited by the genetic potential for milk protein.

CONCLUSIONS

This study confirmed that short-term intake behavior is related to DMI and milk production but also showed that other factors also play a role. The influence of type of roughage on short-term intake behavior was stronger than that off the type of concentrate.

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REFERENCES

Abijaoudé, J. A., P. Morand-Fehr, J. Tessier, P. Schmidely, and D. Sauvant. 2000. Diet effect on the daily feeding behaviour, frequency and characteristics of meals in dairy goats. Livestock Production Science 64: 29-37.

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91: 2033-2045.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83: 1598-1624.

Bannink A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. Journal of Theoretical Biology 238: 36-51.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Reviews of Nutrition 23: 203-227.

Beauchemin, K. A. 1991. Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. Journal of Dairy Science 74: 3140-3151.

Beauchemin, K. A., B. I. Farr, L. M. Rode, and G. B. Schaalje. 1994. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. Journal of Dairy Science 77: 1326-1339.

Bruining, M., R. Bakker, J. van Bruchem, and S. Tamminga. 1998. Rumen digesta kinetics in dairy cows fed grass, maize and alfalfa silage: 1. Comparison of conventional, steady-state and dynamic methods to estimate microbial degradation, comminution and passage of particles. Animal Feed Science and Technology 73: 37-58.

Coppock, C. E., C. H. Noller, and S. A. Wolfe. 1974. Effect of forage-concentrate ratio in complete feeds fed ad libitum on energy intake in relation to requirements by dairy cows. Journal of Dairy Science 57: 1371-1380.

Dado, R. G., and M. S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. Journal of Dairy Science 77: 132-144.

Dado, R. G., and M. S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. Journal of Dairy Science 78: 118-133.

Deswysen, A. G., P. Dutilleul, J. P. Godfrin, and W. C. Ellis. 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: Analysis by finite fourier transform. Journal of Animal Science 71: 2739-2747.

De Vries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. Journal of Dairy Science 86: 3354-3361.

De Vries, T. J., M. A. G. von Keyserlingk, and D. M. Weary. 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behavior of free-stall housed lactating dairy cows. Journal of Dairy Science 87: 1432-1438.

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, ph and rumen liquid volume. British Journal of Nutrition 69: 385-396.

Dulphy, J. P., B. Remond, and M. Theriez. 1980. Ingestive behaviour and related activities in ruminants. Pages 103-122 in Digestive Physiology and Metabolism in Ruminants. Y. Ruckebush and P. Thivend, ed. AVI Publishing Co., Westport, CT.

Friggens, N. C., B. L. Nielsen, I. Kyriazakis, B. J. Tolkamp, and G. C. Emmans. 1998. Effects of feed composition and stage of lactation on the short-term feeding behavior of dairy cows. Journal of Dairy Science 81: 3268-3277.

Givens, D. I., and H. Rulquin. 2004. Utilisation by ruminants of nitrogen compounds in silage-based diets. Animal Feed Science and Technology 114: 1-18.

Grant, R. J., and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. Journal of Animal Science. 73: 2791-2803.

Huzzey, J. M., M. A. G. von Keyserlingk, and D. M. Weary. 2005. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. Journal of Dairy Science 88: 2454-2461.

ISO. 2004. Animal Feeding Stuffs - Enzymatic determination of total starch content. ISO 15914:2004. International Organization for Standardization, Geneva, Switzerland.

Jung, H. G., and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science 73: 2774-2790.

Kertz, A. F., L. F. Reutzel, and G. M. Thomson. 1991. Dry matter intake from parturition to midlactation. Journal of Dairy Science 74: 2290-2295.

Kolver, E. S., and M. J. de Veth. 2002. Prediction of ruminal ph from pasture-based diets. Journal of Dairy Science 85: 1255-1266.
Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using sas procedures. Journal of Animal Science 76: 1216-1231.
Lopez, S., F. D. D. Hovell, J. Dijkstra, and J. France. 2003. Effects of volatile fatty acid supply on their absorption and on water kinetics in the rumen of sheep sustained by intragastric infusions. Journal of Animal Science 81: 2609-2616.
Melin, M., H. Wiktorsson, and L. Norell. 2005. Analysis of feeding and drinking patterns of dairy cows in two cow traffic situations in automatic milking systems. Journal of Dairy Science 88: 71-85.
Miron, J., E. Yosef, M. Nikbachat, A. Zenou, E. Maltz, I. Halachmi, and D. Ben-Ghedalia. 2004. Feeding behavior and performance of dairy cows fed pelleted nonroughage fiber byproducts. Journal of Dairy Science 87: 1372-1379.
Newbold, J. R., E. A. Lewis, J. Lavrijssen, H. J. Brand, H. Vedder, and J. Bakker. 2006. Effect of storage time on ruminal starch degradability in corn silage. Journal of Animal Science 84 (Suppl. 1): 190.
Oldenbroek, J. K. 1984. Holstein friesians, dutch friesians and dutch red and whites on two complete diets with a different amount of roughage: Performance in first lactation. Livestock Production Science 11: 401-415.
Robinson, P. H., S. Tamminga, and A. M. Van Vuuren. 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. Livestock Production Science 15: 173-189.
Tamminga, S., W. M. Van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The dutch protein evaluation system: The DVE/OEB-system. Livestock Production Science 40: 139-155.
Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. Animal Feed Science and Technology 131: 102-119.
Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science 87: 3417-3427.
Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121: 243-256.
Tempelman, R. J. 2004. Experimental design and statistical methods for classical and bioequivalence hypothesis testing with an application to dairy nutrition studies. Journal of Animal Science 82: E162-172.
Tolkamp, B. J., D. J. Allcroft, E. J. Austin, B. L. Nielsen, and I. Kyriazakis. 1998. Satiety splits feeding behaviour into bouts. Journal Theoretical Biology 194: 235-250.
Tolkamp, B. J., N. C. Friggens, G. C. Emmans, I. Kyriazakis, and J. D. Oldham. 2002. Meal patterns of dairy cows consuming mixed foods with a high or a low ratio of concentrate to grass silage. Animal Science 74: 369-382.
Tolkamp, B. J., and I. Kyriazakis. 1999. To split behaviour into bouts, log-transform the intervals. Animal Behavior 57: 807-817.
Van Es, A. J. H. 1975. Feed evaluation for dairy cows. Livestock Production Science 2: 95-107.

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, S. Tamminga and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reproduction, Nutrition and Development 45: 665-688.

Yeates, M. P., B. J. Tolkamp, D. J. Allcroft, and I. Kyriazakis. 2001. The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. Journal of Theoretical Biology 213: 413-425.

CHAPTER 3

Frequent Allocation of Rotationally Grazed Dairy Cows Changes Grazing Behavior and Improves Productivity

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ABSTRACT

Twenty Holstein cows were blocked in 2 groups according to milk yield to evaluate the effect of frequency of allocation to new grazing plots on pasture intake, grazing behavior, rumen characteristics, and milk yield. The 2 treatments, daily allocation to 0.125-ha plots (1D) or allocation every four days to 0.5 ha plots (4D) of *Lolium perenne* L., were tested in a randomized block design (2 rotations with 3 or 4 measuring periods of 4 d each) with mixed model analysis accounting for repeated measures. There were no differences in the chemical composition of offered pasture on offer and in pasture dry matter intake (DMI) between 1D and 4D. However, an interaction between treatment and rotation indicated a difference in pasture DMI between treatments during the first rotation (4D, 16.5 vs. 1D, 18.3 kg/d) but not during the second rotation (4D, 15.0 vs. 1D, 14.7 kg/d), possibly a result of a greater pasture mass in the first rotation. Grazing time (average 562 min/d) and ruminating time (average 468 min/d), observed using IGER graze recorders, were similar between treatments, but grazing time increased numerically (549 to 568 min/d), and ruminating time decreased linearly (471 to 450 min/d) within periods in the 4D treatment. Mean rumen pH (6.16 vs. 6.05) and rumen NH_3-N concentration (113.7 vs. 90.1 mg/L) were higher in 4D than in 1D, and total volatile fatty acids (VFA) concentrations did not differ. Molar proportions of VFA, except butyrate, differed between treatments, causing the non-glucogenic to glucogenic VFA ratio to be greater in 4D than in 1D. Within days in the 4D treatment, the molar proportion of acetate increased and those of all other VFA decreased linearly. Rumen NH_3-N concentration within the 4D treatment declined quadratically from 170.3 mg/L on d 1 to 80.7 mg/L on d 4. In contrast to rumen NH_3-N concentration, milk urea content did not differ between treatments, but decreased quadratically from d1 to d4 in the 4D treatment (from 26.7 to 20.7 mg/dL). Mean fat- and protein-corrected milk was greater in 1D than in 4D (23.5 vs. 22.8 kg/d), mainly due to a difference in milk yield (24.5 vs. 23.7 kg/d). Fat and protein content were slightly lower in the 1D treatment than in the 4D treatment (3.66 vs. 3.76% and 3.28 vs. 3.34%, respectively). This study confirmed that increasing pasture allocation frequency from once every 4 d to every day improved milk production in grazing dairy cows, especially when offered pasture was high.

INTRODUCTION

In grazing systems, pasture DMI is often insufficient to achieve high milk yield (Bargo et al., 2003). Grazing management may influence grazing behavior and thus influence pasture DMI. Grazing is defined as 'the taking of a succession of bites from the surface of the sward, with the depth of each bite into the sward being influenced by the vertical distribution of sward characteristics such as live and dead material and of morphological components such as leaves and pseudostems' (Illius and Gordon, 1987). Pasture DMI of grazing cows is the product of total grazing time (GT, min/d) and intake rate (IR, g/min of grazing), with IR the product of bite rate (BR, bites/min) and bite mass (BM, g of DM/bite). Important factors influencing BM in grazing cows are sward surface height (SSH) and the mass of pasture (PM) offered (Allden and Whittaker, 1970). The effect of SSH on BM is curvilinear, with successively smaller increments in BM for each increment in SSH (Chilibroste, 2005).

Pasture DMI is related to the chemical composition of pasture, which is affected by, among others, days of regrowth, season, and time of day. Pasture DMI increased when a high-sugar

and low-NDF ryegrass cultivar was offered to zero-grazed cows in early lactation (Moorby et al., 2006). In contrast, cultivars with an elevated water-soluble carbohydrate content did not consistently result in greater pasture DMI in grazing dairy cattle (Tas et al., 2006) or in zero-grazed dairy cattle (Miller et al., 2001; Taweel et al., 2005).

Variation in chemical composition of pasture also occurs within the vertical distribution of pasture offered to cows as the proportion of lamina material decreases, and the proportion of stem and dead material increases as the animal grazes progressively down through the sward. Delagarde et al. (2000) showed an enrichment of DM and structural carbohydrates in the lower layers of the sward whereas the upper layers were enriched in CP and sugar. Thus, due to modified morphology and chemical composition, successive defoliations of the same area will result in a reduction of BM, IR, and CP and sugar content, but an increase in structural carbohydrate content of the material ingested. Such differences in pasture composition may be involved in the control of short-term daily pasture intake through effects on the concentrations of fermentation end products, rumen fill, and clearance rate of feed from the rumen, as these have been suggested as a combination of signals that control intake (Forbes, 1996). Indeed, an increased GT and decreased BM on short swards as compared with longer swards, as well as increased rumen fill and increased pool sizes of fermentation end products, were found after longer regrowth of pasture (Chilibroste et al., 2000). Under a continuous stocking system, BR, chewing rate and BM were increased as the day progressed, showing the potential to maximize intake through intake behavior, whereas rumen fermentation end products did not play an important role in initiating or terminating a grazing bout (Taweel et al., 2004).

Different rotational grazing strategies might result in changes in grazing behavior by affecting the chemical and morphological characteristics of the sward within and between days. It has been postulated that these rotational grazing systems result in a more efficient utilization of grassland, increase pasture DMI, and enhance productivity (Holecheck et al., 1995). In various countries, rotational grazing involves access of cows to fresh pasture once or twice daily to benefit from assumed increased productivity on fresh pasture. In other rotational grazing systems, fresh pasture is provided after several days to save on labor and fencing costs. However, scientific evidence on the effects of allocation frequency is scarce, and Parsons and Chapman (2000) even argued that effects of differences in grazing management on production are more imagined than real. Daily strip grazing resulted in greater milk yield per hectare compared with paddock grazing, although there were no differences in milk yield per cow per day (Kuusela and Khalili, 2002). In contrast, Dalley et al. (2001) found a decrease in milk yield for frequently allocated cows (6 times daily vs. once daily) despite the numerically greater pasture DMI.

The objective of this experiment was to determine the influence of 2 grazing systems (once daily vs. once every 4 d reallocating to a fresh plot) on pasture DMI, grazing behavior, rumen characteristics, and milk yield in grazing dairy cows.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was undertaken between July 6 and August 9, 2004, after approval by the Institutional Animal Care and Use Committee of Wageningen University. The study was conducted as paired comparisons in a randomized block design with repeated measures. After adaptation to grazing during 3 wk, 2 groups of 10 dairy cows were assigned to their

respective treatments and adapted to these treatments during 4 d. The treatments, reallocation after morning milking every day (1D) to a fresh 0.125-ha plot or every 4 days to a 0.5-ha plot (4D), were repeated during 2 rotations with 4 periods of 4 d in the first rotation and 3 periods of 4 d in the second rotation. Water was available *ad libitum*.

Pasture

A uniform stand of perennial ryegrass (*Lolium perenne* L., cultivar Havera, 70:30 mixture of tetraploid and diploid), established in August 2003, was used during the experiment. The fertilizer application rates were 78 kg of N/ha in spring, 52 kg of N/ha before the first rotation, and 54 kg of N/ha before the second rotation. The paddock was divided into 10 plots of 0.5 ha which were stepwise cut (2 plots every 4 d). For treatment 1D, half of the plots were then split into 4 sub plots of 0.125 ha. This procedure was carried out to have approximately equal DM offered per day after 25 d of regrowth preceding the first rotation, and 18 d of regrowth preceding the second rotation.

Pasture mass offered was estimated using a method comparable to the double sample technique described by Chilibroste et al. (2000). Sward surface height was measured in 25 quadrants (0.5 × 0.5 m) with a rising plate meter (weight: 350 g, diameter: 0.5 m, standing pressure ca. 17.5 N/m2; Eijkelkamp, Giesbeek, the Netherlands), and PM above 4 cm from ground level was determined. The r2 of the regression of PM against SSH was 0.52 and 0.92 for the first and second rotation, respectively. Pasture mass per day was calculated using approximately 20 and 40 SSH measurements per milking for 1D and 4D, respectively.

Animals

Twenty Holstein cows, of which 6 were previously fitted with a rumen cannula (10-cm i.d.; Bar

Table 3.1

Ingredient and chemical composition of the concentrate.

Item	
Ingredient, g/kg	
Barley	15.0
Corn	23.2
Beet pulp	22.0
Soya hulls	19.0
Soya	7.0
Coconut expeller	5.0
Molasses	6.0
Premix vitamin/mineral	1.5
Sodium chloride	0.2
Calcium carbonate	0.6
Magnesium oxide 85%	0.2
Alkane + arbocel mix	0.4
Chemical composition, g/kg DM	
DM, g/kg	906
OM	928
CP	144
Crude fat	15
Sugars	112
Starch	223
NDF	280
ADF	188
ADL[1]	12
NE_L[2,3],MJ/kg of DM	7.5
DVE[3,4]	107
OEB[3,5]	-18

[1] Acid detergent lignin.
[2] Net energy for lactation calculated with VEM system (Van Es, 1975).
[3] Provided by the feed manufacturer (Research Diet Services, Wijk bij Duurstede, The Netherlands).
[4] Intestinal digestible protein (Tamminga et al., 1994).
[5] Degraded protein balance (Tamminga et al., 1994).

Diamond Inc., Parma, ID) in the dorsal sac, were paired by parity, DIM, and milk yield during the adaptation period and randomly assigned to the treatments. Rumen cannulated animals were paired and assigned similarly. At the start of the experiment, cows produced 22.4 ± 0.6 kg of milk/d (values expressed as means ± SE), and were 178 ± 12 DIM, BW was 533 ± 14 kg, and BCS was 2.5 ± 0.1 (recorded on a 5-point scale). These variables were similar between treatments.

Cows were milked twice daily at 0600 and 1600 h using a mobile milking parlor and individual milk yield was recorded throughout the experiment. Individual milk samples were collected at each milking and stored no longer than 4 d in a refrigerator at 4°C using sodium azide and bronopol as preservative. Fat, protein, and lactose contents were determined according to ISO 9622 (Melkcontrolestation, Zutphen, the Netherlands; ISO, 1999c) and milk urea was determined using the pH-difference technique (ISO 14637; ISO, 2004) Fat- and protein-corrected milk (FPCM) yield (kg/d) was calculated as [0.337 + 0.116 × fat% + 0.06 × protein% × milk yield (kg/d; CVB 2007)]. Cows received a concentrate (Table 3.1) with alkanes (2.72 kg of DM/d) in 2 equal portions during milking throughout the experiment starting 14 d before the start of the experiment.

The alkane dotriacontane (C_{32}) was dissolved over cellulose powder (arbocel; 1:10) and added to the concentrate feed before pelleting. Daily alkane supplementation was 949 mg/d and concentrate orts were collected daily. Intake of concentrate (2.71 kg of DM/d) was almost complete. Pasture DMI was estimated as described by Taweel et al. (2006). In contrast to the methodology described by Taweel et al. (2006), pasture and feces were sampled twice daily around milking throughout the experiment, and 4 concentrate samples were taken. Pasture and concentrate samples were pooled per treatment per rotation while fecal samples were pooled per animal per rotation. Samples were stored at -20°C, freeze-dried, and ground to pass through a 1-mm sieve before alkane analysis.

Pasture and Concentrate Sampling

During every milking, representative samples from the pasture were randomly collected from both treatments at 4 cm above ground level and oven-dried for 24 h at 70°C. Similarly, residual pasture was randomly sampled and dried immediately after cows were allocated to a new plot. At the end of the experiment, samples were pooled into 2 samples in 1D (offer and residual) and 5 samples in 4D (d 1, 2, 3, 4, and residual) per rotation. Also, 4 representative concentrate samples were taken, dried, and pooled per rotation. Pasture and concentrate samples were ground to pass through a 1-mm sieve and analyzed for DM, inorganic matter (ash), CP (N × 6.25), crude fat, NDF, ADF, acid detergent lignin (ADL), and sugars. The DM content was determined by drying at 103°C (ISO 6496; ISO, 1999a) and ash content was determined by combustion at 550°C (ISO 5984; ISO, 2002). The Berntrop-method (ISO 6492; ISO, 1999b) was used to determine crude fat, and N was determined according to the Kjeldahl method with CuSO4 as the catalyst (ISO 5983; ISO, 1997). Neutral detergent fiber was determined according to a modified method of Van Soest et al. (1991) with additional incubations in alpha amylase and protease as described by Goelema et al. (1998). Contents of ADF and ADL were determined according to Van Soest (1973). Sugar analysis was carried out as described by Van Vuuren et al. (1993) using a 40% ethanol solution. Modifications to this method were as follows: 1) after hydrolysis, 0.25 M K4Fe(CN)6·3H2O (Carrez I) and 0.5 M Zn(C2H3O2)2·2H2O (Carrez II) were used to clarify the solution, which was filtered (595½, 150 mm diameter, Whatman Sleicher & Schuell, Dassel, Germany); 2) dilution depended on

estimated concentrations of sugars, which were dissolved in 10-fold lower concentrations of CuSO4·5H20, Na2CO3·10H2O and neocuproine than described by Van Vuuren et al. (1993); 3) concentrations were measured using a spectrophotometer (model Du 530, Beckman Coulter, Fullerton, CA) at 460 nm and 4) concentrations were estimated by calibration using a standard curve ranging from 10 to 100 mg of glucose/L.
During 2 periods per rotation, samples from the pasture were taken during milking at 4 cm above ground level and pooled per day to determine pasture morphology. Pasture material was distinguished into leaf blade, pseudostem (split at the ligule of each leaf), stem, and dead material (all material without green color).

Grazing Behavior
Grazing behavior of 4 cows per treatment was observed using Institute of Grassland and Environmental Research (IGER) solid-state automatic behavior recorders (Ultra Sound Advice, London, UK; Rutter et al., 1997). Jaw recorders were fitted to the cows after the morning milking and removed after the next morning milking. Recordings were obtained on d 1 and 3 in 1D and every day in 4D. The data were analyzed with the Graze Data Analyses Program (version 8.0, IGER, Devon, UK), identifying jaw movements and different behaviors (grazing, ruminating, idling; Rutter, 2000). Bite mass (per cow per rotation) was calculated as pasture DMI divided by the average number of bites per day.

Rumen Measurements
During the last 3 periods of the first rotation and during all periods of the second rotation, rumen fluid samples were taken after every milking from the 6 rumen-cannulated animals. Equal amounts of rumen fluids were collected from the front and middle of the ventral sac and from the cranial sac using a solid, perforated plastic tube (85 cm long, 2.5 cm in diameter). The pH was measured immediately using an electronic pH meter (pH electrode HI 1230, Hanna Instruments B.V., IJsselstein, the Netherlands). A duplicate sample was taken, either acidified with phosphoric acid or with trichloroacetic acid, and stored at -20°C pending VFA and NH_3-N analysis, respectively, as described by Taweel et al. (2005).

Statistical Analysis
All statistical analyses were carried out by ANOVA using the PROC MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC). Multiple measurements per animal cannot be regarded as independent units of observations (Littell et al., 1998). Therefore repeated measures ANOVA was performed on all data except for pasture chemical composition, with day as the repeated subject. A first-order autoregressive covariance structure [AR(1)] fitted the data best and was used to account for within-cow variation. To determine time-dependent changes, orthogonal contrasts were used. Data are presented similarly for all variables, with treatment means for 1D and 4D and for the 4 different days per period within 4D, SEM for the treatment effects and for the effects of day within 4D, and P-values for treatment effects and linear and quadratic effects of day within 4D. The only exception is the chemical and morphological composition of the pasture offered (Table 3.2) because pasture samples were pooled per rotation for 1D. Therefore the treatment effect was tested after calculating the average composition per rotation in 4D. Differences were considered significant at a probability of $P < 0.05$, and post-hoc analyses were carried out using the Tukey test to test pairwise comparisons. When interactions were not significant ($P > 0.05$), they were excluded from the model.

Table 3.2

Chemical and morphological of pasture of dairy cows allocated every day (1D) or every four days (4D) to a new plot.

	1D		4D			*P*-value	
Variable	offer	residual	offer	residual	SEM	Treatment	Offer/residual
Chemical composition							
DM, g/kg	169.1	192.5	169.8	181.6	5.43	0.455	0.036
OM, g/kg DM	892.8	886.5	892.0	885.5	0.60	0.365	0.005
CP, g/kg DM	147.7	127.7	148.5	130.5	2.62	0.655	0.014
Crude fat, g/kg DM	28.4	22.9	27.4	24.2	0.53	0.877	0.010
NDF, g/kg DM	454.4	501.2	464.3	497.2	3.09	0.546	0.003
ADF, g/kg DM	273.3	296.8	279.8	299.9	2.12	0.205	0.005
ADL[1], g/kg DM	17.2	21.7	18.0	21.9	0.72	0.650	0.026
Sugars, g/kg DM	154.5	134.2	153.1	136.9	1.29	0.733	0.002
Morphological comp.							
Leaf, %	68.7	49.5	65.7	52.5	1.52	1.000	0.005
Pseudostem, %	21.4	35.7	22.4	31.8	1.54	0.556	0.012
Stem, %	5.7	2.9	6.3	5.4	0.60	0.178	0.123
Dead material, %	4.1	11.9	5.6	10.3	0.77	0.977	0.010

[1] ADL = Acid detergent lignin.

Pasture Morphological and Chemical Composition. The average chemical and morphological composition of offered pasture per rotation, per treatment was analyzed with treatment, rotation and sample (offer or residual) as fixed factors. Time of sampling (morning vs. evening milking) was also included in the model for pasture DM content. The interaction between sample and treatment was not significant ($P > 0.05$) and therefore excluded from the model. The effect of day on pasture chemical composition was further analyzed for the 4D treatment with rotation and day as fixed factors.

Pasture mass and SSH data were calculated per treatment per day. The statistical model included treatment, rotation, day nested within treatment, and the interaction between treatment and rotation as fixed factors. Pasture DMI was analyzed similar to the analysis on the chemical composition of pasture, although the fixed factor cow and the interaction between treatment and rotation were included in the model.

Grazing Behavior. Grazing behavior variables were analyzed per day (24 h) as well as after separating the data into the period between morning and evening milking (10 h; between 0600 and 1600 h, AM-PM) and between evening and morning milking (14 h; between 1600 and 0600 h, PM-AM). Grazing behavior was analyzed with treatment, rotation, and day nested within treatment as fixed factors using the repeated-measures procedure with day as the repeated variable. Bite mass was analyzed with rotation and treatment as fixed factors using

the repeated measures procedure with day as the repeated variable.
Rumen Fluid. The rumen fluid variables were tested with treatment, rotation, time of sampling (during morning or evening milking) and day nested within treatment as fixed variables, using the repeated-measures procedure with day as the repeated variable.

Milk Yield and Composition. Before statistical analysis, milk composition was calculated per day as the weighted average of evening and morning samples following allocation to a new plot. The model contained the fixed effects of treatment, rotation, and day nested within treatment using the repeated measures procedure, with day as the repeated variable. The mean values of the tested variables in the last 18 d of the adaptation period before cows were assigned to their respective treatment were used as a covariate in the model.

RESULTS

Pasture

Pregrazing chemical and morphological composition of the pasture was similar between treatments, but all variables except the percentage of stem were different between the offered and residual pasture (Table 3.2).

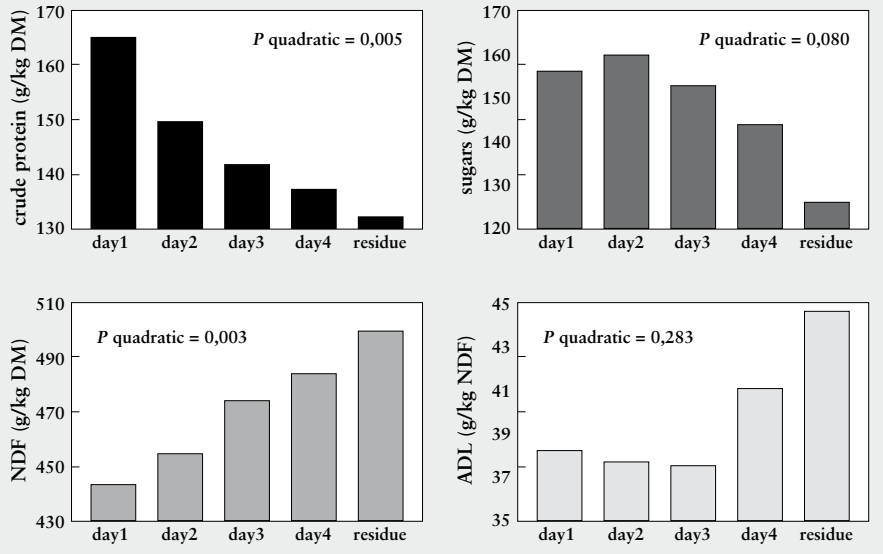

Figure 3.1

Changes in crude protein ■, sugars ■, NDF □, and acid detergent lignin (ADL) in NDF □ content in pasture over days where cows were allocated every four days to a new plot (4D treatment).

Because there was no interaction between treatment and sample (offer or residual), the differences between days in 4D were further studied. Crude protein (P = 0.005) and sugars (trend, P = 0.080) content decreased quadratically and NDF content increased quadratically (P = 0.003; Figure 3.1). The content of ADL in NDF between days in 4D showed a numerical increase on d 3 and 4. The proportion of leaves decreased quadratically during the 4 d in 4D (P = 0.005) and the fractions of pseudostem (P = 0.008) and stem and dead material (P = 0.050) increased quadratically (Figure 3.2).

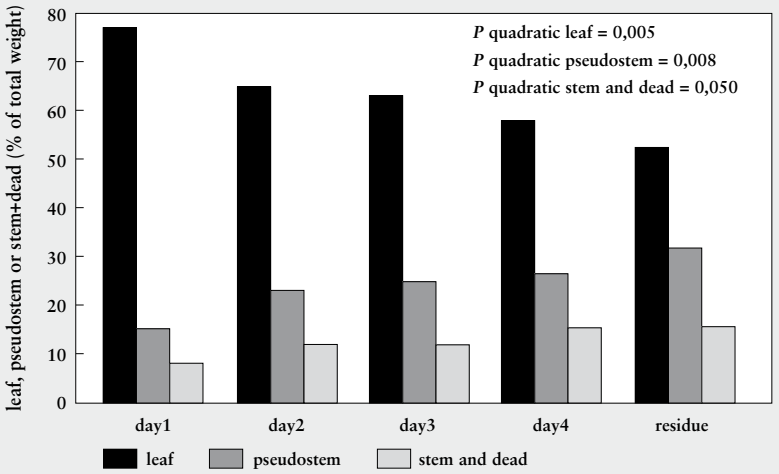

Figure 3.2

Morphological fractions of pasture when cows were allocated every four days to a new plot (4D treatment).

Sward surface height offered on d 1 (P = 1.000), PM offered on d 1 (P = 0.789), and residual SSH on d 4 (P = 0.872) were similar between treatments (results not shown). However, SSH and PM offered were greater, and residual SSH was lesser in 1D than in 4D due to quadratic decreases between days in 4D (P < 0.05; Table 3.3). Pasture DMI did not differ between treatments. Sward surface height and PM offered were greater during the first rotation than the second rotation (P < 0.001). During the first rotation offered SSH and PM were 19.7 cm and 2,834 kg of DM/ha, respectively, and during the second rotation SSH and PM on offer were 14.3 cm and 1,545 kg of DM/ha, respectively. Although pasture DMI was similar between treatments, an interaction between treatment and rotation was observed (P = 0.025), which was caused by an increase of pasture DMI in the 1D treatment during the first rotation (4D, 16.5 vs. 1D, 18.3 kg/d) but not during the second rotation (4D, 15.0 vs. 1D, 14.7 kg/d).

Table 3.3

Mean pasture mass (PM), sward surface height (SSH) on offer, residual SSH and pasture DMI of dairy cows allocated every day (1D) or every four days (4D) to a new plot.

			Days in 4D				SEM		P-value		
Variable	1D	4D	d1	d2	d3	d4	T[1]	D[2]	T[1]	D[2] linear	D[2] quadratic
SSH offer, cm	19.7	14.3	19.4	14.8	12.5	10.5	0.42	0.62	<0.001	<0.001	0.010
Residual SSH, cm	8.9	11.8	14.8	12.5	10.5	9.4	0.29	0.37	<0.001	<0.001	0.025
PM, kg DM/ha[3]	2600	1779	2485	1784	1523	1323	84.4	120.2	<0.001	<0.001	0.010
Pasture DMI, kg/d	16.5	15.8	-	-	-	-	0.65	-	0.426	-	-

[1] Treatment.
[2] Day within 4D.
[3] Pasture mass calculated based on plot sizes and using regression formulas between PM (kg DM/ha) and sward surface height (SSH; cm) per rotation: PM = A × e(B × SSH) where: First rotation: A = 739.6 (± 175.5); B = 0.066 (± 0.013); r2 = 0.52; n = 25 Second rotation: A = 483.9 (±50.9); B = 0.079 (± 0.006); r2 = 0.92; n = 25.

Grazing behavior

Grazing behavior variables were similar between d 1 in 4D and d 1 in 1D (results not shown), and similar between treatments with the exception of the number of chews ($P = 0.045$) and the chew rate per day ($P = 0.007$), which were greater in 4D than in 1D (Table 3.4). Although similar between treatments, the number of bites, BR, and chews per bolus increased and ruminating time (RT) decreased linearly ($P < 0.05$) from d 1 to 4 in the 4D treatment.

In the AM-PM period, all grazing behavior variables were similar between treatments, with the exception of BR being greater in 4D than in 1D ($P = 0.001$). Within the 4D treatment, BR increased linearly ($P = 0.021$). Grazing time and RT AM-PM between days in 4D showed opposite quadratic effects ($P = 0.047$ and $P = 0.015$, respectively), resulting in a similar sum of GT and RT between days.

In the PM-AM period, treatment differences were generally more pronounced than in the AM-PM period, with a longer GT ($P = 0.020$), a greater number of bites ($P = 0.013$) and chews ($P = 0.001$), a higher chew rate ($P = 0.002$), and a lower RT ($P = 0.026$) in 4D than in 1D. Grazing time as well as the number of bites and BR increased linearly between days in 4D in PM-AM whereas RT decreased linearly ($P < 0.05$). Grazing, ruminating, and idling time in PM-AM between days in 4D, expressed as a percentage of available time, is shown in Figure 3.3. The linear increase in GT in PM-AM ($P = 0.006$) coincided with a linear decrease in RT ($P = 0.013$), while idling remained unaltered in time, in line with the sum of GT and RT (Table 3.4).

Table 3.4

Grazing behavior of dairy cows allocated every day (1D) or every four days (4D) to a new plot on a daily basis, between morning and evening milking (0600 – 1600 h; AM-PM) and between evening and morning milking (1600 – 0600 h; PM-AM).

			Days in 4D				SEM		P-value		
Variable	1D	4D	d1	d2	d3	d4	T[1]	D[2]	T[1]	D[2] linear	D[2] quadratic
Per day											
Grazing time, min	557	559	549	546	574	568	9.7	12.6	0.872	0.152	0.890
Bites, no	35375	36638	34487	35178	38778	38111	783.3	1058.2	0.274	0.007	0.497
Chews, no	8477	9655	9242	9956	10044	9379	378.1	559.8	0.045	0.848	0.234
Bite rate, /min	64	65	63	65	67	67	0.7	1.0	0.195	0.006	0.583
Chew rate, /min	15	17	17	18	18	16	0.5	0.8	0.007	0.812	0.166
Bite mass, mg/bite	485	453	-	-	-	-	31.3	-	0.496	-	-
Ruminating time, min	477	460	471	479	441	450	5.7	8.8	0.061	0.010	0.977
Chews per bolus, no	57	58	55	57	60	59	1.3	1.7	0.883	0.039	0.228
G + R[3], h	17.2	17.0	17.1	17.2	17.0	16.9	0.19	0.24	0.532	0.611	0.577
AM-PM											
Grazing time, min	300	289	295	278	288	295	5.6	7.1	0.150	0.786	0.047
Bites, no	18145	18487	18495	17432	18890	19130	431.3	568.6	0.585	0.206	0.207
Chews, no	4975	5221	5380	5175	5165	5165	208.9	284.8	0.420	0.621	0.706
Bite rate, /min	60	64	62	63	65	65	0.6	0.9	0.001	0.021	0.855
Chew rate, /min	16	18	18	19	18	17	0.6	0.8	0.059	0.473	0.513
Ruminating time, min	129	131	128	138	138	123	4.0	5.3	0.711	0.551	0.015
Chews per bolus, no	56	55	50	53	61	57	1.3	1.7	0.888	<0.001	0.014
G + R[3], h	7.2	7.0	7.1	6.8	7.1	7.0	0.08	0.12	0.164	0.995	0.856
PM-AM											
Grazing time, min	255	277	256	271	294	286	6.4	8.2	0.020	0.006	0.123
Bites, no	16833	18552	16553	17738	20306	19612	454.9	609.9	0.013	<0.001	0.107
Chews, no	3617	4694	4004	4992	4925	4857	205.3	292.2	0.001	0.072	0.082
Bite rate, /min	66	67	65	66	68	68	0.7	1.0	0.702	0.010	0.506
Chew rate, /min	14	17	16	18	17	17	0.6	0.8	0.002	0.667	0.153
Ruminating time, min	347	328	341	337	313	322	5.9	7.2	0.026	0.013	0.265
Chews per bolus, no	59	59	58	60	58	59	1.3	1.7	0.996	0.835	0.448
G + R[3], h	10.0	10.0	9.9	10.2	10.1	10.0	0.12	0.15	0.776	0.683	0.277

[1] Treatment.
[2] Day within 4D.
[3] G + R is the sum of grazing time and ruminating time (in hours).

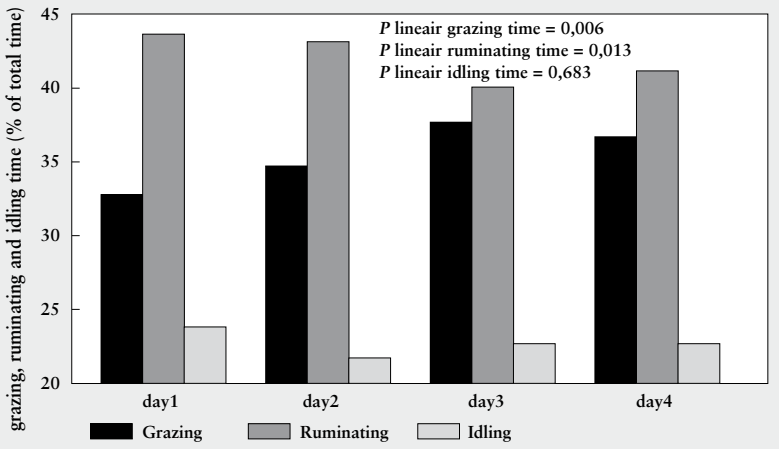

Figure 3.3

Grazing, ruminating and idling time per day when cows were allocated every 4 days to a new plot (4D treatment) as percentage of time between evening and morning milking (1600 to 0600 h; PM-AM).

Rumen Variables

Means per day were similar between days in 1D for all rumen variables except mean molar proportion of isovalerate, which decreased between days in 1D with the largest difference being 1.12 vs. 0.90% ($P = 0.018$). Rumen pH and NH_3-N concentration in rumen fluid were greater ($P < 0.001$) in 4D than in 1D (Table 3.5). The concentration of total VFA was similar between treatments ($P = 0.324$), but mean molar proportions of all individual VFA, with the exception of butyrate proportions, were different between treatments. Proportions of acetate and isobutyrate were greater ($P < 0.01$) in 4D, whereas propionate, valerate, and isovalerate were greater in 1D ($P < 0.05$). These changes in molar proportions of individual VFA resulted in a greater non-glucogenic to glucogenic ratio in 4D than in 1D ($P = 0.003$). This ratio is calculated as [Acetate + 2 (butyrate + isobutyrate) + valerate + isovalerate] / [propionate + valerate + isovalerate].

There were strong day effects in 4D for all rumen variables. Rumen pH and non-glucogenic to glucogenic increased linearly, whereas total VFA concentration decreased linearly ($P < 0.01$). The decrease in NH_3-N was stronger in the first days of the 4 d period resulting in a quadratic effect ($P = 0.006$). During the 4 d period, molar proportions of acetate increased linearly ($P < 0.001$), whereas the molar proportions of the other VFA decreased linearly ($P < 0.05$).

Table 3.5

Rumen pH, ammonia-N (NH_3-N), total VFA and molar proportions of individual VFA of dairy cows allocated every day (1D) or every four days (4D) to a new plot.

			Days in 4D				SEM		*P*-value		
Variable	1D	4D	d1	d2	d3	d4	T[1]	D[2]	T[1]	D[2] linear	D[2] quadratic
pH	6.05	6.16	6.01	6.14	6.19	6.31	0.019	0.037	<0.001	<0.001	0.952
NH_3-N, mg/L	90.1	113.7	170.3	112.9	90.9	80.7	4.18	8.36	<0.001	<0.001	0.006
Acetate, %[3]	64.9	65.8	64.4	65.2	66.2	67.2	0.20	0.39	0.002	<0.001	0.858
Propionate, %[3]	21.1	20.4	20.8	20.5	20.5	19.6	0.17	0.34	0.002	0.012	0.396
Butyrate, %[3]	11.2	11.2	11.6	11.3	10.8	10.9	0.08	0.18	0.979	0.001	0.225
Isobutyrate, %[3]	0.75	0.83	0.92	0.87	0.80	0.72	0.010	0.020	<0.001	<0.001	0.453
Valerate, %[3]	1.05	0.93	1.06	1.00	0.88	0.79	0.024	0.048	0.001	<0.001	0.783
Isovalerate, %[3]	1.02	0.96	1.14	1.03	0.87	0.79	0.022	0.045	0.042	<0.001	0.779
Total VFA, mmol/L	116.5	117.8	127.8	121.6	114.9	107.1	0.97	1.94	0.324	<0.001	0.684
NGR[4]	3.96	4.15	4.00	4.09	4.13	4.37	0.043	0.088	0.003	0.004	0.364

[1] Treatment.
[2] Day within 4D.
[3] % of total VFA.
[4] The non-glucogenic to glucogenic ratio (NGR) was calculated as
[Acetate + 2 (Butyrate + Isobutyrate) + Valerate + Isovalerate]/[Propionate + Valerate + Isovalerate].

Table 3.6

Milk yield and milk composition of dairy cows allocated every day (1D) or every four d (4D) to a new plot.

			Days in 4D				SEM		*P*-value		
Variable	1D	4D	d1	d2	d3	d4	T[1]	D[2]	T[1]	D[2] linear	D[2] quadratic
Milk yield											
Milk, kg/d	24.5	23.7	23.4	24.7	23.8	22.8	0.14	0.20	<0.001	0.005	<0.001
FPCM, kg/d	23.5	22.8	23.0	23.6	22.8	21.8	0.17	0.23	0.003	<0.001	<0.001
Milk composition											
Fat, %	3.66	3.76	3.92	3.69	3.70	3.75	0.028	0.040	0.013	0.004	<0.001
Protein, %	3.28	3.34	3.40	3.33	3.33	3.29	0.016	0.018	0.018	<0.001	0.157
Lactose, %	4.39	4.42	4.40	4.43	4.43	4.43	0.013	0.015	0.066	0.062	0.078
Urea, mg/dl	23.2	23.4	26.7	24.4	21.9	20.7	0.43	0.48	0.774	<0.001	0.005
Amount											
Fat, g/d	892	876	898	898	869	838	9.6	12.9	0.249	<0.001	0.081
Protein, g/d	796	791	795	825	793	750	6.6	8.2	0.607	<0.001	<0.001

[1] Treatment.
[2] Day within 4D.

Milk

Milk and FPCM yield were greater in 1D than in 4D ($P < 0.003$; Table 3.6). Fat and protein content in milk were greater in 4D than in 1D ($P < 0.05$), whereas milk lactose and urea content, and fat and protein yield were similar between treatments.

Milk production and composition changed during the 4 d in 4D ($P < 0.01$) except for lactose content. Milk and FPCM yield increased on d 2 after which they decreased, resulting in a quadratic effect ($P < 0.001$). Milk fat content showed a quadratic effect between days in 4D with the greatest milk fat content on d 1 and the lowest on d 2 ($P < 0.001$). Milk protein content decreased linearly over time ($P < 0.001$). The decrease in milk urea content was smaller during the last day than at the start of the 4 d period (P quadratic = 0.005). Milk fat production per day decreased linearly between days in 4D ($P < 0.001$), and milk protein production per day showed a quadratic response ($P < 0.001$).

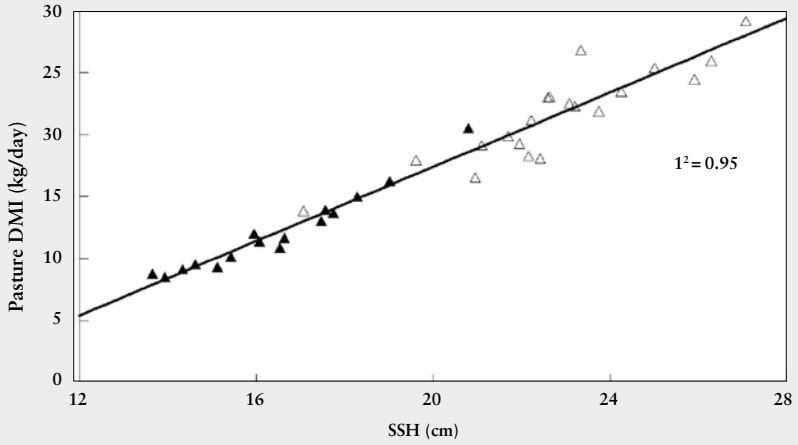

Figure 3.4

Relation between sward surface height (SSH) and pasture DMI in 1D (△rotation 1; ▲rotation 2). The regression formula is Pasture DMI (kg/d) = -12.7 (± 1.2) + 1.51 (± 0.06) × SSH (cm).

DISCUSSION

The objective of this experiment was to determine the influence of daily allocation and allocation every 4 d to new grazing plots on pasture DMI, grazing behavior, rumen characteristics, and milk yield in dairy cows. Frequent allocation of cows to new grazing plots is carried out in grazing management of modern dairy farming in different parts of the world (e.g. Australia, the Netherlands), although hardly any information is available on the effects of grazing behavior, rumen fermentation, and production. Allocating cows once a day to a fresh plot compared to once every 4 d in the current experiment resulted in a greater milk production and a greater

DMI if the pasture allowance was large. On the 4D plots, chemical composition of the pasture and consequently grazing behavior and rumen variables differed greatly across days.

Pasture

A decrease in SSH between days in 4D (Figure 3.2), as well as between offered and residual pasture in 1D (Table 3.2), is related to changes in the morphological fractions and is in line with previous experiments (Wales et al., 1998; Virkajarvi et al., 2002). The resulting changes in proximate chemical composition reflect the higher CP and sugar content and lower concentration of cell-wall constituents in leaves than in stems (Delagarde et al., 2000). The increase in ADL in NDF at d 4 (Figure 3.2) suggests that selection of leafy, high quality pasture must have taken place. This is also indicated by the calculated chemical composition of ingested pasture (data not shown), using the difference in offered and residual PM, and their respective chemical compositions. The quality of ingested pasture was better than the quality of PM offered, as CP and sugars content were greater and NDF content was lower.

Grazing Behavior

Taweel et al. (2006) hypothesized that grazing behavior is influenced by pasture composition. However, tensile strength and grazing behavior did not differ among 4 varieties of perennial ryegrass at similar SSH with varying NDF and water-soluble carbohydrate contents. Sward surface height is expected to influence grazing behavior through effects on IR and consequently GT (Gibb, 2006). At low SSH levels, dairy cattle compensate for the reduced IR by increasing GT at the expense of RT (Gibb et al., 1996). In the present study the differences in pasture composition and SSH between days in 4D did not coincide with significant changes in GT, although there was a numerical increase in GT (P linear = 0.152) and a linear decrease in RT between days in 4D (Table 3.4). These effects were more profound between evening and morning milking than between morning and evening milking. This observation is possibly related to the crepuscular nature of cows, being most active at sunrise and at sunset and the preference of cows not to graze during the night (Albright, 1993; Rutter, 2006). Besides peaks in grazing behavior at sunrise and sunset, Gibb (2006) described that several smaller meals occur between sunrise and evening milking, alternated with ruminating and resting. After evening milking, the greatest amount of daily pasture intake occurred (Gibb, 2006). This was confirmed with findings of Orr et al. (2001) who found short and fragmented meals during the morning following morning allocation to a fresh plot whereas following evening allocation to a fresh plot, the major grazing meal of the day occurred. Diurnal patterns in grazing behavior were not studied although in the current experiment, it seems likely that changes in GT and RT mainly occurred during the major grazing meal and the longest period of ruminating (being the night), resulting in greater differences between evening and morning milking than between morning and evening milking.

Grazing time, as well as the percentage time spent grazing between evening and morning milking (Figure 3.3) increased over days at the expense of time spent ruminating, but time spent idling was not affected. This is in agreement with Gibb (2006), who emphasized the importance of idling (when the animal is not grazing or ruminating; that is, social interaction and resting) in the total time budget per day.

Dry Matter Intake

The differences in grazing behavior between days in 4D probably affected pasture DMI in these days. Studies with dosed alkanes showed that some 5 to 6 d are needed for the fecal alkane concentration to reach equilibrium (Dove and Mayes, 1991). Therefore, pasture DMI was estimated per cow per rotation. Diurnal patterns of fecal n-alkane excretion have always been a major concern for variation in marker studies when alkanes are pulse-dosed (Dove and Mayes, 1991). The fecal concentrations of the (natural) odd-chain alkanes tend to be relatively constant, but due to the dosing schedule, diurnal variation in excretion of the dosed even-chain alkane can occur. Therefore, during the current experiment fecal samples from spontaneous defecations during 1 d in the 1D group were analyzed to investigate diurnal variation in excretion of the dosed alkane in feces (data not shown). Some variation was found in the concentration of dosed alkane in fecal samples. However, the concentrations of dosed alkane in fecal samples at milking, when fecal samples were taken to determine pasture DMI, were similar to the average concentration of dosed alkane over the day. This implies that there was no effect of diurnal variation in alkane excretion in feces on the estimation of pasture DMI.

Pasture DMI was not estimated per day in 4D using the alkane method. Therefore, the relation between SSH and pasture DMI was used in an attempt to discuss possible effects of the imposed treatment on pasture DMI per day within 4D. Sward surface height and pasture DMI usually show a positive relationship (Gibb et al., 1997), which was also found during this experiment (Figure 3.4). Pasture DMI per group per day was estimated using offered and residual SSH data to calculate offered and residual PM using the regression formulas between SSH and PM as given in Table 3.3. The difference between offered and residual PM was assumed to be consumed by the 10 cows per group. The relation between SSH and pasture DMI in the current experiment showed a strong (r2=0.95) linear relationship over the range of SSH applied (13.6 to 27.1 cm). The relation between SSH and BM is expected to be curvilinear, resulting from successive smaller increments in BM at each increment of SSH (Chilibroste, 2005). Because BR generally declines when BM increases (Gibb et al., 1996), this implies that an increase in SSH will not be followed to the same extent by an increase in IR. Pasture DMI is the product of GT and IR (Allden and Whittaker, 1970), implying that the cows were able to (partly) compensate IR at shorter SSH by increasing GT. More importantly, from the decrease in SSH between days in 4D and the relation between SSH and pasture DMI, it is likely that DMI decreased quadratically between days in 4D. This is confirmed by the curvilinear decrease in milk yield between days in 4D. The maximum milk yield was achieved on the second day of the period followed by a decrease thereafter, indicating that milk yield follows offered pasture with a delay of at least 12 hours.

Despite the absence of differences in pasture DMI between treatments, there were differences between both rotations, probably related to SSH. Residual SSH is considered a good indicator for grazing management success, with SSH of 9 to 10 cm as an indication of efficient grassland management (Virkäjarvi et al., 2002). Le Du et al. (1979) found reduced pasture DMI when residual SSH is below 8 to 10 cm. In the current experiment, there was a difference in residual SSH between both rotations (10.9 cm in the first rotation vs. 7.4 cm in the second rotation; $P < 0.001$). This difference coincided with an interaction between treatment and rotation in pasture DMI ($P = 0.025$) with a 1.8 kg/d greater pasture DMI in 1D than in 4D in the first rotation, indicating that frequent allocation to a new plot positively affected pasture DMI if offered pasture was sufficient. The lower pasture DMI and SSH in the second rotation was related to a lower BM compared with the first rotation (432 vs. 505 mg DM/bite, respectively; $P = 0.006$). The expected increase in BR due to a decrease in BM (Gibb, 2006) was not seen

when comparing both rotations. The greater difference in pasture DMI during the first rotation compared with the second rotation corresponded with a larger difference in FPCM production during the first rotation (4D, 24.3 kg/d vs. 1D, 25.9 kg/d) than during the second rotation (4D, 21.2 vs. 1D, 21.7 kg/d), although the interaction between treatment and rotation in milk yield was not significant.

Rumen Fermentation and Milk Composition

Fermentation of NDF results in an increase in acetate and a decrease in propionate production (Bannink et al., 2006). The relation between pasture NDF content and molar proportions of these VFA is demonstrated in the current experiment by the fact that the increase in NDF between d 1 and 4 in the 4D treatment (Figure 3.1) coincided with a strong linear increase in the molar proportion of acetate and a linear decrease in the molar proportion of propionate across days (Table 3.5). This was also related to milk fat content, although the increase in milk fat content appeared to be delayed by 1 d, showing a peak in milk fat content on d 1 of the 4D treatment (Table 3.6). The results also show that changes in milk composition rapidly follow changes in diet composition.

General Treatment Effects

Between both treatments pasture DMI and pasture composition were similar. Therefore production of VFA in the rumen and consequently VFA absorbed from the gastrointestinal tract is assumed to have been rather comparable between 1D and 4D. Indeed, mean total VFA concentration was similar between treatments, but rumen pH was lower in 1D than in 4D. As the fractional rate of absorption of VFA is faster at low rumen pH (Dijkstra et al., 1993), VFA available for metabolism in the animal may have been greater in 1D than in 4D. This, in combination with the numerical greater pasture DMI of 0.7 kg of DM/d in 1D, might have been the reason for a greater milk and FPCM yield in 1D than in 4D.

The greater milk fat content in 4D than in 1D may be explained by the numerically greater pasture NDF content in 4D, increasing lipogenic nutrients in 4D as compared with 1D (Bannink et al., 2006). Alternatively, the lower rumen pH at 1D may have resulted in incomplete rumen biohydrogenation of pasture linolenic acid and an increased formation of *trans* fatty acids that inhibit *de novo* milk fat synthesis in the mammary gland (Baumgard et al., 2002). The concentration of CP in pasture and the concentration of milk urea did not differ between treatments, but the concentration of NH_3-N in the rumen was greater in 4D than in 1D. Taking the (numerically) greater pasture DMI in 1D into account, the supply of CP to cows in this treatment was greater than in 4D. The lower rumen NH_3-N concentration in 1D may be the result of increased microbial protein synthesis. One of the most important factors in microbial efficiency is fractional passage rate (Dijkstra et al., 2002). The numerically greater pasture DMI in 1D, causing greater fractional passage rates of rumen contents, could thus have led to greater microbial protein synthesis in 1D than in 4D. This is in agreement with greater odd- and branched-chain fatty acid proportion of milk fat from cows in 1D than in 4D (unpublished data), because milk odd- and branched-chain fatty acids are closely related to microbial flow to the duodenum (Vlaeminck et al., 2005).

CONCLUSIONS

This study confirmed that increased pasture allocation frequency from once every 4 d to once a day improved milk production in grazing dairy cows, especially when the amount of pasture offered was high. This was mainly the result of a change in grazing behavior, resulting in an increase in pasture DMI.

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REFERENCES

Albright, J. L. 1993. Feeding behavior of dairy cattle. Journal of Dairy Science 76: 485-498.

Allden, W. G., and I. A. McD. Whittaker. 1970. The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. Australian Journal of Agricultural Research 21:755-766.

Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. Journal of Theoretical Biology 238:36-51.

Bargo, F., L. D. Muller, J. E. Delahoy, and E. S. Kolver. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86: 1-42.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, *cis*-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. Journal of Dairy Science 85: 2155-2163.

Chilibroste, P., S. Tamminga, H. Boer, M. J. Gibb, and G. den Dikken. 2000. Duration of regrowth of ryegrass (lolium perenne) effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. Journal of Dairy Science 83:984-995.

Chilibroste, P. 2005. Pasture characteristics and animal performance. Pages 681-706 in Quantitative Aspects of Ruminant Digestion and Metabolism. J. Dijkstra, J. M. Forbes, and J. France, ed. CAB International, Wallingford, UK.

CVB, 2007. Tabellenboek Veevoeding 2000 (Dutch Feeding Tables). Voedernormen landbouwhuisdieren en voederwaarde veevoeders (in Dutch). Central Bureau for Livestock Feeding, Lelystad, the Netherlands.

Dalley, D. E., J. R. Roche, P. J. Moate, and C. Grainger. 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Australian Journal of Experimental Agriculture 41:593-599.

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

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. British Journal of Nutrition 69:385-396.

Dijkstra, J., J. A. N. Mills, and J. France. 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. Nutrition Research Reviews. 15: 67-90.

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

Forbes, J. M. 1996. Integration of regulatory signals controlling forage intake in ruminants. Journal of Animal Science 74: 3029-3035.

Gibb, M. J., C. A. Huckle, R. Nuthall, and P. D. Penning. 1996. Can grazed pasture meet the needs of the high genetic merit dairy cow? In Grass and forage for cattle of high genetic merit, proceedings of the British Grassland Society winter meeting 1996. British Grassland Society, Reading.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass Forage Science 52:309-321.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. Pages 141-157 in Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Goelema, J. O., M. A. M. Spreeuwenberg, G. Hof, A. F. B. van der Poel, and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Animal Feed Science Technolology 76:35-50.

Holecheck, J. L., R. D. Pieper, and C. H. Herbel. 1995. Range management: Principles and practices (2nd ed.). Prentice Hall, Englewood Cliffs, New Jersey.

Illius, A. W., and I. J. Gordon. 1987. The allometry of food intake in grazing ruminants. Journal of Animal Ecology 56:989-999.

ISO. 1999. Whole milk - Determination of milkfat, protein and lactose content - Guidance on the operation of mid-infrared instruments. ISO 9622:1999. International Organization for Standardization, Geneva, Switzerland.

ISO. 2004. Milk - Determination of urea content - Enzymatic method using difference in pH (Reference method). ISO14637:2004. International Organization for Standardization, Geneva, Switzerland.

ISO. 2002. Animal Feeding Stuffs - Determination of Crude Ash. ISO 5984:2002. International Organization for Standardization, Geneva, Switzerland.

ISO. 1999. Animal Feeding Stuffs. Determination of moisture and other volatile matter content. ISO 6496:1999. International Organization for Standardization, Geneva, Switzerland.

ISO. 1997. Animal Feeding Stuffs. Determination of Nitrogen Content and Calculation of Crude Protein content - Kjeldahl method. ISO 5983:1997. International Organization for Standardization, Geneva, Switzerland.

ISO. 1999. Animal Feeding Stuffs. Determination of Fat Content. ISO 6492:1999. International Organization for Standardization, Geneva, Switzerland.

Kuusela, E., and H. Khalili. 2002. Effect of grazing method and herbage allowance on the grazing efficiency of milk production in organic farming. Animal Feed Science and Technology 98:87-101.

Le Du, Y. L. P., J. Combellas, J. Hodgson, and R. D. Baker. 1979. Herbage intake and milk production by grazing dairy cows 2. The effects of level of winter feeding and daily herbage allowance. Grass Forage Science 34:249-260.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science 76:1216-1231.

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

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

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass Forage Science 56: 352-361.

Parsons, A. J., and D. F. Chapman. 2000. The principles of pasture growth and utilisation. Pages 31-89 in Grass, its production and utilisation. A. Hopkins, ed. Blackwell Science, Oxford, UK.

Rutter, S. M., R. A. Champion, and P. D. Penning. 1997. An automatic system to record foraging behaviour in free-ranging ruminants. Applied Animal Behaviour Science 54:185-195.

Rutter, S. M. 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Applied Animal Behaviour Science 97: 17-35.

Rutter, S. M. 2000. Graze: A program to analyze recordings of the jaw movements of ruminants. Behavior Research Methods, Instruments and Computers 32:86-92.

Tamminga, S.. W. M. van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever and M. C. Blok. 1994. The Dutch protein evaluation system: The DVE/OEB-system. Livestock Production Science 40: 139-155.

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

Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science 87: 3417-3427.

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

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

Van Es, A. J. H. 1975. Feed evaluation for dairy cows. Livestock Production Science 2:95-107.

Van Soest, P. J. 1973. Collaborative study of acid-detergent fibre and lignin. Journal of Association of Official Analytical Chemists 56:781-784.

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

Van Vuuren, A. M., C. J. Van Der Koelen, H. Valk, and H. De Visser. 1993. Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. Journal of Dairy Science 76:2982-2993.

Virkajarvi, P., A. Sairanen, J. I. Nousiainen, and H. Khalili. 2002. Effect of herbage allowance on pasture utilization, regrowth and milk yield of dairy cows in early, mid and late season. Animal Feed Science and Technology 97:23-40.

Vlaeminck, B., C. Dufour, A. M. van Vuuren, A. R. J. Cabrita, R. J. Dewhurst, D. Demeyer and V. Fievez. 2005. Use of odd and branched-chain fatty acids in rumen contents and milk as a potential microbial marker. Journal of Dairy Science 88: 1031-1042.

Wales, W. J., P. T. Doyle, and D. W. Dellow. 1998. Dry matter intake and nutrient selection by lactating cows grazing irrigated pastures at different pasture allowances in summer and autumn. Australian Journal of Experimental Agriculture 38:451-460.

CHAPTER 4

The Effect of Allocation Frequency in Rotational Grazing Systems on the Fatty Acid Profile in Milk Fat of Dairy Cows

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Submitted

ABSTRACT

Eight Holstein cows were blocked in 2 groups to evaluate the effect of frequency of allocation to new grazing plots on profiles of fatty acids (FA) in milk fat. The 2 treatments were daily allocation to 0.125-ha plots (1D) or allocation every 4 d to 0.5-ha plots (4D) of *Lolium perenne* L. and were tested in a randomized block design (2 rotations with 2 measuring periods of 4 d each). There was no difference in the FA composition of the offered and residual pasture between 1D and 4D. Within days in the 4D treatment, the proportion of 16:0, 18:0 and 18:2*n*-6 increased, and that of 18:3*n*-3 as well as total FA content of grass linearly decreased. Treatment effects on milk FA composition and secretion were small. In contrast, milk FA composition was largely affected by day within the 4D treatment. Secretion of *de novo* synthesised and C16-FA decreased linearly during the 4 d whereas secretion of odd- and branched-chain and C18-FA were not affected by day in the 4D treatment. Short term variation in pasture quality during the 4 days affected milk FA composition with a greater effect on biohydrogenation intermediates in milk fat compared with its major precursor, 18:3*n*-3. Results from this study suggests that increasing pasture allocation frequency from once every 4 d to every day has no effect on profiles of FA in milk.

INTRODUCTION

Long chain *n*-3 poly-unsaturated FA and *cis*-9, *trans*-11-18:2 have potential human health benefits (Lock et al., 2004, Wahle et al., 2004). Thus, increasing the concentration of these FA in milk is beneficial to public health. Feeding fresh grass is an efficient way to increase 18:3*n*-3 and *cis*-9, *trans*-11-18:2 content in milk fat of dairy cows. Compared with TMR diets, pasture-based diets consistently resulted in higher concentrations of unsaturated long-chain FA and *cis*-9, *trans*-11-18:2 in milk (Dewhurst et al., 2006). Milk 18:3*n*-3 is derived from 18:3*n*-3 bypassing the rumen and being absorbed from the small intestine, whereas milk *cis*-9, *trans*-11-18:2 results from both rumen biohydrogenation of dietary 18:2*n*-6 and desaturation by mammary Δ-9-desaturase of *trans*-11-18:1. The latter is formed during biohydrogenation of dietary 18:2*n*-6 and 18:3*n*-3 in the rumen. More than 80% of *cis*-9, *trans*-11-18:2 in milk fat originates from this endogenous synthesis (Mosley et al., 2006, Shingfield et al., 2007).

Schroeder et al. (2004) compared concentrations of *cis*-9, *trans*-11-18:2 in milk of cows fed pasture-based diets to that of cows fed TMR diets from seven studies, and reported an increase of 134% compared to the control, with considerable variation in the responses ranging from 15 to 396%. Differences in herbage allowance (Stanton et al., 1997; Stockdale et al., 2003; Bargo et al., 2006b), herbage mass (Stanton et al., 1997; Stockdale et al., 2003), time on pasture (Khanal et al., 2007), grass species and cultivar (Dewhurst et al., 2006), growth stage (Griinari et al., 1998) and N fertilization rate (Boufaied et al., 2003) might explain these large variations.

Pasture intake is influenced by many factors like sward surface height (Gibb, 2006), herbage mass on offer (Dalley et al., 1999), and chemical and morphological composition of the sward (Chilibroste, 2005). Grazing strategies, changing grazing behaviour through variation in chemical and morphological characteristics, are reported to alter pasture intake (Pulido and Leaver, 2003). To our knowledge, no experiments describe the effect of allocation frequency in rotational grazing systems on milk FA profile, although effects on DMI and milk production characteristics are reported (Dalley et al., 2001; Abrahamse et al., 2008). The objective of this

experiment was to determine the influence of grazing system (once daily vs. once every 4 d reallocating to a fresh plot) on milk FA profile in dairy cows.

MATERIAL AND METHODS

Grazing Management and Experimental Design

A detailed description of the experimental design is presented by (Abrahamse et al., 2008). Briefly, twenty lactating Holstein dairy cows were paired on the basis of milk yield, parity and DIM and were allocated at random to experimental treatments. One group of 10 cows was daily allocated to a fresh 0.125-ha perennial ryegrass (*Lolium perenne* L.) plot (treatment 1D), while the other group was allocated to a fresh 0.5-ha plot every 4 d (treatment 4D). Cows were milked twice daily at 0600 and 1600 h and allocated to a new plot after the morning milking. Grass height (GH) and grass DM on offer on the first day were equal between groups. After adaptation to grazing during three weeks and further adaptation to the grazing system during 4 d, the treatments were replicated during two rotations with four periods of 4 d in the first rotation and three periods of 4 d in the second rotation. Samples from four cows per treatment during two periods in each rotation (periods 2 and 3) were analysed. At the start of the experiment, the eight cows produced 20.7 ± 0.77 kg of milk/d (values expressed as means ± SE), and were 179 ± 9.0 DIM; BW was 514 ± 15.4 kg, and BCS was 2.4 ± 0.08 (recorded on a 5-point scale). These variables were similar between treatments. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Wageningen University.

Sampling, Measurements and Analysis

A representative grass sample of each plot was taken during the morning milking, by cutting grass at 4 cm above ground level from approximately 20 and 40 sites per plot for 1D and 4D, respectively. In group 1D, grass samples were taken on d 1 and 3, whereas in group 4D grass was sampled during all four days. In addition, residual grass was sampled after cows were removed from the plot (i.e. residual material from d 1 and 3 in group 1D and from d 4 in group 4D). Samples were stored frozen (-20°C) and freeze dried prior to FA analysis. FA extraction followed an adapted Folch method (Folch et al., 1957) with chloroform/methanol (2/1, vol/vol) as described by (Lourenço et al., 2005).

Milk production was recorded daily throughout the study. During both periods per rotation, two 10 ml aliquots of milk were collected at each milking. One aliquot was stored no longer than 4 d at 4°C using sodium azide and bronopol as preservative and analysed for fat, protein and lactose content according to ISO 9622 (Melkcontrolestation, Zutphen, The Netherlands; (ISO, 1999)). The other aliquot of milk was collected without preservative and stored frozen (-20°C). Prior to FA analysis, samples of two consecutive milkings (i.e. evening and subsequent morning milking) were pooled resulting in four milk samples per cow per period. Two milk samples (out of 128 samples total) were lost. The FA composition was determined according to (Vlaeminck et al., 2005). Briefly, in the first step, samples were extracted with ammonium hydroxide solution, ethanol, diethyl ether and petroleum ether. In the second extraction step, ethanol, diethyl ether and petroleum ether were used, and in the final extraction step, the solvents used were diethyl ether and petroleum ether. Extracts from the 3 consecutive steps were combined, evaporated, methylated and analysed separately for short chain FA (4:0 - 10:0) and medium and long chain FA (12:0 - 24:0). Standard curves were used to determine

the response factors for milk short chain FA, using tridecanoic acid (Sigma, Bornem, Belgium) as the internal standard, whereas the other FA were quantified with nonadecanoic acid as the internal standard (Sigma, Bornem, Belgium). Methylation and analysis by GLC were described previously (Vlaeminck et al., 2005).

Calculations

In order to relate the variation in pasture 18:3*n*-3 content in the 4D treatment with milk content and secretion of individual FA, milk content and secretion of the individual FA observed on day i were compared with 18:3*n*-3 content in grass observed at day i-1. This resulted in 7 observations per rotation. The values of milk content and secretion of the individual FA were the average of the four animals on day i whereas 18:3*n*-3 in grass was the average of offer on day i-1 and residual of day i-1 (sampled on day i).

Statistical Analysis

All statistical analyses were performed using the MIXED procedure of SAS (SAS Institute, 2004). Least square means are presented in the tables and significance was declared at $P < 0.05$.

Grass Samples. The effect of grazing management on FA composition of grass samples were analysed as

$Yijk = \mu + Ti + Sj + Rk + TSij + \varepsilon ijk$

with Yijk the individual observation, μ the overall mean, Ti the effect of treatment (i = 1D, 4D), Sj the effect of time of sampling (j = offer, residual), Rk the effect of rotation (k = 1, 2), TSij the interaction between Si and Tj and εijk the residual error. Time of sampling was treated as a repeated measure assuming an autoregressive order one covariance structure. Prior to this statistical analysis, values for offered and residual material of d 1 and 3 were averaged for 1D treatment whereas values for d 1 and the residual of d 4 were retained for the 4D treatment.

Within group 4D, changes in FA composition during the 4 days were analysed as

$Yij = \mu + Di + Rj + \varepsilon ij$

with Yij the individual observation, μ the overall mean, Di the effect of day (i = 1..5, i.e. the morning samples of the four days and the residual of the 4th day), Pj the effect of rotation, and εijk the residual error. The interaction term day × rotation was not significant ($P > 0.05$) and was not included in the model. Day was treated as a repeated measure assuming an autoregressive order one covariance structure. Orthogonal contrasts were used to test for significance of linear and quadratic effects of day.

Milk Samples. The effect of grazing management on milk production characteristics and FA profile and secretion were analysed as

$Yijklm = \mu + Ti + D(T)ij + Rk + TRik + Cl + covm + \varepsilon ijklm$

with Yijkl the individual observation, μ the overall mean, Ti the effect of treatment (i = 1D, 4D), D(T)ij the effect of day (j = 1..4) within treatment, Rk the effect of rotation (k = 1, 2), Cl the random effect of cow, TRik the interaction between treatment and rotation, covm the covariate value of variable Y and εijklm the residual error. As covariate, the values of the parameter under consideration were used, derived from milk samples after adaptation to grazing during three weeks. Day was treated as a repeated measure assuming an autoregressive order one covariance structure. Orthogonal contrasts were used to test for significance of linear and quadratic effects of day within treatment.

The CORR procedure of SAS was used to evaluate correlations between content in grass and secretion in milk of individual FA. Variation in 18:3*n*-3 content in grass between days in the 4D treatment was related to milk content and secretion of individual FA. The relation of total FA content with proportion of 18:3*n*-3 of grass samples was evaluated using all individual observations (n = 36).

RESULTS

Table 4.1

Fatty acid content (g/kg DM) and fatty acid composition (g/100 g fatty acids) of pasture grazed by dairy cows allocated every day (1D) or every four d (4D) to a new plot.

	4D		1D		SEM	*P*-value		
	Offer[1]	Residue	Offer	Residue		Treatment	Time[2]	Treatment×Time
Total fatty acids	22.7	15.9	21.0	13.2	1.07	0.129	<0.001	0.607
14:0	2.58	2.58	2.84	2.87	0.138	0.210	0.796	0.785
16:0	12.9	15.3	12.7	16.4	0.425	0.180	0.001	0.235
18:0	1.86	2.75	1.71	2.62	0.287	0.684	0.012	0.984
***cis*-9-18:1**	1.41	1.98	1.35	2.84	0.366	0.288	0.040	0.284
18:2*n*-6	10.7	14.4	10.2	14.5	0.47	0.548	<0.001	0.653
18:3*n*-3	67.6	59.9	68.6	58.2	1.15	0.638	<0.001	0.384

[1] Offer in 4D treatment represents the first day only.
[2] The effect of offer vs. residue.

FA Composition of Grass

Table 4.1 shows the FA content of grass for both treatments. FA composition of the offered and residual grass was similar ($P > 0.05$) for both treatments. In both treatments, the offered grass had a higher total FA content and proportion of 18:3*n*-3, but lower proportions of 16:0, 18:0, *cis*-9-18:1 and 18:2*n*-6, than the residual grass. The 18:3*n*-3 content averaged 63.6 % of total FA and a strong relation ($r_{pearson} = 0.884$, n = 36, $P < 0.001$) existed between proportion of 18:3*n*-3 and the total amount of FA. Within the 4D treatment, content of total FA ($P = 0.001$) and proportion of 18:3*n*-3 ($P < 0.001$) linearly decreased between the beginning of d 1 and the residue at the end of d 4 whereas proportions of 16:0 ($P = 0.003$), 18:0 ($P = 0.027$) and 18:2*n*-6 ($P < 0.001$) linearly increased between offer and turnout (Table 4.2).

Table 4.2

Fatty acid content (g/kg DM) and fatty acid composition (g/100 g fatty acids) of pasture grazed by dairy cows allocated every four d (4D) to a new plot.

Variable	Day 1	Day 2	Day 3	Day 4	residue	SEM	L[1]	Q[2]
Total fatty acids	22.7	20.8	18.0	16.0	15.9	1.42	0.001	0.271
14:0	2.58	2.49	2.76	2.54	2.58	0.162	0.918	0.582
16:0	12.9	13.5	14.0	14.9	15.3	0.48	0.003	0.957
18:0	1.86	2.11	2.01	2.72	2.75	0.279	0.027	0.666
cis-9-18:1	1.41	1.78	1.78	1.97	1.98	0.210	0.092	0.415
18:2*n*-6	10.7	11.7	12.3	13.8	14.4	0.49	<0.001	0.866
18:3*n*-3	67.6	65.7	63.9	61.1	59.9	1.34	<0.001	0.880

[1] Linear effect of day.
[2] Quadratic effect of day.

Milk Production and Composition

Allocation frequency had no effect ($P > 0.05$) on milk yield and on fat, protein and lactose content and yield (Table 4.3). Only the excretion of C16-FA was affected ($P = 0.024$) by treatment, with a lower secretion of C16-FA in 1D than in 4D. Milk yield, fat and protein content, and fat, protein and lactose yield changed during the 4 d in the 4D treatment (Table 4.3) whereas changes were limited in the 1D treatment ($P > 0.05$, data not shown). Milk yield increased on d 2 and decreased thereafter resulting in a quadratic effect ($P = 0.001$). Milk fat content also showed a quadratic effect ($P = 0.002$) and was greatest on d 1 and lowest on d 3. Milk protein also showed a quadratic effect between days in 4D ($P = 0.030$), with a greater protein content on d 1 than on the other days. Milk secretion of short-chain FA, C16-FA and saturated FA linearly decreased ($P < 0.05$) during the 4 d in 4D whereas secretion of poly-unsaturated FA increased on d 2 and decreased thereafter resulting in a quadratic effect.

FA in Milk Fat

The effect of pasture allocation frequency on the milk FA profile is presented in Table 4.4. Hardly any differences occurred between treatments. Only *iso*-14:0 (lower in 1D than 4D; $P = 0.009$) and 17:0 (higher in 1D than 4D; $P = 0.001$) differed between the treatments. In contrast, milk FA were largely influenced by day within the grazing regime (d 1 to d 4) (Table 4.4). Changes in the milk FA profile were less variable in the 1D (data not shown) compared to the 4D treatment. Milk proportions of C4 to C10 FA, *cis*-9 14:1, *cis*-9 16:1, *cis*-14 + *trans*-16 18:1, trans monoene isomers with a double bond at carbon positions C6-8, C11, C12 and C15, *cis*-9, *trans*-11-18:2, *trans*-10, *cis*-12-18:2, *trans*-11, *cis*-15-18:2 and 18:3*n*-3 increased from d 1 to d 2 in the 4D treatment and decreased on d 4. In contrast, 18:0 decreased on d 2 and increased thereafter. Cis-9-18:1, *cis*-12-18:1 and 18:2*n*-6 linearly increased during the 4 d period whereas 12:0, 14:0 and 16:0 decreased. The milk fat content of short-chain

Table 4.3

Milk production characteristics of dairy cows allocated every day (1D) or every 4 d (4D) to a new plot and changes in milk production characteristics during the 4 d of the 4D treatment.

	1D	4D				SEM		*P*-value		
Variable		Day 1	Day 2	Day 3	Day 4	T[1]	D[2]	T[1]	L[3]	Q[4]
Milk yield	24.6	23.2	25.0	24.0	23.4	0.41	0.48	0.258	0.814	0.001
Milk composition (g/100 g)										
fat	3.28	3.72	3.48	3.40	3.57	0.119	0.131	0.164	0.053	0.002
protein	3.18	3.40	3.27	3.28	3.25	0.087	0.089	0.320	0.001	0.030
lactose	4.32	4.43	4.44	4.45	4.42	0.046	0.048	0.107	0.709	0.294
Milk secretion (g/d)										
fat	829	826	835	790	798	38.0	39.9	0.798	0.037	0.977
protein	781	776	810	779	750	12.5	14.8	0.890	0.014	0.001
lactose	1062	1026	1112	1062	1035	25.7	27.9	0.925	0.715	0.001
SCFA[5]	173	195	200	180	179	15.1	15.6	0.571	0.004	0.383
C16-FA[6]	177	228	221	202	201	8.8	9.9	0.024	0.001	0.530
C18-FA[7]	330	329	332	331	335	12.3	13.8	0.909	0.575	0.957
OBCFA[8]	33.6	32.8	32.3	31.7	32.4	1.08	1.22	0.435	0.559	0.327
SFA[9]	445	514	503	467	471	27.8	29.3	0.391	0.001	0.435
MUFA[10]	245	225	232	230	231	8.0	9.2	0.162	0.472	0.663
PUFA[11]	35.3	31.3	36.9	33.7	30.8	1.29	1.57	0.255	0.326	0.001

[1] Treatment.
[2] Day within 4D.
[3] Linear effect of day within 4D.
[4] Quadratic effect of day within 4D.
[5] Short and medium-chain fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 10:1 + 12:0 + 14:0 + *cis*-9-14:1).
[6] C16-fatty acids (16:0 + *cis*-9-16:1).
[7] C18-fatty acids (18:0 + *trans*-6, -7, -8-18:1 + *trans*-9-18:1 + *trans*-10-18:1 + *trans*-11-18:1 + *trans*-12-18:1 + *trans*-15-18:1 + *cis*-9-18:1 + *cis*-11-18:1 + *cis*-12-18:1 + *cis*-13-18:1 + *cis*-14 + *trans*-16-18:1 + *cis*-15-18:1 + *trans*-10, *cis*-12-18:2 + *cis*-9, *trans*-11-18:2 + *trans*-11, *cis*-15-18:2 + 18:2*n*-6 + 18:3*n*-3).
[8] Odd and branched-chain fatty acids (*iso*-13:0 + *iso*-14:0 + *iso*-15:0 + *iso*-16:0 + *iso*-17:0 + *anteiso*-13:0 + *anteiso*-15:0 + 11:0 + 15:0 + 17:0 + *cis*-9-17:1).
[9] Saturated fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 16:0 + 18:0 + *iso*-13:0 + *iso*-14:0 + *iso*-15:0 + *iso*-16:0 + *iso*-17:0 + *anteiso*-13:0 + *anteiso*-15:0 + 11:0 + 15:0 + 17:0).
[10] Mono-unsaturated fatty acids (10:1 + *cis*-9-14:1+ *cis*-9-16:1+ *trans*-6, -7, -8-18:1 + *trans*-9-18:1 + *trans*-10-18:1 + *trans*-11-18:1 + *trans*-12-18:1 + *trans*-15-18:1 + *cis*-9-18:1 + *cis*-11-18:1 + *cis*-12-18:1 + *cis*-13-18:1 + *cis*-14 + *trans*-16-18:1 + *cis*-15-18:1 + *cis*-9-17:1).
[11] Poly-unsaturated fatty acids (*trans*-10, *cis*-12-18:2 + *cis*-9, *trans*-11-18:2 + *trans*-11, *cis*-15-18:2 + 18:2*n*-6 + 18:3*n*-3).

the four days of the 4D treatment whereas there was a quadratic response for *iso*-14:0 and *iso*-17:0. Anteiso-15:0 was lower on d 1 than d 2 and 3 in the 4D treatment. Of the linear odd-chain FA, 11:0 declined from d 1 to 4 whereas 17:0 and *cis*-9-17:1 initially declined from d 1 to d 2 and thereafter increased again.

Table 4.4

Fatty acids in milk fat (g/100 g fatty acids) of dairy cows reallocated every day (1D) or every 4 d (4D) to a new plot and changes in milk fatty acids during the 4 d of the 4D treatment.

	1D	4D				SEM		*P*-value		
Variable		Day 1	Day 2	Day 3	Day 4	T[1]	D[2]	T[1]	L[3]	Q[4]
4:0	4.11	3.86	4.18	4.14	4.16	0.067	0.086	0.783	0.005	0.017
6:0	2.03	2.03	2.19	2.05	2.02	0.063	0.069	0.697	0.279	0.003
8:0	1.18	1.04	1.11	1.02	0.99	0.050	0.054	0.108	0.060	0.010
10:0	2.30	2.46	2.53	2.29	2.18	0.119	0.127	0.768	0.001	0.025
10:1	0.186	0.191	0.216	0.178	0.176	0.014	0.017	0.865	0.149	0.265
12:0	2.34	2.78	2.78	2.54	2.48	0.188	0.198	0.403	0.001	0.578
14:0	9.53	10.7	10.6	9.86	9.55	0.398	0.421	0.367	0.001	0.584
***cis*-9-14:1**	0.955	0.905	1.022	0.944	0.873	0.026	0.032	0.662	0.133	0.001
16:0	22.9	26.1	25.0	23.7	23.2	0.737	0.798	0.186	0.001	0.327
***cis*-9-16:1**	1.26	1.19	1.24	1.24	1.12	0.031	0.040	0.182	0.195	0.002
18:0	10.4	10.2	9.93	10.6	10.4	0.296	0.336	0.352	0.037	0.001
***trans*-6, -7, -8-18:1**	0.258	0.242	0.261	0.264	0.238	0.007	0.010	0.546	0.752	0.001
***trans*-9-18:1**	0.180	0.181	0.160	0.167	0.168	0.011	0.013	0.578	0.411	0.144
***trans*-10-18:1**	0.197	0.206	0.224	0.234	0.223	0.015	0.019	0.292	0.399	0.270
***trans*-11-18:1**	4.31	4.13	4.60	4.56	3.86	0.394	0.416	0.969	0.323	0.001
***trans*-12-18:1**	0.273	0.300	0.325	0.312	0.261	0.010	0.014	0.056	0.049	0.001
***trans*-15-18:1**	0.289	0.343	0.407	0.392	0.359	0.032	0.034	0.122	0.683	0.001
***cis*-9-18:1**	22.0	19.1	19.1	20.8	21.5	0.978	1.023	0.278	0.001	0.226
***cis*-11-18:1**	0.472	0.459	0.398	0.451	0.432	0.020	0.027	0.230	0.794	0.299
***cis*-12-18:1**	0.127	0.120	0.121	0.131	0.131	0.003	0.004	0.731	0.017	0.801
***cis*-13-18:1**	0.072	0.071	0.072	0.078	0.073	0.004	0.005	0.781	0.450	0.200
***cis*-14 + *trans*-16-18:1**	0.465	0.505	0.531	0.532	0.491	0.031	0.033	0.346	0.579	0.009
***cis*-15-18:1**	0.262	0.262	0.274	0.276	0.256	0.014	0.015	0.830	0.627	0.007
***trans*-10, *cis*-12-18:2**	0.008	0.009	0.010	0.011	0.009	0.001	0.001	0.238	0.488	0.016
***cis*-9, *trans*-11-18:2**	2.05	1.84	2.34	2.28	1.83	0.159	0.174	0.908	0.809	0.001
***trans*-11, *cis*-15-18:2**	0.647	0.678	0.809	0.762	0.634	0.042	0.047	0.214	0.147	0.001
18:2*n*-6	0.842	0.742	0.796	0.776	0.838	0.028	0.035	0.257	0.018	0.856
18:3*n*-3	0.870	0.790	0.827	0.815	0.788	0.034	0.056	0.258	0.835	0.026
Iso-13:0	0.050	0.055	0.054	0.055	0.055	0.002	0.003	0.210	0.966	0.968
Iso-14:0	0.121	0.132	0.130	0.135	0.141	0.004	0.004	0.009	0.008	0.015
Iso-15:0	0.362	0.360	0.359	0.378	0.390	0.005	0.006	0.170	<0.001	0.090
Iso-16:0	0.276	0.280	0.294	0.298	0.304	0.009	0.010	0.187	0.013	0.452
Iso-17:0	0.509	0.540	0.516	0.546	0.573	0.016	0.017	0.172	0.006	0.001
Anteiso-13:0	0.014	0.015	0.015	0.015	0.014	0.001	0.001	0.678	0.252	0.178
Anteiso-15:0	0.762	0.722	0.754	0.766	0.741	0.034	0.036	0.736	0.333	0.018
11:0	0.027	0.034	0.031	0.027	0.025	0.003	0.003	0.672	0.001	0.989
15:0	1.22	1.27	1.25	1.26	1.23	0.033	0.035	0.125	0.215	0.748
17:0	0.648	0.594	0.532	0.575	0.626	0.012	0.014	0.001	0.004	0.001
***cis*-9-17:1**	0.256	0.265	0.251	0.277	0.292	0.018	0.019	0.566	0.002	0.012
SCFA	22.7	23.9	24.5	23.0	22.4	0.71	0.76	0.557	<0.001	0.035
C16-fatty acids	24.2	27.2	26.2	24.9	24.3	0.70	0.77	0.246	<0.001	0.472
C18-fatty acids	44.6	39.3	39.5	41.9	41.7	1.52	1.59	0.147	<0.001	0.687
OBCFA	4.30	4.16	4.08	4.23	4.28	0.114	0.120	0.479	0.039	0.122
SFA	59.2	62.7	61.0	59.2	58.8	0.99	1.10	0.455	<0.001	0.196
MUFA	31.7	28.3	29.1	30.7	30.3	1.03	1.09	0.237	<0.001	0.122
PUFA	4.64	3.82	4.55	4.41	3.87	0.156	0.180	0.070	0.984	<0.001

[1] Treatment.
[2] Day within 4D.
[3] Linear effect of day within 4D.
[4] Quadratic effect of day within 4D.

Treatment effects on the desaturase indices are presented in Table 4.5. The ratio *cis*-9-16:1/(16:0 + *cis*-9-16:1) was lower ($P = 0.019$) in the 4D treatment than the 1D treatment whereas no treatment effects were observed for the other indices. Desaturase indices did not change during the 4 days in the 1D treatment (data not shown) whereas indices increased on d 2 and 3 in the 4D treatment and declined thereafter.

Table 4.5

Desaturase indices of dairy cows allocated every day (1D) or every 4 d (4D) to a new plot and changes in desaturase indices during the 4 d of the 4D treatment.

	1D	4D				SEM		*P*-value		
Variable		Day 1	Day 2	Day 3	Day 4	T[1]	D[2]	T[1]	L[3]	Q[4]
cis-9-14:1/(14:0 + *cis*-9-14:1)	0.089	0.079	0.090	0.089	0.085	0.002	0.002	0.149	0.005	<0.001
cis-9-16:1/(16:0 + *cis*-9-16:1)	0.053	0.043	0.047	0.050	0.046	0.002	0.002	0.019	0.056	0.002
cis-9-17:1/(17:0 + *cis*-9-17:1)	0.289	0.300	0.311	0.316	0.308	0.009	0.009	0.129	0.134	0.008
cis-9-18:1/(18:0 + *cis*-9-18:1)	0.676	0.657	0.680	0.678	0.671	0.005	0.006	0.630	0.026	<0.001
cis-9, *trans*-11-18:1/(*trans*-11-18:1 + *cis*-9, *trans*-11-18:1)	0.327	0.305	0.334	0.330	0.320	0.006	0.007	0.607	0.026	<0.001

[1] Treatment.
[2] Day within 4D.
[3] Linear effect of day within 4D.
[4] Quadratic effect of day within 4D.

Variation coefficients of the secretion of milk FA (g/d) and C18-FA in milk fat (g/100 g of FA) during the 4 d in 4D are presented in Table 4.6 and 4.7. The variation coefficients of the secretion of short-chain FA and C16-FA are related whereas variation in the secretion of C18-FA was positively related with secretion of odd-and branched-chain FA (Table 4.6). Variation in 18:3*n*-3 in milk fat during the 4 d did not differ ($P > 0.05$) from that in *cis*-9-18:1 and 18:2*n*-6, but was lower ($P < 0.05$) compared with 18:0, *trans*-11-18:1, *cis*-9, *trans*-11-18:2 and *trans*-11, *cis*-15-18:2 (Table 4.7). The variation in milk fat proportion of *cis*-9, *trans*-11-18:2 was positively related to that of *trans*-11-18:1 and *trans*-11, *cis*-15-18:2.

Table 4.6

Variation coefficients of the secretion (g/d) of short-chain fatty acids (SCFA), C16-fatty acids (C16-FA), C18-fatty acids (C18-FA) and odd- and branched-chain fatty acids (OBCFA) during the 4 d in the 4D treatment. Values above the diagonal represent correlation coefficients, values below the diagonal represent pairwise differences.

Variable	Mean	SCFA	C16-FA	C18-FA	OBCFA
SCFA	9.88		0.879***	-0.283	-0.106
C16-FA	11.22	-1.35*		-0.276	-0.054
C18-FA	7.54	2.34	3.69		0.881***
OBCFA	6.83	3.05	4.40*	-0.708	

*** $P < 0.001$
* $P < 0.05$

Table 4.7

Variation coefficients of the proportion (g/100 g fatty acids) of C18-fatty acids in milk fat during the 4 d in the 4D treatment. Values above the diagonal represent correlation coefficients, values below the diagonal represent pairwise differences.

Variable	**Mean**	**18:0**	**Trans-11-18:1**	**Cis-9-18:1**	**Trans-11, *cis*-15-18:2**	**18:2*n*-6**	**18:3*n*-3**	**Cis-9, *trans*-11-18:2**
18:0	8.80		-0.412	0.200	0.096	-0.300	0.292	-0.051
Trans-11-18:1	12.2	-3.41		0.283	0.307	-0.002	0.076	0.756***
Cis-9-18:1	7.55	1.26	4.66*		0.419	-0.030	0.233	0.470
Trans-11, *cis*-15-18:2	15.0	-6.16**	-2.75	-7.41***		0.063	-0.173	0.502*
18:2*n*-6	10.8	-2.01	1.40	-3.26	4.15		0.006	-0.137
18:3*n*-3	6.12	2.68*	6.09**	1.43	8.81***	4.69		0.076
***cis*-9, *trans*-11-18:2**	15.7	-6.93**	-3.53*	-8.19***	-0.777	-4.93	-9.62***	

*** $P < 0.001$
** $P < 0.01$
* $P < 0.05$

Relation between Grass Composition and Milk FA

Regression equations relating the gradual decrease in 18:3*n*-3 content of the grazed pasture during the 4 days of the 4D treatment with milk fat content and secretion of some individual FA are presented in Table 4.8. With the exception of *trans*-11-18:1 in milk fat, milk fat content and secretion of the selected FA were strongly and positively related with 18:3*n*-3 content of the grazed pasture.

Table 4.8

Regression equations relating variation in 18:3*n*-3 content in grass (g/kg DM) on day i – 1 during the 4 d in the 4D treatment with milk fat content and secretion of selected fatty acids on day i (n = 14).

	intercept			18:3*n*-3 content in grass				
Variable	estimate	SE[1]	P[2]	estimate	SE	P	R^2	Residual SE
Milk fat content (g/100 g fatty acids)								
trans-11-18:1	3.335	0.527	0.001	0.073	0.042	0.110	0.199	0.393
trans-11, *cis*-15-18:2	0.226	0.080	0.015	0.041	0.006	0.001	0.773	0.059
cis-9, *trans*-11-18:2	1.268	0.314	0.002	0.069	0.025	0.017	0.387	0.234
18:3*n*-3	0.630	0.066	0.001	0.021	0.005	0.002	0.576	0.049
Milk secretion (g/d)								
trans-11-18:1	16.8	3.18	0.001	1.15	0.255	0.001	0.628	2.37
trans-11, *cis*-15-18:2	0.611	1.043	0.569	0.391	0.084	0.001	0.646	0.78
cis-9, *trans*-11-18:2	5.57	1.395	0.002	0.807	0.112	0.001	0.813	1.04
18:3*n*-3	3.21	1.154	0.017	0.274	0.092	0.012	0.423	0.86

[1] Standard error of the regression coefficient.
[2] *P*-value of the regression coefficient.

DISCUSSION

FA Composition of Pasture

The main FA in the grazed pasture is 18:3*n*-3 and levels are similar to those reported by Dewhurst et al. (2006). Total FA content and 18:3*n*-3 content in pasture were strongly related. Similar observations were made by Bauchart et al. (1984), investigating the FA profiles throughout the season and by Boufaied et al. (2003), studying the effect of growth stage and N fertilization. Dewhurst et al. (2006) suggested the common basis for many of the effects on FA and 18:3*n*-3 content in grass appears to be the leaf/stem ratio with lower concentrations of FA in stemmy regrowths. The decrease in leaf/stem ratio during the 4 days in 4D observed by Abrahamse et al. (2008) is in line with the decrease in total FA and 18:3*n*-3 content observed in the present experiment. This variation in leaf/stem ratio and FA content during the 4 days of treatment 4D and between offer and residue in treatment 1D were most likely induced by the eating behaviour of the dairy cows (Abrahamse et al., 2008). Indeed, grazing takes place in horizons, i.e. the grass sward is to a certain extent grazed in horizontal layers. Delagarde et al. (2000) showed that the chemical composition of perennial ryegrass differs over the vertical distribution of grass. This seems also the case for 18:3*n*-3 as illustrated by the strong relationship of grass height with 18:3*n*-3 content (18:3*n*-3 [g/kg DM] = -9.53 (SE = 1.709, $P < 0.001$) + 8.27 (SE = 0.662, $P < 0.001$) × ln(grass height [cm]), $R^2 = 0.821$, $P < 0.001$, n = 36), indicating a higher 18:3*n*-3 content in the leafy top of grass as compared to the lower parts of the grass. This vertical distribution of 18:3*n*-3 in grass has direct consequences on the temporal intake pattern of 18:3*n*-3.

The difference in FA content and composition between days in treatment 4D, related to a decrease in grass height as the cows were grazing down the sward, probably caused a gradual decline in 18:3*n*-3 intake during the 4 days. Pasture intake was not estimated per day in the 4D treatment because of limitations of the alkane method used (Abrahamse et al., 2008). However, based on the relationship between sward surface height and intake, Abrahamse et al. (2008) derived a quadratic decrease in pasture intake between days in 4D. Therefore, a decline in 18:3*n*-3 intake between days in 4D is highly likely. The similarity of the FA composition of the material offered and the residue between treatments suggests the gradual decline in 18:3*n*-3 content and intake during the 4 days of the 4D treatment also occurred in the 1D treatment within 1 day.

Milk FA

The absence of major effects of dietary treatments on milk FA profile was not surprising as proximate chemical composition (Abrahamse et al., 2008) and FA composition of the pasture (Table 4.1) were similar between treatments. Abrahamse et al. (2008) observed a small but significant greater milk production and lower fat and protein content with 1D than 4D. The present results are quantitatively in line with these findings, but the lower number of animals and of periods than in Abrahamse et al. (2008) may have caused these differences not to be significant.

As expected, the FA profile in milk fat in the 1D treatment was less variable between days compared with the 4D treatment. The observed correlation between and the magnitude of the variation coefficients of the yield of short- and medium-chain FA, C16-FA, C18-FA and OBCFA between days in 4D suggests secretion of *de novo* synthesised FA are more susceptible to variation in pasture quality and allowance compared with secretion of preformed FA (C18-FA and microbial derived FA). Changes in short- and medium-chain FA in milk fat during the 4 days in 4D confirm the findings of Stockdale et al. (2003) and Bargo et al. (2006b) reporting that grazing at high pasture allowance usually resulted in a higher milk fat content of short- and medium-chain FA. Similarly, Kay et al. (2007) report a decrease in short- and medium-chain FA when pasture allowance decreased although differences in their study did not reach significance. In three experiments investigating the effects of low or high herbage allowance reported by Stockdale et al. (2003), a difference in pasture DMI of 9.4 or 5.2 kg/d did result in higher levels of milk short- and medium chain FA at the high herbage level, whereas a smaller difference in pasture DMI of 2.1 kg/d did not affect levels of these FA. The much lower DMI levels at low compared with high herbage allowance in two of the three experiments suggest that energy intake did not match energy requirements, and animals at low pasture allowance may have mobilized significant amounts of body fat, which comprises predominantly long chain FA. Van Knegsel et al. (2007) measured energy balance of dairy cattle by indirect calorimetry and observed elevated contents of short chain FA in milk with increasing energy balance. Thus, the likely lower pasture intake level on d 4 than d 1 in the 4D treatment (Abrahamse et al., 2008) may have resulted in body fat mobilisation, and consequently an additional supply of long chain FA for incorporation into milk fat. Another reason for this decrease in short- and medium-chain FA with low pasture allowance (Stockdale et al., 2003; Bargo et al., 2006b) or after 4 days in 4D (current experiment) might be related to a decreased supply of precursors (i.e. acetate and butyrate) through decreased DMI. Indeed, concentrations of acetate and butyrate in rumen fluid decreased during the 4 days of treatment 4D (82.3, 79.3, 76.1 and 77.2 mmol acetate/l and 14.8, 13.7, 12.4 and 12.5 mmol butyrate/l on d 1, 2, 3 and 4, respectively; Abrahamse et al., 2008). The decrease in short- and medium-chain FA was related

with an increase in *cis*-9-18:1 (rpearson = -0.684, $P < 0.001$, n = 126), a response frequently observed with cows in negative energy balance (Palmquist et al., 1993).
Milk fat concentrations of 18:3*n*-3 between days in 4D was highest at d 2, probably reflecting the increased intake of 18:3*n*-3 when cows were allocated to a new plot and assuming a delay in occurrence of effect of approximately 1 d. In the current experiment, variation in 18:3*n*-3 of the grazed pasture between days was related with its content and secretion in milk (Table 4.6). Concentrations of *trans*-11, *cis*-15-18:2 in milk fat showed a similar pattern as 18:3*n*-3 during the 4 days of the 4D treatment. Trans-11, *cis*-15-18:2 is formed during biohydrogenation of 18:3*n*-3 in the rumen (Harfoot and Hazlewood, 1997) and increased 18:3*n*-3 intake is reported to increase duodenal flow and concentration in milk fat of *trans*-11, *cis*-15-18:2 (Loor et al., 2004, Loor et al., 2005). This explains the positive relation between 18:3*n*-3 content of grass and secretion of *trans*-11, *cis*-15-18:2 in the current experiment (Table 4.6). The variation between days in 4D of 18:3*n*-3 in milk fat was smaller compared to *trans*-11, *cis*-15-18:2 potentially reflecting the variation in subsequent biohydrogenation of *trans*-11, *cis*-15-18:2 due to rumen conditions, or the preferential incorporation of 18:3*n*-3 in plasma phosholipids and cholesterol esters (Tyburczy et al., 2008), or both.
Compared with TMR diets, pasture-based diets have resulted in higher concentrations of *cis*-9, *trans*-11-18:2 in milk (Kelly et al., 1998; Dhiman et al., 1999), attributed to an increased rumen supply of *trans*-11-18:1. Recent estimates suggested that 84.4 % (abomasal infusion of lipid supplement enriched in *trans*-11-18:1; Shingfield et al., 2007) and 83 % (abomasal infusion of 13C-labeled *trans*-11-18:1; Mosley et al., 2006) of the *cis*-9, *trans*-11-18:1 in milk is formed by desaturation of *trans*-11-18:1 in the mammary gland with stall fed animals. With dairy cows fed fresh pasture, endogenous synthesis of *cis*-9, *trans*-11-18:2 was responsible for more than 91% of the secretion of this FA in milk fat as estimated by abomasal infusion of sterculic oil (Kay et al., 2004). The major contribution of ruminal *trans*-11-18:1 to milk *cis*-9, *trans*-11-18:2 is supported by the positive relation between milk *trans*-11-18:1 and *cis*-9, *trans*-11-18:2 in milk fat (*cis*-9, *trans*-11-18:2 [g/100 g FA] = 0.207 (SE = 0.085, $P = 0.016$) + 0.432 (SE = 0.019, $P < 0.001$) × *trans*-11-18:1 [g/100 g FA], $R^2 = 0.803$, RMSE = 0.228, $P < 0.001$, n = 126). Similar relations between *trans*-11-18:1 and *cis*-9, *trans*-11-18:2 were described previously with pasture based diets (Bargo et al., 2006a, Bargo et al., 2006b). The stronger relationship between *cis*-9, *trans*-11-18:2 and *trans*-11-18:1 in milk fat than between *cis*-9, *trans*-11-18:2 and Δ-9-desaturase index (rpearson = 0.355, $P < 0.001$, n = 126) indicates that the variation in *cis*-9, *trans*-11-18:2 content of milk fat is primarily resulting from rumen derived *trans*-11-18:1 and, to a lesser extent, by changes in the Δ-9-desaturase system in the udder. Both substrate supply (i.e. 18:2*n*-6 and 18:3*n*-3) and the ruminal formation and hydrogenation of *trans*-11-18:1 are reported to regulate the rumen supply of *trans*-11-18:1. The importance of substrate supply was illustrated by (Stockdale et al., 2003) and (Bargo et al., 2006a) who showed that increased intake of pasture (Stockdale et al., 2003) and 18:3*n*-3 (Bargo et al., 2006a) in grazing dairy cows increased concentrations of *cis*-9, *trans*-11-18:2 in milk fat. In the current experiment, the gradual decline in 18:3*n*-3 content of the pasture during the 4 days was related with decreased milk content and secretion of *cis*-9, *trans*-11-18:2 (Table 4.8), suggesting the variation of *cis*-9, *trans*-11-18:2 during the 4 days in the 4D treatment was associated with an altered supply of 18:3*n*-3. Nevertheless, the decrease in 18:3*n*-3 content of the grass during the 4 days in the 4D treatment was accompanied with an increase in grass NDF content and rumen fluid pH (Abrahamse et al., 2008). An increased dietary NDF and increased rumen pH is frequently reported to stimulate complete biohydrogenation, again resulting in a decreased rumen accumulation of FA intermediates (Harfoot and Hazlewood,

1997). Hence, the increase in NDF content of the pasture with decreasing 18:3*n*-3 might have enhanced the relations found in the current experiment. Moreover, in the present study all desaturation indices were enhanced on d 2 and 3 in the 4D treatment (Table 4.5), indicating increased Δ-9-desaturase activity. In other studies, pasture allowance or proportion of fresh grass in the diet did not affect the C14 or C16 desaturase proxies (Bargo et al., 2006b; Couvreur et al., 2006). Thus, the proxies suggest that variation in Δ-9-desaturase activity does contribute to observed variation in *cis*-9 FA content. The highest Δ-9-desaturase activity (on d 2 and d 3) is associated with the lowest milk fat content. This is in contrast with findings that milk fat depression coincides with reduced Δ-9-desaturase activity, and in line with Harvatine et al. (2009) who concluded in their review that there is no direct evidence to support the inhibition of Δ-9-desaturase activity as a specific causative factor in milk fat depression. In our study, *trans*-10, *cis*-12-18:2 content was highest on d 2 and d 3 and positively associated with Δ-9-desaturase activity, whereas in general this particular CLA isomer inhibits Δ-9-desaturase activity (Harvatine et al., 2009). Further research is required to elucidate the relationships between particular unsaturated FA and Δ-9-desaturase activity in grass-based diets.

Odd- and branched-chain FA in milk fat changed during the 4 days in group 4D, but the magnitude was less compared with the C18-FA. Odd- and branched-chain FA are largely derived from bacteria leaving the rumen, and variation in the odd- and branched-chain FA profile were suggested to be a reflection of changes in the relative abundance of specific bacterial populations in the rumen (Vlaeminck et al., 2006), whereas its secretion in milk reflects duodenal flow of bacterial N (Vlaeminck et al., 2005; Vlaeminck et al., 2006). The small differences between days in the 4D group suggest no major changes in the rumen microbial population. Indeed, the magnitude of variation during the 4 days in group 4D was substantially lower compared with changes brought about by replacing grass silage with maize silage or increasing the dietary forage to concentrate ratio (Vlaeminck et al., 2006). Recent studies have shown that branched-chain FA have anti-cancer activity (Yang et al., 2000; Wongtangtintharn et al., 2004). Interestingly, concentrations of all individual branched-chain FA were generally higher compared with values reported for dairy cows fed TMR (e.g. Vlaeminck et al., 2006) and confirm the increase in branched-chain FA in milk fat after transition to pasture (B. Vlaeminck, unpublished results).

CONCLUSION

This study showed that increasing pasture allocation frequency from once every 4 d to every day has limited effects on the FA profile in milk fat. In contrast, milk FA composition was largely affected by day within the 4d rotation system, with a greater effect on biohydrogenation intermediates in milk fat compared with its major precursor, 18:3*n*-3.

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REFERENCES

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91:2033-2045.

Bargo, F., J. E. Delahoy, G. F. Schroeder, L. H. Baumgard, and L. D. Muller. 2006a. Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. Animal Feed Science and Technology 131(3-4):226-240.

Bargo, F., J. E. Delahoy, G. F. Schroeder, and L. D. Muller. 2006b. Milk fatty acid composition of dairy cows grazing at two pasture allowances and supplemented with different levels and sources of concentrate. Animal Feed Science and Technology 125(1-2):17-31.

Bauchart, D., R. Vérité, and B. Remond. 1984. Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. Canadian Journal of Animal Science 64:330-331.

Boufaied, H., P. Y. Chouinard, G. F. Tremblay, H. V. Petit, R. Michaud, and G. Belanger. 2003. Fatty acids in forages. I. Factors affecting concentrations. Canadian Journal of Animal Science 83(3):501-511.

Chilibroste, P. 2005. Pasture characteristics and animal performance. Pages 681-706 in Quantitative Aspects of Ruminant Digestion and Metabolism. J. Dijkstra, J. M. Forbes, and J. France, ed. CAB International, Wallingford, UK.

Couvreur, S., C. Hurtaud, C. Lopez, L. Delaby, and J. L. Peyraud. 2006. The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. Journal of Dairy Science 89(6):1956-1969.

Dalley, D. E., J. R. Roche, C. Grainger, and P. J. Moate. 1999. Dry matter intake, nutrient selection and milk production of dairy cows grazing rainfed perennial pastures at different herbage allowances in spring. Australian Journal of Experimental Agriculture 39(8):923-931.

Dalley, D. E., J. R. Roche, P. J. Moate, and C. Grainger. 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Australian Journal of Experimental Agriculture 41(5):593-599.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin - cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1-2):49-68.

Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Animal Feed Science and Technology 131(3-4):168-206.

Dhiman, T. R., G. R. Anand, L. D. Satter, and M. W. Pariza. 1999. Conjugated linoleic acid content of milk from cows fed different diets. Journal of Dairy Science 82(10):2146-2156.

Folch, J., M. Lees, and S. G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497-509.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. In Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra, and S. Tamminga, ed. Springer, The Netherlands.

Griinari, J. M., K. Nurmela, A. Sairanen, J. I. Nousiainen, and H. Khalili. 1998. Effect of dietary sunflower oil and pasture forage maturity on conjugated linoleic acid (CLA) content in milk fat from lactating dairy cows. Journal of Dairy Science 81(Suppl. 1):300.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid Metabolism in the Rumen. Pages 382-426 in The Rumen Microbial Ecosystem. P. N. Hobson and D. S. Stewart, ed. Chapman & Hall, London.

Harvatine, K. J., Y. R. Boisclair, and D. E. Bauman. 2009. Recent advances in the regulation of milk fat synthesis. Animal 3(1):40-54.

ISO. 1999. Whole milk. Determination of milkfat, protein and lactose content-Guidance on the operation of mid-infrared instruments. ISO 9622:1999. International Organization for Standardization, Geneva, Switzerland.

Kay, J. K., T. R. Mackle, M. J. Auldist, N. A. Thomson, and D. E. Bauman. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. Journal of Dairy Science 87(2):369-378.

Kay, J. K., T. R. Mackle, D. E. Bauman, N. A. Thomson, and L. H. Baumgard. 2007. Effects of CLA on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. Journal of Dairy Science.

Kelly, M. L., E. S. Kolver, D. E. Bauman, M. E. Van Amburgh, and L. D. Muller. 1998. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. Journal of Dairy Science 81(6):1630-1636.

Khanal, R. C., T. R. Dhiman, and R. L. Boman. 2007. Changes in fatty acid composition of milk from lactating dairy cows during transition to and from pasture. Livestock Science 114:164-175.

Lock, A. L., B. A. Corl, D. M. Barbano, D. E. Bauman, and C. Ip. 2004. The anticarcinogenic effect of *trans*-11 18 : 1 is dependent on its conversion to *cis*-9, *trans*-11 CLA by delta-9-desaturase in rats. Journal of Nutrition 134(10):2698-2704.

Loor, J. J., A. Ferlay, A. Ollier, K. Ueda, M. Doreau, and Y. Chilliard. 2005. High-concentrate diets and polyunsaturated oils alter trans and conjugated isomers in bovine rumen, blood, and milk. Journal of Dairy Science 88(11):3986-3999.

Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage: concentrate ratio and linseed oil in dairy cows. Journal of Dairy Science 87(8):2472-2485.

Lourenço, M., B. Vlaeminck, M. Bruinenberg, D. Demeyer, and V. Fievez. 2005. Milk fatty acid composition and associated rumen lipolysis and fatty acid hydrogenation when feeding forages from intensively managed or semi-natural grasslands. Animal Research 54(6):471-484.

Mosley, E. E., B. Shafii, P. J. Moate, and M. A. McGuire. 2006. *cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. Journal of Nutrition 136(3):570-575.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk-fat composition. Journal of Dairy Science 76(6):1753-1771.

Pulido, R. G. and J. D. Leaver. 2003. Continuous and rotational grazing of dairy cows - The interactions of grazing system with level of milk yield, sward height and concentrate level. Grass and Forage Science 58(265-275).

SAS Institute. 2004. SAS/STAT 9.1 User's Guide. SAS Institute Inc, Cary, NC.

Schroeder, G. F., G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller. 2004. Effects of fat supplementation on milk production and composition by dairy cows on pasture: a review. Livestock Production Science 86(1-3):1-18.

Shingfield, K. J., S. Ahvenjarvi, V. Toivonen, A. Vanhatalo, and P. Huhtanen. 2007. Transfer of absorbed *cis*-9, *trans*-11 conjugated linoleic acid into milk is biologically more efficient than endogenous synthesis from absorbed vaccenic acid in lactating cows. Journal of Nutrition 137(5):1154-1160.

Stanton, C., F. Lawless, G. Kjellmer, D. Harrington, R. Devery, J. F. Connolly, and J. Murphy. 1997. Dietary influences on bovine milk *cis*-9, *trans*-11-conjugated linoleic acid content. Journal of Food Science 62(5):1083-1086.

Stockdale, C. R., G. P. Walker, W. J. Wales, D. E. Dalley, A. Birkett, Z. P. Shen, and P. T. Doyle. 2003. Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. Journal of Dairy Research 70(3):267-276.

Tyburczy, C., A. L. Lock, D. A. Dwyer, F. Destaillants, Z. Mouloungui, L. Candy, and D. E. Bauman. 2008. Characterization of the utilization of trans octadecenoic acids in lactating dairy cows. Journal of Dairy Science 91:3850-3861.

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. Journal of Dairy Science 90(3):1467-1476.

Vlaeminck, B., C. Dufour, A. M. van Vuuren, A. R. J. Cabrita, R. J. Dewhurst, D. Demeyer, and V. Fievez. 2005. Use of odd and branched-chain fatty acids in rumen contents and milk as a potential microbial marker. Journal of Dairy Science 88(3):1031-1042.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131(3-4):389-417.

Wahle, K. W. J., S. D. Heys, and D. Rotondo. 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Progress in Lipid Research 43(6):553-587.

Wongtangtintharn, S., H. Oku, H. Iwasaki, and T. Toda. 2004. Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. Journal of Nutritional Science and Vitaminology 50(2):137-143.

Yang, Z. H., S. P. Liu, X. D. Chen, H. Chen, M. Huang, and J. P. Zheng. 2000. Induction of apoptotic cell death and in vivo growth inhibition of human cancer cells by a saturated branched-chain fatty acid, 13-methyltetradecanoic acid. Cancer Research 60(3):505-509.


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CHAPTER 5

The Effect of Twice Daily versus Once Daily Allocation in Stripgrazing Dairy Cattle

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Submitted

ABSTRACT

Sixteen Holstein cows were blocked in two groups according to parity, lactation stage and milk yield to evaluate the effect of frequency of allocation to new grazing plots on pasture intake, grazing behaviour, rumen characteristics and milk yield. Two treatments, daily allocation to 0.125 ha plots of *Lolium perenne* L. after evening milking (1D) or twice daily allocation to 0.0625 ha plots after both morning and evening milking (2D) were tested in a crossover design. Herbage dry matter intake was greater in 2D than in 1D (15.5 and 14.4 kg/d, respectively), especially when offered herbage and sward surface height (SSH) were high (25.0 kg DM/d and 17.4 cm, respectively), but not when SSH was low and offered herbage was high (13.7 cm and 29.2 kg DM/d, respectively). Grazing behaviour, observed using IGER graze recorders, was more equally distributed in 1D than in 2D. Almost no differences were found in rumen fermentation variables, milk composition and milk yield between treatments, but milk yield was increased in 2D compared to 1D at high SSH. This study confirmed that increased pasture allocation frequency from once to twice daily improved intake and milk yield in grazing dairy cows, especially when offered SSH was high.

INTRODUCTION

Fresh grass is a cheap source of nutrients, resulting in low costs of milk produced by dairy cows during the grazing season (Taweel et al., 2006). In grazing systems, dry matter intake (DMI) of herbage is often insufficient to achieve high milk yield (Gibb et al., 1999; Bargo et al., 2003). When no concentrates are supplemented, milk yield is limited to approximately 25 kg/d (Kolver and Muller, 1998). Intensive rotational systems like daily stripgrazing increase DMI in dairy cows and may result in more efficient utilization of grassland than grazing systems where cows are reallocated less frequently (Barrett et al., 2001). Indeed, especially when offered herbage was high, intake of herbage and simultaneously milk yield was increased when cows were allocated daily to a new stripgrazing plot, in comparison to allocation every four days to a new plot (Abrahamse et al., 2008a). The effect on DMI might be the result from offering a fresh plot more frequently during the day, as shown by Dalley et al. (2001). However, milk yield was reduced during one of their experiment when cows were allocated 6 times daily to a new plot, compared to once daily, and in the second experiment, no differences were found between treatments.

Direct effects of grass chemical composition on intake might also play an important role in grazing animals. The chemical composition of herbage is variable and depends on several factors, including grass variety (Taweel et al., 2005a; Tas et al., 2006a), season, regrowth days, time of allocation (Orr et al., 2005), and management factors (mainly fertilization rate; Peyraud and Astigarraga 1998). High sugar grasses may increase voluntary herbage DMI of cattle. In a preference study with 6 different perennial ryegrass cultivars, dairy cattle preferred the high-sugar varieties (Smit et al., 2006). Herbage DMI indeed increased when a high-sugar cultivar was offered to zero-grazed cows in early lactation (Moorby et al., 2006), but these effects could not be repeated in other experiments (Miller et al., 2001; Taweel et al., 2005a; Tas et al., 2006a). High sugar grasses may also improve the balance of nutrients in grass. In fresh ryegrass, the amount of readily available carbohydrates and that of protein may be imbalanced (Gibb et al., 1999; Barrett et al., 2001). If insufficient energy is available from carbohydrate fermentation, the rapidly degradable proteins in grazed grass are used as an energy source

rather than being assimilated into microbial protein, and ammonia accumulates in the rumen (Kingston-Smith and Theodorou, 2000). Thus, increasing energy content in grass with sugars may increase microbial protein synthesis (Miller et al., 2001; Tas et al., 2006b). This has been investigated in several experiments by comparing allocation of cows in the late afternoon or evening versus in the morning, since sugars accumulate in grass during the day, resulting in higher sugar contents in grass in the evening than in the morning (Van Vuuren et al., 1986; Delagarde et al., 2000). Orr et al. (2001) found no effects on DMI, but showed a tendency to increased milk yield after afternoon allocation in comparison to morning allocation. In a similar setup, also Abrahamse et al. (2009) did not find effects of morning versus afternoon allocation on DMI, but they did observe a reduction in glucogenic volatile fatty acids (VFA) in the rumen of the cows allocated during the afternoon, which was related to an increased milk fat content and a higher FPCM production in the afternoon group than in the morning group. The aim of the current experiment was to investigate the influence of once daily (after evening milking; 1D) vs. twice daily (after morning and evening milking; 2D) allocation to a fresh plot on herbage DMI, grazing behaviour, rumen characteristics and milk yield and milk composition in grazing dairy cattle.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was undertaken between June 19 and September 7, 2006, after approval by the Institutional Animal Care and Use Committee of Wageningen University. The study was conducted as a crossover design with 2 periods and 2 treatments. After adaptation to grazing during six weeks, two groups of 8 dairy cows were assigned to their respective treatments and adapted to these treatments for one week. The treatments, once daily reallocation after evening milking (1D) to a fresh 0.125 ha plot or twice daily reallocation after both morning and evening milking (2D) to a fresh 0.0625 ha plot, were repeated during two periods of 14 and 8 days, respectively. Water was available *ad libitum*.

Herbage

A uniform stand of perennial ryegrass (*Lolium perenne* L.), established in August 2003, was used during the experiment. The mixture used was Havera, a mixture composed of 0.70 *Lolium perenne* tetraploid cvar Elgon and 0.30 *Lolium perenne* diploid cvar Veritas. The fertilizer application rates were 65 kg of N/ha and 15 kg of P/ha in spring, 52 kg of N/ha and 12 kg of P/ha after the first cut, and 59 kg N/ha prior to each period. Total plot size offered every day was 0.125 ha unless herbage allowance was lower than 22.5 or more than 27.5 kg dry matter (DM)/cow/d. If herbage allowance was below 22.5 kg DM/cow/d or above 27.5 kg DM/cow/d, plot size was adjusted to reach herbage allowance of 25 kg DM/cow/d. Herbage mass on offer was estimated using the sward surface height (SSH) and herbage mass double sample technique described in Abrahamse et al. (2008a). Prior to the experiment, plots of 0.125 ha size were stepwise cut, 2 or 3 daily plots for each treatment every 2 or 3 days, and for treatment 2D half of the plots were split into 2 subplots of 0.0625 ha. This procedure was carried out to have approximately an equal amount of DM on offer per day. Plot size was reduced during all days of the first period, while it was increased with 50% during three days and with 100% during 4 days in the second period. This was necessary as grass growth was fast due to extensive rainfall during the first period after a long period of drought, while grass

growth was limited during the second period due to drought. Weather conditions during the experiment as well as average values of 1951 – 2000 are given in Table 5.1.

Table 5.1

Weather conditions during the summer of 2006 as compared to long-term averages*.

	June		July		August		September	
Variable	Average †	2006	Average †	2006	Average †	2006	Average †	2006
Average temperature (°C)	15.4	16.6	17.1	22.4	16.8	16.0	14.2	18.0
Maximum temperature (°C)	20.2	22.0	21.8	28.5	21.8	20.5	18.9	23.0
Minimum temperature (°C)	10.2	10.0	12.2	15.0	11.9	11.9	9.7	13.0
Rainfall (mm/month)	69.2	19.9	73.9	27.1	70.3	214	66.7	9.8
Sunshine (hour/month)	174.0	258.9	169.9	336.3	173.4	123.2	131.1	187.3

* Data from the meteo station Haarweg, Wageningen, The Netherlands.
† Average between 1951 and 2000.

Animals and Milk Yield

Sixteen Holstein cows, of which four were previously fitted with a rumen cannula (10 cm i.d.; Bar Diamond Inc., Parma, ID) in the dorsal sac, were paired by parity, days in milk (DIM), and milk yield during the adaptation period and randomly assigned to the treatments. One pair of cows (one cow in each treatment) was eliminated from the experiment due to low milk yield. At the start of the experiment, cows produced 26.8 ± 1.34 kg of milk/d (values expressed as means ± SE), were 151 ± 20.4 DIM, weighed 575 ± 10.5 kg and had a body condition score (scale 1-5) of 2.2 ± 0.08.

Cows were milked twice daily at 0600 and 1800 h using a mobile milking parlour and individual milk yield was recorded throughout the experiment. Individual milk samples were collected at each milking except for the adaptation periods and stored in a refrigerator at 4°C for no more than 4 d using

Table 5.2

Ingredient and chemical composition of the concentrate.

Item	
Ingredient (% as fed)	
Barley	**15.0**
Corn	**23.2**
Beet pulp	**22.0**
Soya hulls	**19.0**
Soyabean meal	**7.0**
Palm expeller	**5.0**
Molasses	**6.0**
Premix vitamin/mineral	**2.5**
Alkane + arbocel mix	**0.3**
Chemical composition (g/kg DM)*	
Dry matter (g/kg)	**912**
Organic matter	**955**
Crude protein	**135**
Crude fat	**27**
Sugars	**85**
Starch	**244**
Neutral detergent fibre	**288**
Acid detergent fibre	**166**
Acid detergent lignin	**11**
Net energy for lactation (MJ/kg DM) †	**7.3**
Intestinal digestible protein‡	**105**
Degraded protein balance‡	**-22**

* Calculated using the Dutch Feeding Tables (CVB, 2005).
† Calculated with VEM system (Van Es, 1975).
‡ Calculated as in Tamminga et al. (1994).

sodium azide and bronopol as preservative. Fat, protein and lactose contents were determined according to ISO 9622 (Melkcontrolestation, Zutphen, The Netherlands) and milk urea was determined using the pH-difference technique (ISO 14637). Fat and protein corrected milk (FPCM) yield (kg/d) was calculated as [0.337 + 0.116 × fat (%) + 0.06 × protein (%)] × milk yield (kg/d). Milk fatty acid concentrations were determined according to van Knegsel et al. (2007). Herbage DMI was estimated using the alkane technique as described by Abrahamse et al. (2008a). Cows received 2.74 kg DM/d of a concentrate with C_{32} alkanes in two equal portions during milking throughout the experiment. Ingredient and chemical composition of the concentrate was supplied by the concentrate producer (Table 5.2). Concentrate orts were collected daily. Intake of the concentrate was 2.72 kg DM/d. Daily alkane consumption was 858 mg/d.

Herbage Sampling

At 0600, 1000, 1400, 1800 and 2200 h every day, representative herbage samples from the offered plot were taken from both treatments at 4 cm above ground level. Besides, at 0600 (in 2D) and 1800 h (in 1D and 2D) representative samples of residual grass were taken. The grass samples were pooled into two sets of composite samples: set a) a composite sample for each time point×treatment×period combination including residual grass samples, resulting in 7 grass samples per treatment per period (5 samples of offered grass and 2 residual grass samples); set b) a composite sample for each treatment×period, excluding the residual grass samples. The composite grass samples per treatment and period (set b) were used for alkane analyses, near infrared reflectance spectroscopy (NIRS) and estimation of digestibility of OM (DOM). The NIRS analyses were carried out by BLGG in Oosterbeek, The Netherlands. Digestibility of OM was determined by BLGG using the method described by Tilley and Terry (1963). From the samples in set b, net energy for lactation (NE_L) was calculated using the VEM system (Van Es, 1975) and intestinal digestible protein (DVE) and degraded protein balance (OEB) were calculated according to Tamminga et al. (1994). All composite grass samples except for the sub samples taken from set b for NIRS and DOM analyses were oven dried for 24 h at 70°C and ground through a 1-mm sieve. The sub samples for NIRS and DOM analyses were stored at -20°C before analyses. Subsequently, they were analyzed for DM, inorganic matter (ash), CP, crude fat (CFAT), NDF, ADF, acid detergent lignin (ADL), and sugars as described by Abrahamse et al. (2008a). During four days in the first period and during two days in the second period, extra herbage samples were taken for morphological analysis. For this analysis, 50 g of herbage was taken from the representative herbage sample taken at 0600 and 1800 h. Herbage material was distinguished into leaf blade, pseudostem (split at the ligule of each leaf), stem, and dead material (all material without green colour).

Grazing Behaviour

Grazing behaviour of all cows per treatment was monitored using IGER solid-state automatic behaviour recorders (Ultra Sound Advice, London, UK; Rutter et al., 1997). The jaw recorders were fitted to 4 cows of each treatment after allocation to a new plot at 1800 h and removed after 24 h. The consecutive day, the remaining 8 cows were monitored using the jaw recorders. The data were analyzed with the Graze Data Analyses Program (version 8.0, IGER, Devon, UK), identifying jaw movements and different behaviours (grazing, ruminating, idling; Rutter, 2000).

Rumen Measurements

Rumen fluid samples were taken after every milking from the four rumen-cannulated animals. Additional samples were taken during one day per experimental period at 0800, 1000, 1200, 1400, 1600, 2000 and 2200 h. Equal amounts of rumen fluid were collected from the front and middle of the ventral sac and from the cranial sac using a solid, perforated plastic tube (85 cm long; 2.5 cm in diameter). The pH was measured immediately using an electronic pH meter (pH electrode HI 1230, Hanna Instruments B.V., IJsselstein, The Netherlands). A duplicate sample was taken, and either acidified with phosphoric acid or with trichloroacetic acid, and stored at -20°C pending volatile fatty acids (VFA) and ammonia-nitrogen (NH_3-N) analysis, respectively, as described by Taweel et al. (2005b). During the last day, 5 rumen evacuations were done on all 4 cannulated animals, viz. directly after morning and evening milking, and after the first grazing bout on pasture in their respective treatment following this evacuation, and finally after a period of 12 h fasting following the last evacuation. The first grazing bout was defined as the period of grazing following allocation, until either cows stopped grazing during at least 10 min, or until 2 h after the start of grazing. The evacuation procedure is described in detail in Abrahamse et al. (2008b), except that only the rumen DM pool was determined at each rumen evacuation.

Statistical Analysis

All statistical analyses were carried out by ANOVA using the PROC MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC). Multiple measurements per animal cannot be regarded as independent units of observations (Littell et al., 1998). Therefore, repeated measurements ANOVA was performed on all grazing behaviour data, rumen and milk variables with day as the repeated subject. A first-order autoregressive covariance structure [AR(1)] fitted the data best and was used to account for within-cow variation. When cow was included in the model, it was included as a random factor. Differences were considered significant at a probability of $P < 0.05$ and post-hoc analyses were carried out using the Tukey test to test pair wise comparisons. When interactions were not significant ($P > 0.05$), they were excluded from the model, except for the interaction between treatment and period, which was included in all models in view of the strongly significant period effect.

After averaging DM content of herbage per treatment, period and time of sampling, the average chemical composition of offered grass per period per treatment per time point was analyzed with treatment, period and time of sampling as fixed factors. The interaction between treatment and period was also included in the model. Grass height (both offered- and residual grass height) as well as offered herbage per treatment per day were analysed similarly, although time of sampling was not included in the model. Since samples for determination of NE_L, OEB, VEM and DOM were analysed per treatment per period, a simplified model including only the effects of treatment and period was used.

Herbage DMI was analyzed with treatment, period and the interaction between both. Grazing behaviour variables were analysed per day as well as per period of the day (between 1800 h and midnight, between midnight and 0600 h, between 0600 h and noon, and between noon and 1800 h). The statistical model included treatment, period, day and the interaction between treatment and period. Bite mass was calculated from the difference in rumen DM content between both rumen evacuations carried out immediately before and after a grazing bout following both morning and evening milking in all rumen cannulated animals, to calculate DM intake during a meal. To correct intake for disappearance of rumen contents due to degradation and passage during the time between both evacuations, the fractional clearance

rate (kcl) of DM was calculated according to the procedure given in Abrahamse et al. (2008b). Intake was then calculated assuming a constant kcl of material from the rumen according to Chilibroste (1999). The number of bites was measured using the IGER behaviour recorders as described before. The statistical model was similar to that of all other grazing behaviour variables, except that day was not included in the model.
The model for rumen fluid variables was similar to the model for grazing behaviour but also included time (morning vs. evening) and the interaction between treatment and time. Milk data were pooled per day and subsequently analyzed with treatment, period, treatment by period interaction, day and the value of each of the variables measured during the adaptation period as covariate.

RESULTS

Herbage

Herbage chemical composition was similar between treatments, except for higher ($P < 0.05$) NE_L and DOM values in 2D than in 1D (Table 5.3). Large differences existed between both periods, with higher DM, CP, CFAT, NDF, ADF and ADL contents but lower sugar, NE_L and DOM contents in grass in the first period as compared to the second period.

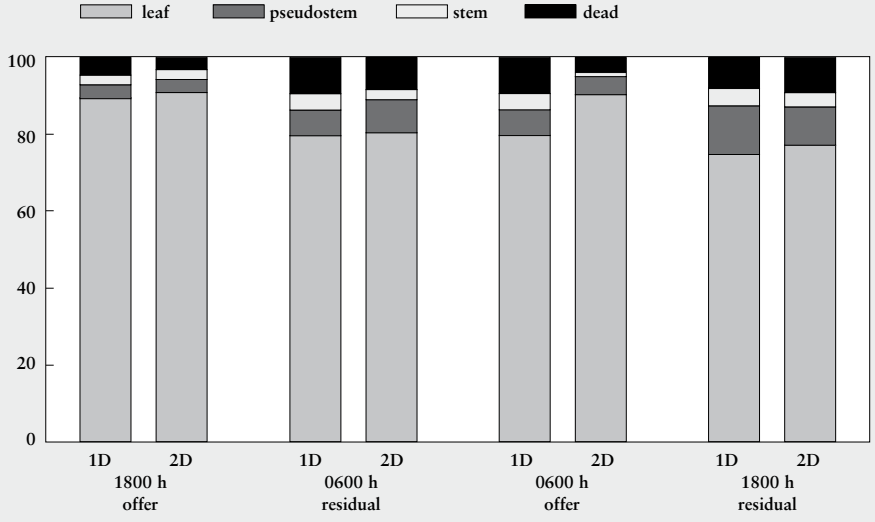

Figure 5.1

Morphological fractions of grass when cows were allocated either once (at 1800 h; 1D) or twice (at 0600 and 1800 h; 2D) per day to a new plot of grass.

The interaction between treatment and period was not significant, except for CFAT content in grass ($P = 0.015$; period 1 1D 37.3 vs. 2D 37.9 g/kg DM, period 2 1D 35.9 vs. 2D 33.4 g/kg DM). There was always a significant effect of time of sampling, except for OM and NDF,

but the interaction between time of sampling and treatment was not significant ($P > 0.05$). Diurnal variation in grass morphological fractions and in sugar and CP content of grass are presented in Figure 5.1 and 5.2, respectively.

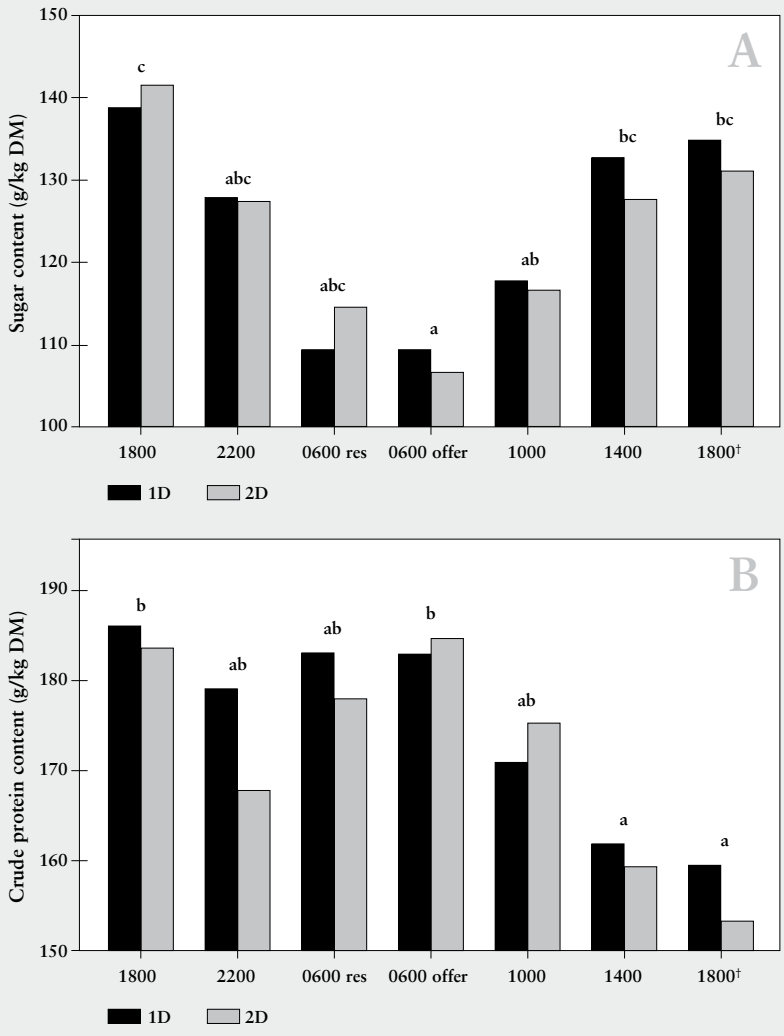

Figure 5.2

Sugar (A) and crude protein (B) content in grass of cows allocated either once (at 1800 h; 1D) or twice (at 0600 and 1800 h; 2D) per day to a new plot of grass*.

*** Letters above bars show a significant difference between different time points ($P < 0.05$).**
† Residual grass sample taken when cows were taken out of the plot.

Since there were no effects of treatment and treatment × period in morphological fractions (data not given), differences between different time points and treatments were tested using the CONTRAST statement in SAS (version 9.1; SAS Inst. Inc., Cary, NC). Leaf fraction of grass on offer showed a difference between treatments at 0600 h (79.6 vs. 90.2 % for 1D and 2D respectively, P = 0.042). A clear decrease in leaf fractions was found between allocation and turnout in 1D and 2D. In 1D, the leaf fraction decreased from 89.1 % to 74.6 % (P = 0.002) and in 2D it decreased during the night from 90.8 % to 80.4 %, and during the day from 90.2 % to 77.0 % (P = 0.002 for average decrease). Similarly, an increase was found between allocation and turnout in the pseudostem and stem fractions, although the increase of the stem fraction was not significant (P > 0.05). Sugar content decreased between 1800 h and 0600 h and increased thereafter until 1800 h, while CP decreased after allocation to a new plot in both treatments (Figure 5.2). The only exception was an increase in CP between 2200 and 0600 h in both treatments. The decrease between 1800 and 2200 h was larger in 2D than in 1D.

Table 5.3

Herbage chemical composition (in g/kg DM) in grass of cows allocated either once (1D) or twice (2D) per day to a new plot of grass*.

	Treatment		Period			*P*-value	
	1D	2D	1	2	SEM	Treatment	Period
DM (g/kg)	165.6	165.9	170.3	161.2	1.22	0.834	<0.001
OM	912.0	907.5	912.3	907.2	3.35	0.367	0.308
CP	176.2	174.3	180.0	170.6	2.27	0.559	0.012
CFAT	36.6	35.6	37.6	34.7	0.38	0.099	0.001
NDF	460.6	455.4	499.7	416.3	2.90	0.226	<0.001
ADF	263.9	262.7	281.2	245.4	1.13	0.473	<0.001
ADL	19.2	19.4	22.7	15.9	0.46	0.820	<0.001
Sugars	125.4	124.0	86.0	163.4	2.02	0.679	<0.001
NFC†	238.5	242.2	195.0	285.7	2.20	0.257	<0.001
NE_L‡ **(/kg DM)**	6.32	6.46	6.06	6.72	0.05	0.034	0.007
Intestinal digestible protein□	90.5	92.5	86.5	96.5	0.71	0.295	0.064
Degraded protein balance □	21.5	18.0	24.5	15.0	4.60	0.686	0.382
DOM+ **(%)**	77.1	78.6	74.7	81.0	0.04	0.021	0.005

* Data presented as least square means. The interaction between treatment and period was NS, except for CFAT (P = 0.015; period 1 1D 37.3 vs. 2D 37.9 g/kg DM, period 2 1D 35.9 vs. 2D 33.4 g/kg DM)
† Non-fibrous carbohydrates, calculated as 1000 – (NDF + CFAT + CP + Ash) (NRC, 2001)
‡ Calculated with VEM system (Van Es, 1975)
□ Calculated as in Tamminga et al. (1994)
+ Digestible organic matter as in Tilley and Terry (1963)

Grazing Behaviour

Although offered grass height and the amount of herbage offered to the cows was similar between treatments, residual grass height was lower in 2D than in 1D ($P = 0.017$), and herbage intake was greater in 2D than in 1D ($P = 0.001$) (Table 5.4). The interaction between treatment and period showed a trend ($P = 0.091$) with a larger difference in herbage intake in period 1 (14.8 vs. 17.0 kg/d) than in period 2 (14.0 vs. 14.1 kg/d). There were no differences in grazing behaviour between treatments, except for a larger number of chews per bolus ($P = 0.045$) and a tendency for a longer rumination time ($P = 0.051$) and for a larger number of ruminations per day ($P = 0.079$) in 2D compared to 1D. Almost all variables showed a difference between periods (Table 5.4), but no variable showed a significant interaction between treatment and period (data not shown). The distribution of grazing and ruminating over a 24-h period is presented in Figure 5.3. Clearly, grazing time is longer during the day (the period between morning and evening milking) than during the night (the period between evening and morning milking). Grazing time in 2D was longer during the day, but shorter during the night, than in 1D. Ruminating time was similar during the day, but was higher during the night in 2D than in 1D.

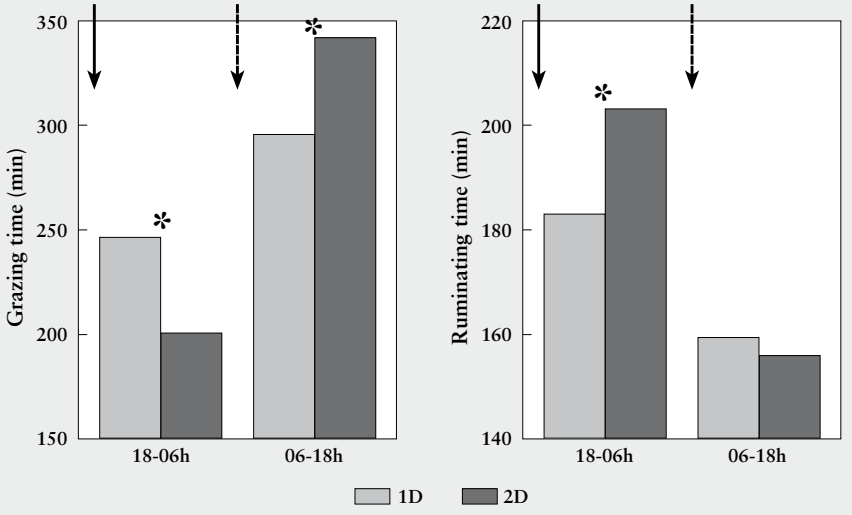

Figure 5.3

Grazing and ruminating time of cows allocated either once (at 1800 h; 1D) or twice (at 0600 and 1800 h; 2D) per day to a new plot of grass.

* shows a significant difference between treatments during this period ($P < 0.05$);
The arrow shows the moment of reallocation to a new plot, the arrow with the dotted line shows the extra moment of reallocation to a new plot in 2D.

Table 5.4

Sward surface height (SSH) of offered and residual herbage, herbage on offer, herbage intake and grazing behaviour of cows allocated either once (1D) or twice (2D) per day to a new plot*.

	Treatment				Period			
Variable	1D	2D	SEM	P-value	1	2	SEM	P- value
General								
Offered SSH (cm)	15.5	15.6	0.25	0.744	17.4	13.7	0.24	<0.001
Residual SSH(cm)	10.3	9.6	0.19	0.017	10.6	9.3	0.19	<0.001
Offered herbage (kg DM/d)	26.9	27.3	0.93	0.802	25.0	29.2	0.92	0.003
Herbage intake (kg DM/d)	14.4	15.5	0.31	0.001	15.9	14.0	0.31	<0.001
Grazing and ruminating time								
Grazing time (min/d)	545	549	11.7	0.785	524	570	12.5	0.003
Ruminating time (min/d)	369	382	13.6	0.051	409	342	13.9	<0.001
Idling time (min/d)	527	510	19.1	0.185	507	530	19.6	0.143
Grazing time (%)	37.9	38.1	0.81	0.785	36.4	39.6	0.87	0.003
Ruminating time (%)	25.6	26.5	0.95	0.051	28.4	23.7	0.96	<0.001
Idling time (%)	36.6	35.4	1.33	0.185	35.2	36.8	1.36	0.143
Grazing variables								
Bites (/d)	36152	36108	1642.3	0.973	32884	39375	1732.0	<0.001
Chews (/d)	9736	10516	671.9	0.183	9964	10287	689.3	0.630
Bite rate (/min)	66	66	2.8	0.932	64	69	3.0	0.042
Chew rate (/min)	18	19	1.2	0.183	19	18	1.2	0.219
Bite mass (mg/bite)†	614	582	37.8	0.612	606	589	38.0	0.803
Ruminating variables								
Ruminations (/d)	27179	28456	1138.4	0.079	31032	24603	1172.1	<0.001
Boli (/d)	497	506	22.5	0.379	540	463	22.8	<0.001
Chews per bolus (number)	54	57	1.7	0.045	58	53	1.7	<0.001

* Data presented as least square means. The interaction between treatment and period was NS, except for herbage intake (P = 0.020; period 1 1D 14.8 vs. 2D 17.0 kg DM/d, period 2 1D 14.0 vs. 2D 14.1 kg DM/d).

† Bite mass determined directly after allocation at 0600 and 1800 h, in the 4 rumen cannulated cows only.

Rumen Variables

All rumen variables were similar between treatments, except for the molar proportion of isobutyrate being higher in 1D than in 2D (P = 0.019) (Table 5.5). There were tendencies (P < 0.10) for higher NH_3-N concentration and molar proportions of butyrate and isovalerate, and a lower molar proportion of acetate, in 1D than in 2D. Large differences existed between both periods, and in many variables a significant interaction between treatment and period is found.

Table 5.5

Rumen pH, NH_3-N, total VFA (mmol/L), and molar proportions of individual VFA of dairy cows allocated either once (1D) or twice (2D) per day to a new plot*.

	1D		2D		T×P interaction†		Treatment		Period	
	1st period	2nd period	1st period	2nd period	SEM	P- value	SEM	P- value	SEM	P- value
pH	6.35c	6.03a	6.30bc	6.21b	0.04	0.002	0.03	0.094	0.03	<0.001
NH_3-N (mg/L)	185.4c	140.9b	191.8c	101.3a	8.68	0.009	6.19	0.059	6.14	<0.001
Total VFA (mmol/L)	127.7a	139.9b	133.5ab	127.6a	2.84	0.002	2.02	0.253	2.01	0.268
Acetate (%)‡	65.4	63.4	66.5	63.5	0.31	0.096	0.22	0.054	0.22	<0.001
Propionate (%)‡	19.4b	21.3c	18.5a	21.8c	0.22	<0.001	0.18	0.353	0.18	<0.001
Butyrate (%)‡	12.3	12.4	12.2	11.8	0.17	0.106	0.12	0.099	0.12	0.355
Isobutyrate (%)‡	0.95	0.76	0.89	0.73	0.019	0.579	0.013	0.019	0.013	<0.001
Valerate (%)‡	1.16	1.53	1.06	1.54	0.042	0.224	0.030	0.288	0.029	<0.001
Isovalerate (%)‡	1.21	1.06	1.11	1.03	0.034	0.298	0.024	0.090	0.024	<0.001
NGR□	4.28b	3.82a	4.55c	3.69a	0.056	<0.001	0.045	0.113	0.045	<0.001

* Data presented as least square means.
† Treatment × Period interaction.
‡ Percentage of total VFA.
□ The nonglucogenic to glucogenic VFA ratio (NGR) was calculated as [acetate + 2 x (butyrate + isobutyrate) + valerate + isovalerate] / [propionate valerate + isovalerate].

Table 5.6

Milk yield and milk composition of cows allocated either once (1D) or twice (2D) per day to a new plot of grass*.

	1D		2D		T×P interaction†		Treatment		Period	
	1st period	2nd period	1st period	2nd period	SEM	P- value	SEM	P- value	SEM	P- value
Milk yield (kg/d)	24.0b	22.3a	25.8c	21.2a	0.40	<0.001	0.31	0.374	0.30	<0.001
FPCM‡ yield (kg/d)	23.6c	22.0b	25.4d	20.1a	0.39	<0.001	0.29	0.984	0.29	<0.001
Fat (%)	4.01	3.98	4.03	3.71	0.074	0.138	0.052	0.004	0.051	<0.001
Protein (%)	3.25	3.39	3.24	3.31	0.030	0.217	0.021	0.073	0.020	<0.001
Lactose (%)	4.44b	4.37b	4.45b	4.27a	0.024	0.041	0.018	0.077	0.017	<0.001
Urea (mg/dL)	46.0	29.8	47.8	29.8	1.26	0.487	0.94	0.460	0.92	<0.001
Fat amount (g/d)	941c	869b	1022d	763a	17.9	<0.001	12.4	0.467	12.4	<0.001
Protein amount (g/d)	764bc	723ab	807c	694a	15.9	0.022	12.0	0.678	11.7	<0.001

* Data presented as least square means.
† Treatment × Period interaction.
‡ Fat- and protein-corrected milk.

Concentrations of NH_3-N and VFA, as well as pH differed between treatments in the second period while they were similar between treatments in the first period. The propionate ratio in total VFA as well as NGR showed the opposite effect with differences between treatments in the first period, but similar results between treatments in the second period. The effect of time of day on rumen variables is presented in Figure 5.4. Total VFA and pH showed large differences between both treatment and time of day combinations. The interaction between time of day and treatment in NH_3-N concentration (mg/L) tended (P = 0.061) to be significant. No differences existed between treatments at 1800 h, but at 0600 h pH was greater in 2D than in 1D while VFA concentration was lower in 2D than in 1D.

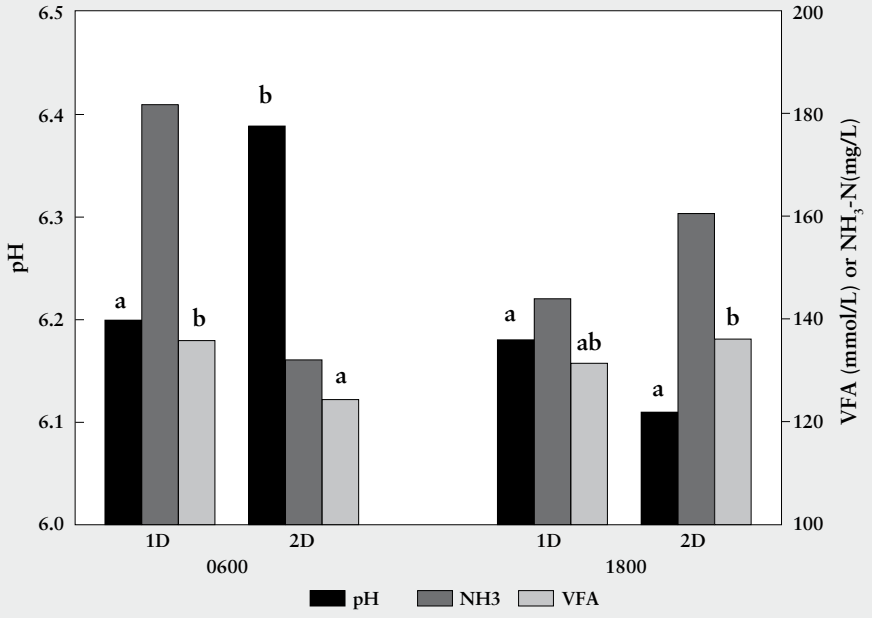

Figure 5.4

Rumen pH, ammonia (NH_3-N) and total VFA concentration at 0600 and 1800 h of cows allocated either once (at 1800 h; 1D) or twice (at 0600 and 1800 h: 2D) per day to a new plot of grass.

Milk

No differences in milk yield, FPCM and milk constituents were found between treatments, except for milk fat concentration being higher in 1D than in 2D (P = 0.004) (Table 5.6). There was a significant effect of period on all variables. However, milk and FPCM yield, as well as milk lactose content and milk fat and protein production per day showed an interaction between treatment and period (P < 0.05). In the first period, milk and FPCM yield were higher

in 2D than in 1D, whereas in the second period, milk yield did not differ between 2D and 1D whereas FPCM yield was lower in 2D than in 1D.
Milk fatty acid composition (Table 5.7) showed a lower concentration of C8:0 and short-chain fatty acids (SCFA; sum of all fatty acids between and including C4 and C13) in 1D than in 2D. Large differences existed between periods, with among others a higher concentration of C14:0, *cis*-9, *trans*-11-C18:2 and *trans*-11-C18:1 and lower concentration of C18:0, *cis*-9-C18:1, C18:2*n*-6 and C18:3*n*-3 in period 2 than in period 1.

DISCUSSION

The objective of this experiment was to determine the influence of daily allocation after morning milking vs. after evening and morning milking to new grazing plots on intake and grazing behaviour, rumen fermentation characteristics, and milk yield in dairy cattle. In an earlier experiment, intake of herbage was found to be increased when cows were allocated daily compared to allocation every four days to a new plot, when offered herbage and SSH were high (Abrahamse et al. 2008a). Similarly, in the present experiment intake of herbage was greater in 2D than in 1D when offered herbage and SSH were high (period 1), but this was not true when SSH was low and offered herbage was still high (period 2). Grazing behaviour was more equally distributed during the day in 1D than in 2D. Almost all rumen fermentation variables, and also milk yield, were similar between treatments, but milk yield was increased in 2D compared to 1D when SSH was high in period 1.

Grass Composition

Grass chemical composition is affected by defoliation, transportation within the plant and the effects of photosynthesis and respiration (Fulkerson and Donaghy, 2001). As cows defoliated the grass during both treatments, grass height decreased after allocation, with similar grass fractions between grass on offer in 1D (1800 h) and 2D (0600 and 1800 h) and also similar grass fractions in residual grass in both treatments (Figure 5.1). The decrease in grass height between allocation and turnout coincided with a decrease in the leaf:stem ratio, similar to findings by McGilloway et al. (1999) and Abrahamse et al. (2008a). This affected the chemical composition of the grass, since the relative proportions of the different morphological components are known to play an essential role in controlling the chemical composition of grass. After regrowth without defoliation, Delagarde et al. (2000) observed that from the top to the base of the sward, levels of CP, OM and OMD generally decrease and levels of DM, NDF and ADL increase. In our experiment, CP in both treatments also showed a decrease between allocation and turnout (Figure 5.2). If undefoliated, the CP content is likely to passively increase during the night and decrease during the day due to changes in sugar content (Delagarde et al., 2000), explaining the increase in CP content between 2200 and 0600 h in both treatments. With leafs containing more CP than stems (Smith et al., 2002), CP contents in grass decreased between 1800 and 2200 h and between 0600 and 1800 h (Figure 5.2). However, although grass fractions showed a larger variation in 2D during the day than in 1D (Figure 5.1), no interaction was found between treatment and time point in grass composition. Apparently, the difference in stem:leaf ratio between the two treatments at different time points during the day was not large enough to result in differences in the composition of grass between treatments. The processes of transportation within the plant, photosynthesis and respiration mainly affect sugar contents in grass. If the intensity of solar radiation, influenced by factors including time

Table 5.7

Fatty acids in milk fat (g/100 g identified fatty acids) of cows allocated either once (1D) or twice (2D) per day to a new plot of grass*.

	1D		2D		T×P interaction†		Treatment		Period	
	1st period	2nd period	1st period	2nd period	SEM	P- value	SEM	P- value	SEM	P- value
C4:0	4.9	4.5	4.9	4.6	0.08	0.338	0.06	0.224	0.06	<0.001
C6:0	2.6a	2.3a	2.4a	2.5a	0.07	0.040	0.05	0.220	0.05	0.066
C8:0	1.3a	1.2a	1.2ab	1.4b	0.05	0.032	0.04	0.007	0.04	0.209
C10:0	2.6	2.5	2.4	2.9	0.13	0.108	0.09	0.066	0.09	<0.001
C11:0	0.3	0.3	0.3	0.4	0.02	0.571	0.02	0.233	0.02	0.013
C12:0	2.9	2.9	2.7	3.3	0.15	0.151	0.11	0.410	0.11	<0.001
C13:0	0.1	0.1	0.1	0.2	0.02	0.234	0.01	0.627	0.01	0.005
***iso*-C13:0**	0.1	0.1	0.1	0.1	0.02	0.900	0.01	1.000	0.01	0.455
C14:0	10.6	10.9	10.4	11.2	0.28	0.550	0.19	0.931	0.19	0.005
***iso*-C14:0**	0.1	0.1	0.1	0.1	0.02	0.731	0.01	0.663	0.01	0.207
***cis*-9-C14:1**	1.0	1.1	1.0	1.1	0.06	0.795	0.04	0.894	0.04	0.163
C15:0	1.1	1.3	1.1	1.4	0.04	0.486	0.03	0.193	0.03	<0.001
***iso*-C15:0**	0.4	0.4	0.3	0.3	0.02	0.953	0.02	0.357	0.02	1.000
***anteiso*-C15:0**	0.6	0.6	0.6	0.7	0.03	0.484	0.02	0.093	0.02	<0.001
C16:0	26.9	27.0	26.7	26.5	0.70	0.839	0.49	0.602	0.49	0.916
***iso*-C16:0**	0.3	0.4	0.3	0.3	0.02	0.176	0.02	0.154	0.02	0.765
***cis*-9-C16:1**	2.2	2.2	2.3	2.1	0.07	0.128	0.05	0.897	0.05	0.072
C17:0	0.7	0.6	0.7	0.6	0.02	0.312	0.02	0.503	0.02	0.062
***cis*-9-C17:1**	0.3	0.3	0.3	0.3	0.02	0.178	0.02	0.730	0.02	0.730
C18:0	11.3	10.3	11.5	10.3	0.54	0.936	0.38	0.763	0.38	0.028
***cis*-9-C18:1**	22.8	22.5	23.4	20.9	0.62	0.193	0.43	0.227	0.43	0.003
***trans*-9-C18:1**	0.4	0.4	0.4	0.8	0.21	0.403	0.15	0.343	0.15	0.377
***trans*-11-C18:1**	3.4ab	3.9ab	2.9a	4.6b	0.29	0.030	0.20	0.840	0.20	0.006
***cis*-9, *trans*-11-C18:2**	1.4ab	1.7bc	1.1a	2.1c	0.11	0.015	0.08	0.580	0.08	<0.001
***trans*-11, *cis*-15-C18:2**	0.5	0.5	0.5	0.5	0.03	0.555	0.02	0.801	0.02	0.801
C18:2*n*-6	1.1	0.9	1.1	0.8	0.03	0.233	0.02	0.054	0.02	<0.001
C18:3*n*-3	0.7	0.6	0.7	0.5	0.03	0.553	0.02	0.432	0.02	<0.001
C20:0	0.1	0.1	0.1	0.1	0.01	0.099	0.01	0.595	0.01	0.595
SCFA‡	14.8	13.8	14.0	15.4	0.47	0.091	0.33	0.034	0.33	0.212
MCFA□	42.9	44.1	43.0	43.3	0.99	0.727	0.68	0.656	0.68	0.353
LCFA+	42.3	42.0	43.0	41.3	1.23	0.657	0.85	0.974	0.85	0.266

* Data presented as least square means.
† Treatment × Period interaction.
‡ Short chain fatty acids (C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + *iso*-C13:0).
□ Medium chain fatty acids (C14:0 + *iso*-C14:0 + *cis*-9-C14:1 + C15:0 + *iso*-C15:0 + *anteiso*-C15:0 + C16:0 + Iso-C16:0 + *cis*-9-C16:1).
+ Long chain fatty acids (C17:0 + *cis*-9-C17:1 + C18:0 + *trans*-9-C18:1 + *trans*-11-C18:1 + *cis*-9-C18:1 + *cis*-9, *trans*-11-C18:2 + *trans*-11, *cis*-15-C18:2 + C18:2*n*-6 + C18:3*n*-3 + C20:0).

of the day and cloud cover, is high enough, in the process of photosynthesis sugars are formed from CO_2 and H_2O. During hours of darkness, the plant uses sugar for respiration, decreasing sugar content in grass during the night (Fulkerson and Donaghy, 2001). This generally results in increasing sugar contents from the bottom to the top of the plant in the evening, while in the morning sugar contents are highest in the stems of grass, where sugars are stored (Delagarde et al., 2000; Smit and Elgersma, 2004). Indeed, sugar content decreased between 1800 and 0600 h and increased between 0600 and 1800 h (Figure 5.2). Sugar content in residual grass at 0600 h was numerically higher in 2D than in 1D. This is in line with results of earlier research (Abrahamse et al., 2009) where sugar content of grass at 1600 h was higher when cows were allocated to a new plot at 0600 h than allocation at 1600 h. Both effects in the current experiment as well as in Abrahamse et al. (2009) are explained by the fact that transportation of sugars from leaves to the lower parts of the plant takes place after photosynthesis during daylight, and because of the nature of grazing cows that graze down the sward. Since SSH was lower in 2D than in 1D at 0600 h, sugar content was higher in 2D. Similarly, cows that were allocated in the evening to a new plot had higher SSH than cows that returned in the evening to a plot that was grazed down already and sugar content at 1800 h was higher in that case (Abrahamse et al., 2009).

Despite the effects of defoliation, transportation, photosynthesis and respiration described above, there were no general treatment differences in grass composition, except for NE_L and DOM (Table 5.3). The latter two are correlated, since in the Dutch system NE_L is calculated based on DOM content (van Es, 1978). In the period between 0600 and 1800 h, the grass contained more leaves in 2D than in 1D, and as grazing time was greater in 2D than in 1D between 0600 and 1800 h (Figure 5.3), this may explain the higher DOM in herbage in 2D. However between periods, large differences existed in chemical composition of the grass (Table 5.3), largely influenced by weather conditions during the experiment (Table 5.1) and the longer period of regrowth before the start of period 1 (41 days) than before period 2 (18 days). Offered grass height was higher in period 1 than in period 2 (17.4 vs. 13.7 cm, $P < 0.001$) and the sum of stem and dead material in grass was larger in period 1 than in period 2 (11.8 vs. 2.5 %), indicating that grass was more mature in period 1 than in period 2. With increasing maturity, the NDF concentration in grass is expected to increase while CP and OMD in grass is expected to decrease due to an increased stem:leaf ratio (Beever et al., 2000). Such changes were also present upon comparison of grass composition in period 1 with period 2.

Grazing Behaviour and Intake

Although frequent allocation to a fresh pasture plot is assumed to increase productivity of dairy cows, scientific evidence is scarce. In two grazing experiments of Dalley et al. (2001), different effects on DMI and milk yield were found when cows were allocated to a fresh plot six times daily compared to once daily. In the first experiment, intake was numerically increased when allocating frequently (16.3 vs. 15.2 kg DM/d), but milk yield was lower when allocating frequently. In the second experiment however, no differences were found between treatments. This might be related to SSH which was higher in the first experiment than in the second experiment (9.7 vs. 7.4 cm). Abrahamse et al. (2008a) found a higher herbage intake when allocating cows daily to a new plot as compared to allocating every four days when pasture mass on offer and SSH were high, but not when offered pasture mass and SSH were low, whereas milk yield was significantly higher in the daily allocation treatment. In the current experiment, herbage intake was greater in 2D than in 1D ($P = 0.001$) (Table 5.4). This effect was only found during period 1, when offered SSH was high. Since offered herbage was similar

between both periods, SSH had a larger influence on herbage intake than offered herbage. This is in agreement with research by McGilloway et al. (1999), who found SSH to be the principal factor controlling intake. At low sward height in period 2, the potential stimulating effect of the 2D treatment on DMI might thus have been nullified by SSH limitations on DMI.
Herbage intake can be decreased when either grazing time or intake rate, being the result of the number of bites and bite mass, are reduced (Gibb et al., 1997). Bite mass was numerically lower on low SSH in period 2 than in period 1, and bite rate was higher ($P < 0.001$) in period 2 than in period 1. Gibb (2006), Barret and co-workers (2001) and Orr et al. (2001) described similar results, indicating that the increased bite rate to reduced bite mass did not fully compensate for the effect of shorter SSH in their research and as a result, intake rate was reduced. Indeed, intake rate calculated from herbage intake and grazing time in Table 5.4 was lower in period 2 than in period 1 (30.3 vs. 24.6 g/min), but higher than values presented at different SSH in Gibb (2006). Pulido and Leaver (2001) found that herbage intake was reduced when sward height decreased, coinciding with a decrease in intake rate and an increase in grazing time. Similar findings were observed by Abrahamse et al. (2008a), where the inverse relation between SSH and herbage intake was clear between days in the treatment where cows were allocated every four days. Bite mass also showed a numerical decrease between 1800 and 0600 h in 2D (712 vs. 452 mg/bite) while in 1D it was similar (605 vs. 622 mg DM/bite). This is not in line with the results above, since measurements on bite mass were taken after allocation to a new plot at 0600 h in 2D. The most probable explanation for these results is the higher DM density in the lower layers of the sward (< 10 cm), as described by Delagarde et al. (2000), since SSH was lower in 1D than in 2D at 0600 h, and was similar between 0600 and 1800 h in 2D.
Although grazing behaviour was similar between treatments, there was a clear effect of allocating cows twice daily on grazing and ruminating time (Figure 5.3). Cows allocated to a new plot at 0600 h (2D) had a longer grazing time between 0600 and 1800 h and a shorter grazing time between 1800 and 0600 h than cows in 1D. Pulido and Leaver (2003) observed similar effects, and suggested that this was due to cows anticipating the timing of movement to another plot, reducing intake prior to this change.

Rumen Fermentation
The concentration of VFA in the rumen is influenced by both production and clearance rate of VFA, the latter being the result of passage rate and absorption rate (Bergman, 1990). Since herbage intake and DOM was greater in 2D, and the concentration of total VFA was similar between treatments, clearance rate is expected to have been higher in the 2D treatment. This is especially expected since the rumen fluid pool (the total fresh rumen pool minus the rumen DM pool, data not given) was numerically higher in 1D (86.2 l) than in 2D (83.5 l). Indeed, clearance rate of OM was higher in 2D than in 1D (5.69 vs. 5.26%/h). During the night, little grazing occurs (Gibb et al., 1997), temporarily decreasing the availability of substrate in the rumen. High substrate concentrations generally increase VFA production and shift the fermentation pattern from acetate to propionate and butyrate, while low substrate concentrations promote fermentation to acetate (Dijkstra et al., 1994). Especially in 1D, with longer grazing time after allocation at 1800 h than in 2D (Figure 5.3), this change would be expected. Indeed, during morning sampling, in 1D VFA was higher (Figure 5.4) and molar proportions of propionate (20.0 vs. 19.7 %, respectively) and butyrate (12.2 vs. 11.9 %, respectively) were numerically higher than in 2D, while in 2D acetate molar proportion was numerically higher than in 1D (65.6 vs. 64.7 %, respectively). This did not result in treatment effects, however, since only the molar proportion of isobutyrate was different between treatments.

Between periods, clear differences existed in grass composition (Table 5.3), resulting in large effects on rumen fluid variables (Table 5.5). Concentration of NFC and OMD were greater and cell wall fractions were smaller and less lignified in period 2 than in period 1, expected to give rise to a more rapid fermentation in the rumen. This is indicated by the lower pH in period 2, although the concentration of VFA was only numerically higher in period 2 than in period 1 (133.7 vs. 130.6 mmol/l, $P = 0.268$). The lower pH and higher level of rapidly fermentable carbohydrates in period 2 is expected to decrease production of acetic acid and increase that of propionic and butyric acid (Bannink et al., 2008), explaining the higher molar proportions of propionate and butyrate in period 2. This is related to the higher fat percentage in milk in period 1 and the higher protein percentage in period 2 (Table 5.6) (Thomas and Martin, 1988).
When the supply of degradable protein exceeds that of degradable carbohydrates required for microbial protein synthesis, ammonia will be formed (Tan and Murphy, 2004). The ratio of N from protein to carbohydrates for optimal microbial protein synthesis is not fixed, but depends on factors including amount of energy used for maintenance, energetic uncoupling, and predation of bacteria by protozoa (Dijkstra et al., 2002). Upon evaluating in sacco degradation characteristics of 8 ryegrass cultivars, Tas et al. (2006b) observed a much higher ratio of effective degradation of protein to OM immediately after start of incubation than several hours later. Indeed, rumen NH3 concentration increased after allocation to a new plot (Figure 5.5), with an exception at 2000 h in 1D. The concentration of NH3 in the rumen in 1D tended to be higher than in 2D cows (Table 5.5). In 1D, after allocation between 1800 and 0600 h, CP was higher (Figure 5.2) and grazing time was longer (Figure 5.3) indicating that cows in 1D ingested more CP than cows in 1D. This is confirmed with a numerically higher NH3 concentration at 0600 h in 1D than in 2D. When monitoring the NH3 concentration of frequently taken samples on the last day of the experiment (Figure 5.5), the NH3 concentration showed a large variation during the day. The concentration of NH3 in 2D was high between 1000 and 1800 h compared to 1D (on average 124 mg/L in 2D and 95 mg/L in 1D), probably related to the estimated higher CP intake in 2D than in 1D during this period.

Milk Yield and Milk Composition

Since grass composition was similar between treatments with the exception of NE_L and DOM (Table 5.3), and herbage intake was greater in 2D (Table 5.4), 2D was expected to result in higher milk yield than 1D. However, milk yield was similar between treatments (Table 5.6). An interaction was found between treatment and period in milk and FPCM yield, with greater milk yield in 2D than in 1D in period 1 but similar milk yield between treatments in period 2. This was in line with differences in herbage intake between treatments in period 1, when SSH was high, which were absent in period 2 with low SSH. Similarly, Abrahamse et al. (2008a) observed more pronounced differences in milk yield due to more frequent allocation to a new plot in the period when SSH was high compared to when SSH was low.
In general, an increase in short-chain fatty acids in milk fat (SCFA) indicates increased *de novo* synthesis of fatty acids. In ruminants, acetate in the rumen is an important source of carbon for *de novo* synthesis of fatty acids in the udder (Bauman and Griinari, 2003). Since pH was higher in 2D than in 1D, and because higher pH values shift fermentation of sugars towards acetic acid whereas type of VFA formed from fermentation of NDF is not affected by pH, this may explain the higher molar proportion of acetic acid in 2D (Bannink et al., 2008), potentially increasing *de novo* fatty acid synthesis. However, although SCFA in milk fat was higher in 2D, milk fat was lower in 2D than in 1D. Likewise, the inhibitory effect

of fat content in the ration on *de novo* fat synthesis in the udder, described by Vlaeminck et al. (2006), is not seen in the current experiment as a tendency (P = 0.099) to reduced fat content in grass in 2D was found. The relationship between acetic acid and milk fat during milk fat depression has been questioned though, and alternatively the lower pH may have reduced the relative rate of biohydrogenation of unsaturated fatty acids and stimulated the formation of specific *trans*-fatty acids that inhibit fatty acid synthesis in the mammary gland (Bauman and Griinari, 2003). The negative effect of *trans*-10-C18:1 on *de novo* fat synthesis (Bauman and Griinari, 2003) could not be investigated since this fatty acid was not identified in the current experiment. The other well-known fatty acid in this respect, *trans*-10, *cis*-12-C18:2, was below detection limit in milk.

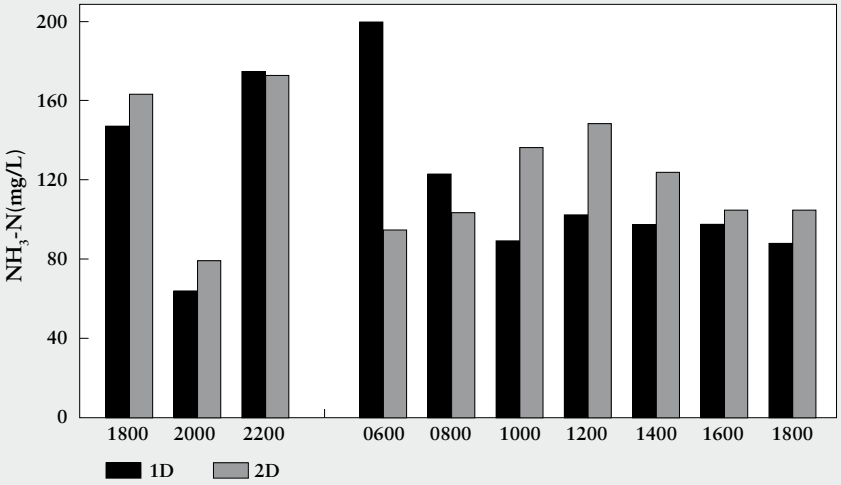

Figure 5.5

Rumen ammonia (NH_3-N) concentration throughout the day of cows allocated either once (at 1800 h; 1D) or twice (at 0600 and 1800 h; 2D) per day to a new plot of grass.

No differences could be found between treatments in *iso*-C14:0 and *iso*-C15:0, indicating cell wall degrading bacteria (Vlaeminck et al., 2006). Between both periods, a significant difference in NDF intake (7.4 kg DM in period 1 and 5.8 kg DM in period 2, calculated from herbage intake and NDF content of herbage in each period) did not result in differences in *iso*-C14:0 and *iso*-C15:0 between treatments. In period 2, the number of regrowth days was lower and the grass was higher in leaf fraction than in period 1. Fatty acids in grass mainly (50-75%) consist of C18:3 (Elgersma et al., 2006). As herbage DMI and CFAT content in herbage was lower in period 2 then in period 2, intake of C18:3*n*-3 from grass is expected to be lower in period 2 than in period 1 , reducing the supply of this fatty acid to the rumen. An indication for a lower intake of C18:3*n*-3 is the lower amount of this fatty acid in milk in period 2

(Table 5.7), although this fatty acid is also partially biohydrogenated via *trans*-11, *cis*-15-C18:2 into *trans*-11-C18:1 and ultimately C18:0 in the rumen and by desaturation of *trans*-11-C18:1 to *cis*-9, *trans*-11-C18:2 in the udder (Vlaeminck et al., 2006). The lower pH in rumen fluid in period 2 than in period 1 might have inhibited the last step of biohydrogenation in the rumen, resulting in higher *trans*-11-C18:1 in period 2 than in period 1 (Kolver and de Veth, 2002). The turnover from *trans*-11-C18:1 to C18:0 in the rumen, and finally by desaturase activity to *cis*-9-C18:1 in the udder is probably inhibited, as indicated by lower values of C18:0 and *cis*-9-C18:1 and higher value of *trans*-11-C18:1 in milk fat in period 2 (Table 5.7).

CONCLUSIONS

In conclusion, herbage intake was higher when allocating cows twice daily compared with once daily to fresh pasture when sward surface height was high, but allocation frequency did not affect intake when sward surface height was low. Similarly, milk yield was higher upon more frequent allocation only when sward surface height was high.

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REFERENCES

Abrahamse, P. A., S. Tamminga, and J. Dijkstra. 2009. Effect of daily shift of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. Accepted by Journal of Agricultural Science.

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008a. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91(5):2033-2045.

Abrahamse, P. A., B. Vlaeminck, S. Tamminga, and J. Dijkstra. 2008b. The Effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. Journal of Dairy Science 91(12):4778-4792.

Bannink, A., J. France, S. Lopez, W. J. J. Gerrits, E. Kebreab, S. Tamminga and J. Dijkstra. 2008. Modeling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology 143:3-26.

Bargo, F., L. D. Muller, J. E. Delahoy, and E. S. Kolver. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86(1):1-42.

Barrett, P. D., A. S. Laidlaw, C. S. Mayne, and H. Christie. 2001. Pattern of herbage intake rate and bite dimensions of rotationally grazed dairy cows as sward height declines. Grass and Forage Science 56(4):362-373.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23(1):203-227.

Beever, D. E., N. Offer, and M. Gill. 2000. The feeding value of grass and grass products. Chapter 7 in Grass its production and utilization. A. Hopkins, ed. Blackwell Science UK.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews 70(2):567-590.

Chilibroste, P. 1999. Grazing time: the missing link? A study on the plant-animal interface by integration of experimental and modelling approaches. PhD Thesis, Wageningen University, Wageningen, The Netherlands.

CVB. 2005. Tabellenboek veevoeding (Dutch feeding tables). Voedernormen landbouwhuisdieren en voederwaarde veevoeders (in Dutch). Central Bureau for Livestock Feeding, Lelystad, the Netherlands.

Dalley, D. E., J. R. Roche, P. J. Moate, and C. Grainger. 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Australian Journal of Experimental Agriculture 41(5):593-599.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1-2):49-68.

Dijkstra, J., J. A. N. Mills, and J. France. 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. Nutrition Research Reviews 15:67-90.

Elgersma, A., S. Tamminga, and J. Dijkstra. 2006. Lipids in herbage. Pages 175-194 in Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Fulkerson, W. J. and D. J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Australian Journal of Experimental Agriculture 41(2):261-275.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. Pages 141-157 in Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Science 52(3):309-321.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science 63(4):269-287.

Kingston-Smith, A. H. and M. K. Theodorou. 2000. Post-ingestion metabolism of fresh forage. New Phytologist 148(1):37-55.

Kolver, E. S. and M. J. de Veth. 2002. Prediction of ruminal pH from pasture-based diets. Journal of Dairy Science 85(5):1255-1266.

Kolver, E. S. and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science 81(5):1403-1411.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science 76(4):1216-1231.

McGilloway, D. A., A. Cushnahan, A. S. Laidlaw, C. S. Mayne, and D. J. Kilpatrick. 1999. The relationship between level of sward height reduction in a rotationally grazed sward and short-term intake rates of dairy cows. Grass and Forage Science 54:116-126.

Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Milk production from late-lactation dairy cows. Grass and Forage Science 56(4):383-394.

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

Orr, R. J., J. E. Cook, K. L. Young, R. A. Champion, and S. M. Rutter. 2005. Intake characteristics of perennial ryegrass varieties when grazed by yearling beef cattle under rotational grazing management. Grass and Forage Science 60(2):157-167.

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56(4):352-361.

Peyraud, J. L., and L. Astigarraga. 1998. Review of the effect of nitrogen fertilization on the chemical composition, intake, digestion and nutritive value of fresh herbage: Consequences on animal nutrition and N balance. Animal Feed Science and Technology 72(3-4):235-259.

Pulido, R. G., and J. D. Leaver. 2001. Quantifying the influence of sward height, concentrate level and initial milk yield on the milk production and grazing behaviour of continuously stocked dairy cows. Grass and Forage Science 56:57-67.

Pulido, R. G. and J. D. Leaver. 2003. Continuous and rotational grazing of dairy cows - The interactions of grazing system with level of milk yield, sward height and concentrate level. Grass and Forage Science 58(3):265-275.

Smit, H. J. and A. Elgersma. 2004. Diurnial fluctuations in vertical distribution of chemical composition in a perennial ryegrass (*Lolium perenne* L.) sward during the season. Land use systems in grassland dominated regions. Pages 21-24 in the Proceedings of the 20th General Meeting of the European Grassland Federation. A. Lúscher, B. Jeangros, W. Kessler, O. Huguenin, M. Lobsiger, N. Millar, and D. Suter, ed. Luzern, Switzerland.

Smit, H. J., S. Tamminga, and A. Elgersma. 2006. Dairy Cattle Grazing Preference among six cultivars of perennial ryegrass. Agronomy Journal 98(5):1213-1220.

Smith, K. F., R. A. Culvenor, M. O. Humphreys, and R. J. Simpson. 2002. Growth and carbon partitioning in perennial ryegrass (*Lolium perenne*) cultivars selected for high water-soluble carbohydrate concentrations. Journal of Agricultural Science 138(4):375-385.

Tamminga, S., W. M. Van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: The DVE/OEB-system. Livestock Production Science 40:139-155.

Tan, Z. and M. R. Murphy. 2004. Ammonia production, ammonia absorption, and urea recycling in ruminants. A review. Journal of Animal and Feed Sciences 13(3):389-404.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006a. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. Journal of Dairy Science 89(9):3494-3500.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006b. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. Animal Feed Science and Technology 131(1-2):102-119.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005a. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121(3-4):243-256.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005b. Improving the quality of perennial ryegrass (*Lolium perenne* L.) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fiber. Livestock Production Science 96(2-3):239-248.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Grazing behaviour, intake, rumen function and milk production of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates. Livestock Science 102(1-2):33-41.

Thomas, P. C. and P. A. Martin. 1988. The influence of nutrient balance on milk yield and composition. Pages 97-118 in Nutrition and lactation in the dairy cow. P.C. Garnsworthy, ed. Butterworths, London, UK.

Tilley, J. M. A. and R. A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18(2):104-111.

Van Es, A. J. H. 1975. Feed evaluation for dairy cows. Livestock Production Science 2(2):95-107.

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. Journal of Dairy Science 90(3):1467-1476.

Van Vuuren, A. M., C. J. van Der Koelen, and J. Vroons- de Bruin. 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. Netherlands Journal of Agricultural Science 34:457-467.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131(3-4):389-417.

CHAPTER 6

Effect of Daily Movement of Dairy Cattle to Fresh Grass in Morning or Afternoon on Intake, Grazing Behaviour, Rumen Fermentation and Milk Production

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ABSTRACT

Twenty Holstein cows were split into two equal groups to test the effect of daily move to a previously ungrazed strip after morning milking (MA) or afternoon milking (AA) on herbage intake, grazing behaviour, rumen characteristics and milk production using a randomized block design with three periods of 14 days each. Milking took place at 06.00 and 16.00 h. The chemical composition of grass was similar between treatments, but an interaction between treatment and time of sampling was found in all variables except acid detergent lignin (ADL). The most pronounced differences existed in sugar content. Grass sugar content was greatest following afternoon milking. However, the difference in sugar content in grass was much larger in MA (158 v 114 g/kg dry matter (DM) at 16.00 and 06.00 h, respectively) than in AA (147 v 129 g/kg DM at 16.00 and 06.00 h, respectively). Neutral detergent fibre (NDF) was significantly higher at 06.00 h than at 16.00 h (469 v 425 g/kg DM) in AA, but was equal between morning and afternoon in MA (453 g/kg DM). Herbage intake, determined using the n-alkane technique, did not differ between treatments. Grazing behaviour observed using IGER graze recorders were similar between treatments, except for ruminating time, bite rate and the number of ruminations and boli per period of the day. However, interactions between treatment and time in grazing behaviour variables were found. Grazing time was longer and number of bites was greater following allocation to a new plot (after milking in the morning in MA or milking in the afternoon in AA) when compared to allocation to the same plot after the subsequent milking per treatment (after milking in the afternoon or morning in MA and AA, respectively). In comparison to AA, grazing time in MA was more evenly distributed during the day but lower during the night. The combined effects of differences in grazing behaviour and chemical composition of the grass between treatments in different periods of the day probably caused higher intake of sugars in AA, resulting in a significantly higher non-glucogenic to glucogenic volatile fatty acid ratio (NGR) in the rumen in AA than MA. Milk fat content was lower in MA than AA, but milk production and milk protein and lactose content did not differ. In conclusion, time of allocation to a fresh plot altered the distribution of grazing behaviour variables over the day, and affected NGR and milk fat content, but herbage intake and milk production were not changed.

INTRODUCTION

Low dry matter intake (DMI) has been identified as a major factor limiting milk production of highly productive dairy cattle in grazing systems, as reviewed by Bargo et al. (2003) and Wales et al. (2005). Numerous factors influence grass intake by cattle, such as plant characteristics including cultivar and chemical composition and management practices including grazing intensity and herbage allowance (Chilibroste 2005; Rearte 2005; Wales et al. 2005).

The soluble sugar content of grass is of particular interest with respect to DMI, profile of nutrients available for absorption in the cow and ultimately milk production. Sugars are a readily available source of energy for rumen microbes (Boudon et al. 2002). Since leaf proteins in grass are also rapidly degraded in the rumen, matching the energy supply from sugars with the protein supply may increase rumen microbial protein synthesis and reduce ammonia levels and losses of N with urine (Miller et al. 2001; Tas et al. 2006b). Dairy cattle prefer grass with a high sugar content and high digestibility of organic matter (DOM) (Smit et al. 2006). Herbage intake increased when a high-sugar and low-neutral detergent fibre (NDF) ryegrass

cultivar was offered to zero-grazed cows in early lactation, but milk production was not affected (Moorby et al. 2006). However, cultivars with elevated water-soluble carbohydrate content did not consistently result in greater herbage intake and milk production in grazing dairy cattle (Tas et al. 2006a) or in zero-grazed dairy cattle in mid and late lactation (Miller et al. 2001; Taweel et al. 2005).
Sugars in grass are produced in the leaves and stored in the stem and pseudostem (Fulkerson and Donaghy 2001). More than most other nutrients in the plant, sugar concentrations undergo diurnal fluctuation. During the day, sugars accumulate and during the night they are consumed during respiration. This results in a higher sugar content in grass in the afternoon than in the morning (Van Vuuren et al. 1986; Delagarde et al. 2000; Orr et al. 2001). In general, cows tend to have patterns of peak grazing activity during the day and the major grazing event and highest intake occurs around dusk (Rook and Huckle 1997; Taweel et al. 2004). In view of the diurnal pattern of grass sugar content and cow intake behaviour, provision of a fresh plot of grass allowance following afternoon milking rather than morning milking may increase intake of the relatively sugar-rich grass and thus may increase nutrients available for milk production by the cow. Orr et al. (2001) aimed to maximize the use of high-sugar afternoon grass by offering cows new areas of grass in a strip grazing system after the afternoon milking, when water soluble carbohydrate content was 204 g/kg DM, rather than after the morning milking when this content was 175 g/kg DM. Grass intake did not differ between treatments, but milk production was increased by 5 % in the afternoon allocation group (Orr et al. 2001). In addition, Gibb (2006) reported milk fat and protein contents from the experiment by Orr et al. (2001) and these were increased by 4.7 and 0.4 g/kg, respectively, in AA. Cows receiving their fresh allocation in the afternoon spent more time grazing between allocation and the next milking (16.45 to 07.45 h) than cows receiving fresh allocation in the morning between allocation and the next milking (07.45 to 16.45 h). Similarly, in beef heifers, afternoon allocation resulted in longer grazing time in the afternoon and improved average daily gain, compared to morning allocation, but herbage intake did not differ (Gregorini et al. 2006). In those experiments, however, no rumen fermentation data were available.
The aim of the current experiment was to determine the influence of grazing management, viz. daily allocation to a new plot of ryegrass after morning or after afternoon milking in a strip grazing system, on intake, intake behaviour, rumen fermentation characteristics and milk production in grazing dairy cows.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was carried out between 13 July and 1 September 2005 after approval by the Institutional Animal Care and Use Committee of Wageningen University. The study was conducted as paired comparisons in a randomized block design with repeated measurements. After adaptation to grazing for 2 weeks, two groups of 10 dairy cows were assigned to their respective treatments and adapted to these treatments for 1 week. The treatments, daily move to a previously ungrazed strip (hereafter termed 'move') after morning milking (MA; 06.00 h) or after afternoon milking (AA; 16.00 h) to a fresh 0.125 ha plot, were repeated during three rotations. Each of the repetitions lasted 14 days. Water was available *ad libitum*.

Herbage

A uniform stand of perennial ryegrass (*Lolium perenne* L.), established in August 2003, was used during the experiment. The mixture used was Havera, a mixture composed of 0.70 *Lolium perenne* tetraploid cultivar Elgon and 0.30 *Lolium perenne* diploid cultivar Veritas (proportions by seed number). The fertilizer application rates were 95 kg N/ha as ammonium nitrate and 23 kg P/ha in the form of pentoxide in spring and 75 kg N/ha as potassium ammonium sulphate prior to each rotation. The paddock was divided into 42 plots of 0.125 ha that were stepwise cut to approximately 40 mm height (three plots for both treatments every 2 or 3 days), to have approximately equal DM on offer per day after 21 days of regrowth. Herbage mass on offer was estimated using the sward surface height and pasture mass double sample technique as described in Abrahamse et al. (2008). Briefly, within on average 20 quadrats of 0.5 × 0.5 m during each rotation, herbage height was measured and pasture mass was determined after cutting grass at 40 mm from ground level prior to each rotation. The regression of pasture mass against herbage height was used to calculate herbage mass from observations of herbage height during the experiment (on average 15 SSH measurements per plot per day).

Animals

Twenty Holstein cows, of which six were previously fitted with a rumen cannula (100-mm i.d.; Bar Diamond Inc., Parma, Idaho, USA) in the dorsal sac, were paired by parity, days in milk (DIM), and milk yield during the adaptation period and randomly assigned to the treatments. At the start of the experiment, cows produced 31.2 ± 1.3 kg of milk/day (values expressed as means ± S.E.), were 127 ± 11 DIM, body weight (BW) was 536 ± 13 kg, and body condition score (BCS) was 2.2 ± 0.3 (recorded on a 5-point scale). Cows were milked twice daily at 06.00 and 16.00 h using a mobile milking parlour, and cows were let out on pasture around 1h after the start of milking. Individual milk yield was recorded throughout the experiment and individual milk samples were collected at each milking and stored in a refrigerator at 4 °C using sodium azide and bronopol as preservative. Fat, protein and lactose contents were determined according to ISO 9622 (Melkcontrolestation, Zutphen,

Table 6.1

Ingredient and chemical composition of the concentrate.

Item	
Ingredient	
Barley (g/kg)	150
Corn (g/kg)	234
Beet pulp (g/kg)	220
Soya hulls (g/kg)	190
Soya-bean meal (g/kg)	70
Palm expeller (g/kg)	50
Molasses (g/kg)	60
Premix vitamin/mineral (g/kg)	25
Alkane + arbocel mix (g/kg)	4
Chemical composition	
Dry matter (g/kg)	901
Organic matter (g/kg DM)	928
Crude protein (g/kg DM)	131
Crude fat (g/kg DM)	16
Sugars (g/kg DM)	115
Starch (g/kg DM)	248
Neutral detergent fibre (g/kg DM)	263
Acid detergent fibre (g/kg DM)	173
Acid detergent lignin (g/kg DM)	14
Net energy for lactation* (MJ/kg DM)	7.4†
Intestinal digestible protein‡ (g/kg DM)	102†
Degraded protein balance‡ (g/kg DM)	-22†

* Calculated with VEM system (Van Es 1975).
† Provided by the feed manufacturer (Research Diet Services, Wijk bij Duurstede, The Netherlands).
‡ Calculated as in Tamminga et al. (1994).

The Netherlands) and milk urea was determined using the pH-difference technique (ISO 14637). Fat and protein corrected milk (FPCM) yield (kg/day) was calculated as (0.337 + 0.0116 × fat (g/kg) + 0.006 × protein (g/kg)) × milk yield (kg/d). Herbage intake per animal per rotation was estimated using the alkane technique as described by Abrahamse et al. (2008). Cows received 2.70 kg DM/day of a concentrate with C_{32} alkanes in two equal portions during milking throughout the experiment (Table 6.1). Intake of concentrate was complete, and daily C_{32} alkane supplementation was 897 mg/d. Intake of herbage was calculated based on C_{32} and C_{33} alkane concentrations in feed and in faecal samples taken twice daily around milking from each cow.

Herbage and Concentrate Sampling

During every milking, around 40 representative herbage samples were randomly taken from both treatments at 40 mm above ground level and oven dried for 24 h at 70 °C. Similarly, samples from residual grass after the move to a new plot were taken. At the end of the experiment, samples were pooled into three samples per treatment per rotation (morning, afternoon and residual). Also, a representative concentrate sample was taken and dried per rotation. Herbage and concentrate samples were ground through a 1 mm sieve and analysed for DM, inorganic matter (ash), crude protein (CP), crude fat (CFAT), NDF, acid detergent fibre (ADF), acid detergent lignin (ADL), sugars (soluble in 0.40 (w/w) ethanol) and starch as described by Abrahamse et al. (2008). Net energy for lactation (NE_L) was calculated using the net energy for lactation (VEM) system (Van Es 1975) and intestinal digestible protein (DVE) and degraded protein balance (OEB) were calculated according to Tamminga et al. (1994). Data used for these calculations were obtained from the concentrate supplier (concentrates) and from near infrared reflectance spectroscopy (NIRS) carried out by BLGG in Oosterbeek, The Netherlands (grass samples). Also, digestibility of organic matter (DOM) was determined using NIRS by BLGG in Oosterbeek, The Netherlands.

Grazing Behaviour

Temporal patterns of grazing behaviour of all cows per treatment were recorded using IGER solid-state automatic behaviour recorders (Ultra Sound Advice, London, UK; Rutter et al. 1997). The 10 available jaw recorders were fitted to five cows of each treatment after moving to a new plot and removed after 24 h. The consecutive day, the remaining 10 cows were monitored using the jaw recorders. The data were analysed with the Graze Data Analyses Program (version 8.0, IGER, Devon, UK), identifying jaw movements and different behaviours (grazing, ruminating, idling; Rutter, 2000).

Rumen Measurements

Rumen fluid samples were taken after every milking from the six rumen-cannulated animals. A solid, perforated plastic tube (850 mm long; 25 mm in diameter) was used to collect equal amounts of rumen fluid from the front and middle of the ventral sac and from the cranial sac. The pH was measured immediately using an electronic pH meter (pH electrode HI 1230, Hanna Instruments B.V., IJsselstein, The Netherlands). Duplicate samples were taken, either acidified with phosphoric acid or with trichloroacetic acid, and stored at –20 °C pending volatile fatty acids (VFA) and ammonia-nitrogen (NH_3-N) analysis, respectively, as described by Taweel et al. (2005).

Statistical Analysis

All statistical analyses were carried out by ANOVA using the PROC MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC). Repeated measurements ANOVA was performed on all data except for offered herbage, herbage chemical composition and herbage intake, with day as the repeated subject, since multiple measurements per animal cannot be regarded as independent units of observations (Littell et al. 1998). A first-order autoregressive covariance structure [AR(1)] fitted the data best and was used to account for within-cow variation. Data are presented similarly for all variables, with treatment means for morning and afternoon samples for both MA and AA, except for milk yield and composition, with S.E.M. values for the interaction between treatment and time and P-values for treatment effects and for the interaction between treatment and time. Effects of rotation and the interaction between rotation and treatment are not discussed as these were of minor interest in view of the aim of the current paper. Differences were considered significant at a probability of $P < 0.05$ and post-hoc analyses were carried out using the Tukey test for pairwise comparisons. When interactions were not significant ($P > 0.05$), they were excluded from the model.

After averaging DM content of herbage per treatment, rotation and sample time combination, the average chemical composition of offered grass per rotation per treatment was analysed with treatment, rotation and time of sampling (morning, afternoon and residue) as fixed factors. The interaction between treatment and time of sampling was also included in the model. After averaging offered herbage per treatment and rotation, offered herbage and NE_L, DVE, OEB and DOM, these variables were tested using a simpler model including only the effects of treatment and rotation.

Herbage intake was analysed similarly, although as herbage intake was determined per cow per rotation, day was excluded from the model and cow was included as a random factor in the model. Grazing behaviour was analysed with treatment, rotation, day, time between milkings (time denotes period between two milkings: from 06.00 to 16.00 (the time between the morning and afternoon milking) and from 16.00 to 06.00 (the time between afternoon and morning milking)) and the interaction between treatment and time.

The model for rumen fluid variables was similar to the model for grazing behaviour but also included the interaction between treatment and rotation, since this interaction was significant for most rumen fluid variables, and included time denoting actual sampling time at milking. Milk data from the two milkings following a move to a fresh plot were pooled per animal and values were analysed with treatment, rotation, day and the value of each of the variables measured during the adaptation period as covariate. As the interaction between treatment and rotation showed no significant differences, it was not included in the model.

RESULTS

Pasture Composition and Intake

Chemical composition of pasture was similar between treatments, except for a higher DM, sugar, NE_L, DVE and DOM content but lower NDF and ADF content in AA than MA ($P < 0.05$, Table 6.2). All variables differed between both times of sampling (06.00 and 16.00 h, $P < 0.05$, data not shown). Most interesting, however, was the significant interaction between treatment and time of sampling in all variables, except for ADL content of pasture. Pasture CP and CFAT contents were lower after the first 10 or 14 h grazing than directly after moving to a new plot. Grass NDF content was lower immediately after the afternoon move to

Table 6.2

Offered herbage and herbage chemical composition of cows moved after morning (MA) or afternoon milking (AA) to a new plot*.

	MA			AA			S.E.M.	*P*	
Variable	06.00	16.00	residue†	16.00	06.00	residue†		Treatment	Treatment × time
Offered herbage (kg DM/day)	23.9			22.6			0.12	0.016	-
Dry matter (g/kg)	151a	189bc	158a	186b	153a	201c	4.0	0.002	<0.001
Organic matter (g/kg DM)	902a	908b	907ab	905ab	906ab	910b	1.1	0.127	0.047
Crude protein (g/kg DM)	193c	162ab	154a	187c	170b	151a	2.5	0.837	0.033
Crude fat (g/kg DM)	40.6c	32.1ab	30.6a	38.2c	34.6b	29.3a	0.59	0.440	0.005
Neutral detergent fibre (g/kg DM)	452b	455b	490c	425a	469bc	468bc	4.8	0.016	0.003
Acid detergent fibre (g/kg DM)	255ab	260b	278c	241a	266bc	267bc	3.3	0.034	0.023
Acid detergent lignin (g/kg DM)	14.9	15.4	17.8	14.0	15.7	16.7	0.41	0.122	0.232
Sugars (g/kg DM)	114a	158cd	135b	147c	129b	163d	2.6	0.004	<0.001
Net energy for lactation (MJ/kg DM)	6.7			6.9			0.02	0.039	-
Intestinal digestible protein (g/kg DM)	96			100			0.6	0.039	-
Degraded protein balance (g/kg DM)	10			18			2.9	0.186	-
Digestibility of organic matter	0.82			0.84			0.002	0.029	-

* Means within the same row with different letters differ ($P < 0.05$).
† The residue was taken from the plot at turnout.

The most pronounced differences existed in sugar contents between the four treatment and time combinations. Sugar content was greatest around afternoon milking, although differences between maximum and minimum sugar content were larger in MA than in AA, and pasture in MA that had already been grazed for 10 h showed a higher sugar content than grass offered fresh at 16.00 h in AA. The amount of pasture offered was greater in MA than AA ($P = 0.016$) due to an unexpected difference in grass height (169 mm in AA and 176 mm in MA, $P = 0.009$), but intake of pasture did not differ between treatments (Table 6.3).

Grazing Behaviour

All rumination variables and the bite rate during grazing differed between treatments, with a longer rumination time (417 v 407 min/day, $P = 0.004$) in MA compared with AA, more ruminations (30500 v 29500 per day, $P = 0.047$) and also more rumination boli (588 v 538 per day, $P < 0.001$) (Table 6.3). However, there was an interaction between treatment and period of the day for all variables. Grazing time, bites and chews were greater in the period immediately after the move to a fresh plot of grass (i.e in AM-PM, the period between morning and afternoon milking in MA and in PM-AM, the period between afternoon milking and morning milking in AA) with a larger difference in chews between AM-PM and PM-AM in MA than in AA. When expressing grazing time as proportion of available time in both treatments and periods of the day, the time budget of the cows shows, on average per treatment, a similar distribution between grazing (0.36), ruminating (0.29) and inactivity (0.35). When examining differences between AM-PM and PM-AM, a shift occurs in that grazing time per period of

the day is higher in AM-PM (0.44) compared to PM-AM (0.31, $P < 0.001$) at the expense of ruminating (0.21 v 0.34 in AM-PM and PM-AM, respectively, $P < 0.001$). Ruminating time (in minutes as well as in proportion of available time), the number of ruminations and the number of boli was greater during PM-AM than during AM-PM. The differences between AM-PM and PM-AM were much larger in MA than in AA (Table 6.3). To further investigate differences within the day between MA and AA, grazing and ruminating time was separated into five periods of 4.5 h (Fig. 1). To group data into these periods, it was necessary to split the time between morning and afternoon milking into two periods while the time between afternoon and morning milking was split into three periods. The time that was excluded from this analysis (06.00–06.00 h and 15.30–16.30 h) was during milking and so chosen to minimize the loss of data. Clearly, grazing time is larger directly after the move following morning milking in MA and following afternoon milking in AA, while rumination time shows the opposite effect. Simultaneously, Fig. 1 shows a diurnal effect in grazing and ruminating, with most grazing taking place between 06.00 and 21.00 h and most rumination time between 21.00 and 06.00 h.

Table 6.3

Herbage intake and intake behaviour of dairy cows moved after morning (MA) or afternoon milking (AA) to a new plot*.

Variable†	MA		AA		S.E.M.	*P*	
	AM-PM	PM-AM	PM-AM	AM-PM		Treatment	Treatment × time
General							
Intake (kg DM/day)	16.3		15.4		0.68	0.321	-
Bite size (mg/bite)	525		509		26.5	0.680	-
Grazing time (min/period)	297b	220a	298b	227a	4.5	0.341	<0.001
Ruminating time (min/period)	104a	313d	256c	151b	2.7	0.004	<0.001
Idling time (min/period)	199a	307d	285c	222b	4.7	0.441	<0.001
Grazing time (fraction of total)	0.50c	0.26a	0.36b	0.38b	0.007	0.110	<0.001
Ruminating time (fraction of total)	0.17a	0.37d	0.31c	0.25b	0.004	0.818	<0.001
Inactive time (fraction of total)	0.33a	0.37bc	0.34ab	0.37c	0.007	0.119	<0.001
Grazing variables							
Bites (number/period)	17900b	13500a	17600b	12780a	283.9	0.169	<0.001
Bite rate (/min)	62b	61b	59b	56a	0.8	0.007	0.039
Chew rate (/min)	24c	18a	22bc	20ab	0.6	0.959	<0.001
Ruminating variables							
Ruminations (number/period)	7470a	22700d	18700c	10800b	231.0	0.047	<0.001
Boli (number/period)	155a	433d	338c	200b	4.6	<0.001	<0.001

* Means within the same row with different letters differ ($P < 0.05$).
† AM-PM indicates the period between morning and afternoon milking, PM-AM indicates the period between afternoon and morning milking.

Rumen Variables and Milk Production

Rumen fluid pH, NH_3-N and total VFA were similar between treatments (Table 6.4). In MA,

the proportions of acetate and butyrate were lower but those of propionate, valerate and isovalerate were higher, resulting in a lower NGR than in AA (Table 6.4). Again, for all rumen variables, a significant interaction between treatment and time was found, mainly caused by the larger differences between morning and afternoon sampling in MA than in AA.

Table 6.4

Rumen pH, ammonia-nitrogen (NH_3-N) and molar proportions of individual volatile fatty acids (VFA) of dairy cows moved after morning (MA) or afternoon milking (AA) to a new plot*.

	MA		AA		S.E.M.	*P*	
Variable	06.00	16.00	16.00	06.00		Treatment	Treatment × time
pH	6.6d	5.9a	6.3c	6.2b	0.02	0.430	<0.001
NH_3-N (mg/L)	50a	132d	73b	106c	2.7	0.486	<0.001
Total VFA (mmol/L)	103a	133d	111b	125c	1.3	0.709	<0.001
Acetate (molar proportion)	0.673d	0.632a	0.664c	0.656b	0.0014	0.001	<0.001
Propionate (molar proportion)	0.195ab	0.218c	0.195a	0.198b	0.0010	<0.001	<0.001
Butyrate (molar proportion)	0.104a	0.118c	0.115b	0.116bc	0.0007	<0.001	<0.001
Isobutyrate (molar proportion)	0.0076a	0.0078bc	0.0076ab	0.0081c	0.0001	0.189	<0.001
Valerate (molar proportion)	0.010b	0.013d	0.009a	0.012c	0.0002	<0.001	<0.001
Isovalerate (molar proportion)	0.011b	0.011b	0.009a	0.011b	0.0002	0.016	<0.001
NGR†	4.3bc	3.8a	4.4c	4.2b	0.03	<0.001	<0.001

* Means within the same row with different letters differ ($P < 0.05$).
† The nonglucogenic to glucogenic VFA ratio (NGR) was calculated as [acetate + 2 x (butyrate + isobutyrate) + valerate + isovalerate] / [propionate + valerate + isovalerate].

Milk yield, milk protein, lactose and urea content were similar between treatments, but milk fat content was higher in AA than in MA ($P < 0.001$, Table 6.5). Because of this higher milk fat content, milk fat and FPCM production were greater in AA than in MA ($P = 0.006$ and $P = 0.002$, respectively).

DISCUSSION

The aim of the current experiment was to determine the effect of daily move to fresh pasture after either morning or afternoon milking on intake, intake behaviour, rumen fermentation characteristics and milk production in grazing dairy cows. Daily strip grazing is a frequently adopted grazing strategy in modern dairy farming and has been shown to improve productivity of dairy cows when compared with a move to a fresh plot every 4 days (Abrahamse et al., 2008), although the role of timing of a move in herbage allocation has not received much attention in dairy nutrition. Little information is available on the combined effects of grazing behaviour, rumen fermentation and milk production in such grazing systems. In the present experiment, it was shown that cows have longer grazing times immediately after the move to a fresh plot than in the hours preceding the move to a new plot. Such grazing behaviour, in

combination with changes in grass composition due to daytime variation and cows grazing down the sward, resulted in a higher NGR in rumen fluid in cows when moved following afternoon milking than morning milking. This was accompanied by an increased milk fat content in AA, resulting in a higher FPCM production.

Table 6.5

Milk yield and milk composition of dairy cows moved after morning (MA) or afternoon milking (AA) to a new plot.

	Treatment			*P*
Variable	MA	AA	S.E.M.	Treatment
Milk yield				
Milk (kg/d)	26.3	26.0	0.19	0.465
FPCM* (kg/d)	24.8	25.6	0.20	0.002
Milk composition				
Fat (g/kg)	36.5	40.4	0.31	<0.001
Protein (g/kg)	32.2	32.9	0.25	0.816
Lactose (g/kg)	45.5	45.2	0.18	0.277
Urea (mg/l)	313	299	6.5	0.129
Amount				
Fat (g/d)	949	1028	8.5	0.006
Protein (g/d)	838	838	8.4	0.607

* Fat- and protein-corrected milk.

Grazing Behaviour

It is well known that grazing dairy cows consume the largest part of their intake during daylight hours. Rook et al. (1994) found 0.88 of intake occurred during daylight hours, while Penning et al. (1991) found 0.90 of intake occurred during the 17 hours of daylight. This decreased to 0.72 later in the season, when daylight was reduced to 12 h. Also in the current experiment, cows were found to show large differences in grazing time between AM-PM and PM-AM. Although grazing time did not differ between treatments, the average proportion of time spent on grazing was much larger AM-PM (0.44) than PM-AM (0.31). There are limited data available on cows allocated fresh pasture after morning or afternoon milking in strip grazing systems. Gregorini et al. (2006) investigated grazing behaviour in beef cattle, allocating animals at 07.00 h or 15.00 h. Their findings show similar effects on grazing time to those found in the current experiment. Total grazing time was much lower, however, caused by their observational method (visual observations during daylight) and lower herbage intake in these animals (on average 5.1 kg DMI/heifer/day) (Gregorini et al. 2006). A similar experiment was carried out with dairy cattle (Gregorini et al. 2008). The proportion of time spent eating was found to be greatest following the movement of cows to a new plot. However, these results cannot directly be compared with the results from the current experiment as Gregorini and co-workers allocated cows to a fresh plot either at 08.00 or 15.00, but grazing was limited in both treatments to the period between 08.00 and 19.00. Orr et al. (2001),

in a similar experimental setup as the current experiment, showed large differences in the time spent grazing during day and night upon a move to a fresh grazing plot after afternoon milking, although these differences after morning move were smaller. The difference between the current findings and those presented by Orr et al. (2001) may be explained by differences in both the duration of the periods between milking and timing of the move to a fresh plot, which was at 07.45 or 16.45h in the experiment by Orr et al. (2001). As mentioned above, cows eat more during daylight hours, implying that grazing time AM-PM would be increased if the duration between morning and afternoon milking is increased from the 9 h in Orr et al. (2001) to 10 h in the current experiment. Also, cows are known to have their main grazing bout during dusk, indicated clearly by Taweel et al. (2004) in a continuous stocking system, showing a linear increase in grazing time between dawn (06.00 to 12.00 h), afternoon (12.00 to 18.00 h) and dusk (18.00 to 24.00 h). Similar findings can be found in Fig. 1, with a longer grazing time at the end of the day in both treatments. This might also have played a role in the larger differences found between AM-PM and PM-AM in AA and the smaller difference in MA in Orr et al. (2001) than in the current experiment.

Sugar Content in Grass

The sugar content of grass depends on the balance between synthesis due to radiation on the one hand and growth and maintenance (during respiration), utilizing sugars to grow new shoots and hence regain photosynthetic capacity, on the other. Since sugars are produced during photosynthesis and respiration occurs mainly during the night, substantial amounts of sugars are transported down the plant during the day and stored in the stem and pseudostem (Fulkerson and Donaghy 2001). Sugar content in grass is also influenced by removal of leaves due to grazing, since sugars are produced in the top layer of the sward, where most radiation is intercepted (Delagarde et al. 2000; Smit and Elgersma, 2004). Indeed, sugar increased during the day (121 g/kg DM at 06.00 h v 153 g/kg DM at 16.00 h, $P < 0.001$) as expected. The concentration of sugars at 16.00 h in the current experiment was numerically higher in MA (158 g/kg DM) than in AA (147 g/kg DM). Since grass is defoliated with a gradual decrease in grass height, and sugars show the highest variability in the top layer of the sward as described by Delagarde et al. (2000), one could argue that sugar content would be expected to be highest at 16.00 h in AA. Although the concentration of sugars during the evening increased between the lowest and highest layer of the sward, during the morning it is lowest in the lowest part of the grass (0–50 mm; 175 g/kg OM) but highest in the second layer from the bottom (50–100 mm; 212 g/kg OM) (Delagarde et al. 2000). This is probably due to transportation of sugars to the lower layers from the bottom of the plants (Fulkerson and Donaghy 2000). The fact that sugar content at 16.00 h in MA (grass already grazed for 10 h) was higher than in AA (grass in a fresh plot) suggested that the effect of transportation of sugars played a larger role in the final sugar content of grass than photosynthesis.

Nutrient Intake and Rumen Fermentation

Both treatment groups spent most of their time grazing during the period of the day following afternoon milking, but grazing time in AA during this period was longer than grazing time in MA in this same period (Figure 6.1). Combining the grazing times AM-PM and PM-AM in both treatments in this experiment (Table 6.3) with the chemical composition of grass during these periods of the day (averaged between the move to a fresh plot and turnout), intake of sugars is expected to be higher in AA than in MA. A better estimation of intake of specific components would be a calculation based on the number of bites and bite size. However,

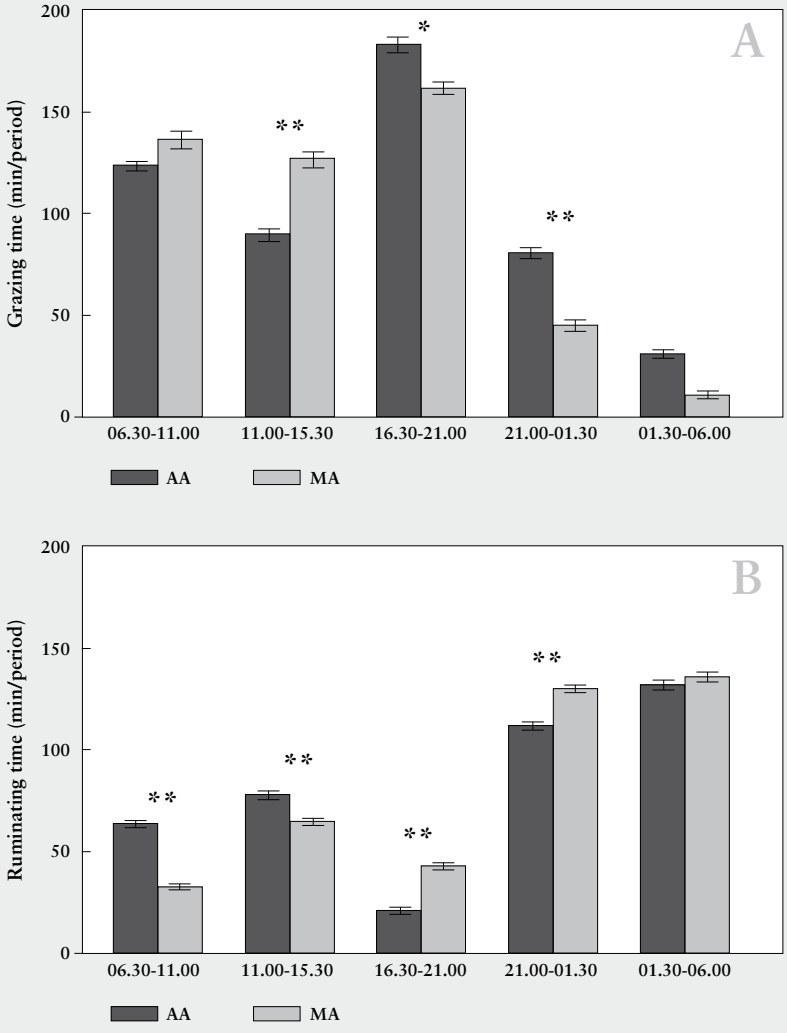

Figure 6.1

Grazing time (a) and ruminating time (b) during different periods of the day when dairy cows are moved after morning (MA) or afternoon (AA) milking to a new plot. Asterisks above the columns indicate the significance of the difference between treatments per period of the day (* $P < 0.01$; ** $P < 0.001$) and bars representing SEM are given.

herbage intake was not estimated in AM-PM or PM-AM separately. However, in earlier experiments, both Gibb et al. (1998) and Taweel et al. (2004) reported larger bite mass in the evening than in the morning. When using the bite mass of either Gibb et al. (1998) or of Taweel et al. (2004) (after averaging bite size during dawn and afternoon to 406 mg/bite and used together with 563 mg/bite during dusk) to calculate sugar intake by multiplying bites and bite size, daily sugar intake is larger in AA than MA in the current experiment (on average 149 g sugar or 7.7 % higher sugar intake) while ADF intake is slightly lower (on average 75 g ADF or 2.1 % lower ADF intake). This is related to a higher NGR in rumen fluid in AA than in MA ($P < 0.001$), since on roughage diets increased fermentation of sugar results in increased production of acetate and reduced production of propionate compared with starch or fibre (Bannink et al., 2006). Indeed, when the effect of the different chemical components of grass in MA and AA on NGR is estimated using the stoichiometric coefficients given by Bannink et al. (2006), NGR is expected to be 0.19 higher in AA than in MA. However, the lower NGR in the current experiment in MA might also have been influenced by the longer interval between the large first meal after the move to a new plot and time of sampling rumen fluid, which was longer in AA (14 h) than in MA (10 h). Besides, the large meal in AA was terminated more hours before rumen fluid sampling than in MA as cows tend to eat the largest part of their grass during daylight hours. This effect can also be observed from the pH of rumen fluid. Although grazing time was longest in AA between 16.30 and 21.00 h, the pH at 06.00 was higher than the pH in MA at 16.00 h. The higher NGR was related with a higher milk fat content in AA than in MA, resulting in more FPCM being produced in AA than in MA. The higher milk fat content in AA was in line with the higher fat content in the afternoon treatment reported by Gibb (2006). The efficiency of FPCM production (expressed as kg FPCM production per kg of DM herbage intake) shows a tendency to be higher in AA than in MA (1.7 v 1.5, $P = 0.079$), while the efficiency of milk production (expressed as kg milk/kg of DM herbage intake) only shows a numerical increase in AA as compared to MA (1.7 v 1.6, $P = 0.536$). Milk urea content was similar between treatments. However, when investigating the separate milk urea values per milking, larger differences appear between the morning and afternoon milking in MA (289 and 335 mg/l, respectively) than in AA (314 and 283 mg/l, respectively). These values were related to rumen NH_3-N values and the ratio of CP to sugar in grass, calculated by averaging the values for CP and sugar at the time of NH_3-N and urea sampling with the value of 12 h earlier. This is in line with findings of Gustafsson and Palmquist (1993), who found that changes in rumen fluid NH_3-N content were quickly observed in milk urea. This shows that indeed, matching energy and protein supply in the rumen to reduce NH_3-N production influences excretion of urea in milk. However, it does not prove that movement strategies in grazing management do have the opportunity to reduce emission of N in grazing systems, as no treatment effect on milk urea content was found, and urinary losses of N as well as microbial protein yield were not determined during this experiment.

CONCLUSION

Time of allocation to a fresh plot altered grazing behaviour over the day. In combination with the variation in chemical composition of the grass, this probably resulted in a larger intake of sugars in AA than in MA. Indeed, these observations were accompanied by an increased NGR in rumen fluid, and an increase in milk fat content, of AA cows. However, herbage intake and milk production were similar between treatments.

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REFERENCES

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91(5):2033-2045.

Bannink, A., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren, and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. Journal of Theoretical Biology 238: 36–51.

Bargo, F., L. D. Muller, J. E. Delahoy, and E. S. Kolver. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86(1):1-42.

Boudon, A., J. L. Peyraud, and P. Faverdin. 2002. The release of cell contents of fresh rye-grass (*Lolium perenne* L.) during digestion in dairy cows: effect of the intracellular constituents, season and stage of maturity. Animal Feed Science and Technology 97(1-2):83-102.

Chilibroste, P. 2005. Pasture characteristics and animal performance. Pages 681-706 in Quantitative Aspects of Ruminant Digestion and Metabolism. J. France, M. Forbes and J. Dijkstra, ed. CAB International, Wallingford, UK.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1-2):49-68.

Fulkerson, W. J. and D. J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Australian Journal of Experimental Agriculture 41(2):261-275.

Gibb, Huckle, and Nuthall. 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science 53(1):41-46.

Gibb, M.J. 2006. Pages 141-147 in Grassland management with emphasis on grazing behaviour. Fresh Herbage for Dairy Cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, Dordrecht, The Netherlands.

Gregorini, P., M. Eirin, R. Refi, M. Ursino, O. E. Ansin, and S. A. Gunter. 2006. Timing of herbage allocation in strip grazing: Effects on grazing pattern and performance of beef heifers. Journal of Animal Science 84(7):1943-1950.

Gregorini, P., S. A. Gunter, and P. A. Beck. 2008. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: Timing of herbage and fasting allocation. J. Anim Sci. 86(4):1006-1020.

Gustafsson, A.H., and D.L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76:475–484.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science 76(4):1216-1231.

Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Milk production from late-lactation dairy cows. Grass and Forage Science 56(4):383-394.

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

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56(4):352-361.

Penning, P. D., A. J. Rook, and R. J. Orr. 1991. Patterns of ingestive behaviour of sheep continuously stocked on monocultures of ryegrass or white clover. Applied Animal Behaviour Science 31(3-4):237-250.

Rearte, D. H. 2005. New insights in the nutritive value of grass. In: Utilization of grazed grass in temperate animal systems. Pages 49-60 in Proceedings of a satellite workshop of the XXth International Grassland Congress, Cork, Ireland. J. J. Murphy, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

Rook, A. J. and C. A. Huckle. 1997. Activity bout criteria for grazing dairy cows. Applied Animal Behaviour Science 54(2-3):89-96.

Rook, A. J., C. A. Huckle, and P. D. Penning. 1994. Effects of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. Applied Animal Behaviour Science 40(2):101-112.

Rutter, S. M. 2000. Graze: A program to analyze recordings of the jaw movements of ruminants. Behavior Research Methods, Instruments, and Computers 32(1):86-92.

Rutter, S. M., R. A. Champion, and P. D. Penning. 1997. An automatic system to record foraging behaviour in free-ranging ruminants. Applied Animal Behaviour Science 54(2-3):185-195.

Smit, H. J. and A. Elgersma. 2004. Diurnial fluctuations in vertical distribution of chemical composition in a perennial ryegrass (*Lolium perenne* L.) sward during the season. Pages 21-24 in Land use systems in grassland dominated regions. Proceedings of the 20th General Meeting of the European Grassland Federation. A. Lúscher, B. Jeangros, W. Kessler, O. Huguenin, M. Lobsiger, N. Millar, and D. Suter, ed. Luzern, Switzerland.

Smit, H. J., S. Tamminga, and A. Elgersma. 2006. Dairy cattle grazing preference among six cultivars of perennial ryegrass. Agronomy Journal 98(5):1213-1220.

Tamminga, S., W. M. Van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: The DVE/OEB-system. Livestock Production Science 40:139-155.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006a. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. Journal of Dairy Science 89(9):3494-3500.

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006b. Rumen degradation characteristics of perennial ryegrass cultivars during the growing season. Animal Feed Science and Technology 131(1-2):102-119.

Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science 87(10):3417-3427.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121(3-4):243-256.

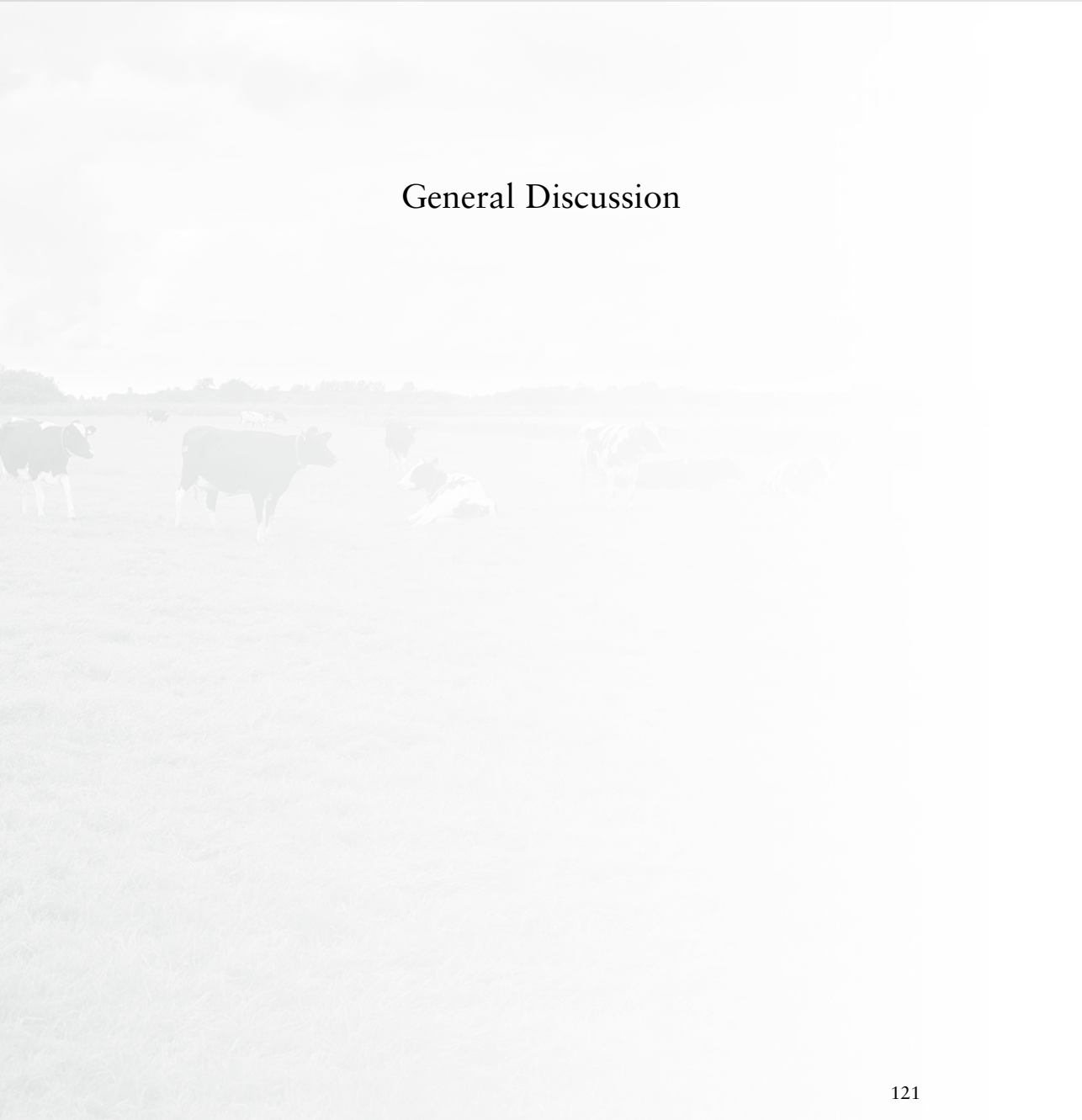
Van Es, A. J. H. 1975. Feed evaluation for dairy cows. Livestock Production Science 2(2):95-107.

Van Vuuren, A. M., C. J. Van Der Koelen, and J. Vroons- De Bruin. 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. Netherlands Journal of Agricultural Science 34:457-467.

Wales, W. J., C. R. Stockdale, and P. T. Doyle. 2005. Plant and sward characteristics to achieve high intake in ruminants. Pages 37-47 in Utilization of grazed grass in temperate animal systems. Proceedings of a satellite workshop of the XXth International Grassland Congress. J. J. Murphy, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

CHAPTER 7

General Discussion



In high producing dairy cows, sufficient feed intake is crucial to make dairy farming profitable without compromising the animals' health and welfare or the environment. Feed intake is distributed in time over the day by the feed intake pattern, consisting of discrete meals alternated with periods of ruminating and idling. Daily dry matter intake (DMI) can therefore be described in terms of the number of meals per day, the length of meals and the intake rate (IR) during meals (Dado and Allen, 1994). The number of meals per day and their length results in grazing or eating time, while intake rate is the product of bite rate (BR) and bite mass (BM). Manipulation of any of these variables may result in a change in DMI.
In stall fed animals, manipulating DMI through feed intake behavior can be achieved by changing the type and/or quality of forage (Chapter 2; Deswysen et al. 1993; Greter et al. 2008), the forage to concentrate ratio (Friggens, et al., 1998) or the composition of the concentrates fed in addition to the forage (Chapter 2; Allen et al. 2000; Miron et al. 2004), while during grazing, grazing management can be used as a tool to influence the feed intake pattern (Chapter 3; Gibb et al., 1997).
Grazing suffers from the major disadvantage that dairy cows are unable to consume enough dry matter (DM) to achieve and maintain high levels of milk production (Gibb, et al., 1999). Dry matter intake is in general affected by physical limitations in the rumen, although this seems not the case in cows fed high quality forage (Chilibroste et al., 2000). Constraints on total time spent eating (Ungar, 1996; Gibb et al., 1997) and the animal's limited ability to modify its grazing behavior (Agnew, et al., 2004; Taweel, et al., 2004) are reasons for insufficient DMI to achieve high milk yield responses. Changing any of these, for instance by changing grazing management, could influence herbage DMI and thereby productivity.

Herbage Dry Matter Intake Estimation
To measure potential effects of grazing management on herbage DMI, an accurate estimation of herbage intake is crucial. In stall fed animals, measuring feed intake and its pattern is relatively easy by regularly weighing the feed bin. In grazing animals measuring feed intake is more complicated. An indirect estimation of herbage intake is necessary since herbage intake cannot be determined directly (Dillon, 1993). Several methods are available, which are mostly separated into 'animal-based techniques' and 'plant-based techniques'. Methods in the latter class generally rely on estimations of herbage mass on offer and refused herbage, corrected for regrowth of the sward (Lantinga et al., 2004). In groups of grazing animals however, plant-based techniques suffer from the disadvantage that no individual animal intake is estimated (Mayes and Dove, 2000). The disadvantage of animal-based techniques however, is that measurements are not completely independent of each other (Rook, 1999; Iason and Elston, 2002; Phillips, 2002). There are four types of animal-based techniques to estimate herbage DMI, based on: i) weighing animals, ii) calculating energy expenditure, iii) feces output and diet digestibility, and iv) rumen emptying corrected for clearance during grazing (Chilibroste et al., 1997; Penning, 2004). The method using n-alkanes as a marker should be classified as an alternative type of animal-based technique to estimate herbage DMI. Unlike the fecal output – diet digestibility method, the quantitative fecal output (usually estimated using indigestible markers) and the actual digestibility (often estimated *in vitro*) is not required, but the assumption has to be made that digestibility of the natural odd-chain alkanes and that of the dosed even-chain alkanes of related chain length is equal.
Plants are protected by a waxy surface that contains a wide range of long chain hydrocarbons (n-alkanes), carboxylic acids, ketones and alcohols that vary between species. Hydrocarbons are organic compounds that contain only the two elements hydrogen (H) and carbon (C).

In most higher plants, n-alkanes with odd-numbered C chains in the range of 21 to 37 C atoms comprise most of the hydrocarbons of the cuticular wax, but alkane composition varies per plant species (Dillon, 1993). It is therefore necessary to take grass samples that represent the whole diet and in non-uniform grasslands, this requires also an estimation of the herbage composition (Mayes and Dove, 2000).
The n-alkane methodology of intake estimation used in the grazing experiments described in this thesis was a slightly modified protocol to the alkane method described by Taweel et al. (2006). This alkane method estimates herbage DMI from a known dose of an even-chain alkane (C_{32}) and compares the concentration in feces of this dosed C_{32} alkane with the natural odd-chain alkane (C_{33}) occurring in plants. The choice of these (C_{32} and C_{33}) alkanes is based on the similarity in the recovery of alkanes in feces. This recovery is generally high but not complete, and depends on chain length of the alkane (Mayes and Dove, 2000). Recoveries of C_{32} and C_{33} were found to be similar in different studies, so, intake estimation is not biased (Dove and Mayes, 1991; Dove and Mayes, 1996).
The n-alkane method has sofar only been validated in indoor experiments. Diurnal variation in the fecal excretion of n-alkanes could increase the error in herbage intake estimation (Mayes et al., 1986). Fecal grab samples should therefore be taken at times when the concentration of the marker is equal to the mean daily value (Dillon, 1993). In a grazing experiment with dairy cows by Dillon and Stakelum (1989), substantial daily variation was observed when concentrate (with dosed even-chain alkanes) was dosed twice daily, which was even increased when concentrate was dosed once daily. However, in Mayes et al. (1986), in a stall-fed experiment with sheep, no evidence was found for systematic diurnal variation.

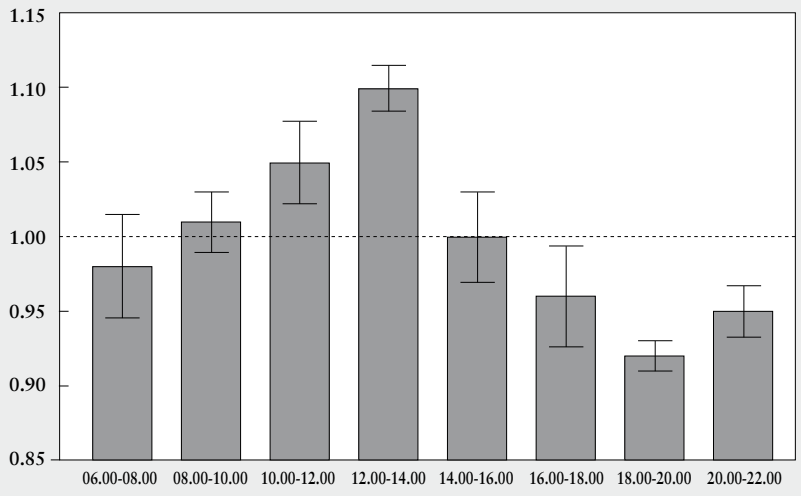


Figure 7.1

Average relative deviation of the average ratio between C_{33} and C_{32} (error bars represent standard error).

To use the method in grazing experiments, it is necessary to rule out systematic errors like diurnal variation in the excretion pattern of the dosed alkanes. Therefore, as part of the experiment described in Chapter 3, data was collected to determine the daily variation of dosed C_{32} alkane. Samples were taken from all defecations of the 10 animals in 1D during one day during daylight from morning milking (0600 h) until approximately sunset at 2200 h. The average variation throughout the day in the ratio of C_{33}:C_{32}, expressed as proportion of the average daily value, varied between 0.92 and 1.10. This variation is greater than that observed by Mayes et al. (1986) but less than the variation observed by Dillon and Stakelum (1989), resulting in a theoretical maximum error in the estimation of herbage DMI of 10%. However, at the time of actual feces sampling upon which herbage DMI intake estimates of Chapter 3 were based (0600 and 1600 h), the ratio of C_{33} to C_{32} was close to 1 (Figure 7.1), which supports the validity of our approach.

Grazing Behavior Measurements

Regardless the way feed is offered to the animal, cows ingest their feed in a number of discrete meals that are alternated with periods of rumination and periods of idling (Forbes, 1995). Idling is behavior that is not related with feed intake, the time in which a cow does not eat nor ruminate. To measure feed intake behavior, several methods can be applied. Eating and ruminating activities can be monitored by visual observations, which are laborious and time-consuming (Beauchemin et al., 1989). A system based on recordings of jaw movements was therefore developed using a small rubber balloon (Penning, 1983), a flexible silicone pipe (Abijaoudé et al., 1999) or a silicone noseband filled with carbon granules (Rutter et al., 1997). The latter method was used in the experiments described in Chapter 3, 5 and 6. The differences in air pressure or in electrical resistance generated by the balloon, pipe and noseband are converted to an electric signal and stored in a data logger and from the jaw movements feed intake behavior can be measured.

Meal Criterion Estimation in Stall-fed Cows

In any of the methods to measure feed intake behavior, only feeding events, also called bouts or visits, are measured. These feeding events are the moments where the cow consumes an amount of food. However, at the end of a feeding event, the cow may not be satiated and starts eating after a short time. This clustered type of feeding behavior is frequently observed, i.e. in cases where cows are disturbed in their grazing behavior by other cows in the flock (Tolkamp et al., 2000; Allcroft et al., 2004). Tolkamp et al. (2000) found meals rather than visits to be the relevant unit for the analysis of feed intake behavior since the probability of cows ending a meal increased with meal duration, while an increase in the probability of cows ending a visit was not observed with increasing visit durations (Tolkamp et al., 2000).

To be able to analyze the effect of different experimental treatments on meal size and meal frequency, it is necessary to merge feeding events into meals. In various experiments that measure feed intake patterns (Forbes et al., 1986; Rook and Huckle, 1997), the meal criterion (MC) has been found a useful tool to merge feeding events into meals before analysis of data. The MC is defined as the longest interval between feeding events that belongs to the same meal (Tolkamp and Kyriazakis, 1999a).

There are several methods to estimate the MC. The first method is to plot the (log) frequency of occurrence of the different intervals between feeding events using a "broken stick" model. In this model, the change in slope indicates the boundary between within-meal pauses and true between meal intervals (Forbes et al., 1986; example in Figure 7.2).

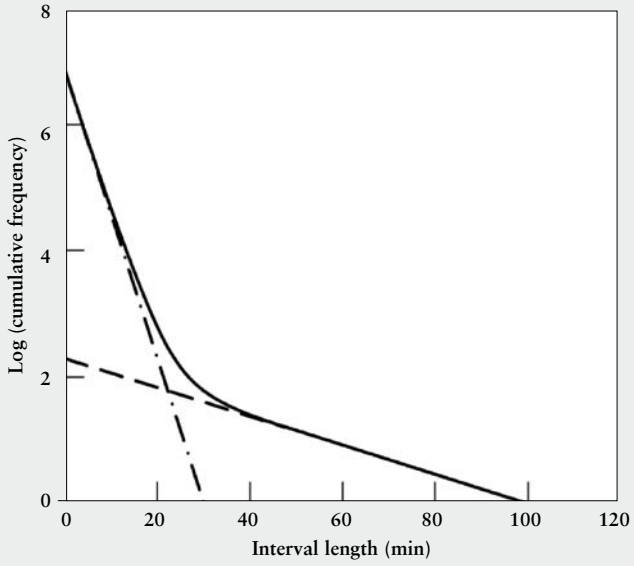


Figure 7.2

Broken stick model to estimate the meal criterion (Tolkamp et al., 1998).

Sibly et al. (1990) suggested another method, based on the concept that there are two randomly distributed processes involved: a distribution of short intervals generating within-meal intervals and a distribution of long intervals generating between-meal intervals. Both intervals are assumed to be Gaussian (normal distribution) processes with two probabilities per unit of time: λ_f for the within-meal interval and λ_s for the between-meal interval, the latter distribution representing the probability per unit of time that a new meal starts. This method was further improved by Tolkamp et al. (1998), taking into account a biological concept: the satiety concept. This concept of satiety implies that the probability of an animal initiating a meal is dependent of the duration of the non-feeding interval (Tolkamp et al., 1998). They therefore plotted the frequency of the logarithm of interval length between visits, rather than the logarithm of the frequency of interval length between visits. The log transformation resulted in two Gaussian distributions, the first one representing intervals between feeding events within a meal and the second one representing intervals between meals. The MC was estimated at the point in the graph where the two log-normal curves crossed (Tolkamp et al., 1998). This model can be expanded with a third normal distribution between the two other distributions, to describe the intervals within meals during which animals drink (Tolkamp and Kyriazakis, 1999b; Figure 7.3).

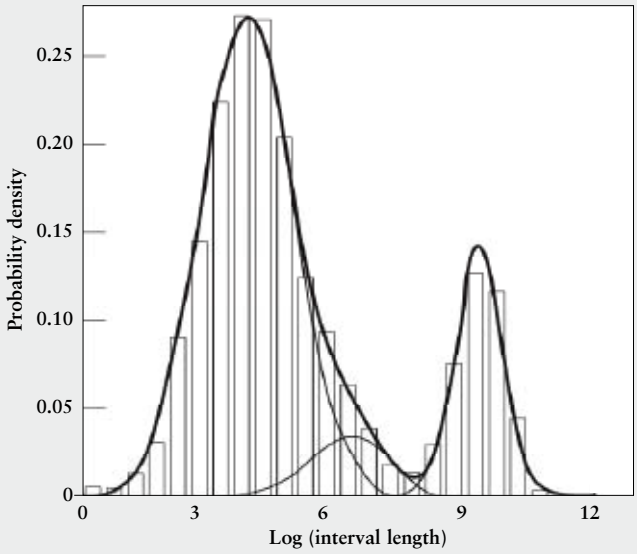


Figure 7.3

Triple log-normal model (Tolkamp and Kyriazakis, 1999b). Interval length was measured in seconds.

Yeates et al. (2001) found that the last Gaussian distribution in the method of Tolkamp et al. (1998), which represents the intervals between meals, to have a better fit using a Weibull distribution. A Weibull distribution is able to describe non-symmetrical frequency distributions. This method resulted in an improved description of the observations from a statistical and biological point of view, and also a better determination of the MC (Yeates et al., 2001).
In the stall-feeding experiment (Chapter 2), the Gaussian-Gaussian-Weibull (GGW) method described in Yeates et al. (2001) was used. This model fitted the data best, based on the Minimal Function Value (MFV) (Yeates et al., 2001). To decide which estimated meal criterion should be used for grouping visits to the feeding bins into meals in the experiment described in Chapter 2, meal criteria per cow per period were calculated and statistically analyzed. Because both lactation stage and the interaction between treatment and lactation stage were significant, meal criteria per treatment and lactation stage were used to pool intake data. Our estimated meal criteria (on average 17.7 min) were somewhat lower than values reported in literature also using the GGW model (28.9 min in Yeates et al. (2001); 39.9 min in Melin et al. (2005)). Because MC influences meal frequency and the duration of a meal as shown for several stall-feeding experiments in Figure 7.4, a uniform and standardized method for estimating MC is required.

However, the MC estimation may be influenced by factors influencing visits, since meal criteria are calculated using the distribution of intervals between visits. These factors include the feeding system, the social hierarchy within a group of cows, competition for feed and physical characteristics of the feed (Grant and Albright, 1995; Tolkamp et al., 2000). Different experiments have used different feeding systems (feeding a TMR from feeding bins in Chapter 2 and in Yeates et al. (2001); feeding concentrates separately from a basal diet in Zom and van Duinkerken (2006), or separately from forage in Melin et al. (2005); and the GrowSafe System using transponders and transponder mats to determine visits to the feed alley in De Vries et al. (2003) and Huzzey et al. (2005)).

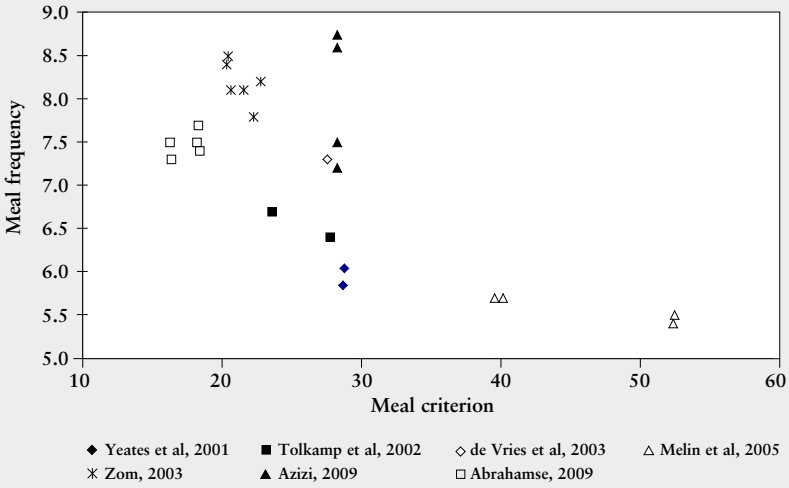


Figure 7.4

Meal frequency and meal criterion determined in different experiments (Yeates et al., 2001; Tolkamp et al., 2002; De Vries et al., 2003; Melin et al., 2005; Zom and Van Duinkerken 2006; Abrahamse et al., 2008; Azizi et al., 2009).

Besides, MC were estimated using different models when using different feeding systems. Hence, the effect of these factors on MC estimations between experiments cannot be determined. Competition for feed is stronger when the number of animals per feeding bin or per meter of feed bunk space is increased. However, this did not result in lower MC estimations due to an increase in the number of short visits. At a higher stocking density in the experiment of Huzzey et al. (2005) compared to a similar experimental setup (De Vries et al., 2003), both using the Gaussian-Gaussian (GG) model to estimate MC, similar MC were found (26.4 vs. 27.7 min, respectively). Also no relation was found between the MC and the number of cows per feeding bin when the MC was estimated using the GGW-model in Chapter 2 compared to the experiments carried out by Yeates et al. (2001) and Melin et al. (2005). Finally, the physical characteristics of the feed did not show a relation with MC estimations, either.

Concentrate is generally eaten quicker than roughage, and as a result, the population of intervals between meals could shift leftwards on the x-axis in graphs like Figure 7.3, resulting in a lower estimation of MC. Huzzey et al. (2005), using a GG-model, indeed found that the average MC pre-calving was higher (34.7 min) than post-calving (24.7 min). This effect coincided with higher inclusion rates of a protein-mineral supplement in the TMR (26.3 % vs. 38.6 % of DM, respectively) fed post-calving, but also with differences in the physiological state of the animals, making it impossible to conclude that the inclusion rate of concentrate caused the observed effects. When estimating the MC using a GGW model, no relation was found between the inclusion rate of concentrate in the diet of the different experiments and the estimated MC in Chapter 2, Huzzey et al. (2005), and Yeates et al. (2001).
Since DMI can be described in meals per day, the length of meals and IR per meal, it is more important if the number of meals, clustered by the MC, differs under different circumstances. To keep the discussion concise, only effects on meal frequency are discussed. As shown in Chapter 2, the number of meals was influenced by lactation stage with more meals in late lactation than in early lactation. This is not in line with Huzzey et al. (2005) who related their increase in meal number per day between the dry period and early lactation to increased energy demand. In fact, although DMI was lower in late lactation than in early lactation in Chapter 2 (19.5 vs. 19.0 kg/d), this decrease was much lower than the decrease in milk yield between early and late lactation (31.5 vs. 19.1 kg/d), showing that cows did not respond to their decreased energy demand in late lactation anyway. Meal frequency was similar at different concentrate to forage ratios in Tolkamp et al. (2002) but the effect of relative amount of concentrate was confounded by the energy content of the diets. Zom and Van Duinkerken (2006) found no effect of concentrate type on meal frequency, but they did find that meal frequency was lower when the ratio of maize to grass silage was 80:20 than when this ratio was 40:60. These results are not in line with the results in Chapter 2, where no effect of roughage nor concentrate type on meal frequency were found. As already explained in Chapter 2, type of supplemented concentrate influenced meal frequency in Miron et al. (2004), although meal frequency was similar between different types of concentrates in Zom and Van Duinkerken (2006) and in Chapter 2. Parity showed to influence daily meal frequency with more meals in primiparous cows than in multiparous cows, probably related to social hierarchy (Azizi et al., 2009). In conclusion, several aspects influence meal frequency in dairy cows, but no firm conclusions can be drawn because of the dependency of meal frequency on MC and many effects on meal frequency are not yet known.

Meal Criterion Estimations in Grazing Cows

Unfortunately, almost all MC estimations have been carried out on feed intake behavior data of dairy cows in stall-feeding situations. There is only one study available that describes an estimation of the MC for dairy cows during grazing (Rook and Huckle, 1997), estimating the MC to be 5 minutes using the broken stick model. Although in Rook and Huckle (1997) both a two- and a three population Gaussian model was also used to estimate the MC, these did not result in a true estimation since these methods failed to converge. The authors describe that the underlying Gaussian may in fact not occur, however, these models could also have failed to converge due to a lack of data. Although quite a number of grazing recordings were analyzed, with measurements 16 days on between 3 and 8 cows, daily measurements were taken on a minute-by-minute basis, excluding intervals shorter than 30 seconds, which led to a substantial decrease in the number of intervals.
Since the methodology of MC estimation in stall-feeding situations has revealed that the

method described by Yeates et al. (2001) did give the best estimations of the MC, grazing data of the experiment described in Chapter 3 has been used to estimate meal criteria for grazing dairy cows. After calculation of the intervals between different grazing events, the frequency of these intervals was plotted against the logarithm interval length for both the total group (Figure 7.5) as well as for the different treatments. As clearly only two populations were visible after plotting the frequency distribution of the logarithm of interval lengths, curve regression using GG (Tolkamp and Kyriazakis 1999a), Gaussian-Weibull (GW, Yeates et al. 2001) and Weibull-Weibull (WW) models were carried out using SAS. The fits of these models was compared using the MFV (Yeates et al., 2001), and are given in Table 2.

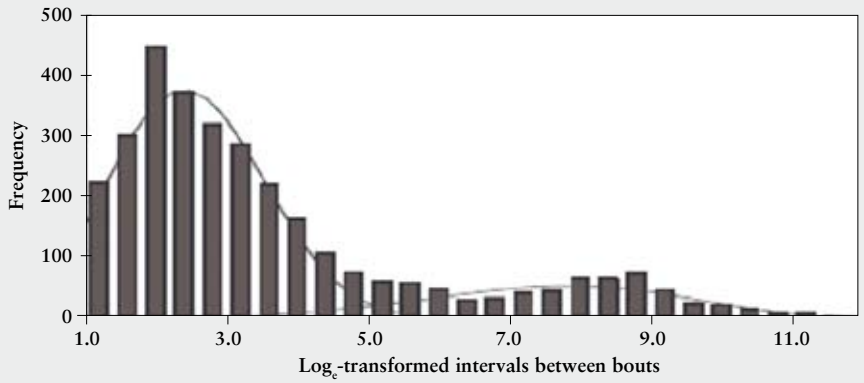


Figure 7.5

Frequency distribution of intervals between bouts. Interval length was measured in seconds.

Table 7.2

MFV values and MC-estimation of different subsets.

Variable	MFV value			Meal Criterion WW
	GG	GW	WW	Minutes
Total dataset	34237	34188	33860	2.1
1D	26426	26388	26158	2.1
4D	7738	7714	7650	1.8
Period 1	16388	16364	16171	2.3
Period 2	17814	17788	17655	1.9
Period 1 1D	12021	12004	11872	2.3
Period 2 1D	14383	14362	14262	2.0
Period 1 4D	4323	4300	4267	2.5
Period 2 4D	3405	3399	3373	1.5

The WW-model of the loge-transformed inter-bout intervals fitted the frequency distribution best. The WW-model is superior to the broken stick model used by Rook and Huckle (1997) in biological as well as in statistical perspective, as in the broken stick model, data points are not independent of each other (Sibly et al., 1990).
The MC is estimated between 1.5 and 2.5 minutes (Table 2). These differences were not tested, statistically, but the MC was numerically different between different subsets. Although differences are small, one could argue that they are in line with the expectations. The MC could be influenced by herbage on offer, resulting in a lower MC if herbage on offer is restricted: cows need to make a larger effort and walk more (pausing grazing for a short period of time) to achieve the required herbage intake level. This effect could be increased on low herbage availability during the last days of grazing on a specific plot in 4D and result in a lower expected MC, especially during the second period (called rotation in Chapter 3), when herbage allowance was lower than in the first period. Indeed, a reduction in MC was seen between period 1 and period 2, and the reduction in MC was larger in 4D than in 1D. Besides the treatment differences it is also clear that these estimations of MC are lower than the values reported in Rook and Huckle (1997), but even much lower than estimations of MC in stall-fed animals. This is probably caused by the method of observations: in stall-fed animals the time cows enter and leave the feed bin are registered, while in grazing animals wearing IGER grazing recorders, direct observations on the animal itself are taken. This means that cows, temporarily stopping eating from the feed bin due to any reason is generally not observed, while with IGER grazing recordings, this would be observed. Therefore, the amount of inter-meal intervals are much higher in grazing animals than in stall-fed animals, caused by the method of observation. Although Figure 7.3 and 5 cannot directly be compared, it is clear that the population representing inter-meal intervals is relatively large in Figure 7.5 as compared to Figure 7.3. This might have implications for the applicability of current estimation models for MC in grazing dairy cows. When using their estimated MC in grazing dairy cows, Rook and Huckle (1997) found an increase in grazing time at the expense of idling time when using the MC to calculate meals. They concluded that it is sound to use MC to calculate the number of meals, but grazing time should be reported from original data without merging feeding events into meals using a MC. When comparing total grazing time per day from the number of meals and meal duration during grazing, as well as from untreated data in Chapter 3, a similar conclusion should be drawn. The average grazing time with untreated data was 558 min/day (Chapter 3), while it was 527 min/day when using the MC (11.8 meals/day and 44.9 min/meal). Because no straightforward conclusions could be drawn on MC and meal frequency from the stall feeding experiments discussed in the previous paragraphs and because of the combination of very low MC estimations and lower grazing time per day when using the MC, it was decided not to use the estimated MC in the grazing behavior experiments in Chapter 3, 5 and 6.

Influencing Grazing Behavior and Herbage Intake with Grazing Management
Pasture DMI of grazing cows is given in equation 2, with the calculation of intake rate (IR) given in equation 3.

Eq. 2: Pasture DMI (kg/d) = grazing time (GT, min/d) * intake rate (IR, kg DM/min of grazing).

Eq. 3: IR (g/min of grazing) = bite rate (BR, bites/min) * bite mass (BM, g of DM/bite).

It is known that BM in grazing cows is influenced by sward surface height (SSH) and the amount of herbage on offer (Allden and Whittaker, 1970). Different rotational grazing strategies might therefore, by influencing different aspects of grazing behavior, result in changes in herbage DMI within and between days. Holecheck et al. (1995) indicated that rotational grazing systems result in, among others, increased pasture DMI and productivity, compared with continuous grazing systems. Rotational grazing is used as a grazing management tool to enhance productivity in various farms around the world, whereas the increased amount of labor and fencing costs compared to continuous grazing or allocation after several days are the reason not to do so in many other farms. There is a renewed interest in rotational grazing systems to increase the daily DMI of high producing dairy cows (Pulido and Leaver, 2003). In a rotational grazing system, the cows are restricted to a paddock for a short period, (e.g. half a day or one day) at regular intervals following a period of re-growth (Mayne et al., 2000). Although in theory, the potential effects of allocation frequency are sound, scientific proof is scarce. Parsons and Chapman (2000) argued that effects of differences in grazing management on production are more imagined than real.

The grazing experiments described in Chapter 3, 5 and 6 provide more insight in the effect of different rotational grazing systems on grazing behavior, herbage DMI, rumen fermentation and productivity of dairy cows. In Table 3, an overview is given of a selection of grazing behavior variables, herbage DMI and milk yield of these experiments.

Treatment effects, as well as day or period of the day effect within treatments as well as period effects are given. The work in Chapter 3 showed increased (numerical) herbage DMI when cows were allocated more frequently (once daily compared to once every four days: 16.5 vs. 15.8 kg DM/d). Herbage intake showed an interaction between treatments and rotations: intake in 1D was increased compared to 4D in the first rotation (18.3 vs. 16.5 kg DM/d) when pasture mass on offer and SSH were high (19.7 cm) but not in the second rotation when SSH was lower (14.3 cm). Milk production was higher in the daily allocation treatment. In Chapter 5, herbage intake was still increased when increasing the allocation frequency from once daily to twice daily (15.5 vs. 14.4 kg DM/d), and again this effect was mainly found during the period where offered sward surface height (SSH) was high (period 1, 17.4 cm) and not in the other period with lower SSH (13.7 cm). In line with these results in herbage DMI, milk yield showed a significant interaction between period and treatment with a greater milk yield in 2D than in 1D in the first period (25.8 kg vs. 24.0 kg/d) but similar milk yield between treatments in the second period. In contrast with the experiment described in Chapter 3, this experiment was designed and resulted in similar amounts of offered herbage between both periods. This leads to the conclusion that SSH rather than offered herbage or grazing management apparently was the first constraint on herbage intake and milk production. This is in agreement with research by McGilloway et al. (1999), who found SSH to be the principal factor controlling intake. In two grazing experiments of Dalley et al. (2001), frequency of allocation to a fresh plot of herbage was increased to six times daily and compared to once daily allocation. Although in one of their experiments intake was numerically increased when allocating frequently (16.3 vs. 15.2 kg DM/d), milk production was lower when allocating frequently. In the other experiment described in the same manuscript, herbage intake and milk production did not differ between treatments. Sward surface height was lower in the experiment showing no differences between both treatments (7.4 cm) than in the experiment with increased herbage DMI (9.7 cm) but much lower than in the experiments described in Chapter 3 (19.6 cm on d 1) and Chapter 5 (15.6 cm).

Table 7.3

Overview of grazing behavior, herbage DMI and milk yield responses during different grazing experiments .

	Chapter 3									Chapter 5						Chapter 6					
		Day within 4D					Period				Period within 1D			Period					Period		
	4D	d1	d2	d3	d4	1D	1	2	1D	18-6h	6-18h	2D	1	2	MA	AA	1	2	3
SSH[1], cm	14.3	19.4	14.8	12.5	10.5	19.7	19.7	14.3	15.5	15.5	11.8	15.6	17.4	13.7	17.6	16.9	16.4	18.0	17.3
Grazing time min/d	559	549	546	574	568	557	564	553	545	246[2]	296[2]	549	542	570	517	525	523	555	487
G+R[3], %	70.8	71.3	71.7	70.8	70.4	71.7	72.4	70.3	63.5	63.1	59.6	64.6	64.8	63.3	65.0	65.0	65.8	67.6	61.1
Bite rate, /min	65	63	65	67	67	64	65	64	66	-	-	66	64	69	62	58	59	59	61
BM calc[4], mg/bite	453	-	-	-	-	485	505	432	398	-	-	429	484	356	525	509	531	493	527
BM evac[5], mg/bite	-	-	-	-	-	-	-	-	641	636	646	609	642	608			-	-	-
IR (g/min)	29.4	-	-	-	-	31.0	32.8	27.6	26.3	-	-	28.3	31.0	24.6	32.6	29.5	31.3	29.1	32.0
Chews/bolus, no.	58	55	57	60	59	57	58	57	54	54	55	57	58	53	51	55	52	54	54
Herbage DMI, kg/d	15.8	-	-	-	-	16.5	17.4	14.8	14.4	-	-	15.5	15.9	14.0	16.3	15.4	16.2	16.0	15.4
Milk yield	23.7	23.4	24.7	23.8	22.8	24.5	25.9	22.3	22.9	-	-	23.5	24.6	21.8	26.3	26.0	25.4	26.5	26.5
Fat (%)	3.76	3.92	3.69	3.70	3.75	3.66	3.71	3.72	4.00	-	-	3.87	4.02	3.85	3.65	4.04	3.78	3.81	3.93
Protein (%)	3.34	3.40	3.33	3.33	3.29	3.28	3.34	3.28	3.32	-	-	3.28	3.25	3.35	3.22	3.29	3.11	3.22	3.41
Urea (mg/dl)	23.4	26.7	24.4	21.9	20.7	23.2	22.6	24.1	37.9	-	-	38.8	46.9	29.8	31.3	29.9	29.0	31.7	31.1
FPCM[6], kg/d	22.8	23.0	23.6	22.8	21.8	23.5	24.9	21.4	22.8	-	-	23.5	24.5	21.0	24.8	25.6	24.3	25.4	25.8

1 Sward surface height at allocation.
2 Grazing time during half a day.
3 G+R is the sum of grazing time and ruminating time.
4 Bite mass calculated from herbage DMI and the number of bites.
5 Bite mass estimated from rumen evacuations as described in Chapter 5.
6 Fat- and protein corrected milk.

This leads to the conclusion that more frequent allocation results in higher herbage intake, if SSH is not restricting herbage intake.

Looking at the underlying effects of grazing behavior on herbage DMI, some general trends can be observed. The increase in herbage DMI with increased allocation frequency in both experiments described in Chapter 3 and 5 was mainly caused by an increase in bite mass

(Table 3), since grazing time (GT) and bite rate (BR) were not or were to lesser extent affected. The methodology of bite mass determinations differs between the calculation based on herbage DMI and the number of bites vs. the estimation based on the rumen evacuations and grazing recordings. Not only the methodology is different, also timing of the measurement differed. While the calculated BM was calculated from data throughout the day, the BM estimated using the rumen evacuations was carried out directly after milking, when cows were allocated to the plots. Although the methodology of determination of BM differs, it is clear that at the start of grazing a new plot in both treatments, BM is larger than when averaged over the day. It was however not anticipated that BM would be higher when allocating at 0600 h (646 mg/bite) than when allocating to a new plot at 1800 h (636 mg/bite), nor was it anticipated that BM would be larger in 1D than in 2D. The decrease within the day in 1D is not in line with the general decreasing trend of BM with decreasing GH (Gibb, 2006), a trend that was also observed on data of calculated BM (Figure 7.6).

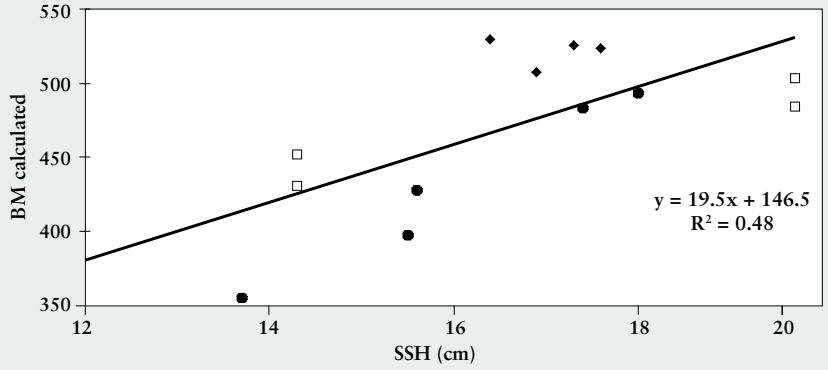


Figure 7.6

The relation between sward surface height (SSH) and bite mass (BM) calculated from herbage DMI and the number of bites in Chapter 3 (□), 5 (◆) and 6 (●).

When looking closer to the shift in grazing behavior between days in 4D, it becomes clear that with a decrease in SSH, grazing time increases at the expense of ruminating time, while bite rate increases (Chapter 3), in contrast to earlier findings (Gibb, 2006) and the general trend over the different treatment means given in Table 3.

Although the general trend of increasing BM with a decrease in BR (Figure 7.7) is also in line wit earlier finding (Gibb, 2006), it is clear that not all experiments show the same trend. The data from Chapter 6 are not in line with the apparent trend in both Figure 7.6 and Figure 7.7. The same applies to the resulting relation between SSH and IR (data not shown).

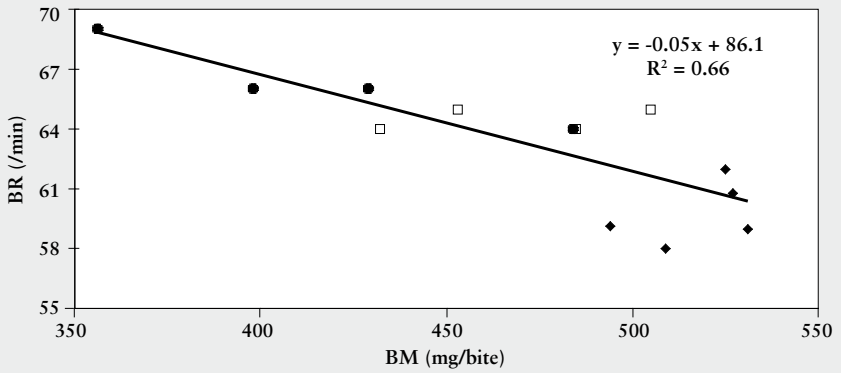


Figure 7.7

The relation between bite mass (BM) and bite rate (BR) in Chapter 3 (□), 5 (◆) and 6 (●).

Possible effects on the number of chews per bolus remain unclear. Although it increases when cows decrease their ruminating time due to increased grazing time between d1 and d4 in 4D and between period 2 and 1 in Chapter 5, no such observations are found in Chapter 6 or between periods in Chapter 3.
In general, it seems that the cow is able to adjust its grazing behavior to the circumstances, and generally, the relations as depicted in Gibb (2006) apply. However, there are several exceptions, leading to the conclusion that cows are quite flexible in their adjustment of grazing behavior to maximize herbage DMI.

Nutrient Imbalance in Grazing Dairy Cows – Sugar Content as Solution?
Besides herbage DMI, also the nutrient balance with high concentrations of highly degradable protein in comparison with carbohydrate content and degradability in grass is a major constraint for high productivity in grazing dairy cows (e.g . Van Vuuren et al., 1993; Tas et al., 2006; Hersom, 2008). In forage-fed cows, protein and carbohydrate contents are the most imbalanced aspect of the diet (Hersom, 2008), with in ryegrass the supplied amount of N often exceeding the supplied amount of energy from carbohydrates, required for microbial protein synthesis in the rumen (Tas et al., 2006). Consequently, the degraded protein that is not used as substrate for microbial protein synthesis is deaminated. Excess ammonia (NH3) is absorbed across the rumen wall and converted into urea by the liver (Van Duinkerken et al., 2005). Urea can be excreted in the urine and the milk or it can be brought back in the rumen by N recycling, re-entering the rumen by diffusion from blood urea and diffusion from the blood into saliva that re-enters the rumen during eating and ruminating. Nitrogen re-entering the rumen can be incorporated into microbial protein if energy is available, otherwise N will largely be lost for the animal via urea in the urine (Miller et al., 2001). Van Vuuren et al. (1991) found degradation rates of 9 to 14%/h for N and 7%/h for OM in ryegrass. An increase in the concentration of quickly fermentable carbohydrates like sugars in grass-fed cows (Gillilland et al., 2002) can improve the balance between carbohydrates and protein by increasing the digestibility of total carbohydrates, as sugars are a readily available source of energy for rumen

microbes (Boudon et al., 2002).
Different experiments investigating the effect of sugar content in grass on productivity have been carried out in the past. Miller et al. (2001) found in a zero grazing experiment that less N was excreted in the urine (25 vs. 35%) for cows fed fresh grass with an elevated sugar content than cows fed fresh grass with a low sugar content (165 vs. 126 g/kg DM), while NDF also differed between treatments (544 vs. 589 g/kg DM, respectively). Grass with high sugar content had a more efficient use of feed N for milk production (30 vs. 23 %) and milk yield and milk protein yield were higher on the grass with high sugar content. However, in a zero-grazing study with the same two grass varieties as in Miller et al. (2001) but in early rather than late lactating cows, Moorby et al. (2006) reported no increase in milk yield and milk composition, although there was a significantly lower proportion of dietary N in urine for high sugar (20%) than for low sugar (27%) cultivar. Similarly, Taweel et al. (2005) did not find improved productivity in dairy cows in a stall-feeding experiment, possibly as differences in sugar content between varieties were relatively small and restricted to maximally 31 g/kg DM. In a grazing experiment, Tas et al. (2006) found inconsistent effects of high-sugar varieties of perennial ryegrass on herbage DMI and milk yield between years in a two-year experiment. In an experiment of Lee et al. (2002), herbage DMI was significantly increased in steers stall-fed high sugar than low sugar content (243 vs. 161 g/kg DM) pasture, although the herbage DMI response coincided with an elevation in grass DM concentration.
Besides providing high-sugar varieties, it is also possible to utilize the variation in chemical composition of grass to reduce the imbalance in nutrients. As already described in Chapter 5 and 6, the sugar content of grass depends on the balance between synthesis due to radiation on the one hand, and growth and maintenance (during respiration) utilizing sugars to grow new shoots and hence regain photosynthetic capacity on the other hand (Fulkerson and Donaghy, 2001). Sugars are produced during photosynthesis in the leaves if the intensity of solar radiation, influenced by time of day and cloud cover, is high enough. This results in higher sugar contents in grass in the afternoon than in the morning (Van Vuuren et al., 1986; Delagarde et al., 2000; Orr et al., 2001). Sugars are stored in the stem and pseudostem, and utilized during respiration occurring mainly during the night. Consequently, substantial amounts of sugars are transported down the plant during the day (Fulkerson and Donaghy, 2001). This generally results in increasing sugar contents from the bottom to the top of the plant in the evening, while in the morning sugar contents are highest in the stems of grass, where sugars are stored (Delagarde et al., 2000; Smit and Elgersma, 2004).
Sugar content in grass is also influenced by removal of grass due to grazing, since sugars are produced in the top layer of the sward, where most radiation is intercepted (Delagarde et al., 2000; Smit and Elgersma, 2004). As grass is defoliated with a gradual decrease in grass height, and sugars show the highest variability in the top layer of the sward (Delagarde et al., 2000), one would expect sugar content to be highest when cows are allocated to a fresh plot after evening milking. The effect of transportation of sugars to the lower parts of the plant however showed to be of greater importance, as sugar content at 1600 h in MA was higher than in AA (Chapter 6) and sugar content at 0600 h was numerically higher in 2D than in 1D (Chapter 5).
Optimizing grazing behavior and the chemical composition of grass to achieve an increase in sugar intake has been one of the aims in the experiments described in Chapter 5 and 6. In general, cows tend to have patterns of peak grazing activity during the day, and the major grazing event and highest intake occurs around dusk (Rook and Huckle, 1997; Taweel et al., 2004). In view of the larger sugar content in grass during the evening, provision of fresh grass allowance following afternoon milking rather than morning milking was thought to increase

intake of sugar, not having realized the effect of transportation of sugars as described above. Both in the experiment described in Chapter 6, as well as in Orr et al. (2001) and in beef heifers in an experiment by Gregorini et al. (2006), cows offered a fresh plot of grass after the afternoon milking showed similar herbage DMI compared to allocation after morning milking However, milk yield was numerically increased by 5% in the afternoon allocation group in Orr et al. (2001) and milk fat content was increased in the experiment by Orr et al. (2001), as described by Gibb (2006) and in Chapter 6. Cows receiving their allocation in the afternoon had a longer evening meal in Orr et al. (2001), Gregorini et al. (2006) and Chapter 6 compared with those receiving their allocation in the morning. In conclusion however, the treatment aiming at increasing herbage DMI and milk production in Chapter 6 did not result in these positive effects, showing that opportunities to increase production by utilizing sugar content in grass is limited in stripgrazing management systems discussed in this thesis. This might be related with SSH as sugars are transported down the sward after they are produced, implying that allocation in the evening could result in increased productivity when SSH is lower.

Implications for the Dairy Farmer

One of the most important findings in this thesis is the beneficial effect of frequent reallocation on herbage DMI, when SSH is large enough. At SSH below some 15 cm, no effects were found of more frequent allocation, whereas above some 17 cm it had clear effects on herbage DMI and milk production. When attempting to reduce feed costs, frequent allocation of cows to a fresh plot might thus help to optimize grass intake and thereby increase milk yield. From a cost-benefit point of view, this might be interesting, especially if labor on the dairy farm is not limiting. Since fencing is a substantial capital cost also, smart fencing systems could be used. These include the use of division fences that can easily be moved, using so-called pigtail posts and polywire fences. Also, the use of tumble-wheel fences is a simple tool to easily reallocate dairy cows. Furthermore, new systems might be coming available in future facilitating intensive rotational grazing systems, including a system where the location of animals is monitored using GPS giving even the opportunity to use 'stimulatory means' to keep cows in a specified geographical area (US patent 5868100).

It seems of no use for dairy farmers in intensive stripgrazing systems to reallocate dairy cows in the evening, rather than after morning milking. In Chapter 6, it is shown that neither herbage DMI nor milk yield was increased when cows were allocated in the evening as compared to morning allocation. Milk fat content increased when cows were reallocated to a new plot in the evening, which (at least in the Dutch system of milk payments) may result in lower income per kg of milk.

With the aim of improving the quality of milk fat, there is room for the dairy farmer to increase the content of unsaturated fatty acid in milk fat with grazing as compared to stall-feeding, and also when the proportion of leaves in the grazing system is increased (Chapter 4). However, grazing management did not result in general treatment differences in milk fatty acid profile (Chapter 4, Chapter 5), implying that frequent allocation of plot to cows will not result in altered milk fatty acid composition.

Under stall-feeding conditions, increasing the amount of corn silage in the ration at the expense of grass silage resulted in a significant increase in DMI, a substantial numerical increase of 2.6 kg of milk per day, with reduced milk fat content and increased milk protein content. This coincided with lower ruminal pH and an increase in the time below pH 5.8, but did not result in a decrease in OM degradability. Increasing the amount of corn silage at the expense of grass silage is thus a tool to improve productivity of dairy cows.

REFERENCES

Abijaoudé, J. A., P. Morand-Fehr, J. Tessier, D. Sauvant, G. Béchet, and J. P. Brun. 1999. A method to record the feeding behaviour of goats. Small Ruminant Research 33(3):213-221.

Abrahamse, P.A., B. Vlaeminck, S. Tamminga, and J. Dijkstra. 2008. The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. Journal of Dairy Science 91: 4778-4792.

Agnew, R. E., R. S. Park, C. S. Mayne, and A. S. Laidlaw. 2004. Potential of near infrared spectroscopy to predict the voluntary intake of grazed grass. Animal Feed Science and Technology 115(1-2):169-178.

Allcroft, D. J., C. A. Glasbey, B. J. Tolkamp, and I. Kyriazakis. 2004. The importance of 'memory' in statistical models for animal feeding behaviour. Behavioural Processes 67(1):99-109.

Allden, W. G. and I. A. M. Whittaker. 1970. The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. Australian Journal of Agricultural Research 21(5):755-766.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83(7):1598-1624.

Azizi, O., A. Kaufmann, and L. Hasselmann. 2009. Relationship between feeding behaviour and feed intake of dairy cows depending on their parity and milk yield. Livestock Science 122:156-161.

Beauchemin, K. A., S. Zelin, D. Genner, and J. G. Buchanan-Smith. 1989. An automated system for quantification of eating and ruminating activities of dairy cattle housed in stalls. Journal of Dairy Science 72:2746-2759.

Boudon, A., J. L. Peyraud, and P. Faverdin. 2002. The release of cell contents of fresh rye-grass (*Lolium perenne* L.) during digestion in dairy cows: effect of the intracellular constituents, season and stage of maturity. Animal Feed Science and Technology 97(1-2):83-102.

Chilibroste, P., S. Tamminga, and H. Boer. 1997. Effects of length of grazing session, rumen fill and starvation time before grazing on dry-matter intake, ingestive behaviour and dry-matter rumen pool sizes of grazing lactating dairy cows. Grass and Forage Science 52(3):249-257.

Chilibroste, P., S. Tamminga, H. Boer, M. J. Gibb, and G. d. Dikken. 2000. Duration of regrowth of ryegrass (*Lolium perenne*) effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. Journal of Dairy Science 83:984-995.

Dalley, D. E., J. R. Roche, P. J. Moate, and C. Grainger. 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Australian Journal of Experimental Agriculture 41(5):593-599.

Delagarde, R., J. L. Peyraud, L. Delaby, and P. Faverdin. 2000. Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84(1-2):49-68.

Deswysen, A. G., P. Dutilleul, J. P. Godfrin, and W. C. Ellis. 1993. Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. Journal of Animal Science 71(10):2739-2747.

De Vries, T.J., M. A. G. Von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. Journal of Dairy Science 86: 3354-3361.

Dillon, P. 1993. The use of n-alkanes as markers to determine herbage intake, botanical composition of available or consumed herbage and in studies of digesta kinetics with dairy cows. Ph.D. thesis, National University of Ireland.

Dillon, P. and G. Stakelum. 1989. Herbage and dosed alkanes as a grass measurement technique for dairy cows. Irish journal of agricultural research 28(1):104.

Dove, H. and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Australian Journal of Agricultural Research 42:913-952.

Dove, H. and R. W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. Journal of Nutrition 126:13-26.

Forbes, J. M. 1995. Voluntary feed intake and diet selection in farm animals. CAB International, Wallingford, UK.

Forbes, J. M., D. A. Jackson, C. L. Johnson, P. Stockill, and B. S. Hoyle. 1986. A method for the automatic monitoring of food intake and feeding behaviour of individual cattle kept in a group. Research and Development in Agriculture 3(3):175-180.

Friggens, N. C., G. C. Emmans, I. Kyriazakis, J. D. Oldham, and M. Lewis. 1998. Feed Intake Relative to Stage of Lactation for Dairy Cows Consuming Total Mixed Diets with a High or Low Ratio of Concentrate to Forage. Journal of Dairy Science 81(8):2228-2239.

Fulkerson, W. J. and D. J. Donaghy. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. Australian Journal of Experimental Agriculture 41(2):261-275.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. Pages 141-157 in Fresh herbage for dairy cattle. A. Elgersma, J. Dijkstra and S. Tamminga, ed. Springer, The Netherlands.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Science 52(3):309-321.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science 63(4):269-287.

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

Grant, R. J. and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. Journal of Animal Science 73(9):2791-2803.

Gregorini, P., M. Eirin, R. Refi, M. Ursino, O. E. Ansin, and S. A. Gunter. 2006. Timing of herbage allocation in strip grazing: Effects on grazing pattern and performance of beef heifers. Journal of Animal Science 84(7):1943-1950.

Greter, A. M., T. J. DeVries, and M. A. G. von Keyserlingk. 2008. Nutrient intake and feeding behavior of growing dairy heifers: Effects of dietary dilution. Journal of Dairy Science 91(7):2786-2795.

Gustafsson, A. H. and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. Journal of Dairy Science 76(2):475-484.

Hersom, M. J. 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in forage-fed ruminants. Journal of Animal Science 86(Suppl. 1):E306-E317.
Holecheck, J. L., R. D. Pieper, and C. H. Herbel. 1995. Page 526 in Range Management: Principles and Practices . Prentice Hall, Englewood Cliffs, New Jersey.
Huzzey, J. M., M. A. G. von Keyserlingk, and D. M. Weary. 2005. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. Journal of Dairy Science 88(7):2454-2461.
Iason, G. R. and D. A. Elston. 2002. Groups, individuals, efficiency and validity of statistical analyses. Applied Animal Behaviour Science 75(3):261-265.
Lantinga, E. A., J. H. Neuteboom, and J. A. C. Meijs. 2004. Sward methods. Pages 23-52 in Herbage intake handbook. P.D. Penning, ed. British Grassland Society, UK.
Lee, M. R. F., L. J. Harris, J. M. Moorby, M. O. Humphreys, M. K. Theodorou, J. C. MacRae, and N. D. Scollan. 2002. Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate. Animal Science 74(3):587-596.
Mayes, R. W. and H. Dove. 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. Nutrition Research Reviews 13(1):107-138.
Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for determining of herbage intake. Journal of Agricultural Science 107:161-170.
Mayne, C. S., I. A. Wright, and G. E. J. Fisher. 2000. Grassland management under grazing and animal responses. Pages 247-291 in Grass its production and utilization. Blackwell Science, Oxford, UK.
McGilloway, D. A., A. Cushnahan, A. S. Laidlaw, C. S. Mayne, and D. J. Kilpatrick. 1999. The relationship between level of sward height reduction in a rotationally grazed sward and short-term intake rates of dairy cows. Grass and Forage Science 54:116-126.
Melin, M., H. Wiktorsson, and L. Norell. 2005. Analysis of feeding and drinking patterns of dairy cows in two cow traffic situations in automatic milking systems. Journal of Dairy Science 88(1):71-85.
Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Milk production from late-lactation dairy cows. Grass and Forage Science 56(4):383-394.
Miron, J., E. Yosef, M. Nikbachat, A. Zenou, E. Maltz, I. Halachmi, and D. Ben-Ghedalia. 2004. Feeding behavior and performance of dairy cows fed pelleted nonroughage fiber byproducts. Journal of Dairy Science 87(5):1372-1379.
Moorby, J. M., R. T. Evans, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 61:52-59.
Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56(4):352-361.
Parsons, A. J. and D. F. Chapman. 2000. The principles of pasture growth and utilisation. Pages 31-89 in Grass, its production and utilisation. A. Hopkins, ed. Blackwell Science, Oxford.
Penning, P. D. 1983. A technique to record automatically some aspects of grazing and ruminating behaviour in sheep. Grass and Forage Science 38(2):89-96.

Penning, P. D. 2004. Animal-based techniques for estimating herbage intake. Pages 53-92 in Herbage intake handbook. P. D. Penning, ed. British Grassland Society, UK.

Phillips, C. J. C. 2002. Further aspects of the use of individual animals as replicates in statistical analysis. Applied Animal Behaviour Science 75(3):265-269.

Pulido, R. G. and J. D. Leaver. 2003. Continuous and rotational grazing of dairy cows - The interactions of grazing system with level of milk yield, sward height and concentrate level. Grass and Forage Science 58(3):265-275.

Rook, A. J. 1999. The use of groups or individuals in the design of grazing experiments. Applied Animal Behaviour Science 61:357-358.

Rook, A. J. and C. A. Huckle. 1997. Activity bout criteria for grazing dairy cows. Applied Animal Behaviour Science 54(2-3):89-96.

Rutter, S. M., R. A. Champion, and P. D. Penning. 1997. An automatic system to record foraging behaviour in free-ranging ruminants. Applied Animal Behaviour Science 54(2-3):185-195.

Sibly, R. M., H. M. R. Nott, and D. J. Fletcher. 1990. Splitting behaviour into bouts. Animal Behaviour 39(1):63-69.

Smit, H. J. and A. Elgersma. 2004. Diurnial fluctuations in vertical distribution of chemical composition in a perennial ryegrass (*Lolium perenne* L.) sward during the season. Pages 21-24 in Land use systems in grassland dominated regions, Proceedings of the 20th General Meeting of the European Grassland Federation. A. Lúscher, B. Jeangros, W. Kessler, O. Huguenin, M. Lobsiger, N. Millar, and D. Suter, ed. Luzern, Switzerland

Tas, B. M., H. Z. Taweel, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Effects of perennial ryegrass cultivars on milk yield and nitrogen utilization in grazing dairy cows. Journal of Dairy Science 89(9):3494-3500.

Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behavior of dairy cows under continuous stocking. Journal of Dairy Science 87(10):3417-3427.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2005. Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Animal Feed Science and Technology 121(3-4):243-256.

Taweel, H. Z., B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga. 2006. Grazing behaviour, intake, rumen function and milk production of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates. Livestock Science 102(1-2):33-41.

Tolkamp, B. J., D. J. Allcroft, E. J. Austin, B. L. Nielsen, and I. Kyriazakis. 1998. Satiety splits feeding behaviour into bouts. Journal of Theoretical Biology 194(2):235-250.

Tolkamp, B. J., N. C. Friggens, G. C. Emmans, I. Kyriazakis, and J. D. Oldham. 2002. Meal patterns of dairy cows consuming mixed foods with a high or a low ratio of concentrate to grass silage. Animal Science 74(2):369-382.

Tolkamp, B. J. and I. Kyriazakis. 1999a. A comparison of five methods that estimate meal criteria for cattle. Animal Science 69(3):501-514.

Tolkamp, B. J. and I. Kyriazakis. 1999b. To split behaviour into bouts, log-transform the intervals. Animal Behaviour 57(4):807-817.

Tolkamp, B. J., D. P. N. Schweitzer, and I. Kyriazakis. 2000. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. Journal of Dairy Science 83(9):2057-2068.

Ungar, E. D. 1996. The Ecology and Management of Grazing Systems. Pages 185-218 in Ingestive behaviour. J. Hodgson and A. W. Illius, ed. CAB International, Wallingford, UK.

Van Duinkerken, G., G. Andre, M. C. J. Smits, G. J. Monteny, and L. B. J. Sebek. 2005. Effect of rumen-degradable protein balance and forage type on bulk milk urea concentration and emission of ammonia from dairy cow houses. Journal of Dairy Science 88(3):1099-1112.

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

Van Vuuren, A. M., C. J. Van Der Koelen, H. Valk, and H. De Visser. 1993. Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. Journal of Dairy Science 76(10):2982-2993.

Van Vuuren, A. M., C. J. Van Der Koelen, and J. Vroons- De Bruin. 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. Netherlands Journal of Agricultural Science 34:457-467.

Yeates, M. P., B. J. Tolkamp, D. J. Allcroft, and I. Kyriazakis. 2001. The use of mixed distribution models to determine bout criteria for analysis of animal behaviour. Journal of Theoretical Biology 213(3):413-425.

Zom, R. L. G., and G. Van Duinkerken. 2005. Voeropnamepatronen van melkvee. Praktijkrapport Rundvee no. 93. Animal Sciences Group, Lelystad, The Netherlands.

SUMMARY

Summary / Samenvatting

SUMMARY

An adequate feed intake is an important prerequisite to realize high milk production in dairy cows, especially during grazing. Cows ingest their feed within a day in a number of discrete meals, alternated with periods of rumination and periods of "idling". Daily intake depends on the number of meals and intake per meal, and during grazing, feed intake is the result of grazing time, bite mass and bite rate. The analysis of feed intake behaviour can assist in understanding variation in daily intake and in improving its prediction.
Grazing management results in variation in grassland characteristics including herbage mass, sward surface height (SSH) and regrowth duration. Besides increased herbage intake, proper grazing management can also result in higher intake of nutrients from herbage by improved nutritional composition of herbage. Stripgrazing, for instance, is believed to be an efficient grazing system due to a constant grass quality and quantity between days, but scientific substantiation is scarce. Furthermore, it is anticipated that besides improving herbage DMI, the concentration of sugar in grass increases the energy available for rumen microbes. Hence a higher sugar concentration is expected to increase rumen fermentation during grazing. Sugar concentration in grass is strongly influenced by physiological conditions of growth, and variation in sugar content exists not only due to photosynthesis and respiration, but also due to transportation of sugars from the leaves to the stem and pseudostem after photosynthesis. This offers opportunities to improve productivity by increased sugar intake by means of altering grazing management.
The aim of this thesis was to determine the effects of quantitative and qualitative changes in nutrient supply on feed intake, feed intake behaviour, the resulting rumen fermentation parameters and milk yield and composition in grazing and stall-fed animals. The hypotheses of this thesis were that diet composition and feed intake level strongly determine the type of nutrients supplied to the intermediary metabolism of a dairy cow, with feed intake level in turn believed to be strongly being influenced by feed intake pattern. When fed indoors, feed intake pattern changes in dependence of diet composition and also due to stage of lactation, while during grazing feed intake pattern depends on grazing management, with grass supply, plant density and grass height as determining factors. In Chapter 2, the stall-feeding experiment is presented, and in Chapters 3 to 6, various grazing experiments are described.
In Chapter 2 the effects of type and concentration of roughages and concentrates were studied in stall-fed animals. Type of roughage was grass silage or maize silage, whereas concentrate composition varied in the ratio between structural or cell wall carbohydrates and non-structural carbohydrates (mainly starch and sugars). Total mixed rations (TMR) differing in structural and non-structural carbohydrates were fed to 15 dairy cows in early and late lactation in two 5 × 5 Latin square experiments. Meal criteria, determined using the Gaussian-Gaussian-Weibull method per animal per treatment, showed an interaction between lactation stage and treatment. Differences in feed intake behaviour were more pronounced between treatments differing in type of roughage than between treatments differing in concentrate composition. This was probably related to larger differences in chemical composition and particle size between the maize silage and grass silage used than between the two concentrates that were fed. These effects were in line with a reduced DMI of the ration high in grass silage compared to the other treatments, although milk production did not differ between treatments. The number of meals was similar between treatments, but eating time was greater in the high grass silage ration (227 min/d) and lower in the high maize silage ration (177 min/d) than the other treatments. There was no effect of type of concentrate on milk composition, but the diet high in maize silage

resulted in a lower milk fat content and higher milk protein content than the diet high in grass silage. Besides, lactation stage affected short-term feed intake behaviour and DMI. The results indicate that short-term feed intake behaviour is related to DMI and therefore may be a helpful tool in optimizing DMI and milk production in high-production dairy cows.

Subsequently a series of experiments were carried out to test the effects of different grazing management systems. In Chapter 3, the allocation to a new grazing plot once every 4 days (4D) was compared with daily allocation (1Da) to a new plot using twenty Holstein cows in a randomized block design during 2 rotations. Herbage DMI differed between treatments during the first rotation when pasture mass on offer and SSH were higher than during the second rotation when herbage DMI was similar between treatments. Grazing and ruminating time was similar between treatments, but grazing time increased numerically and ruminating time decreased between days in the 4D treatment. This coincided with differences between days in the 4D treatment in rumen fermentation characteristics and milk composition. Milk yield was greater in 1Da than in 4D. Therefore, this study confirmed that increasing pasture allocation frequency from once every 4 days to every day improved milk production in grazing dairy cows, especially when offered pasture was high. Since milk fatty acid (FA) composition might influence human health, milk samples from the experiment in Chapter 3 were taken and analysed for their FA composition. The results are discussed separately in Chapter 4. Milk fat samples of eight of the 20 cows in Chapter 3 showed hardly any difference in milk FA composition between treatments. In contrast, milk FA composition was largely affected by day within the 4D treatment. Short-term variation in pasture quality occurring during the 4 days affected milk FA composition. Secretion of *de novo* synthesized and C16-fatty acids decreased linearly during the 4 days. The effect of variation induced by pasture quality during the 4 days on biohydrogenation intermediates originating from rumen fermentation and subsequently excreted in milk fat was greater than the effect on its major precursor in grass, 18:3*n*-3. Thus, although increasing pasture allocation frequency from once every 4 days to every day improved milk production, allocation frequency did not affect the profiles of fatty acids in milk.

Chapter 5 presented the results of a comparison between once daily (1Db) and twice daily (2D) allocation of a new grazing plot using sixteen cows. Herbage DMI was greater in 2D than in 1Db, especially when offered herbage and SSH were high but not when SSH was low and offered herbage was still high. Grazing behaviour was more equally distributed in 1Db than in 2D. Almost no differences were found in rumen fermentation variables, milk composition and milk yield between treatments, but milk yield was increased in 2D compared to 1Db at high SSH. Milk fat concentration was higher in 1Db than 2D, but except for a lower proportion of short chain fatty acids in 1D than in 2D, milk FA composition was not changed. This study confirmed the results of Chapter 2 that increased pasture allocation frequency improved intake and milk yield in grazing dairy cows, and added that this is especially true when offered SSH was high enough.

Finally, Chapter 6 compared morning allocation (MA) with afternoon allocation (AA) of a new grazing plot in an experiment with twenty Holstein cows. Grazing behaviour did not, and herbage intake hardly differed between treatments, but cows receiving a fresh plot in the afternoon had a longer evening meal than cows receiving a fresh plot in the morning. Sugar content in AA was unexpectedly lower in the evening than in MA, probably because of transportation of sugars to the lower parts of the plants in both treatments while samples of herbage were taken from herbage on offer. The combined effects of differences in grazing behaviour and chemical composition of the grass between treatments in different periods of

the day probably caused higher intake of sugars in AA, resulting in a significantly higher non-glucogenic to glucogenic volatile fatty acid ratio in the rumen in AA than MA and consequently higher milk fat content. However, milk production did not differ between treatments.

In the General Discussion, the use of the meal criterion (MC) and the methodology to determine this 'longest interval between feeding events that belongs to the same meal' was discussed extensively. Because MC influences meal frequency and the duration of a meal, it was concluded that a uniform and standardized method for estimating MC is required. From stall-feeding experiments published in literature as well as our own experiments, it was concluded that from a statistical and biological point of view, the model used in Chapter 2, containing two Gaussian and one Weibull distribution, is the best method for MC determination. Several factors influence meal frequency in dairy cows, but no firm conclusions could be drawn because of the dependency of meal frequency on MC. Since only one study described an estimation of the MC for dairy cows during grazing, using a less sophisticated model than the model described above, grazing data of the experiment described in Chapter 3 was used to estimate meal criteria for grazing dairy cows. A model including two Weibull populations of the loge-transformed inter-bout intervals fitted the data best and gave MC-estimations of between 1.5 and 2.5 minutes. However, when comparing total grazing time per day calculated from the number of meals and meal duration during grazing, with the data obtained with grazing recorders in Chapter 3, the average grazing time obtained with grazing recorders appeared to be substantially higher than when using the MC. Therefore, no MC estimation could be applied in the grazing behaviour experiments in Chapter 3, 5 and 6, but further research on MC estimations in grazing dairy cattle is necessary.

Furthermore, the effects of the different rotational grazing systems applied in Chapter 3, 5 and 6 on grazing behaviour and herbage DMI were discussed in the General Discussion. From the effects of more frequent allocation in Chapter 3 and 5 on herbage DMI and milk yield, the conclusion is drawn that SSH rather than offered herbage apparently was the first constraint on herbage intake and milk production, and more frequent allocation results in higher herbage intake only if SSH is not restricting herbage intake. The increase in herbage DMI with increased allocation frequency in both experiments described in Chapter 3 and 5 was mainly caused by an increase in bite mass, although grazing behaviour between days in 4D showed that with the decrease in SSH, grazing time increased at the expense of ruminating time, while bite rate increased (Chapter 3). In general, it seems that cows are quite flexible in their adjustment of grazing behaviour to maximize herbage DMI. Chapter 6 showed that opportunities to increase production by utilizing sugar content in grass are limited. This might be related with SSH as sugars are transported down the sward after they are produced, implying that allocation in the evening could result in increased productivity when SSH is lower.

In conclusion, frequent reallocation to a fresh grazing plot positively influences herbage DMI, provided SSH is large enough. When attempting to reduce feed costs, frequent allocation of cows to a fresh plot might thus help the farmer to optimize grass intake and thereby increase milk yield. However, effects on labour and fencing should be taken into account when investigating the economical benefits of these grazing management systems. It seems of no use for dairy farmers in intensive stripgrazing systems to reallocate dairy cows following afternoon milking, rather than after morning milking. Neither herbage DMI nor milk yield was increased when cows were allocated in the evening as compared to morning allocation. The quality of milk fat can be improved by increasing the content of unsaturated fatty acids in milk fat during grazing. This is true when the proportion of leaves in the grazing system is increased (Chapter 4), but grazing management did not result in general treatment differences in the milk fatty acid profile

(Chapter 4, Chapter 5). Under stall-feeding conditions, increasing the amount of maize silage in the ration at the expense of grass silage resulted in an increase in DMI, with reduced milk fat content and increased milk protein content. Increasing the amount of maize silage at the expense of grass silage is thus a tool to improve productivity of dairy cows.

SAMENVATTING

Een adequate voeropname is bij melkkoeien een belangrijke vereiste om hoge melkproducties te realiseren, vooral indien beweiding wordt toegepast. Koeien nemen hun voer binnen een dag op in een aantal discrete maaltijden, afgewisseld met periodes van herkauwen en perioden waarin de koeien rusten, ofwel geen voeropnamegedrag vertonen. De dagelijkse voeropname hangt af van het aantal maaltijden en de opgenomen hoeveelheid voer per maaltijd. Tijdens beweiding is voeropname het resultaat van graastijd, hapgrootte en hapsnelheid. De analyse van voeropnamegedrag kan behulpzaam zijn bij het begrijpen van variatie in dagelijkse voeropname en bij het voorspellen ervan.
Graslandmanagement resulteert in variatie in graslandkenmerken zoals gewasmassa, gewashoogte en hergroeiduur van het gewas. Naast een verhoogde voeropname kan goed graslandmanagement ook resulteren in verhoogde opname van nutriënten uit gras door verbeterde samenstelling van het gewas. Van stripgrazen wordt bijvoorbeeld aangenomen dat het een efficiënt beweidingssysteem is door een constante graskwaliteit en -kwantiteit tussen dagen. Wetenschappelijk bewijs hiervoor is echter schaars. Ook wordt aangenomen dat de hoeveelheid suiker in gras de grasopname en de hoeveelheid energie die beschikbaar komt voor microben in de pens verhoogt. Zodoende wordt aangenomen dat een verhoogd suikergehalte in gras de pensfermentatie tijdens beweiding verhoogt. Het suikergehalte in gras wordt sterk beïnvloed door de fysiologische condities tijdens groei. Variatie in het suikergehalte van gras ontstaat niet alleen door fotosynthese en respiratie, maar ook door transport van suiker van het blad naar de stengel en de schijnstengel na fotosynthese. Dit biedt mogelijkheden om de productiviteit van melkvee te verhogen door verhoogde suikeropname, bijvoorbeeld door beweidingsmanagement.
Het doel van dit proefschrift was de effecten van kwantitatieve en kwalitatieve veranderingen in het aanbod van nutriënten te bepalen op voeropname, voeropnamegedrag, pensfermentatie en melkproductie en -samenstelling bij grazende en stalgevoerde koeien. De hypotheses hierbij waren dat de rantsoensamenstelling en het voeropnameniveau het type nutriënten dat beschikbaar komt voor het intermediaire stofwisselingssysteem van de koe in sterke mate beïnvloeden, waarbij de voeropname zelf sterk beïnvloed wordt door het voeropnamepatroon. Bij stalgevoerde dieren verandert het voeropnamepatroon afhankelijk van de rantsoensamenstelling en het lactatiestadium, terwijl tijdens beweiding het voeropnamepatroon afhankelijk is van graslandmanagement met gewasaanbod, plantdichtheid en gewashoogte als bepalende factoren. In Hoofdstuk 2 zijn de resultaten van de stalproeven weergegeven, terwijl in Hoofdstuk 3 tot en met 6 de beweidingsproeven beschreven zijn.
In Hoofdstuk 2 zijn de effecten van het type en het aandeel ruwvoer en krachtvoer bestudeerd in stalgevoerde dieren. Het type ruwvoer was grassilage of maissilage, terwijl de krachtvoersamenstelling varieerde in het aandeel structurele ofwel celwand koolhydraten ten opzichte van niet-structurele koolhydraten (voornamelijk zetmeel en suikers). Totaal gemengde rantsoenen (TMR) die verschilden in structurele en niet-structurele koolhydraten werden aan 15 koeien gevoerd in vroege en late lactatie in twee experimenten die uitgevoerd werden als 5 × 5 Latijns vierkant. Maaltijdcriteria (MC), gedefinieerd als het langste interval tussen vreetmomenten die nog tot dezelfde maaltijd behoren, werden bepaald met de Gaussian-Gaussian-Weibull methode, een combinatie van twee normale en één Weibull distributie, per dier per behandeling. De MC's lieten een interactie zien tussen lactatiestadium en behandeling. Verschillen in voeropnamegedrag waren duidelijker tussen behandelingen die verschilden in ruwvoertype dan tussen behandelingen die verschilden in krachtvoertype. Dit was waarschijnlijk

gerelateerd aan grotere verschillen in de chemische samenstelling en deeltjesgrootte tussen maissilage en grassilage dan tussen de verschillende krachtvoeders. Deze resultaten waren in lijn met een verlaagde drogestof (DS) opname van het TMR hoog in graskuil ten opzichte van de andere behandelingen, alhoewel melkproductie niet verschilde tussen de behandelingen. Het aantal maaltijden was gelijk tussen de behandelingen, maar de vreettijd was langer in het rantsoen met veel grassilage (227 min/d) en korter in het rantsoen met veel maissilage (177 min/d) dan bij de andere behandelingen. Er was geen effect van het type krachtvoer op melksamenstelling, maar het rantsoen met veel maissilage resulteerde in een lager melkvetgehalte en een hoger melkeiwitgehalte dan het rantsoen met veel grassilage. Daarnaast had het lactatiestadium een effect op voeropnamegedrag en voeropname. De resultaten geven aan dat voeropnamegedrag gerelateerd is aan DS-opname en daardoor behulpzaam kan zijn in de optimalisatie van DS-opname en melkproductie in hoogproductief melkvee.

Vervolgens is een serie experimenten uitgevoerd om de effecten van beweidingssystemen te onderzoeken. In Hoofdstuk 3 is dagelijks omweiden (1Da) vergeleken met elke 4 dagen omweiden (4D) in een proef die volgens geward blokkenschma uitgevoerd werd met 20 Holstein koeien gedurende twee rotaties. Grasopname verschilde tussen beide behandelingen tijdens de eerste rotatie, toen het gewasaanbod en de gewashoogte hoger waren dan tijdens de tweede rotatie. Tijdens de tweede rotatie was de DS-opname gelijk tussen beide behandelingen. Graas- en herkauwtijd was gelijk tussen de twee behandelingen, maar de graastijd nam numeriek toe en de herkauwtijd daalde met het toenemen van het aantal dagen in de 4D behandeling. Dit ging gelijk op met verschillen tussen dagen in 4D in pensfermentatie kenmerken en melksamenstelling. De melkproductie was hoger in 1Da dan in 4D. De studie bevestigde dat vaker omweiden tussen 4D en 1Da de melkproductie verhoogde in grazende koeien, in het bijzonder wanneer het gewasaanbod voldoende hoog was. Omdat de vetzuur samenstelling van melkvet de humane gezondheid kan beïnvloeden, werden melkmonsters genomen tijdens het experiment in Hoofdstuk 3 en geanalyseerd op melkvetsamenstelling. De resultaten zijn beschreven in Hoofdstuk 4. Monsters melkvet van acht van de 20 koeien in Hoofdstuk 3 vertoonden nauwelijks verschillen in het melkvetzuur patroon tussen behandelingen. De melkvetzuur samenstelling varieerde echter sterk tussen de 4 dagen in 4D, wat werd toegeschreven aan de variatie in gewaskwaliteit tijdens deze 4 dagen. Uitscheiding van *de novo* gesynthetiseerde vetzuren en C16 vetzuren vertoonden een lineaire daling gedurende de 4 dagen. Het effect van variatie in gewaskwaliteit gedurende de 4 dagen op biohydrogenatie intermediairen afkomstig van pensfermentatie en de uitscheiding ervan via melkvet was groter dan het effect op de belangrijkste bron in gras, linoleenzuur (18:3*n*-3). Zodoende bleek dat, hoewel vaker omweiden tussen 4D en 1Da de melkproductie verhoogde, dit geen effect had op de melkvetzuur-samenstelling.

In Hoofdstuk 5 zijn de resultaten weergegeven van een vergelijking tussen dagelijks (1Db) en tweemaal dagelijks (2D) omweiden met 16 Holstein koeien. Gewasopname was hoger in 2D dan in 1Db, in het bijzonder wanneer zowel gewasaanbod als gewashoogte hoog waren, maar niet wanneer gewashoogte laag en gewasaanbod hoog was. Graasgedrag was meer evenredig verdeeld in 1Db dan in 2D. Er werden geen verschillen in pensfermentatie kenmerken, melksamenstelling en melkhoeveelheid tussen de behandelingen gevonden, maar melkproductie was verhoogd in 2D ten opzichte van 1Db wanneer het gewas hoog was. Het vetgehalte in melk was hoger in 1Db dan in 2D, maar melkvetsamenstelling was gelijk tussen behandelingen. De uitzondering daarop was een lager aandeel kortketen vetzuren in 1Db dan in 2D. Deze studie bevestigde de resultaten uit Hoofdstuk 2 dat vaker omweiden van koeien de opname en melkproductie van koeien tijdens beweiding verhoogt, en voegde daaraan toe dat dit vooral

het geval is wanneer de gewashoogte voldoende is.
Tenslotte werd in Hoofdstuk 6 inscharen tijdens ochtend (MA) of middag (AA) in een nieuw perceel onderzocht met 20 Holstein koeien. Het graasgedrag verschilde niet, en de gewasopname nauwelijks tussen beide behandelingen, maar koeien die 's middags ingeschaard werden hadden een langere avondmaaltijd dan koeien die 's ochtends ingeschaard werden. Het suikergehalte in AA was 's middags, tegen de verwachting in, lager dan in MA, waarschijnlijk door transport van suikers naar de lagere delen van de plant in beide behandelingen, terwijl gewasmonsters genomen werden van het volledige aangeboden gewas. De gecombineerde effecten van verschillen in graasgedrag en chemische samenstelling van het gras tussen behandelingen in verschillende periodes van de dag zorgden waarschijnlijk voor een hogere opname van suikers in AA. Dit resulteerde in een significant hogere niet-glucogene tot glucogene vluchtige vetzuurverhouding in de pens en een hoger melkvetgehalte in AA dan in MA. De melkproductie was echter gelijk tussen beide behandelingen.
In de Algemene Discussie (Hoofdstuk 7) zijn het gebruik van het maaltijdcriterium en de methode om dit 'langste interval tussen voeropname momenten dat tot dezelfde maaltijd behoren' te bepalen uitvoerig bediscussieerd. Omdat het MC de maaltijdfrequentie beïnvloedt evenals de maaltijdduur, was de conclusie dat een uniforme en gestandaardiseerde methode voor de bepaling van het MC noodzakelijk is. Uit in de literatuur gepubliceerde stalexperimenten en onze eigen experimenten, werd geconcludeerd dat uit statistisch en biologisch standpunt het model beschreven in Hoofdstuk 2 met twee Gaussian- en één Weibull-verdeling de beste methode voor MC bepaling is. Verschillende factoren beïnvloeden de maaltijdfrequentie bij melkkoeien, maar er kan geen duidelijke conclusie getrokken worden vanwege de afhankelijkheid van maaltijdfrequentie van het MC. Doordat slechts één studie het MC bij grazende koeien beschreven heeft, met een minder ver ontwikkeld model dan het model dat hierboven beschreven werd, zijn de graasgedrag gegevens uit Hoofdstuk 3 gebruikt om het MC voor grazende koeien te schatten. Een model met twee Weibull populaties beschreef de loge-getransformeerde tussenvreetintervallen het beste en gaf MC-schattingen tussen 1.5 en 2.5 minuten. Wanneer echter de totale graastijd per dag berekend werd uit het aantal maaltijden en de maaltijdduur, en vergeleken werd met de gegevens uit de graasrecorders in Hoofdstuk 3, bleek de gemiddelde graastijd bepaald met behulp van de graasrecorders substantieel langer te zijn dan wanneer het MC gebruikt werd. Daarom is het MC niet geschat en gebruikt in de graasexperimenten in Hoofdstuk 3, 5 en 6. Vervolgonderzoek naar MC schattingen bij grazende melkkoeien is noodzakelijk.
In de Algemene Discussie zijn ook de effecten beschreven van verschillende beweidingssystemen op graasgedrag en grasopname, zoals getest in Hoofdstuk 3, 5 en 6. De gevonden effecten van vaker omweiden in Hoofdstuk 3 en 5 op grasopname en melkproductie, leidden tot de conclusie dat de gewashoogte een sterkere invloed heeft op grasopname en melkproductie dan het gewasaanbod. Ook werd geconcludeerd dat vaker omweiden resulteert in een hogere grasopname als de gewashoogte niet beperkend is. De stijging in grasopname bij verhoogde frequentie van omweiden in beide experimenten in Hoofdstuk 3 en 5 zijn voornamelijk veroorzaakt door een verhoogde hapgrootte. Een uitzondering daarop was het graasgedrag tussen dagen bij 4D, dat liet zien dat bij een verlaging van de gewashoogte de graastijd toenam ten koste van de herkauwtijd, terwijl de hapsnelheid eveneens toenam (Hoofdstuk 3).
In het algemeen lijken koeien redelijk flexibel in de aanpassing van hun graasgedrag om zodoende de grasopname te maximaliseren. Hoofdstuk 6 liet zien dat de mogelijkheden om de productiviteit van melkvee te verhogen via het suikergehalte in gras beperkt zijn. Dit is mogelijk gerelateerd aan de gewashoogte, omdat suikers na productie in het blad van gras naar

beneden getransporteerd worden. Dit impliceert dat inscharen na avondmelking kan resulteren in verhoogde productiviteit indien de gewashoogte lager is.
De conclusies van het onderzoek beschreven in dit proefschrift zijn dat vaker omweiden naar een nieuw stuk gras de grasopname positief beïnvloeden, mits de gewashoogte voldoende is. Vaker omweiden kan de boer dus helpen de gewasopname te optimaliseren, waardoor de melkproductie stijgt en de voerkosten verlaagd worden. De effecten op arbeidskosten en kosten voor omheining moeten uiteraard meegenomen worden bij het onderzoek naar economische voordelen van deze beweidings strategieën. Het lijkt weinig zin te hebben voor melkveehouders om koeien na avondmelken in te scharen in een nieuw perceel ten opzichte van inscharen na de ochtendmelking. Grasopname en melkproductie waren niet verhoogd wanneer 's avonds ingeschaard werd ten opzichte van 's ochtends. De kwaliteit van melkvet kan verhoogd worden door het hogere aandeel onverzadigde vetzuren in melkvet tijdens beweiding. Dit is het geval wanneer het opgenomen aandeel blad in het beweidingssysteem verhoogd is (Hoofdstuk 4), maar het beweidings management had geen effecten op het vetzuur-profiel van melkvet (Hoofdstuk 4 en 5). Bij stalgevoerde dieren kan het aandeel maissilage in het rantsoen ten opzichte van het aandeel grassilage resulteren in een verhoging van de DS-opname, met een verminderd melkvetgehalte en een verhoogd melkeiwitgehalte. Het verhogen van het aandeel maissilage ten koste van grassilage is daarom een mogelijkheid de productiviteit van melkkoeien te verhogen.

Curriculum Vitae

Curriculum Vitae

ABOUT THE AUTHOR

Pieter Alexander (Sander) Abrahamse werd op 17 november 1977 geboren te Middelburg. Hij groeide op op de boerderij van zijn ouders, Molenperk, te Serooskerke. In 1996 behaalde hij zijn VWO diploma aan de Christelijke Scholengemeenschap te Middelburg. In september 1996 begon hij aan de studie Biologie aan Wageningen Universiteit te Wageningen. Na een jaar wisselde hij van studie en vervolgde hij zijn opleiding aan de Hogere Agrarische School te Den Bosch. De studie Dier- en Veehouderij werd binnen 3.5 jaar afgerond. De daaropvolgende MSc-opleiding Animal Nutrition aan Wageningen Universiteit werd in 2002 afgerond met een afstudeervak voor Provimi BV bij het Grasslands Research Centre van AgResearch Ltd. in Palmerston North, Nieuw-Zeeland. In September 2003, na een korte periode als onderzoeksassistent bij Provimi BV, werd hij aangesteld als assistent in opleiding (AIO) bij de leerstoelgroep Diervoeding van Wageningen Universiteit. Per oktober 2006 werd hij part-time aangesteld bij Provimi BV als productmanager Rundvee, waarnaast hij zijn promotieonderzoek aan de leerstoelgroep Diervoeding afrondde.

Pieter Alexander (Sander) Abrahamse was born on 17 November 1977 in Middelburg. He grew up on his parents' farm, Molenperk, in Serooskerke. In 1996 he graduated from the Christelijke Scholengemeenschap Walcheren in Middelburg, after which in September 1996 he started a MSc in Biology at Wageningen University, Wageningen. After one year, he continued his education at the Hogere Agrarische School in Den Bosch. He obtained his BSc in Animal Husbandry within 3.5 years. The subsequent MSc in Animal Nutrition at Wageningen University was finalized in 2002 with a thesis for Provimi BV in the Grasslands Research Centre of AgResearch Ltd. in Palmerston North, New Zealand. In September 2003, after a short period as a research assistant at Provimi BV, he started his PhD at the Animal Nutrition Group of Wageningen University. As from October 2006 onward, he was appointed as product manager Ruminants, part-time, while finalizing his PhD at the Animal Nutrition Group.

PUBLICATIONS

Refereed Scientific Publications

Kool, D. M., E. Hoffland, P. A. Abrahamse and J. W. van Groenigen. 2006. What artificial urine composition is adequate for simulating soil N2O fluxes and mineral N dynamics? Soil Biology and Biochemistry 38:1757-1763.

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2007. Frequent reallocation of strip grazing cows improves productivity. Journal of Animal Science 85 (suppl 1):636.

Abrahamse, P. A., B. Vlaeminck, S. Tamminga and J. Dijkstra. 2008. The effect of silage and concentrate type on intake behavior, rumen function, and milk production in dairy cows in early and late lactation. Journal of Dairy Science 91:4778-4792.

Dijkstra, J., E. Kebreab, A. Bannink, L. A Crompton, S. López, P. A. Abrahamse, P. Chilibroste, J. A. N. Mills and J. France. 2008. Comparison of energy evaluation systems and a mechanistic model for milk production by dairy cattle offered fresh grass-based diets. Animal Feed Science and Technology 143:203-219.

Abrahamse, P. A., S. Tamminga and J. Dijkstra. 2009. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. Journal of Agricultural Science, doi: 10.1017/S0021859609990153, published online by Cambridge University Press 6 August 2009.

Contributions to Conferences and Symposia

Abrahamse, P. A., D. Reynaud, J. Dijkstra and S. Tamminga, S. 2005. The meal criterion estimated in grazing dairy cattle: evaluation of different methods. Page 198 in Utilisation of Grazed Grass in Temperate Animal Systems. J. Murphy, ed. Wageningen Academic Publishers, Wageningen.

Abrahamse, P. A., D. Reynaud, J. Dijkstra and S. Tamminga, S. 2005. The meal criterion estimated in grazing dairy cattle - evaluation of different methods. Page 53-54 in Proceedings 30ste Nederlandstalige Voedingsonderzoekers Dag (NVO Meeting), Ghent, Belgium.

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2006. The possibilities of rotational grazing systems: effects on grazing behaviour, rumen characteristics and milk production in dairy cattle. Page 8-9 in Proceedings 31ste Nederlandstalige Voedingsonderzoekers Dag (NVO Meeting), Rotterdam, The Netherlands.

Dijkstra, J., E. Kebreab, E., A. Bannink, A., L. A. Crompton, L.A., S. Lopez, S., P. A. Abrahamse, P. Chilibroste, J. A. N. Mills and J. France, J. 2006. Supply and requirement of nutrients predicted by feed evaluation systems and a mechanistic model for dairy cattle offered grass-based diets. Canadian Journal of Animal Science 86 (4):569.

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Animal Science 85 (suppl 1) / Journal of Dairy Science 91 (suppl 1):2033-2045.

Abrahamse, P. A., J. Dijkstra, J. and S. Tamminga, S. 2007. Effects of morning versus evening reallocation on grazing behavior, rumen fermentation and milk production in strip grazing cows. Page 576 in Proceedings of the 7th International Symposium on the Nutrition of Herbivores: Herbivore Nutrition for the Development of Efficient, Safe and Sustainable Livestock Production. Q. X. Meng, L. P. Ren and Z. J. Cao, ed. Beijing: China Agricultural University Press.

Jonker, A., P. A. Abrahamse, J. Dijkstra and S. Tamminga. 2007. Effects of reallocating dairy cows once or twice daily to a fresh paddock on grass characteristics and milk production. In Proceedings 32ste Nederlandstalige Voedingsonderzoekers Dag (NVO Meeting), Ghent, Belgium.

Pellikaan, W. F., P. A. Abrahamse, S. M. Borgijink, L. J. G. M. Bongers, S. van Laar-Schuppen, W. Hendriks, W. 2007. In vitro fermentation and methane production kinetics of grass and sainfoin using rumen inoculum from dairy cows receiving diets differing in quality. Page 578 in Proceedings of the 7th International Symposium on the Nutrition of Herbivores: Herbivore Nutrition for the Development of Efficient, Safe and Sustainable Livestock Production. Q. X. Meng, L. P. Ren and Z. J. Cao, ed. Beijing: China Agricultural University Press.

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2008. Variatie in nutriëntenaanbod bij melkvee in relatie tot voeropname en opnamepatronen bij beweidings. In Proceedings studiedag 'Voeding van hoogproductief melkvee, een uitdaging voor nutritionisten, Nederland, Wageningen.

Dijkstra, J. Crompton, L. A., Kebreab, E. Bannink, A., Lopez, S., Abrahamse, P. A., Chilibroste, P., France, J. & Mills, J. A. N. (2008). Comparison of three requirements-based energy evaluation systems and a mechanistic model for estimating nutritional requirements for milk production from grass- based diets fed to dairy cattle. Journal of Agricultural Sciense 146: 729-730.

Vlaeminck, B., P. A. Abrahamse, V. Fievez, J. Dijkstra and S. Tamminga. 2009. The effect of allocation frequency in rotational grazing systems on the fatty acid (FA) profile in milk fat of dairy cows. Journal of Animal Science 87 (suppl 1): 92.

Reports

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2007. De invloed van dagelijks of 4-daags omweiden op voeropnamepatroon, pensfermentatie-karakteristieken en melkproductie bij melkvee. Leerstoelgroep Diervoeding, Wageningen.

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2007. De invloed van de samenstelling van het winterrantsoen op het voeropnamepatroon van melkkoeien in verschillende lactatiestadia en het effect hiervan op penskarakteristieken. Leerstoelgroep Diervoeding, Wageningen.

Abrahamse, P. A., J. Dijkstra and S. Tamminga. 2007. De invloed van 's ochtends of 's avonds inscharen op het voeropnamepatroon van melkkoeien en het effect hiervan op penskarakteristieken. Leerstoelgroep Diervoeding, Wageningen.

Abrahamse, P. A., S. Tamminga and J. Dijkstra. 2007. De invloed van eenmaal daags of tweemaal daags inscharen in een nieuw perceel op voeropnamepatroon, pensfermentatiekarakteristieken en melkproductie bij melkvee. Leerstoelgroep Diervoeding, Wageningen.

Training and supervision plan

Name	Sander Abrahamse
Group	Animal Nutrition Group
Daily supervisor	Dr. ir. J. Dijkstra
Supervisor	Prof. dr. ir. S. Tamminga

The basic package	*year*	*credits* *
WIAS Introduction course	2005	1,5
Philosophy of science and ethics	2006	1,5
International conferences		
IGC Cork 2005	2005	1,2
ASAS/ADSA San Antonio	2007	1,2
ISNH 2007	2007	1,2
Seminars and workshops		
PhD Retreat	2004	0,6
WIAS Science Day 2004, 2005, 2006 and 2007	2004-2007	1,2
NVO 2005, 2006 and 2007	2005-2007	0,9
Farewell seminar Seerp Tamminga	2005	0,2
Farewell seminar Martin Verstegen	2006	0,2
Seminar 'Perennial ryegrass for dairy cows'	2005	0,2
Seminar 'Nutritional influences on rumen development and glucose metabolism in calves'	2006	0,2
Presentations		
WIAS Science Day (oral presentation)	2005	1,0
NVO 2005 and 2006 (oral presentation)	2005	2,0
IGC Cork 2005 (poster presentation)	2005	1,0
ISNH Beijing 2007 (oral presentation)	2007	1,0
ASAS/ADSA San Antonio (oral presentation)	2007	1,0
In-depth studies		
Regulation of food intake and its implications for nutrition and obesity	2006	1,0
Advances in feed evaluation science	2006	1,5
Ecophysiology of the gastrointestinal tract	2007	1,3
Design of animal experiments	2005	1,0
Nutrient dynamics, modelling part	2005	4,0
Statutory courses		
Use of laboratory animals	2002	4
Professional skills support courses		
Course Techniques for scientific writing	2005	1,2
Course Supervising MSc thesis work	2004	1,0
WIAS midterm job assessment	2005	0,5
Scientific publishing: an introductory workshop for PhD students and young authors	2004	0,3
Scientific writing	2005	1,8
Didactic skills training		
Toegepaste Dier Biologie 2004, 2005, 2006 and 2007	2004	1,5
MSc-theses (10 major, 2 minor)	2003-2007	23,0
BSc-theses (9)	2003-2007	9,0
Inleiding dierwetenschappen (praktijkproject)	2003	0,5
Management skills training		
WIAS Science Day	2006	1,0
Promovendi Netwerk Nederland	2005	0,5
Total	69,0	

* one ECTS credit equals a study load of approximately 28 hours

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