Influence of carbohydrates on feed intake, rumen fermentation and milk performance in high-yielding dairy cows

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw en milieuwetenschappen,
op gezag van de rector magnificus,
Dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 20 oktober 1993
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

Stellingen

- De verschillen in melkproduktie tussen melkkoeien wordt in de eerste twee weken van de lactatie niet bepaald door verschillen in voeropname, maar door de capaciteit van individuele dieren om lichaamsreserves aan te spreken.
  Dit proefschrift

- De lagere voeropname in de eerste weken van de lactatie van winterrantsoenen met een laag drogestofgehalte is het gevolg van een combinatie van factoren; een grotere bulk en een verminderde beschikbaarheid van energie voor microbiële eiwitsynthese
  Dit proefschrift

- Optimale microbiële eiwitsynthese in de pens is niet alleen afhankelijk van de totale hoeveelheid koolhydraten en stikstof, maar ook van de onderlinge beschikbaarheid ervan.
  Dit proefschrift

- De hoeveelheid melkvet op basis van *de novo* synthese is afhankelijk van de produktie aan ketogene vluchtige vetzuren in de pens.
  Dit proefschrift

- De ranking van zetmeelbestendigheid op basis van *in situ* incubaties suggereert grotere verschillen in produktie van glycogene nutriënten, dan op grond van modelberekeningen met *vivo*-waarnemingen kon worden vastgesteld.
  Dit proefschrift

- Inoculanten kunnen de kwaliteit van natte graskuilen verhogen, maar als gevolg van pensfysiologisch ongewenste substraatverschuivingen leidt dit tot negatieve effecten voor de melkproduktie.
  E.J. Smith, ea., 1993. Animal Production 56: 301

- Het veevoedingsonderzoek is meer gebaat bij microbiologisch onderzoek naar de rol van micro-organismen in de verschillende compartimenten van het maagdarmkanaal, dan naar het functioneren van micro-organismen in silages.
  B. Ullrich, 1984. Thesis, Universität Hannover

- Echte doorbraken op het gebied van veevoedkundig onderzoek zijn pas te verwachten, wanneer er goede meetmethoden beschikbaar zijn voor niet steady-state condities.

- Naast de beoogde doelstelling zijn de anatomische bouw en chirurgische bereikbaarheid van organen de belangrijkste criteria, die de methode van bloedflowmeting bepalen.

- De grootste verdienste van modelmatige beschrijvingen van biologische processen is niet het uiteindelijk verkregen wiskundige model, maar het gedocumenteerde denkproces dat eraan ten grondslag ligt.

- Uit oogpunt van rijcomfort is een ongeblazen 5 liter motor te prefereren boven een 2.5 liter motor met turbo-charger met hetzelfde vermogen.

- Het Amerikaanse systeem van bewegwijzering op basis van wegnummers en indicatie van de rijrichting (noord, zuid, oost, west) is voor een vreemdeling eenvoudiger dan een systeem op basis van plaatsnamen.

- De vorming van slechts enkele zeer grote verzelfstandigde DLO-onderzoeksinstituten maakt Centraal DLO volledig overbodig.

ɜnk de Visser
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ɲce in high-yielding dairy cows
ɟeningen, 20 oktober 1993

Voorwoord

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Content

Chapter 1

Introduction

Introduction

During recent decades the average milk production of the Dutch dairy herd has increased considerably. This increase in production, yield and composition, has been realized as a result of a combination of an increased genetic potential, due to breeding and advances in nutrition. Within the area of nutrition better quality roughage was the first step towards higher milk outputs. The increase in energy density of roughage was accomplished by change-over from hay-making to ensiling, earlier cutting of grass, the use of nitrogen fertilizers and other advances in grassland husbandry (Prins and Van Burg, 1979; Salette, 1982; McDonald, 1983; Thomas and Thomas, 1985; Frame, Harkess and Talbot, 1989). In general, grassland management has improved substantially.

Despite all the progress which has been made in roughage quality, the energy and protein intake from forage cannot yet meet the increased requirements of high producing dairy cows, because feed intake remains a constraining factor in high-level dairy performance (Bines, 1976). Addition of concentrates to the basal diets is therefore unavoidable for improving of the energy density of dairy diets in an attempt to exploit the higher genetic potential for milk production. Milk yield is considered an important factor in controlling feed intake, due to its role in defining the physiological status of the animal on the one hand (Bauman and Currie, 1980; Neal, Thomas and Cobby, 1984; Bosch, 1991) and the extraction of large quantities of nutrients from the metabolic pool required for synthesis and subsequent excretion of milk (MacRay, Buttery and Beever, 1988) on the other. The nutritive requirements of the mammary gland are of such magnitude relative to total metabolism in a high yielding dairy cow, that the cow could be considered an appendage on the udder instead of the reverse (Brown, 1969).

At first, small amounts of concentrates were fed, mainly from home grown grains, e.g. wheat, oats or rye, and completed with oil cakes, e.g. linseed, coconut. However, due to developments in the food industry an increasing number of by-products of various origin became available as compound feed ingredients (De Boer, 1985). When given the opportunity to choose between available feedstuffs, adequate yardsticks are required to evaluate quality. "Quality" can be defined in various ways. Traditionally, the initial factors to be estimated are the energy and protein value of a feedstuff. Variation in energy values of the grains and oil cakes was slight (CVB, 1991). However, by-products derived from the food-industry show considerable variation in chemical composition and energy value, due mainly to the origin of the by-product as well as the processes involved (De Boer, 1985; CVB, 1991). Energy and protein values (dcp) are still the only criteria used in formulating concentrates and estimating the energy and protein values of total diets. The Net Energy system (Van Es, 1978) which estimates energy requirements for maintenance and milk production, with appropriate adjustments for tissue gain or loss and foetal

demands is generally acknowledged to be useful, but has recognized limitations in predicting animal performances. These energy and protein systems have been developed independantly and hence have little chance of coping with the complex dynamics and interrelated processes involved in the metabolism of energy and protein in the lactating animal (MacRae, Buttery and Beever, 1988). The recently introduced Dutch protein system (DVE; CVB nr7, 1991) makes an attempt to interrelate energy and protein available for microbial protein synthesis in the rumen taking into account the site of digestion of both protein and carbohydrates (Van Straalen and Tamminga, 1991). Although far from being perfect it is a first step in a systematical approach to interrelate energy and protein metabolism in dairy cow nutrition.

The most important group of energy carriers in dairy cow feeding are the carbohydrates. Dairy feedstuffs, being mainly of botanical origin, consist for more than 70% of carbohydrates. The major carbohydrates of by-product ingredients are starch and cell wall constituents. Both carbohydrates can be degraded in the rumen relatively very rapidly or more slowly.

This study will discuss in seven chapters the progress which has been made over a period of 15 years in the characterization of carbohydrates in relation to feed intake and milk performance in an attempt to describe the consequences to milk production of changes in nutrient availability from various dairy diets. The discussion will emphasize changes in the chemical composition of carbohydrates in both concentrates and roughages, their rate of degradation, their site of digestion, the balance between carbohydrates and nitrogen available for microbial protein synthesis and their influence on nutrient availability for milk production.

Chapter 2 describes the possibilities for defining iso-caloric concentrates, using by-products, varying in carbohydrate composition and dry matter content and their effect on feed intake, with emphasis on feed intake disturbances immediately after parturition.

The third chapter considers the effects of the origin of cell wall constituents (beet pulp and maize silage) on digestibility, feed intake and milk production performance. Chapter 4 deals with the effects of the diets, presented in chapter 3, on rumen fermentation and rumen degradation of the organic matter in relation to feeding level and milk composition.

Both roughage and by-product ingredients become available to dairy cows as products with a high dry matter content or as moist ensiled ingredients. The effects of dry matter content or the amount of fermentation end products in moist ensiled diets were investigated and the results are discussed in relation to feed intake and milk production (chapter 5). Chapter 6 deals subsequently with the relationship between dry matter content and the amount of fermentation end products on degradability, rumen fermentation and kinetics.

The influence of the type of carbohydrate and resulting differences in degradability on feed intake and milk performance was investigated and the results are

presented in chapter 7. The subsequent chapter (8) deals with the effects of the carbohydrate type and degradation characteristics on rumen fermentation and kinetics.
The general discussion (chapter 9), gives an integrated resumé of the results of all experiments and gives an evaluation of the topics discussed in the preceding chapters.

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Chapter 2

Utilization of by-products for dairy cow feeds

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Utilization of by-products for dairy cow feeds

H. de Visser & A. Steg

Introduction

Food for human consumption originates from plants directly, after processing, or indirectly by conversion of plant material into food of animal origin through livestock. However, only part of the plant materials produced is suitable for direct utilization. De Boer (1985) analysing food crops in the Netherlands concluded that, except for potatoes, less than 50% of dry matter produced ha^{-1} is converted into food. Therefore large quantities of by-products, varying in origin, are produced as a consequence of food production. Without alternative use these by-products would be wasted and could easily lead to environmental pollution. Fortunately, many by-products of food production have considerable value as feedstuffs and several have a long tradition as a component of the diet, especially for ruminants and pigs, thereby leading to a reduction in costs of food production and reducing feed costs in animal husbandry (De Boer, 1980). Utilizing non-traditional by-products as feedstuffs is a possibility, sometimes turning a waste into a valuable feedstuff (Steg, Smits & Spoelstra, 1985). In the Netherlands, utilization of by-products for livestock feeding is very important. The Netherlands is a small country with a considerable number of livestock. Animal density ha^{-1}, calculated in livestock units is almost four times the average of the European Economic Community (EEC). Over the last 25 years, only limited amounts of home grown feedstuffs like roughages and cereals were available to feed the steadily increasing number of pigs, poultry and dairy cattle. As a result the Netherlands now occupies a special position within the EEC as

Table 2.1 ***Available feedstuffs in EUR-9 and the Netherlands***

Product	% of total feed units (EUROSTAT, 1982)	
	EUR-9	Netherlands
Fresh and preserved forage	55	36
Cereals and pulses	24	14
Cassava	2	9
Oil seed by-products	8	12
Products of animal origin	4	7
Miscellaneous	7	22

After Steg (1983)

regards feedstuff usage (Table 2.1).
While in most EEC countries forages provide more than 45% of feedstuffs energy, Denmark and the Netherlands are exceptions with 33 and 36% respectively. Denmark, however, provides 42% of feedstuffs energy from cereals and the Netherlands 14%. When forages, cereals and cassava are considered primary products, the percentage of energy in feedstuffs originating from by-products was 19, 14 and 41% for EUR-9, the UK and the Netherlands respectively. Interest in the utilization of by-products as feedstuffs for dairy cattle feeding has increased in the EEC as a whole. By-products from brewing, distilling, starch and sugar industries and -most of all- the oil extracting industries are particularly important (De Boer, 1985). Most of the by-products are produced within the EEC except for by-products of oil extraction (De Boer, 1985). In this respect the Netherlands has a special position. As little home-grown concentrates are produced, over 90% (Steg, 1983) of the concentrates needed for supplementing grass, grass silage and maize silage in diets for dairy cows are provided by the compound feed industry. This industry has been active in selecting ingredients with lowest costs per unit of feeding value and this has resulted in concentrates with extremely high contents of by-products (Table 2.2).

Table 2.2 ***Percentage composition of British (1978) and Dutch (1980) compound feeds for dairy cattle***

	Netherlands	UK
Cereals	1	28
Cereal by-products		
- maize gluten feed	25	6
- other	3	24
Vegetable proteins	19	14
Animal proteins	-	4
Molasses/condensed mol.sol.	7	7
Beet pulp and citrus pulp	36	10
Cassava	3	-
Minerals	2	3
Miscellaneous	4	4

After Steg, Van der Honing and De Visser (1985)

The data demonstrate striking differences between Dutch and British compound feeds.
Recently, direct on-farm use of by-products has aroused interest. This applies

especially to high-moisture by-products, like ensiled pressed beet pulp, moist maize gluten feed and brewing and distilling by-products.
In the remainder of this chapter comments are made on the feeding value of dry by-products, effects on rumen fermentation, intake and milk production response. In addition attention is given to specific aspects of feeding moist instead of dry by-products to dairy cows.

Dry by-products for dairy cows

When moving away from a tradition of livestock feeding based upon well known roughage, cereals and some oil seed cakes to feed strongly based on by-products, an increasing demand for correct feed evaluation is evident, because the feeding value of by-products can vary considerably. Part of the variation in feeding value is connected with the origin of the product (which basic product was used and which production process was involved). In an attempt to characterize the feeding value aspects of by-products, classification according to the method of processing is often employed (e.g. De Boer, 1985) (Table 2.3).

Table 2.3 ***Classes of by-products***

Processing by-products (Agri-industrial by-products)	Examples
1. Milling	Middlings, bran
2. Brewing	Brewers'grains, yeast
3. Distilling	Distillers'grains, yeast
4. Starch	Gluten feed, potato pulp
5. Sugar	Beet pulp, molasses, bagasse
6. Oil extracting	Soyabean meal, coconut expeller
7. Others	Citrus pulp
8. Fishmeal	Fishmeal
9. Slaughter	Feather meal, blood meal

After De Boer (1985)

Indeed knowledge about origin and production processes can help to obtain an initial idea of feeding value. For example, the by-products of sugar and starch production from tubers, bulbs, carrots and beets are normally digested very well. By-products of oil production from sunflower seed, on the other hand, can vary strongly in cell wall constituents and hence feeding value. However, sometimes by-products of the same origin and, in principle, the same producti-

on process vary substantially in feeding value, e.g. different batches of rice bran. By-products may become available after additional mixing of several types of processing. For instance during wet milling of maize several by-products are produced, such as steep water, maize germs, bran, maize gluten meal and corn syrup. Some of these by-products are additionally mixed to maize gluten feed, so variation in the characteristics is highly probable. By-products can be further processed themselves, leading to others, for instance maize germ is processed to maize oil and maize germ meal (Boucqué and Fiems, 1987). In an attempt at further classification of the feeding value of by-products, Lonsdale (1986) grouped products on the basis of metabolizable energy (ME) together with crude protein (Table 2.4). Although this classification gives more information than that based upon production processes, prediction of feeding value for a particular batch of a by-product is still far from accurate.

Energy and protein value

The energy value of a feedstuff is a very important parameter of feeding value in general. In most energy evaluation systems the value for a particular feedstuff is calculated from its content of apparently digestible organic components (Van der Honing and Steg, 1984), determined with sheep fed at maintenance. So information is needed on chemical composition as well as digestibility. For many by-products, variation in chemical composition and digestibility is considerable, as demonstrated in various feed tables (CVB, 1986; MAFF, 1986). Fortunately, relationships between chemical composition and digestibility make it possible to explain part of the variation in the content of digestible components and hence in energy and protein value from variation in chemical characteristics, for types of feeds or sometimes for feeds of the same origin. So, correction for differences in chemical composition can lead to a more accurate prediction of feeding value. Such a type of correction may be reflected in recognizing subclasses of by-products, as is practised in various feed tables (Table 2.5).

An alternative is to apply regression equations for prediction of feeding values from chemical composition. The Dutch feed table (CVB, 1986) mentions separate equations for by-products of maize, wheat and cassava. The application of such equations for other types of by-products is hampered by lack of sufficient data on digestibility of those products in sheep.

Feeding values predicted purely from chemical analyses (mainly some determination of cell wall content) are still fairly inaccurate, with a residual coefficient of variation of 4-5% (Steg, 1981). For by-products, as for roughages, a further improvement in accuracy of prediction of digestibility and feeding value is possible by determining organic matter digestibility *in vitro* with rumen fluid or (combinations of) enzymes (Feedingstuffs Evaluation Unit, 1984; Van der Meer, personal communication). It is advocated that any procedure *in vitro* is standardized by calibration, within each run, against similar samples with known

digestibility *in vivo* (Van der Meer, 1980). Chemical analyses and in particular digestibility determination *in vitro* are laborious, a considerable drawback especially in commerce.

Table 2.4 ***Classes of by-products***

Crude protein ($g\ k^{-1}$ DM)	Metabolizable energy ($MJ\ kg^{-1}$ DM)		
	High (>12)	Medium (9-12)	Low (<9)
High (>200)	Blood meal	Fishmeal	Meat and bone meal
	Maize gluten meal	Cotton seed expeller	Sunflower seedmeal
	Soyabean meal	Brewers'yeast	
	Distillers'grain	Rape seed meal extr.	
	Maize gluten feed	Brewers'grains	
	Pot ale syrup	Palm kernel expeller	
	Whey	Grain screenings	Wheat bran
Medium (120-200)		Steamed potato feed	Rice bran
		Wheat feeds	Grape pulp
Low (<120)	Sugar beet pulp	Soyabean hulls	Olive pulp
	Citrus pulp	Molasses/CMS blends	Grape seeds
	Molasses		

After Lonsdale (1986)

Recently, advances have been made with the application of near infra-red reflectance spectroscopy (NIRS) for the prediction of chemical characteristics of feedstuffs. Van Es, Wolsink and Vedder (1987) also studied the possibility of using NIRS for prediction of digestible components in roughage and concentrate ingredients. When further research confirms the applicability of this method for direct prediction of feed value, this will certainly help in reducing inaccuracies in formulating rations based on by-products. The protein value of a feedstuff is essentially determined by the amount of amino acids absorbed from the small intestine together with the relative proportion of amino acids absorbed. For prediction of protein value, knowledge of protein content as well as its degradability in the rumen is important. It is obvious that by-products are normally more variable in protein content than primary products, but determination of crude protein via N-analyses is simple. It may be expected that the degradability of the by-product in the rumen shows considerable variation too, depending upon the production procedure. However, information on variation in degradability is

still insufficient and, in addition, there is lack of practical information on how degradabilities can be predicted. In some countries new protein evaluation systems are using degradability as a factor (ARC, 1980, 1984; Verité, Journet and Jarrige, 1979), but due to lack of information in other countries, the protein value of feedstuffs is still predicted from apparently digestible protein. For most products an accurate prediction of digestible protein content is possible via proximate analysis (CVB, 1986).

Table 2.5 ***Recognized subclasses of concentrate ingredients***

Product	No. of subclasses	Criteria	Source
Maize gluten feed	4	Crude protein, crude fibre	CVB[1]
	3	Crude protein	DLG[2]
	1		MAFF[3]
Beet pulp	4	Sugar	CVB
	1		DLG
	2	Molassed/unmolassed	MAFF
Soyabean meal solvent extract	4	Crude fibre, origin	CVB
	5	Heat treated, coated, decorticated	DLG
	1		MAFF
Palm kernel expeller	2	Crude fibre	CVB
	3	Crude fat, origin	DLG
	1		MAFF
Cassava	3	Starch	CVB
	2	Variety	DLG
	1		MAFF

[1] CVB table (1986) the Netherlands
[2] DLG table (1982) Germany
[3] MAFF (1986) UK

Rumen fermentation

In feeding the high-yielding dairy cow, optimum rumen fermentation is essential to reach optimum feed intake, utilization of energy and milk performance.

In this respect the effects on rumen fermentation of including large quantities of by-products in a ration are important. De Visser and De Groot (1980) fed increasing levels of compound feeds, containing variable proportions of by-products to rumen cannulated dairy cows, in addition to 7.5 kg dry matter of grass silage or grass hay. The compound feeds varied in cell walls and cell contents. These differences were characterized as variation in starch and sugar content (S+S) -217, 440 g kg^{-1} DM- and amounts of concentrates containing higher S+S content (Figure 2.1).

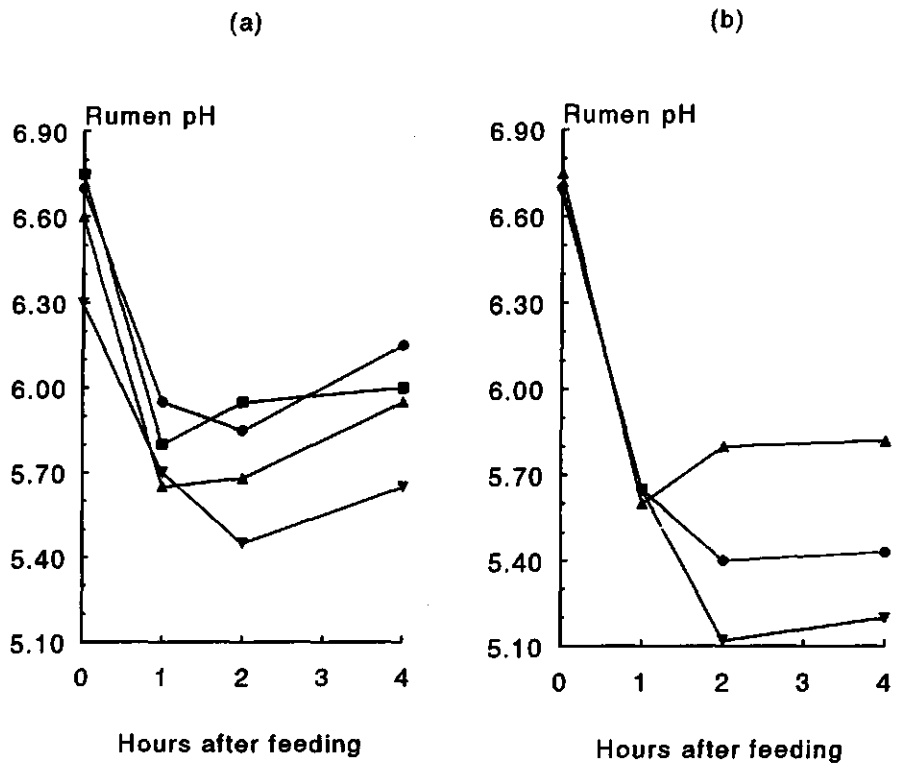

Figure 2.1 ***pH measurement in rumen fluid as affected by the quantity and composition of the concentrate mixture (a) 200 g kg^{-1} S+S; (b) 400 g kg^{-1} S+S (after De Visser and De Groot, 1980).***
▲-▲ = 10 kg conc.; ●-● = 12 kg conc.; ▼-▼ = 14 kg conc.; ■-■ = 16 kg conc.; S+S = starch and sugars

Allowance to concentrates with high levels of S+S had to be restricted, as off feed problems occurred. Therefore in Figure 2.1 no curve is given for an allowance of 16 kg of concentrates with 440 g kg^{-1} DM S+S in the dry matter. Robinson, Tamminga and Van Vuuren (1986) also found decreases in ruminal pH when feeding variable contents of starch, although the differences were smaller than those found by De Visser and De Groot (1980). This was probably due to the lower level of concentrates fed and the lower amount of starch (320 g kg^{-1} dry matter) in the compound feed compared with 440 g kg^{-1} DM in the experiment of De Visser and De Groot (1980).

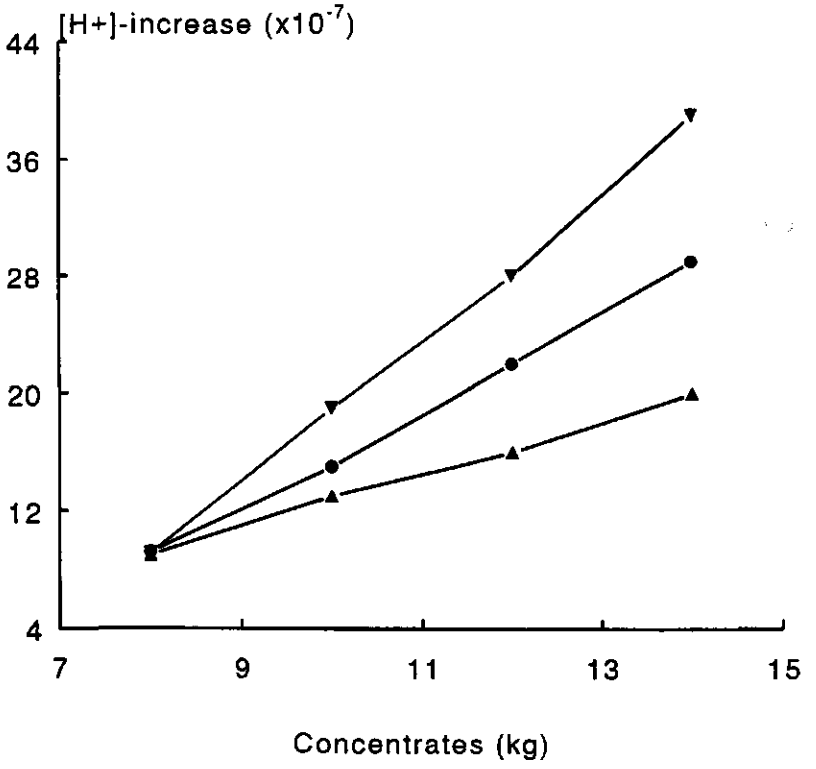

Figure 2.2 ***The effects of chemical composition and quantity of concentrates on the maximal [H^+] increase of rumen fluid (after De Visser, 1984). S+S, starch and sugars (g kg^{-1})***
▲-▲ = 150 g S+S; ●-● = 300 g S+S; ▼-▼ = 450 S+S.

In an experiment with cannulated dairy cows, De Visser (1984) fed increasing amounts of compound feeds, based on by-products, containing 150, 300 and 450 g S+S kg^{-1} DM in addition to grass hay, maize silage or grass hay + maize silage (8 kg DM per animal day^{-1}). At the lower levels (<10 kg) of concentrates no significant difference in ruminal pH was measured. Increasing the amount of compound feed showed effects on ruminal pH when higher contents of S+S were fed (Figure 2.2). At low intake levels no substantial differences appeared in volatile fatty acid ratios.
However, when large amounts of concentrates were consumed these ratios remained almost constant for rations containing low levels of S+S, while at higher levels relatively more propionic acid was produced, changing the C2:C3 ratio considerably. These results confirmed similar ones from De Visser and De Groot (1980) and Robinson, Tamminga and Van Vuuren (1986). Based on these results it was suggested that restricting the level of starch and sugar in the concentrates will affect rumen fermentation positively and thereby reduce off feed problems.
Malestein *et al.* (1982) questioned the level of S+S being the crucial factor in optimizing rumen fermentation. In their experiments different combinations of ingredients were fed, either in compounds or separately, in addition to a basal ration of low quality grass hay. Ruminal pH was affected by the level of S+S, but the magnitude depended upon the ration fed. Unfortunately, in these trials, several of the rations studied were unbalanced with regard to digestibility and protein content.The effects of reducing the starch and sugar contents of concentrates are probably due to changes in cellulolytic activity and changes in microbial population in the rumen (Giesecke *et al.*, 1976; Russell, Sharp and Baldwin, 1979; Kaufmann, Hagemeister and Dirksen, 1980; Goetsch *et al.*, 1983; Hiltner and Dehority, 1983). As many by-products have a higher ratio of cell walls to cell contents relative to primary products, the inclusion of by-products in rations for dairy cows may positively influence rumen fermentation. Protein fermentation may also be affected by by-product feeding, as the overall degradability may be influenced (Lindberg, 1981; Kristensen, Moller and Hvelplund, 1982; Tamminga, Van Diepen and Hoekstra, personal communications).

Feed intake and milk performance

Most by-products are characterized by a high ratio of cell wall constituents (neutral detergent fibre and acid detergent fibre) to cell contents (starch and sugars). Other chemical parameters can be higher or lower than the primary product, e.g. protein in soyabean meal or soya hulls; lipids in brewers'grains or soyabean meal, etc.
When a sufficient spectrum of feedstuffs is available, it is possible to compose compound feeds with similar calculated energy and protein values, but with a considerable difference in the level of cell wall constituents. A higher cell wall content in the compound feed leads, in most cases, to a lower level of easily

fermentable carbohydrates, as there is a close negative relationship between these two. Several authors have studied the effect of the fibre level in compound feeds on animal performance.

De Visser and De Groot (1980) fed compound feeds varying in S+S content (217, 308, 440, 516 g kg^{-1} DM) to dairy cows in early lactation, along with 7.5 kg DM of roughage (hay or wilted grass silage) (Table 2.6). Concentrate intake was highest when S+S content was low, leading to higher energy intakes and milk production. Milk fat content tended to be lower as S+S were increased. No effects were observed on milk protein content.

Similar effects on feed intake were reported by Thomas *et al.* (1986) comparing concentrates rich in starch (barley) with those rich in fibre (beet pulp, rice bran), 591 and 136 g S+S kg^{-1} DM respectively, along with grass silage *ad libitum*. When fed 6 or 11 kg DM concentrates day^{-1} the roughage intake was 0.9 and 1.2 kg DM higher for fibrous diets. Meijs (1985) used equal amounts of starch rich or fibrous supplements (350 and 200 g S+S kg^{-1} DM respectively) in addition to grazing fresh grass at pasture. The compound feeds supplied equal quantities of calculated energy and protein. The cows receiving the fibrous concentrates showed higher herbage intake and, as a result, milk production of these animals was higher as well as milk fat content. Milk protein content was not affected. Both the trials of Thomas *et al.* (1986) and Meijs (1985) indicated higher roughage intakes when cows are fed *ad libitum* with the more fibrous concentrates. Similar results were reported on replacing high starch concentra tes for more fibrous ones (Tremère *et al.*, 1968; Lees, Garnsworthy and Oldham, 1982; Sutton *et al.*, 1984; Thomas *et al.*, 1984).

Table 2.6 ***Feed intake and milk production when feeding variable starch and sugar (S+S) contents in the first 13 weeks of lactation***

	S + S in concentrate DM (%)			
	20	30	40	50
Number of animals	30	30	30	30
Roughage intake (kg DM day^{-1})	7.4	7.4	7.5	7.5
Concentrate intake (kg DM day^{-1})	11.2	10.4	9.3	8.3
Total intake (kg DM day^{-1})	18.6	17.7	16.8	15.8
Milk production (kg day^{-1})	32.0	31.0	30.3	27.7
Fat (g kg^{-1})	41.5	41.2	40.3	38.1
Protein (g kg^{-1})	31.8	31.9	31.9	32.0
4% fat corrected milk (kg day^{-1})	32.6	31.5	30.3	26.9

After De Visser and De Groot (1980)

Higher cellulolytic activity of microbes, due to better conditions in the rumen is probably the reason for the higher intakes (Porter *et al.*, 1973; Russell, Sharp and Baldwin, 1979; Counotte, 1981; Hiltner and Dehority, 1983). Sometimes milk production was lower when highly fibrous concentrates were fed (Thomas *et al.*, 1984; Sutton, personal communication). This was probably due more to a lower actual energy intake than was predicted from the average quality of the concentrate ingredients.

De Visser (1984) fed extremely fibrous concentrates (including 400 to 500 g kg^{-1} soyabean hulls), with between 100 and 200 g S+S kg^{-1} DM, and 7.5 kg DM of roughage (grass hay or maize silage). Compound feeds with 100 g S+S contained 468 g NDF kg^{-1} DM, with an energy value comparable to commercial compound feed in the Netherlands (7.5 MJ NE kg^{-1} DM). Feed intake was not influenced by the type of roughage or concentrate. Milk production and composition were not affected by the type of ration (Table 2.7).

Feeding of by-products can also help in supplying dairy cows with sufficient amounts of undegradable, but digestible protein, as was demonstrated by Bakker *et al.* (1979, 1980, 1981). They studied the effect of variation in degradability of protein in compound feeds on animal performance in early lactation. Differences in degradability were entirely the result of the choice of ingredients (maize gluten, brewers'grains, coconut expeller, soyabean meal, sesame expeller).

Table 2.7 ***Feed intake and milk production when feeding extremely fibrous concentrates (first 13 weeks of lactation)***

Treatment	Maize 100[1]	Maize 200	Hay 200
Number of animals	32	32	32
Roughage intake (kg DM day^{-1})	7.4	7.1	7.2
Concentrate intake (kg DM day^{-1})	12.1	11.6	11.5
Total intake (kg DM day^{-1})	19.5	18.7	18.7
Milk production (kg day^{-1})	34.5	33.5	33.2
Fat (g kg^{-1})	39.9	40.5	40.5
Protein (g kg^{-1})	30.9	31.2	30.2
4% fat corrected milk (kg day^{-1})	34.2	33.6	33.2

[1] 100 = 100 g kg^{-1} starch and sugars in the dry matter of compound feed
After De Visser (1984)

Concentrates with lower degradability increased milk volume, milk fat content and milk protein content. However, Erdman and Vandersall (1983) were unable to demonstrate a positive effect on protein degradability of concentrate supple-

ment, when feeding a low (52.9%) or a highly degradable (72.8%) concentrate to dairy cows. No effects on milk yield and milk fat content were measured.
Milk protein content was decreased with low degradable concentrates. Ørskov, Reid and McDonald (1981) fed two diets of different degradability based on fishmeal or ground nut meal. With animals in negative energy balance a larger response in fat corrected milk and milk protein content was observed than during positive energy balance.
The effect of degradability of protein in by-products on intake and milk production seems to be complicated by other factors, such as stage of lactation, energy balance and the amino acid composition of the undegraded protein.

Low *versus* high dry matter of by-product ingredients for dairy cows

In recent years there has been a tendency to use more wet by-products in rations for dairy cows. Increased costs of fossil energy has made alternatives to artificial drying economically attractive. These by-products originate from so-called wet processing, such as sugar production from sugar beets, starch production from maize, etc. Depending upon the processes, the dry matter contents of the by-products may vary between 50 and 450 g kg^{-1}. The products can be fed as such (fresh material) or after ensiling. When considering the production and feeding of wet by-products instead of predried ones, information is needed on several aspects, such as extra transport costs, losses during feeding (fresh), losses during ensiling, extra storage costs, introduction of other feeding systems, etc. Quantitatively the most important wet by-products are pressed beet pulp, maize gluten feed, brewers'grains and whole grain stillage. However, a broad range of other by-products rich in moisture can be profitably fed to dairy cows, sometimes including products recently considered to be a waste (Steg, Oostendorp and Smits, 1984). It is impossible to discuss the advantages and constraints in usage of all types of wet products. However, some general comments are appropriate, discussing similarities and differences with dry-product evaluation.
As for the dry by-products, it is very important to have information on the production processes used. Knowledge of these processes is not only important to estimate energy and protein value, but also for appropriate storage, when necessary; effects of the process on chemical composition may affect the possibilities or constraints of preservation by ensiling. In most cases additional preservation is necessary. Some by-products, such as maize gluten feed and brewers'grains are produced throughout the year, which enables them to be used 'fresh'. However, great care should be taken to restrict deterioration of material to a minimum. Deterioration will lead to appreciable losses in feeding value and may result in excessive mould growth and possibly the formation of mycotoxins. On arrival at the farm some by-products, such as maize gluten feed

and whole grain stillage, possess a lower pH due to their organic acid content, thus delaying the onset of deterioration. For other by-products, acidification via organic acids or mineral acids may be employed (e.g. spent mycelium sludge reported by Steg, Oostendorp and Smits, 1984). When using mineral acids, special attention must be given to maintain a correct acid-base equilibrium at the animal level. For many wet by-products, however, preservation by ensiling is most appropriate. Losses to be considered can be divided into those due to fermentation, seepage, and aerobic deterioration after opening. Depending upon the product characteristics, these losses could be considerable, in excess of 25% of the initial feeding value, when poor technology is provided. Therefore special attention should be given to the minimization of the losses when these products are ensiled (Cotto, 1976, 1977; Rombouts, Geraerds and Haaksma, 1984; Vandergeten and Vanstallen, 1986). A correct estimation of the energy and protein value of moist by-products depends on the variability in digestible components and the accuracy of prediction of this variation from chemical characteristics. Estimation of feeding values of the moist products is further complicated by variable losses, due to seepage, fermentation and deterioration. Some reports comparing the feeding value of moist with dry by-products mention only minor differences in energy and protein values under good preservation conditions (Murdock, Hodgson and Riley, 1981; Steg and Haaksma, 1982; Firkins, Berger and Fahey, 1985; Polan *et al.*, 1985; De Visser and Tamminga, 1987; Hindle, personal communication).

Rumen fermentation

Feeding small amounts of wet instead of dry by-products to dairy cows normally has little effect on rumen fermentation. If, however, preservation was not adequate, rumen fermentation may be negatively influenced, due to larger energy and protein losses. Feeding larger quantities of fermented material to dairy cows (roughage and ensiled by-products), may affect total rumen fermentation, due to a shortage of easily fermentable substrate (Miller, 1982; Van Soest, 1982; ARC, 1984). The results from Robinson, Tamminga and Van Vuuren (1987), comparing high levels of wet *versus* dry by-products (45% of total DM) in rations containing 400 g kg^{-1} DM of ensiled roughages (wilted grass silage and maize silage), showed lower rumen ammonium concentrations for the diet with dry by-products, despite a higher N to net energy ration, indicating that more ammonia was captured by bacteria, resulting in a larger ruminal bacterial organic matter pool with the dry diet. Firkins *et al.* (1984) reported no differences in degradability between wet or dry distillers' grains or maize gluten feed. Armentano *et al.* (1986) found reduced degradability of protein for wet brewers' grains. The latter showed that large differences in degradability of dry matter occurred between batches, ranging from 17 to 30%. Veen and Haaksma (1984) reported that in comparison with dry beet pulp the protein degradability of ensiled pressed beet pulp is reduced.

Feed intake and milk performance

When comparing the effect of diets based on moist by-products with dried ones, special attention should be given to the disturbing influence of differences in calculated energy values. As mentioned above, under good preservation conditions only minor differences in calculated energy and protein values are to be expected, but in practice this is not always so. Substantial information is available on the feeding of ensiled pressed beet pulp. High intakes were reported without a specific negative effect on roughage intake (De Brabander *et al.*, 1980; Haaksma, personal communication). The results of Cotto (1979); De Brabander *et al.* (1980); Potthast, Heiting and Nasser (1980) indicated an increased milk production when feeding ensiled pressed beet pulp (or dried beet pulp), when replacing low energy concentrates or roughages. The fat content of milk from cows fed ensiled pressed beet pulp was decreased and the protein content increased simultaneously when high amounts were fed (De Brabander *et al.*, 1980; Andries *et al.*, 1986). Andries *et al.* (1984), experimenting with dairy cows in the second half of lactation found no influence on milk yield, but even at low levels of production an increase in milk protein content and a decrease in milk fat content were measured. In several of these trials the higher milk production from feeding beet pulp was due to higher energy intakes, because beet pulp replaced roughage or low energy feedstuffs or because a higher overall intake was achieved.

However, when comparing dried with moist beet pulp in rations for dairy cows, only minor differences were noticed. De Visser and Hindle (1987) found no difference in total dry matter intake of dried *versus* ensiled pressed beet pulp from the same batch, feeding approximately 5.5 kg DM per animal day^{-1}. At the excellent production level achieved (on average 33 kg milk daily in the first 100 days of lactation) no significant differences were noticed in milk volume nor in fat or protein content of the milk. Similar findings were made by Hemingway, Parkins and Fraser (1986) and Andries *et al.* (1984) experimenting with dairy cows in the second half of lactation.

Information on experiments with moist maize gluten feed is limited. Staples *et al.* (1984) observed decreased total dry matter intake, when maize gluten feed (MGF) comprised 0, 200, 300, 400 g kg^{-1} DM. According to those authors the lower intake might be due to reduced digestibility. Energy intake was decreased due to a lower intake and milk production was also reduced. Milk fat content was increased with higher amounts of MGF. This coincided with the higher molar proportions of acetic acid in the rumen fluid. Milk protein content was reduced at increased proportions of MGF, probably due to the lower energy intakes. Several authors fed brewers'grains to dairy cows. Davis, Grenawalt and McCoy (1983) fed rations containing 200, 300 and 400 g kg^{-1} DM of moist brewers'grains. At the highest level feed intake was reduced. Polan *et al.* (1985) found no differences between dried or moist brewers'grains.

The amounts fed were lower than the highest levels fed by Davis, Grenawalt

and McCoy (1983). Murdock, Hodgson and Riley (1981) fed two levels of wet or dried brewers'grains, and found no differences in dry matter intake, milk production and composition. Higher energy values were calculated for wet brewers'grains than as advised by the National Research Council.
Information on experiments with moist maize distillers'grains is limited. Schingoethe, Clark and Voelker (1983) fed wet maize distillers'grains *ad libitum* to dairy cows. No effects on dry matter intake, milk production and composition were found.
Combinations of moist *versus* dry by-products were studied by De Visser and Tamminga (1987). They fed three by-products (beet pulp, maize gluten feed and brewers'grains) to dairy cows in early lactation. The total amount of by-product was 45% of total dry matter, with 40% of the dry matter from roughage (wilted grass silage and maize silage) and 15% from concentrates. The combination of moist by-products resulted in a reduced dry matter intake, milk production and milk protein content (Table 2.8).

Table 2.8 ***The effects of a combination of moist versus dry by-products on daily feed intake, milk production and composition***

	By-product	
	Dry	Moist
Roughage intake (kg DM)	8.4	8.2
Beet pulp (kg DM)	4.0	3.6
Maize gluten feed (kg DM)	3.0	2.7
Brewers'grains (kg DM)	2.0	1.8
Concentrate (kg DM)	3.6	3.3
Total dry matter intake (kg)	21.1	19.6
Net energy intake (MJ NE)	143	128
Milk (kg)	31.7	30.9
Milk fat (g kg^{-1})	43.1	42.9
Milk protein (g kg^{-1})	32.1	31.3

After De Visser and Tamminga (1987)

In general it may be concluded, that provided a correct estimation of energy values of the products is made, feeding moist by-products instead of dry by-products does not substantially affect feed intake, milk production or milk composition, when the level of inclusion in the rations is below 20% of total dry matter. When the aim is to include more than 250 g kg^{-1} moist by-products in the ration, special consideration should be given to other components of the ration.

Conclusions

For profitable utilization of by-products in feeding dairy cows a correct evaluation of their feeding value is essential, because of the high degree of variation encountered. Product classification according to the methods of processing might help, but considerable emphasis should be given to proximate analysis and the prediction of feeding value from that or alternative information.
Considerable information is available on the use of dry by-products for dairy cows, in particular as a component of compound feeds. The results of feeding trials suggest that, provided the energy value of the concentrates is equivalent, more by-product based concentrates may slightly stimulate overall feed intake and milk production and positively affect milk fat content. These effects may be explained by a decrease in the level of easily fermentable carbohydrates, due to more by-products in the concentrates. As a result, rumen fermentation will be optimal for maintaining good ruminal cellulolytic activity, which is supported by a more stable and higher pH and C2:C3 ratio. Besides affecting carbohydrates, by-product feeding may affect the level and characteristics of protein consumed by dairy cows. Changes in the ingredient composition of the concentrates may greatly affect protein degradability in the rumen.
Information on the use of moist by-products for dairy cows is more recent. Compared to feeding dried products, special attention should be given to proper storage of the wet product to avoid high losses from deterioration. The variable storage losses may complicate the correct evaluation of moist products. Feeding trials with moist by-products suggest that effects on intake and production are comparable to dried products, provided adequate storage of the moist product and provided the level of inclusion in the ration is kept below 20-25% of total dry matter. At high levels of moist by-product inclusion in the ration, reductions of intake and resulting milking performance are noticed, probably as a result of suboptimum rumen fermentation, due to lack of easily fermentable energy.

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Chapter 3

Dried beet pulp, pressed beet pulp and maize silage as substitutes for concentrates in dairy cow rations. 1. Feeding value, feed intake, milk production and milk composition

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Abstract

A feeding trial was carried out with 48 dairy cows in second or later lactation. The experiment started immediately after parturition and lasted for 13 weeks. Basal diets consisted of a mixture of artificially dried grass and maize silage as roughage components and concentrates based on a mixture of by-product feeds. Additional to the basal diet, dried beet pulp, ensiled pressed beet pulp or maize silage was fed. Two groups received either dried beet pulp (DP) or ensiled pressed beet pulp (PP). The third group received extra maize silage (M). All rations were fed as totally mixed rations (TMR) to prevent selection. Digestibility of the ration components was determined with in vitro and in vivo methods. The feeding value of both types of beet pulp and maize silages agreed with that reported in literature. Total dry matter intake differed significantly between DP and the other groups (PP and M). Energy intake was highest for DP and lowest for group M. Milk yield was similar for all groups. However, group PP displayed an apparently different pattern with higher peak production than group M. Fat content and fat yield were similar for all groups. Milk protein yield did not differ significantly between groups, group M tended to have the lowest production. Milk protein percentage did not differ between the groups, although group M showed a tendency to be lowest. The calculated energy balance agreed with liveweight changes.

Introduction

Use of by-product feeds as animal feedstuffs is very common in the Netherlands. Concentrates used in dairy cow feeding consist almost exclusively of by-products. Particularly by-products from the sugar industry, such as beet pulp, are highly appreciated feedstuffs, because of their quality and high level of digestibility. Increasing drying costs have led to the introduction of alternative methods of storage and feeding. The moist pulp, directly after processing, has a dry matter content of approximately 10%. Pressing this material increases the dry matter content to between 18 and 25%, reducing transport costs and improving ensiling characteristics.

Concentrates provide the animal with energy and protein in addition to that supplied by the roughage part of the diet. Part of the concentrate mixture can

be replaced by single energy sources, such as dried or moist ensiled beet pulp. An alternative to such energy sources is the increase of an energy-rich roughage component, such as maize silage. Although a fair amount of information is available on the nutrition characteristics, such as palatability, digestibility and feeding value of a new feedstuff, the information on dried and ensiled pressed beet pulp originating from the same batch is very limited.
Therefore, it was decided to make a comparative study of the influence of ensiled pressed beet pulp, dried beet pulp and maize silage on production characteristics of dairy cows in early lactation. Objectives of this study were:

1. To measure the nutritive value of ensiled pressed beet pulp *in vitro* and *in vivo*.
2. To evaluate pressed beet pulp as a dietary ingredient in a feeding trial with dairy cows.
3. To measure the effects of introduction of pressed beet pulp in dairy rations on rumen fermentation characteristics.

This paper deals with the results of the first two objectives. The results of the rumen fermentation study will be reported in a subsequent paper.

Materials and methods

A feeding trial was performed with 3 groups of 16 animals of the Dutch Friesian Black and White or Dutch Friesian x Holstein Friesian breed. The animals were in second (approx. 40%) or later lactation. The average age of the animals at the start of the experiment was 4 years and 6 months. The animals were grouped in blocks, based on their previous performances (milk production and composition). Within each block, animals were allocated at random to one of the treatment groups. The trial started immediately after parturition and lasted for 13 weeks. Experimental treatments were the diets. In addition to a basal diet of artificially dried grass (20% of DM), maize silage (20% of DM) and a concentrate mixture (35% of DM), 25% of the dry matter was fed as pressed beet pulp, dried beet pulp or extra maize silage (treatment groups PP, DP and M, respectively). To avoid selection by the animals the rations were offered as completely mixed diets and prepared using a mixing feeding wagon. Feed was offered twice daily; 40% at 5:00 h and 60% at 15:00 h. To compensate for the lower energy value of maize silage compared to both beet pulps, the amount of extra maize silage was reduced by 2% and substituted with concentrates. The concentrate mixture consisted of coconut expeller (5%), maize gluten feed (38.5%), palm kernel expeller (8.3%), soya bean meal solv. extract (24.3%), soya bean hulls (15.0%), cane molasses (5%), salt (1%), calcium carbonate (0.3%), dicalcium phosphate (1.3%), vitamins and minerals (0.5%), magnesium oxide (0.8%).
Ration ingredients were analysed for dry matter, ash, nitrogen, crude fat, neutral detergent fibre (NDF), starch and sugars. Ensiled pressed beet pulp and

maize silages were also analysed for volatile fatty acids, lactic acid and pH. DM was determined at 105°C and subsequently corrected for losses through volatiles. OM was calculated as weight loss upon ashing at 550°C. Nitrogen was determined by the Dumas method (Merz, 1979). NDF was determined by the procedure described by Robinson *et al.* (1986). Starch, crude fat and sugars were determined according to the procedures of the Netherlands Normalisation Institute (NEN 3574, NEN 3576 and NEN 3571, respectively). Volatile fatty acids and lactic acid were analysed using gaschromatography as described by Robinson *et al.* (1986).
In vitro digestibility of organic matter was determined for all components (Tilley & Terry, 1963; and IVVO modifications, Van der Meer, pers. comm.). Artificially dried grass, dried beet pulp, pressed beet pulp, and some of the maize silages were also investigated for *in vivo* digestibility using wethers fed at maintenance, according to the method described by Van Es & Van der Meer (1980). The amount of feed offered to dairy cows in the feeding trial was based on restricted *ad libitum* feeding (approx. 2% residues). Animals were fed individually and daily refusals recorded. Milk yield was measured each week on two consecutive days by means of a Tru tester (Moderniek, Soest). At the same time samples were collected for analysis of milk fat and protein. Milk samples were preserved with sodium azide and analysed at a commercial laboratory (Stichting melkcontrolestation 'Noord', the Netherlands). Liveweight of the animals was measured the day after parturition and once a week for the duration of the trial. Bodyweight losses were calculated. The energy balance was calculated and adjusted for mobilization of body reserves. Statistical examination of the results using variance analysis techniques was accomplished using the statistical programme Genstat (Alvey *et al.*, 1982).

Results

The chemical compositions of the roughages are shown in Table 3.1 and those of the concentrate mixture, dried beet pulp and pressed beet pulp in Table 3.2.
Dried and pressed beet pulp had similar proportions of ash, N-Dumas, crude fibre and crude fat. The sugar content was lowest in the pressed beet pulp, this is probably as a result of the conversion of sugars into volatiles during the ensiling process and due to losses of sugar during the pressing process. The latter would also explain the difference in NDF content between the dried and pressed beet pulp. Maize silages from different batches were similar in ash, N-Dumas and crude fat contents, but varied in crude fibre and NDF, due to time of harvesting and/or stage of maturity. The *in vitro* determinations for the organic matter digestibilities of dried beet pulp, ensiled pressed beet pulp and maize silage (Table 3.3) agreed favourably with previous studies (Verité, 1975; Kelly, 1983; De Visser & Tamminga, 1987; Moran *et al.*, 1988).

The *in vivo* digestibility of the organic matter of the dried beet pulp and ensiled pressed beet pulp from the same origin was high and showed a relatively low variation (Table 3.4). The results agreed with the literature (Kelly, 1983; De Visser & Tamminga, 1987).

Table 3.1 ***Chemical composition of artificially dried grass and maize silages in feeding trial (g/kg^{-1} DM)***

	Artificially dried grass	Maize silage				
		11	12	B	D	large silo
Dry matter	835	265	242	264	286	256
Ash	127	57	56	58	52	60
N-Dumas	36	15	16	14	12	15
Crude fibre	261	209	232	236	215	249
Crude fat (40/60 PE)	30	29	28	29	29	28
Neutral detergent fibre	553	442	464	456	457	495
Starch	-	216	220	232	284	226
Sugars	55	-	-	-	-	-
Acetic acid	-	27	32	14	9	17
Lactic acid	-	-	-	46	-	-
pH	-	4.0	4.1	4.0	3.9	4.1

Table 3.2 ***Chemical composition of the concentrate mixture, dried beet pulp and pressed beet pulp in feeding trial (g/kg^{-1} DM)***

	Concentrate	Dried pulp	Pressed pulp
Dry matter	864	897	185
Ash	102	86	86
N-Dumas	38	16	16
Crude fibre	137	206	206
Crude fat (40/60 PE)	36	4	5
Neutral detergent fibre	395	450	530
Starch	84	-	-
Sugars	74	111	35
Acetic acid	-	-	7
Lactic acid	-	-	35
pH	-	-	4.1

The digestibility of the crude protein of both the dried beet pulp and the ensiled pressed beet pulp was low (68 and 69, respectively), yet agreed favourably

Table 3.3 ***In vitro digestibility and energy value of maize silage, dried beet pulp and pressed beet pulp***

Product		*In vitro* digestibility	SE	NEL[1] (MJ)
Maize silage	11	72.8	0.8	6.12
	12	72.8	0.8	6.15
	B	69.1	0.5	5.99
	D	73.3	1.1	6.27
	large silo	69.9	1.4	5.87
Dried beet pulp		89.5	0.5	7.32
Pressed beet pulp		87.5	0.1	7.18

[1] NEL = Net Energy Lactation, according to the Dutch energy system (Van Es, 1978) by means of *in vitro* organic matter digestibility

Table 3.4 ***The in vitro digestibility and calculated net energy values and digestible crude protein values of some of the maize silages, dried beet pulp and pressed beet pulp***

Product		Digestibility coefficient[1]						NEL[2] MJ/kg^{-1}	dcp[3] g/kg^{-1}
		DM	OM	cp	cfat	cf	NFE	DM	DM
Maize silage	11	71	74	61	78	66	78	6.22	57
	D	71	73	55	78	67	78	6.29	41
	large silo	68	71	55	79	63	76	5.95	52
Dried beet pulp		83	89	68	-25	87	95	7.24	68
Pressed beet pulp		84	90	69	-82	88	95	7.30	69

[1] DM = dry matter; OM = organic matter; cp = crude protein; cfat = crude fat; cf = crude fibre; NFE = N-free extract
[2] NEL = Net Energy Lactation, according to the Dutch energy system (Van Es, 1978)
[3] dcp = digestible crude protein

with previous experiments (Kelly, 1983; De Visser & Tamminga, 1987). Especially the digestibility coefficients for N-free extracts and crude fibre were high, yet in agreement with the results found by Cottyn *et al.* (1980) and De Visser & Tamminga (1987). In spite of small differences in digestibility and chemical composition between the dried beet pulp and ensiled pressed beet pulp used in this experiment, the net energy (NEL) and digestible crude protein values

(dcp) were almost equal. Using the actual *in vivo* digestibility values to calculate NEL and dcp the results were 7.24, 7.30 NEL and 68, 69 dcp for dried beet pulp and ensiled pressed beet pulp, respectively. The *in vitro* digestibilities of the maize silages used were similar (Table 3.3). The lower digestibility of the maize silage from the large silo could be explained from its chemical composition, because this silage was very high in crude fibre content compared to the other batches. The average digestibility was similar to what was found in other experiments (Steg, unpublished; Verité, 1975; Moran *et al.*, 1988). The *in vitro* digestibility of maize silage was lower than that of the dried or pressed beet pulp (Table 3.3). These results agree with other experiments (Steg, unpublished; Cottyn *et al.*, 1980). The *in vivo* digestibility of the different batches of maize silage were similar (Table 3.4). Again the maize silage from the large silo was lower than the other batches that were investigated. The *in vitro* and *in vivo* organic matter digestibilities were comparable with each other. The beet pulps showed a better *in vivo* digestibility than the maize silage, confirming earlier findings (Verité, 1975; Cottyn *et al.*, 1980; Steg, unpublished). The most important differences in digestibility between beet pulp and maize silage were found in the crude fibre and N-free extracts. Both fractions were highly digestible in beet pulp, but to a lesser extent digestible in maize silage (Table 3.4).
This could be explained by the fact that maize silage contains a larger rumen undegradable organic matter fraction compared to beet pulp, as shown by the results of incubations into the rumen, using nylon bag incubations (Table 3.5).

Table 3.5 ***Characteristics of degradability of the organic matter***

Rations	Soluble fraction (%)	Digestible fraction (%)	Undigestible fraction (%)	Rate of degradation (% per hour)
Dried beet pulp	6.3	88.0	5.7	5.59
Pressed beet pulp	34.5	62.4	3.1	6.16
Maize silage	33.0	48.6	17.7	2.49

The estimated net energy and the digestible crude protein (dcp) values of the maize silages, dried beet pulp and pressed beet pulp are shown in Table 3.4. The values for the artificially dried grass and the concentrates were 5.36 and 6.96 MJ/kg^{-1} DM and 164 and 191 dcp/kg^{-1} DM, respectively.
The average results concerning feed intake, milk production and composition during the first 13 weeks of the lactation are displayed in Table 3.6. Total dry matter intake averaged approximately 22 kg DM per animal per day. The level of intake was equal to that observed in previous trials, when diets were also largely based on by-product ingredients (De Visser & Tamminga, 1987; De

Visser & Steg, 1988). Total dry matter intake differed significantly between DP and the other treatments. Group M showed a slightly different pattern (Fig. 3.1), while the intake of group PP remained lower for the duration of the experiment.
Because of a lower DM intake the net energy intake was significantly lower for group PP compared to DP (Table 3.6). The energy intake was significantly lower

Tabel 3.6 ***Feed intake, milk production, milk composition and body-weight for the experimental period of 13 weeks (mean from 16 animals)***

	DP[1]	PP[2]	M[3]	SEM	Sign
Roughage (kg DM)	9.2[a4]	8.8[a]	13.4[b]	0.10	P<0.01
Concentrates (kg DM)	13.7[a]	13.3[a]	8.6[b]	0.11	P<0.01
Beet pulp (kg DM)	5.7	5.6	-	0.04	NS
Total intake (kg DM)	22.9[a]	22.1[b]	22.0[b]	0.21	P<0.05
Net energy intake (MJ/d^{-1})[5]	148[a]	144[b]	140[c]	1.36	P<0.01
Protein intake (g dcp)[6]	2871	2768	2788	26.10	NS
Milk (kg)	32.9	33.5	32.5	0.51	NS
Fat (g)	1459	1473	1458	24.61	NS
Protein (g)	1084	1098	1031	14.87	P<0.10
Fat (%)	4.44	4.41	4.52	0.05	NS
Protein (%)	3.32	3.30	3.19	0.03	P<0.10
FCM 4 % (kg)	35.0	35.5	34.9	0.54	NS
Net energy req. (MJ)	148	150	148	1.94	NS
Protein req. (g dcp)	2607	2638	2598	35.32	NS
Energy intake/req. (%)[7]	100.6[a]	97.2[a]	94.9[b]	1.12	P<0.05
Protein intake/req.(%)[8]	111.3	106.3	108.1	1.29	NS
Body weight (kg)	624	627	626	6.25	NS
Body weight changes (kg)	-4[a]	-11[ab]	-18[b]	2.34	P<0.05

[1] Dried beet pulp
[2] Pressed ensiled beet pulp
[3] Maize silage
[4] Figures with a different superscript differ significantly from each other.
[5] Net energy intake = net energy intake according to the Dutch Energy System (Van Es, 1978)
[6] dcp = digestible crude protein
[7] Energy intake/req. = ratio between energy intake and energy requirements
[8] Protein intake/req. = ratio between protein intake and protein requirements

for group M compared to both beet pulp groups (DP and PP). This can be accounted for by the lower DM intake and the lower energy value of maize silage compared to both beet pulps (Table 3.4). Compensation for the lower energy value of the maize silage with extra concentrates proved to be insufficient. The digestible crude protein intake did not differ significantly between groups.

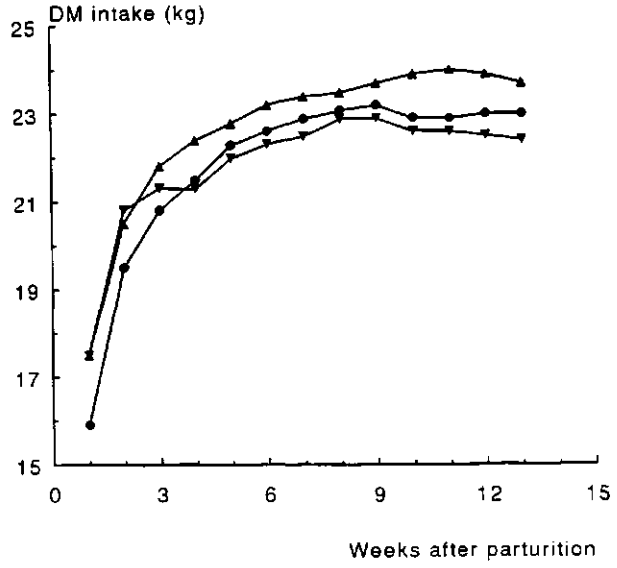

Figure 3.1 ***Dry matter intake (kg).*** ▲-▲ ***dried beet pulp (DP);*** ●-● ***pressed beet pulp (PP);*** ▼-▼ ***maize silage (M).***

Milk yield did not differ between groups, although production in relation to weeks *post partum* suggested a flatter curve for cows fed maize silage, while cows fed pressed beet pulp had a more pronounced peak (Fig. 3.2). Milk fat yields and percentages were not significantly different between groups, although group M tended ($P<0.10$) towards a higher fat percentage. Milk protein yield and percentage did not differ significantly among groups, although cows fed maize silage tended to have the lowest protein yield and percentage ($P<0.10$). Protein percentage did differ significantly from week 7 *post partum* onwards, being 3.27, 3.27 and 3.09 for DP, PP and M respectively ($P<0.05$; Fig. 3.3).

Figure 3.2 ***Milk production (kg).*** ▲-▲ ***dried beet pulp (DP);*** ●-● ***pressed beet pulp (PP);*** ▼-▼ ***maize silage (M).***

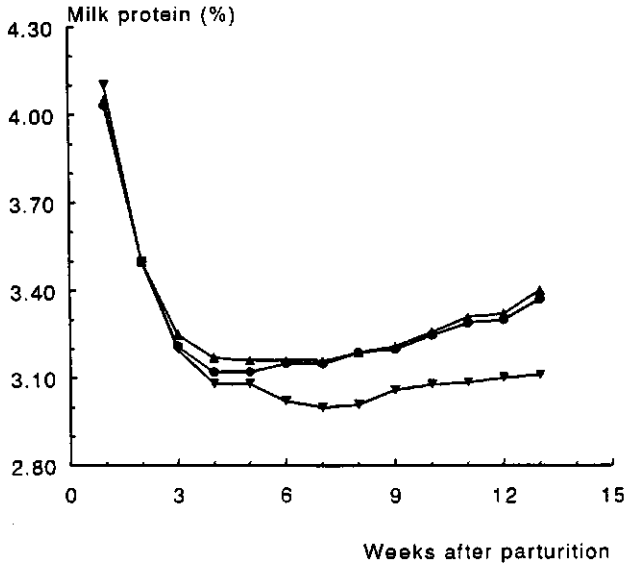

Figure 3.3 ***Milk protein percentage.*** ▲-▲ ***dried beet pulp (DP);*** ●-● ***pressed beet pulp (PP);*** ▼-▼ ***maize silage (M).***

The energy intake/requirement ratio of all groups were in agreement with the body weight changes, based on group averages and according to the Dutch net energy lactation system (Van Es, 1978). Individual energy balances and body weight changes were poorly related.

Discussion

The significantly lower DM intake of group PP compared to DP can be made plausible by two explanations. Firstly, due to the lower dry matter content of diet PP, animals had to consume larger quantities, which is very difficult during early lactation (Lahr *et al.* 1983). Secondly, due to the ensiling process, easily fermentable carbohydrates were used by micro organisms in the silo during the ensiling process, which may have led to changes in the sources of energy available for micro-organisms in the rumen (carbohydrates *versus* volatile fatty acids and lactic acid), as suggested by Van Soest (1982). Hemingway *et al.* (1986) fed similar amounts of dried and pressed beet pulp (5 kg DM). The total amount of feed consumed in their trial was lower than in our experiment, because animals were not fed *ad libitum*, but according to their requirements (milk production and bodyweight). In literature, several authors reported much higher intakes (approaching 10 kg DM) of dried or pressed beet pulp. They did not mention any problems with regard to intakes at such high levels (De Brabander *et al.* (1980); Andries *et al.* (1984); Haaksma, pers. comm.). In these trials beet pulp was fed *ad libitum* and separately from the other ingredients. The significantly lower intake of group M compared to DP can be explained by the higher undegradable fraction and the lower rate of degradation of the potential degradable fraction of the OM (Table 3.5). Snijders (pers. comm.) found variable results when comparing rations with maize silage or beet pulp. In his experiments the DM intake was negatively influenced, when maize silages were fed with a lower quality (stage of maturity). Several researchers reported reductions in total dry matter intakes, when the roughage/concentrate ratio was increased (Broster *et al.*,1978; Kirchgessner & Schwarz, 1984). In these experiments the undegradable fraction and/or the rate of fermentation were probably negatively influenced, leading to a reduction of total DM intake.
Milk yield did not differ significantly between dried and pressed beet pulp groups, which confirmed the findings from other experiments (Hemingway *et al.*, 1986; Snijders, pers. comm.) when direct comparisons were made between both forms of beet pulp fed in early lactation. Comparing dried and pressed beet pulp in late lactation did not result in production differences either (Ronning & Bath, 1962; Castle, 1972; Parkins *et al.*, 1986). However, increasing responses of milk yield due to higher energy intakes are limited in late lactation (Broster, 1972). De Brabander *et al.* (1980) obtained higher milk yields when feeding pressed beet pulp in early lactation. However, in their trial low-energy concen-

trate components and even roughage was replaced by pressed beet pulp. The net energy intake was thus increased, leading to a higher milk output. After correction for higher energy intakes, differences were no longer significant. Milk yield did not differ significantly between either beet pulp groups and the maize silage group, in spite of the lower energy intake. Due to the lower energy intake the animals of group M showed a lower milk yield peak than both the beet pulp groups. Snijders (pers. comm.) results showed occasional negative effects, when feeding maize silage instead of beet pulp, but these differences could be explained by lower energy intakes. When balanced rations containing large amounts of maize silage were fed, no negative effects on milk yield were found (Parrassin *et al.*, 1979; De Visser & Steg, 1987).

The similarity in fat percentage and yield of both beet pulp groups in our experiment confirmed findings elsewhere (Ronning & Bath, 1962; Castle, 1972; Hemingway *et al.*, 1986; Parkins *et al.*, 1986). However, De Brabander *et al.* (1980) found lower milk fat percentages when feeding pressed beet pulp, probably caused by a higher roughage/concentrate ratio in the rations containing pressed beet pulp. The fat percentage was not significantly different between beet pulp groups and maize silage, but group M tended to be highest ($P < 0.10$). This is in agreement with Snijders (pers. comm.) who also found a higher fat percentage for those groups fed maize silage. The companion study with rumen cannulated animals, fed with equal amounts of feed, showed no difference in total concentration of major volatile fatty acids or in their ratio. This indicates that no differences in milk fat yield and percentage might be expected, due to ruminal events (De Visser, unpublished results).

The milk protein percentage and yield did not differ significantly among groups fed beet pulp, which agreed with the results found by Ronning & Bath (1962), Castle (1972), Hemingway *et al.* (1986), Parkins *et al.* (1986). However, De Brabander *et al.* (1980) found an increase in milk protein content and milk protein yield, but in their experiment energy intakes were higher, which might explain the higher milk protein output. The milk protein percentage over the total experimental period tended to differ between groups fed beet pulp and those fed maize silage, which agreed with the results of Snijders (pers. comm.). The lower energy intake is one of the possible explanations for the lower production and percentage of protein. The results of the accompanying fermentation study showed significantly increased concentrations of iso-acids and ammonia for the maize silage diet. Since the protein/energy ratio did not differ between treatments, this could be an indication of lower microbial protein synthesis (Miller, 1982; Van Soest, 1982; Robinson *et al.*, 1987).

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Chapter 4

Dried beet pulp, pressed beet pulp and maize silage as substitutes for concentrates in dairy cow rations. 2. Feed intake, fermentation pattern and ruminal degradation characteristics

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Abstract

Four rumen-cannulated dairy cows were fed twice daily with a totally mixed ration (TMR), in which the basal diet consisted of artificially dried grass, maize silage and concentrates. In addition, 25% of total dry matter intake was dried beet pulp (BP), ensiled pressed beet pulp (PP) or extra maize silage (M). The measurements were performed at approximately 15 (LLI) and 25 kg (HLI) dry matter feed intake respectively in a 2x3x4 latin square design. Measurements were made of organic matter degradation (nylon bag) of diet ingredients, while during 48 hour periods the pattern of intake, pH and osmolality, as well as the concentration of volatile fatty acids (VFA), lactic acid (HL) and ammonia (NH_3) were determined.

The pH and concentration of the major VFA's did not differ significantly between treatments. The concentration of the branched chain fatty acids (BCFA), valerate and ammonia were significantly higher for diet M. The level of intake significantly influenced the pH, concentration of the major VFA's, 2-methyl-butyrate and valerate. The feed intake pattern differed between intake levels, but were similar for the three diets. The results of the degradability of treatments were calculated from individual degradability measurements of diet ingredients.

The undegradable fraction (U) was highest for diet M. The water-soluble fraction (S) was lowest for treatment DP, whereas the rate of degradation (k_d) was lowest for treatment M.

Introduction

Beet pulp is a by-product from the sugar beet industry, which is available to farmers either moist or as a dehydrated product. The moist product involves lower production costs (no drying costs), but is bulky (transport) and must be ensiled. There is a shift in utilization of beet pulp in Dutch dairy feeding from dried beet pulp to pressed ensiled beet pulp. Recently reported information suggests, that the energy values of both beet pulps are high and do not differ significantly from each other (Hemingway *et al.*, 1986; De Visser & Tamminga, 1987; De Visser & Hindle, 1990).

Dutch dairy cow rations usually contain large quantities of partly fermented roughage(s), grass silage and/or maize silage. In early lactation, large amounts of compound feeds are fed as supplement to the roughage-based diets of high-

yielding dairy cows in order to meet requirements. These concentrates can be partially replaced by moist-ensiled by-products, such as pressed ensiled beet pulp, ensiled maize gluten feed or moist ensiled brewers'grains. Dried beet pulp on the other hand is used by the compound feed industry as an important energy rich ingredient in concentrate mixtures. An alternative for dairy farmers may be the use of an energy-rich roughage, such as maize silage.
Replacing concentrates with pressed ensiled beet pulp or maize silage (both partly fermented by micro-organisms in the silo) may have a negative influence on rumen fermentation. During the ensiling process part of the energy in the feed (carbohydrates) is converted into the end-products of fermentation such as volatile fatty acids and lactic acid. These fermentation products cannot be used as efficiently as the original carbohydrates by the micro-organisms in the rumen (Van Soest, 1982; Robinson *et al.*, 1987).
Therefore a comparative study was performed, in which pressed ensiled beet pulp was compared to dried beet pulp and maize silage. The objectives were:

1. To measure the influence of pressed ensiled beet pulp, dried beet pulp or maize silage on rumen fermentation patterns.
2. To measure the influence of two feeding levels (15 and 25 kg DM per animal per day) on these patterns.
3. To measure the degradation characteristics of the organic matter of pressed ensiled beet pulp, dried beet pulp and maize silage using nylon bag incubations.
4. To explain the results of a feeding trial, in which the same dietary ingredients were applied. (De Visser & Hindle, 1990).

Materials and methods

Rumen fermentation

The 4 animals used in this experiment were of the Dutch Friesian and Dutch Friesian x Holstein breed and were each equipped with a rumen cannula. One cow was fitted with a PVC cannula of 5 cm i.d. (Eriks, Alkmaar, the Netherlands). The other three animals were equipped with a large rumen cannula of 10 cm i.d. (Bar-Diamond Inc., Parma, ID, USA.). The animals were housed in a tie stall and in order to avoid selection they were offered a totally mixed ration (TMR), using a mixer-forage wagon. The diets corresponded to those of the accompanying feeding trial (De Visser & Hindle, 1990) and are shown in Table 4.1. The treatments were dried beet pulp (DP), pressed ensiled beet pulp (PP) and extra maize silage (M). The animals were fed individually twice daily; at 5:00 h (40% of the diet) and at 14:00 h (60% of the diet).
The composition of the concentrate mixture was as described by De Visser & Hindle (1990) and consisted of coconut expeller (5%), maize gluten feed (38.5%),

palm kernel expeller (8.3%), soya bean meal solv. extracted (24.3%), soya bean hulls (15%), cane molasses (5%), minerals and vitamins (3.9%). Rumen fermentation pattern was investigated at two feeding levels. During the first 5 months after parturition the animals were fed at a high level (approx. 25 kg DM) (HLI), while a low level (approx. 15 kg DM) (LLI) was fed between the 6 and 11th month after parturition.

Table 4.1 ***Diet ingredients and chemical composition, after correction for volatiles (g/kg^{-1} DM)***

	Dried beet pulp (DP)	Pressed beet pulp (PP)	Maize silage (M)
Diet ingredient			
Artificially dried grass	200	200	200
Maize silage	200	200	430
Dried beet pulp	250	-	-
Pressed beet pulp	-	250	-
Concentrate mixture[1)]	350	350	370
Chemical composition			
Dry matter	746	568	599
Ash	94	94	88
N-Dumas	27	28	28
Crude fat	25	25	31
Crude fibre	198	198	202
Neutral detergent fibre	455	475	459
Starch	76	76	132
Sugar	64	45	38

[1] De Visser & Hindle, 1990

The experiment was performed as a change over 3x4 latin square design, which is outlined in Table 4.2. It was not possible to perform a complete 6x4 latin square design, because the animals may have refused the high level of intake during the second part of lactation, or probably had suffered from ketosis when feeding the low level of intake during early lactation. Each period lasted at least 5 weeks. Measurements were made on two consecutive days during the last week of each experimental period. These measurements involved taking 9 samples per day of the rumen fluid at 5:00, 7:00, 10:00, 14:00, 16:00, 19:00, 22:00, 2:00 and 5:00 h

using a plastic tube 60 cm long with a diameter of 4 cm, which was perforated over the last 40 cm with holes of 1 mm. A Record syringe was used for the suction of 100 ml rumen fluid, from the inside of the tube. Samples were directly analysed for pH and osmolality, using a pH meter (Philips 2000) and an osmometer (Osmomat 300, Gonotec). Subsamples were taken for analysis of volatile fatty acids (VFA), lactic acid (HL) and ammonia (NH_3). The preservation of these subsamples and the analytical methods used were as described by Robinson *et al.* (1986).

Table 4.2 ***Experimental design***

Period	Cow no.			
	744	773	829	1338
1	H[1] PP[2]	L DP	H DP	L M
2	H M	L PP	H PP	L DP
3	H DP	L M	H M	L PP
4	L DP	H DP	L PP	H M
5	L PP	H PP	L M	H DP
6	L M	H M	L DP	H PP

[1] Feeding levels: H = HLI (high level); L = LLI (low level)
[2] Diets: DP = dried beet pulp, PP = pressed ensiled beet pulp, M = maize silage

The results of the individual volatile fatty acids were used to calculate the ratio between the lipogenic and glucogenic volatile fatty acids (NGR) as described by Ørskov (1975). The diets were analysed for dry matter (DM), ash, nitrogen (N), crude fibre, Neutral Detergent Fibre (NDF), sugar and starch as described by De Visser & Hindle (1990).

During the fermentation study the feed intake pattern of the diets at the two feeding levels was measured by recording the amount of feed consumed between the various rumen sampling times.

All data of the daily rumen samples were used to calculate daily mean values (weighted for time intervals), while the variation during the day was expressed as the standard error of the values measured at the various sampling times during the day.

Variance analysis on mean daily values and daily variations in rumen parameters, using the statistical package Genstat (Alvey *et al.*, 1982) were performed using diet and intake levels as independent variables.

Rumen degradation

Three cows fitted with a large rumen cannula were used. Two of the animals were in early lactation, while the third one was in mid lactation. The animals were fed the intermediate diet (1/3 DP + 1/3 PP + 1/3 M) from the fermentation study (Table 4.1). The two animals in early lactation were fed 24 and 22 kg DM per day, respectively, while the third animal was fed 15 kg DM per day. The animals were housed in the same stall and fed as the animals in the rumen fermentation study. Rumen incubations were performed for 0, 3, 6, 12, 24, 48 and 216 hours to determine the degradation of organic matter (OM). Rumen degradability was measured for all dietary ingredients (Table 4.1), used in the fermentation study and the feeding experiment of De Visser & Hindle (1990).

The results of the individual feedstuffs were used to calculate the degradability of the three treatment diets (DP, PP, M), using the original data at the various incubation times.

Small samples (approximately 5 g DM) of all ingredients were put into nylon bags measuring 10x19 cm with a pore size of 41 nm. The dried beet pulp and the compound feed were ground through a 5 mm sieve. The pressed beet pulp and the maize silage were homogenized with a meat cutter (Duker); the artificially dried grass was chopped into particles of approx. 10 mm length with a paper guillotine. The procedure for nylon bag incubations and the analytical and mathematical methods employed were as described by Van Vuuren *et al.* (1989).

As a result of these procedures the OM of the feedstuffs was divided into the water-soluble fraction (S), the potentially degradable fraction (D) and the undegradable fraction (U), while the rate of degradation (k_d) was estimated as the first derivative of the degradation curve. The total potentially fermentable fraction (F), was defined as the sum of S and D. Significance tests were performed for differences between feedstuffs and between diets with analysis of variance using the statistical package Genstat (Alvey *et al.*, 1982).

Results

Rumen fermentation

The results obtained from the rumen fermentation study are shown in Table 4.3. The mean pH, osmolality, HL, total VFA, NGR, and the major VFA's did not differ significantly between diets. The daily variation in these parameters did not differ significantly either.

The concentrations of NH_3, the branched-chain fatty acids (BCFA) (iso-butyrate, 2-methyl-butyrate, 3-methyl-butyrate) and valerate were significantly higher for diet M, compared to both beet pulp diets (DP and PP), as was daily variation of 2-methyl-butyrate and valerate.

Table 4.3 ***Rumen pH and concentrations of volatile fatty acids, lactic acid, ammonia (mmol/l[1]) and osmolality (meq l[1]), between diets containing dried beet pulp (DP), pressed beet pulp (PP) or extra maize silage (M) and the effects due to level of feed intake (LLI versus HLI)***

Component		Rations			SED[1]	Level of intake		SED
		dried pulp	pressed pulp	maize silage		HLI high	LLI low	
pH,	mean	6.03	6.00	6.11	0.05	5.93[a2]	6.19[b]	0.04
	range[3]	0.38	0.38	0.32	0.03	0.32[a]	0.41[b]	0.02
Osmolality	mean	0.304	0.311	0.310	0.01	0.313	0.303	0.01
	range	0.022	0.022	0.021	0.002	0.021	0.023	0.001
Ammonia	mean	7.32[a]	6.93[a]	9.06[b]	0.88	7.36	8.29	0.73
	range	5.31	5.26	5.21	0.56	4.71[a]	5.94[b]	0.46
Lactate	mean	1.46	1.75	1.38	0.24	1.26[a]	1.87[b]	0.19
	range	2.25	2.91	2.40	0.57	1.47[a]	3.84[b]	0.46
Total VFA	mean	118	120	119	4.21	126[a]	110[b]	3.46
	range	15.7	16.4	15.4	1.43	13.7[a]	18.7[b]	1.17
NGR[4]	mean	4.60	4.52	4.38	0.19	4.26[a]	4.80[b]	0.15
	range	0.50	0.41	0.47	0.07	0.33[a]	0.62[b]	0.06
Acetate	mean	74.7	76.0	73.3	2.42	78.7[a]	69.7[b]	1.98
	range	9.45	9.80	8.77	1.19	8.1[a]	10.8[b]	0.98
Propionate	mean	22.7	23.1	23.3	1.02	25.3[a]	19.6[b]	0.84
	range	4.16	4.25	4.28	0.38	3.50[a]	5.15[b]	0.31
Isobutyrate	mean	0.71[a]	0.71[a]	1.00[b]	0.11	0.84	0.77	0.09
	range	0.21	0.19	0.24	0.08	0.23	0.19	0.06
Butyrate	mean	16.3	15.9	16.4	0.75	17.2[a]	14.9[b]	0.61
	range	2.00	2.19	2.31	0.20	2.15	2.19	0.12
2-Methyl-butyrate	mean	0.32[a]	0.48[a]	1.12[b]	0.15	0.80[a]	0.43[b]	0.12
	range	0.17[a]	0.22[a]	0.61[b]	0.16	0.42	0.24	0.14
3-Methyl-butyrate	mean	0.54[a]	0.53[a]	0.86[b]	0.10	0.67	0.62	0.08
	range	0.20	0.22	0.27	0.06	0.23	0.24	0.05
Valerate	mean	1.54[a]	1.57[a]	1.92[b]	0.16	1.89[a]	1.41[b]	0.13
	range	0.38[a]	0.37[a]	0.61[b]	0.09	0.45	0.46	0.08

[1] SED = Standard error of difference; [2] Figures with a different superscript are significantly ($P<0.05$) different; [3] Range = calculated as standard error during the day (SE); [4] NGR = Nonglucogenic Glucogenic Ratio (Ørskov, 1975)

There was no significant interaction between diet and feeding level. However, feeding level itself significantly influenced most of the rumen parameters. At HLI the concentration of the major VFA's, 2-methyl-butyrate and valerate was significantly higher, while the pH of the rumen fluid was significantly lower. The daily variation on LLI was significantly higher for pH, NH_3, HL, total VFA, NGR, acetate, propionate and butyrate.
Feed intake pattern did not differ between diets, but feeding level had a large influence on feed intake pattern. Animals fed the LLI consumed their feed within one hour, while those at HLI consumed their feed in several smaller meals (Figure 4.1).

Table 4.4 ***The soluble (S), potentially fermentable (D), undegradable fraction (U) and total potentially rumen fermentable fraction (F) (%), rate of degradation (k_d) (% h^{-1}) and lag time (L) (h) in the organic matter of various feeds and rations as measured or estimated in sacco***

Component	Fraction				k_d	L
	S	D	U	F		
Ingredient						
Dried beet pulp	6.3[a3]	88.0[a]	5.7[a]	94.3[a]	5.59[a]	3.36
Pressed beet pulp	34.5[b]	62.4[b]	3.1[a]	96.9[a]	6.16[a]	0.53
Maize silage no. 11[1]	38.8[b]	33.1[c]	28.1[b]	71.9[b]	3.45[b]	3.82
no. 12	32.4[b]	41.5[c]	26.1[b]	73.9[b]	2.62[b]	0.00
no. D	27.8[b]	47.2[c]	25.0[b]	75.0[b]	2.15[b]	0.27
Artificially dried grass	27.5[b]	55.9[b]	16.6[c]	83.4[b]	3.09[b]	1.19
Concentrate	51.0[c]	42.2[c]	5.7[a]	93.2[a]	4.48	0.39
SED[2]	2.09	2.62	3.72	3.76	0.25	
Diets (average)						
Dried beet pulp (DP)	31.5[a]	56.1[a]	12.4[a]	86.6[a]	4.40[a]	1.49
Pressed beet pulp (PP)	38.6[b]	49.7[b]	11.7[a]	88.3[a]	4.40[a]	0.78
Maize silage (M)	38.6[b]	44.3[b]	17.2[b]	82.9[b]	3.40[b]	0.97
SED	1.40	1.45	1.81	1.94	0.23	

[1] Maize silage no. 11 = maize silage used in fermentation and feeding trial which was stored in silo no. 11
[2] SED = standard error of difference
[3] Figures with a different superscript are significantly ($P<0,05$) different

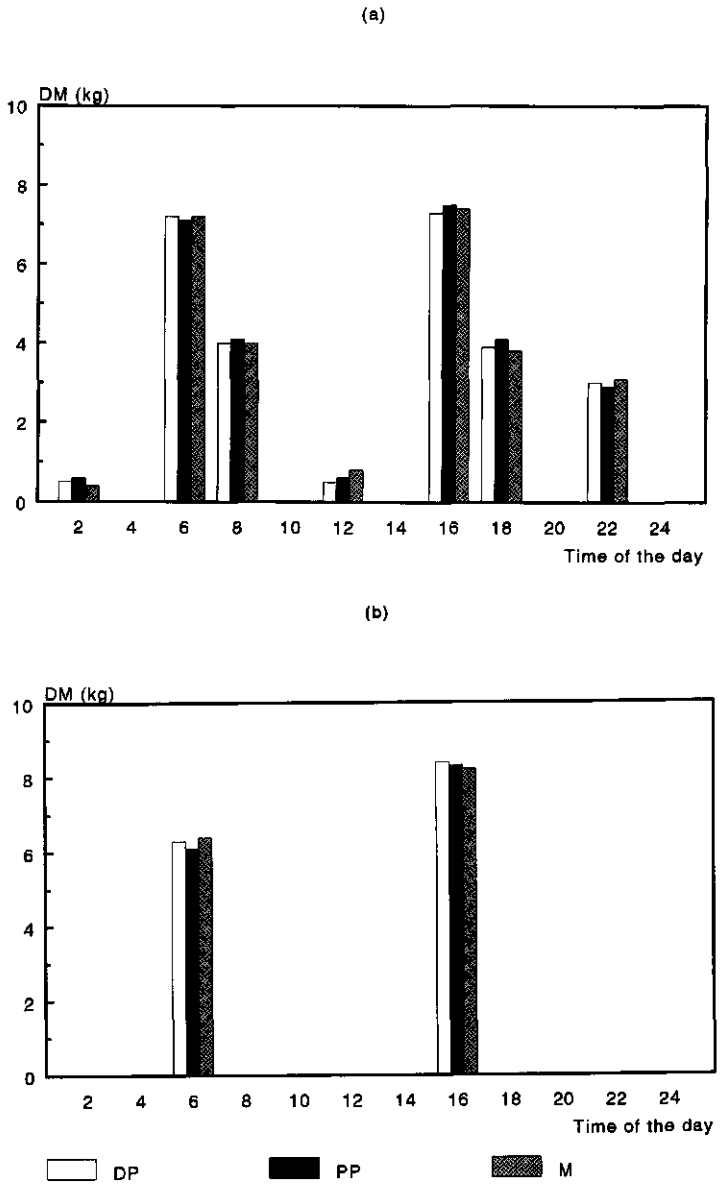

Figure 4.1 ***Feed intake pattern during the day***
Dried beet pulp (DP)
Pressed ensiled beet pulp (PP)
Maize silage (M)
(a) High level of intake (HLI)
(b) Low level of intake (LLI)

Degradability

The fractions S, D, U and F together with k_d of the OM of the dietary ingredients and the calculated values for the total diets are shown in Table 4.4.

The S fraction was much lower for dried beet pulp than for pressed beet pulp or maize silage. As a result the calculated S fraction of diet DP was also lower. The U fractions of the maize silages used were significantly higher than that of dried beet pulp or pressed beet pulp, resulting in a higher U fraction for diet M, which resulted in a lower F fraction for diet M. The rate of OM degradation was lowest for maize silage and slightly higher yet similar for both beet pulps. The calculated k_d was lowest for diet M, while diets DP and PP were similar.

Discussion

The three diets compared favourably in their ash, N, crude fibre and NDF contents. The differences observed in sugar and starch contents are not considered to be influential (Table 4.1), because of the relatively low levels of these components in total rations. The rumen fermentation characteristics of the three TMR rations did not differ significantly in pH, total VFA, NGR and the major VFA's. This is in agreement with the chemical composition of the diets presented to the animals (Table 4.1; Table 4.3). The results of this experiment agreed with previous findings. Only small, if any differences were observed in rumen fermentation characteristics for dried beet pulp and pressed beet pulp diets (Bhattacharya & Lubbadah, 1971; Castle, 1972; Huhtanen, 1988) or between diets based on beet pulp or maize silage (Verité, 1975).

Results from the nylon bag incubation study showed differences in the S fraction between diet DP on the one hand and the diets PP and M on the other. The chemical composition of the S fraction was not analysed, but the fraction in dried beet pulp was very small and probably consisted mainly of sugars. The ground dry material probably swelled during the washing programme, increasing particle size and therefore reducing the number of very small particles, which could be rinsed out of the nylon bags. Pressed beet pulp and maize silage had higher S fractions, yet again the chemical composition of these fractions was not analysed. However, the remaining sugars, part of the starch and the fermentation end-products of pressed ensiled beet pulp and maize silage may have been removed from the nylon bags during washing. Furthermore, these components do not account for all the material which was measured as the soluble fraction. Fermentation processes in the silo may convert other components into more soluble fractions and/or reduce their particle size, increasing their possibility of being washed out of the bags. Courtin & Spoelstra (1989) suggested that pectins, present to a large extent in beet pulp, could be degraded by enzymes produced by yeasts and species of the bacterial genus *Bacillus* into smaller particles during the ensiling process, which could explain differences in the S fraction between dried beet pulp and pressed

beet pulp. A larger quantity of soluble OM, which is assumed to be available for fermentation immediately after feed intake, was expected to affect rumen fermentation characteristics. The S fraction was higher for diets PP and M than for diet DP. Nevertheless, this was not reflected in the concentration of the major VFA's, the pH of the rumen fluid, or the daily variation of these parameters.
When using the results of the degradation study to explain rumen fermentation characteristics, it seems therefore acceptable to use the total rumen degradable fraction (F), because this fraction was similar for both beet pulp diets. The results of the nylon bag incubation study showed a significant difference in the U fraction between maize silage and both beet pulps. This is in agreement with differences in organic matter digestibility measured *in vivo* and *in vitro*, for maize silage (72%) and beet pulp (89%) (De Visser & Hindle, 1990). The higher U-fraction for diet M, in comparison to both beet pulp diets (Table 4.4) did not result in differences in pH or concentration of the major VFA's (Table 4.3).
A lower rate of degradation (k_d) was calculated for diet M, when compared to both beet pulp diets (Table 4.4), but it did not affect rumen fermentation, with regard to pH and the concentration of the major VFA's (Table 4.3).
In this experiment only the OM degradation was measured.
Probably the difference in fermentation pattern between NDF and starch, the increase of VFA, due to a reduced liquid/OM ratio (Sutherland, 1988) and the negative influence of a lower degradability were compensating each other, resulting in similar concentrations of VFA between both beet pulp and the maize silage diets.
The higher U fraction of the OM and the lower rate of OM degradation were thought to be responsible for the reduced total dry matter intake of diet M, compared to both beet pulp rations, as measured in the feeding trial of De Visser & Hindle (1990), because those animals were fed ad libitum. In this experiment the animals were restricted in intake and no differences in DM intake were found.
The lower F fraction and the reduced k_d of diet M resulted in a lower availability of energy components for the synthesis of microbial protein (Van Straalen & Tamminga, 1991), leading to increased concentrations of BCFA, valerate and ammonia (Table 4.3), which agreed with the results of Miller (1982), Van Soest (1982) and Robinson *et al.* (1986). The results of the feeding trial of De Visser & Hindle (1990), in which a reduced milk protein content was found on the maize silage diet, might have been the result of the lower microbial protein synthesis, especially in early lactation, when the animals are fed below both energy and protein requirements.
The level of feed intake significantly influenced rumen fermentation, which can be explained by the larger quantity of rumen fermentable OM, which was available at HLI, compared to LLI (Table 4.3). This agrees with the results of Hodgson *et al.* (1976), who also found increased concentrations of VFA at higher levels of intake. Robinson *et al.* (1986) showed a linear relationship between the OM intake and rumen pool size. The ratio liquid/solids was reduced at higher intakes, increasing concentrations of VFA. At LLI the daily variation of the concentrations were

significantly higher for ammonia, total VFA, NGR, acetate and propionate. This is thought to be a result of the difference in feed intake patterns (Fig.4.1) providing a more constant supply of rumen degradable OM at the high level of intake.
The results of the fermentation and degradation studies confirmed the results of the feeding trial, but more information concerning the degradation of the various OM fractions is required to enable a more precise explanation of fermentation patterns.

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Chapter 5

Autumn-cut grass silage as roughage component in dairy cow rations. 1. Feed intake, digestibility and milk performance

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Autumn-cut grass silage as roughage component in dairy cow rations. 1. Feed intake, digestibility and milk performance

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Abstract

A feeding trial was carried out with 56 dairy cows in second or later lactation. The duration of the experiment was 13 weeks and started immediately after parturition. Basal diets consisted of maize silage, pressed beet pulp, moist ensiled maize gluten feed, ensiled brewers' grains and concentrates, which provided 70% of total dry matter (DM). The remaining 30% of the DM consisted of grass silages, which were fed as supplement to the basal diet. Treatments were wilted grass silage (WGS), moist grass silage ensiled with molasses (MGS), moist grass silage ensiled with formic acid (FGS) and wilted grass silage with added water (WW). The rations were fed as a totally mixed ration (TMR) to avoid selection. All grass silages were harvested between August 30th and September 1st from the same pastures. The moist grass silages differed in chemical composition from the wilted grass silage in ash (higher), crude fibre (lower) and NDF (lower). The in vitro and in vivo digestibility did not differ between silages. Total dry matter intake was lowest for both moist silages (MGS and FGS) and as a consequence net energy intake was also lowest with MGS and FGS. Milk production was highest on WGS and WW, resulting from the higher energy intake. Milk fat production and content did not differ between treatments. Milk protein production was significantly lower for groups fed MGS and FGS. Milk protein content tended to be lower for groups fed FGS and MGS. During the experiment energy balance was negative on all treatments.

Calculation of the Duodenal digestible protein value (DVE), using the recommended equation for grass silage, gave for the WGS silage a better fit with the DVE balance measured in the feeding trial, than found with high moisture silages (MGS, FGS), which appeared to be under-estimated. Bodyweight changes corresponded favourably with net energy balances, after correction of energy values for volatiles in grass silage, using individual net energy values for volatiles instead of an average for grass silage OM.

Introduction

During the winter period wilted grass silage is the most important roughage fed to dairy cows in the Netherlands. Some of the advantages of wilting include the fact that less moisture has to be transported, a lowering in microbial conversions and a reduction in seepage losses. In some countries, e.g. United Kingdom and Ireland

farmers tend to produce moist silages, with or without an additive, due to the climatic conditions which prevail. Several research workers reported good milk performances when feeding high-moisture silages (Gordon, 1980; Burgstaller & Huber, 1984; Honig *et al.*, 1984). In the Netherlands farmers have harvested wilted grass silage for many years with dry matter contents between 35 and 60%. In order to reduce harvesting losses and to limit the risks from bad weather conditions there is an increasing tendency towards more rapid ensiling techniques, one day wilting with or without additives instead of a 2-3 day wilting period. Changing from wilting to wet ensiling systems increases the amount of fermentation products in the silage (Murphy & Gleeson, 1984; Donaldson & Edwards, 1976; Derbyshire *et al.*, 1975). The quality of grass silage with a high-moisture content made without an additive deteriorates rapidly, (Castle & Watson, 1970a, 1970b; Donaldson & Edwards, 1976), due to seepage, increased respiration losses, high pH and increased butyric acid levels. The type of additive used can also influence the amount of fermentation end-products. Using molasses as an additive increases the amount of fermentation end products, due to fermentation of the available sugars (lactic acid and acetic acid), while addition of formic acid reduces the formation of these products, due to reduced activity of micro-organisms as a result of an increase in acidity. Increases in the amount of carbohydrates fermented to lactic acid and volatile fatty acids during ensiling reduce the amount of energy available to microbes in the rumen for synthesis of microbial protein, according to Van Soest (1982) and Miller (1982). Reduction in microbial protein synthesis decreases milk protein percentage and/or production (Robinson *et al*, 1987; De Visser & Tamminga, 1987; De Visser & Hindle, 1990). It was the aim of this study to investigate separately the effects of fermentation during ensiling and dry matter content of grass silages on:

1. Chemical composition and nutritive value (measured *in vivo* and *in vitro*)
2. Silage quality and the amount and type of fermentation products
3. Performance evaluation of grass silages, varying in fermentation products and dry matter, as dietary ingredients in a feeding trial with dairy cows.

Alongside this experiment a comparative study was performed with rumen cannulated dairy cows to investigate the influence of these rations on rumen fermentation characteristics (fermentation pattern, degradability and kinetics). The results of the rumen studies will be reported in a subsequent paper (De Visser *et al.*, 1993).

Materials and methods

On August 30th and September 1st three types of grass silage were made from previously cut pastures, one was a strongly wilted grass silage, the other two were high moisture silages with additives (cane molasses or formic acid). After mowing, one quarter of each pasture was dried to approximately 22 to 25% dry matter in

an attempt to avoid seepage losses. The grass was tedded once prior to harvesting. During harvesting molasses (MGS) was applied at a rate of approximately 40 kg per tonne fresh grass. The second quarter of each pasture was harvested at the same time and ensiled with addition of formic acid (6 litres per tonne fresh grass) (FGS). The remaining half of each pasture was wilted to a dry matter content of approximately 45 to 50% and ensiled without an additive (WGS). The grass was wilted for 36 to 40 h. All silages were harvested with a precision chop harvester and ensiled in long small drive-over silos. Each silo was sealed with two layers of plastic sheeting. The grass was cut at approximately 3000 kg DM per hectare, consisted of approximately 95% of *Lolium perenne* and was fertilized after the previous cut with 80 kg N/ha^{-1}.

During the first 13 weeks of lactation a feeding trial was performed with 56 dairy cows. The animals, Dutch Friesian or Dutch Friesian x Holstein breed, were in second (approx. 60%) or later lactation. A blocked experimental design was used. Each block consisted of four animals grouped in accordance with their previous lactation performances. Within each block the animals were allocated at random to one of the four treatment groups. The animals were kept in a loose housing system with Calan electronic feeding doors.

The basal diet, supplying 70% of the total dry matter, consisted of maize silage, ensiled pressed beet pulp, moist ensiled maize gluten feed, moist ensiled brewers'grains and concentrates. The remaining 30% of the diet was composed of wilted grass silage (WGS), grass silage with molasses (MGS), grass silage with formic acid (FGS), or wilted grass silage with extra water (WW). This water was added during the preparation of the diet. The amount of water added to diet WW was equal to the difference in water content measured between the wilted grass silage (WGS) and the grass silage with molasses (MGS). The dietary compositions are given in Table 5.1. All diets were fed as totally mixed rations (mixer/forage wagon) to avoid selection and fed at 5.00 h (40%) and 15.00 h (60%) respectively. The animals were fed individually and feed refusals recorded daily. The ingredients contained in the concentrate mixture are listed in Table 5.2. Pressed beet pulp, moist ensiled maize gluten feed, moist ensiled brewers' grains and concentrates were sampled once a week and analyzed for dry matter (DM) content. The grass and maize silages were sampled twice weekly and analyzed for DM. These weekly samples were subsampled over a monthly period. These monthly samples were analyzed for ash, nitrogen, crude fat, crude fibre, neutral detergent fibre (NDF), starch and sugars, as described earlier (De Visser & Hindle, 1990). Grass silages, maize silage and ensiled moist by-product feedstuffs were regularly analyzed for volatile fatty acids, alcohols, lactic acid, ammonia and pH to enable corrections to dry matter content and energy value due to losses of volatile components. The analytical methods used for volatile fatty acids, alcohols, lactic acid and ammonia were as described by Robinson *et al.* (1986).

The *in vitro* OM-digestibility was determined for all dietary components using the Tilley & Terry method (1963), as modified by Van der Meer (1980).

In vivo digestibility of the grass silages was also measured, using wethers fed at maintenance, according to the method described by Van Es & Van der Meer (1980). The energy values of the grass silages were calculated, using the *in vivo* digestibility measurements. These energy values were corrected for losses of volatiles using two methods. In the first method the energy of the volatiles was assumed to be 100% digestible grass silage OM, while in the second method the energy value was calculated using the individual energy values for the constituent volatile components as published in the Dutch Feed Table (CVB, 1991a).

Table 5.1 ***Composition of the diets (g/kg dry matter)***

Ingredient	Group			
	WGS	MGS	FGS	WW[1]
Wilted grass silage	300	-	-	300
Grass silage (molasses)	-	300	-	-
Grass silage (formic acid)	-	-	300	-
Maize silage	140	140	140	140
Pressed beet pulp (ensiled)	200	200	200	200
Maize gluten feed (ensiled)	150	150	150	150
Brewers'grains (ensiled)	100	100	100	100
Concentrates	110	110	110	110

[1] Group WW received extra water, which was added to the complete diet

Table 5.2 ***Composition of the concentrate mixture***

Ingredient	g per kg
Coconut expeller	405
Soya bean hulls	100
Linseed expeller	200
Soya bean meal (solv. extr.)	145
Fat (animal)	33
Molasses (cane)	40
Calcium carbonate	17
Vitamins and minerals	20
Salt (NaCl)	20
Magnesium oxide	20

Milk production was recorded at four consecutive milkings, using a Tru tester (Moderniek bv, Soest). Subsamples were taken in which the fat-, protein- and lactose contents were analyzed (Stichting Melkcontrole Station Noord, the Netherlands). Animals were weighed twice weekly to record bodyweight and calculate bodyweight changes during the experiment. Net energy intake, net energy requirement for maintenance and milk production and body weight change were used to calculate energy balance according to the Dutch net energy system (Van Es, 1978).
Animal performances were also used to compare the digestible crude protein (dcp) and the Duodenal digestible protein systems (DVE) (Van Straalen & Tamminga, 1991). The DVE values of the roughages, moist ensiled by-product ingredients and concentrates were calculated according to equations (Anonymous, CVB nr. 7, 1991b) and values recorded in the Dutch Feeding Table (Anonymous, CVB, 1991a). All resulting data were subjected to statistical analysis for a blocked experimental design (Genstat; Alvey *et al.*, 1982).

Results

The chemical composition of the various grass silages is shown in Table 5.3.
WGS silage showed the lowest ash- and highest cell wall contents (crude fibre, NDF). Sugar content was highest in FGS and lowest in MGS. The concentration of volatile components differed between silages. A relatively large proportion of the organic matter consisted of volatiles in MGS silage, compared to WGS and FGS (Table 5.4).
The *in vivo* and *in vitro* OM-digestibility did not differ between silages. The results from both methods showed a high degree of correlation ($R^2 = 0.99$).

Table 5.4 ***Volatile components in grass silage (g per kg DM) and pH***

Grass silage	pH	ac	but	lact	NH_3	alc
Wilted (WGS)	4.22	10.3	0.2	36.1	10	2.2
Molasses (MGS)	3.98	18.4	0.2	118.6	8	7.2
Formic acid (FGS)	4.15	19.4	2.0	25.8	5	6.5

ac = acetic acid
but = butyric acid
lact = lactic acid
alc = alcohols
NH_3 = Ammonia-nitrogen fraction

Table 5.3 ***Chemical composition and in-vitro digestibility of the dietary components (g per kg dry matter)***

Ingredient	DM[1]	ash	N	CF	cfat	NDF	starch	sugar	*vitro*
Grass silages									
Wilted	456±49	113±13	30±4	268±14	39±3	492±20	-	26±12	73±1
Molasses	246±28	156±11	31±1	222±6	42±3	412±9	-	13±2	73±1
Formic acid	233±26	146±16	32±3	229±11	46±2	429±20	-	41±8	73±1
Maize silage	269±11	57± 6	13±1	227±12	25±1	453±17	259±5	-	71±1
Beet pulp	213±11	86±16	16±1	196±5	-	530±19	-	13±4	87±1
Maize gluten f.	401±19	61±1	34±1	103±11	22±1	435±14	168±15	10±2	88±1
Brewers'grains	229±17	53±8	44±4	161±18	100±8	614±40	22±4	-	56±2
Concentrates	898±6	139±12	37±1	127±5	83±5	360±16	17±6	88±7	80±1

Maize gluten f. = ensiled moist maize gluten feed
Beet pulp = ensiled pressed beet pulp
Brewers'grains = ensiled brewers'grains
456±49=456 and a standard deviation of 49
vitro = modified Tilley and Terry *in vitro* digestibility
[1] ensiled products corrected for losses due to volatile components

The *in vivo* digestibilities of nitrogen, crude fibre, crude fat, NDF and N-free extract did not differ between silages (Table 5.5). According to the Dutch Net Energy system the calculated net energy values differed between grass silages, when volatiles were calculated as 100% digestible OM of grass silage (first method), but were similar when using individual energy values for volatile components (Anonymous, 1991b) (second method) (Table 5.6).

Table 5.5 ***In vivo and in vitro digestibility of wilted grass silage and wet grass silages with molasses or formic acid (%), without correction for losses of volatiles***

Grass silage	*vitro*	OM	N	CF	cfat	N-free extract	NDF
Wilted (WGS)	73±1	75±1	75±1	77±3	64±1	74±1	73±3
Molasses (MGS)	73±1	75±1	75±1	77±4	63±1	75±1	72±3
Formic acid (FGS)	73±1	75±1	74±1	78±4	68±1	75±1	72±3

vitro = *in vitro* Tilley & Terry (1963), modified IVVO (1980)
cfat = petroleum ether 40:60°C; 75±1 = 75 and a standard deviation of 1

Table 5.6 ***Net energy values (MJ per kg DM), dcp and DVE values (g per kg DM) of wilted grass silage, high moisture grass silage with molasses or formic acid***

Grass silage	Net energy values		dcp	DVE
	Method 1	Method 2		
Wilted (WGS)	5.86	5.70	141	68
Molasses (MGS)	5.83	5.30	146	47
Formic acid (FGS)	5.63	5.41	148	52

Energy value correction for volatile components:
Method 1: volatiles assumed 100% digestible and allocated an average energy value for OM;
Method 2: volatiles assumed 100% digestible and allocated individual energy values per component, in accordance with CVB Table, 1991;
dcp = digestible crude protein; DVE = Duodenal digestible protein

The protein values of the grass silages were calculated as dcp as well as DVE and are shown in Table 5.6. The dcp values were similar for all silages, but DVE values were highest in WGS silage and lowest in MGS silage. Average dry matter content in the four TMR rations were 399, 336, 331, 336 g per kg for WGS, MGS, FGS and WW diets respectively.

Table 5.7 ***Daily feed intake, milk production, milk composition and bodyweight for the 13 week experimental period (mean of 14 animals)***

	WGS	MGS	FGS	WW	SED
Total DM intake (kg)	21.9^{a}	19.6^{b}	20.0^{b}	21.0^{a}	0.53
Grass silage (kg)	6.5	6.0	6.1	6.3	0.15
Net energy intake (MJ)[1]	143^{a}	130^{b}	129^{b}	137^{a}	3.74
dcp intake (g)	2664^{a}	2466^{b}	2533^{b}	2570^{a}	74.0
DVE intake (g)	1894^{a}	1573^{b}	1636^{b}	1816^{a}	65.0
Milk (kg)	35.9^{a}	34.3^{b}	34.4^{b}	35.8^{a}	0.64
Fat (g)	1640	1570	1572	1664	67.8
Protein (g)	1152^{a}	1077^{b}	1080^{b}	1138^{a}	24.7
Lactose (g)	1680^{a}	1581^{b}	1592^{b}	1682^{a}	32.8
Fat (%)	4.57	4.58	4.57	4.65	0.16
Protein (%)	3.21	3.14	3.14	3.18	0.05
Lactose (%)	4.68^{ac}	4.61^{b}	4.63^{bc}	4.70^{a}	0.03
PFCM (kg)	38.0^{a}	36.2^{b}	36.2^{b}	38.1^{a}	1.02
Energy intake/req. ratio[1]	91^{a}	86^{b}	86^{b}	87^{b}	2.49
Energy intake/req. ratio[2]	90^{a}	83^{b}	84^{b}	86^{b}	2.13
dcp intake/req. ratio	97	93	96	96	2.90
DVE intake/req. ratio	103^{a}	94^{b}	97^{ab}	102^{a}	2.50
Bodyweight (kg)	607	588	581	616	19.0
Bodyweight loss (g)	290	460	440	450	70.6

Figures with a different superscript differ significantly ($P<0.05$)
Net energy intake = intake in Net Energy Lactation (van Es, 1978)
dcp intake = intake of digestible crude protein
[1] Energy intake/req. ratio = intake/requirement ratio (%) calculated with individual energy values of volatiles
[2] Energy intake/req. ratio = intake/requirement ratio (%) calculated with average energy value of OM
dcp intake/req. ratio = intake/requirement ratio (%)
DVE intake/req. ratio = intake/requirement ratio (%)
PFCM = Protein and fat corrected milk; SED = standard error of difference

Feed intake and milk performance results are shown in Table 5.7. Total dry matter intake was significantly lower on both high moisture grass silage diets (MGS, FGS), compared to diets WGS and WW, as was the Dutch net energy (using the second method) and protein intake (dcp, DVE). Milk production was highest for cows fed WGS and WW. Milk fat percentage and production did not differ between treatments. Milk protein production was significantly lower on both of the high moisture silages (MGS, FGS), while milk protein percentage tended to be lower on treatments MGS and FGS, than WGS. Milk production corrected for fat and protein content (PFCM) was lowest on MGS and FGS.
All treatments showed a calculated negative net energy balance for the duration of the trial.

Discussion

Although the ash content in the silages was approximately 140 g per kg DM and as such relatively high, the values fell within the range accepted for autumn grown grass silages (100-155 g per kg DM), as found in commercially analysed field tests in the Netherlands (Van Dijk; Bedrijfslaboratorium voor Grond- en Gewasonderzoek, pers. comm.).
The comparatively lower ash content found in WGS as opposed to the other grass silages (MGS, FGS) was probably caused by the removal of excess soil (ash) during the wilting process. Ash content in MGS silage was 10 g per kg DM higher than that found in FGS silage. This difference in ash content between MGS- and FGS silage could be explained by excess ash present in the molasses, which was added to the MGS silage (Anonymous, 1991b). The higher crude fibre and NDF contents found in WGS silage agreed with results from earlier laboratory studies by Van Vuuren *et al.* (1989). They concluded, that the drying process itself positively influenced NDF levels. Probably, due to inducing the formation of a nitrogen sugar complex (Maillard reaction).
MGS silage contained higher amounts of fermentation end-products compared to WGS and FGS silage, which was in agreement with results from Donaldson & Edwards (1976) and Murphy & Gleeson (1984). This was reflected in the lower amount of sugars measured in MGS silage, in spite of the higher sugar content of MGS silage at the time of ensiling (grass and molasses). Although 6 liters of formic acid were added per ton fresh grass in FGS silage a relatively large proportion of fermentation end products could still be measured, in comparison with the MGS silage, this was in disagreement with Hinks & Henderson (1984).
Several researchers reported lower energy values, as well as higher concentrations of butyric acid in wilted silage compared to wet ensiled grass silage made with an additive (formic acid) (Gordon, 1989; Zimmer & Wilkins, 1984). However, the results of these experiments showed large differences in duration of wilting, weather conditions (temperature, rainfall, etc.) and increased DM-content as a

result of wilting compared to our experiment. Under poor weather conditions the use of formic acid had a positive influence on the quality of wilted grass silage (Gordon, 1989; Zimmer & Wilkins, 1984). When comparing wilted silages with high moisture silages ensiled using an additive the weather conditions should be taken into account, because it is evident that poor weather conditions have a negative influence on the results.

The *in vitro* and *in vivo* OM-digestibilities were highly correlated and fell well within the range of reported results (Burgstaller & Huber, 1984; Donaldson & Edwards, 1976; Murphy & Gleeson, 1984 and Steg *et al.*, 1990). The *in vivo* digestibilities of nitrogen, crude fibre, crude fat, NDF and N-free extract were similar for all silages (Table 5.5) and agreed with the results of Burgstaller *et al.* (1984) and Steg *et al.* (1990). The energy values of the grass silages were substantially influenced by the calculation method employed to estimate the energy value of the volatiles (Table 5.6). Energy values are under-estimated (first method), when grass silages contain large quantities of volatiles, especially alcohols. This observation agreed with Zimmer & Wilkins (1984), reviewing data of various experiments comparing wilted to wet silages. They also concluded that energy values as well as DM content will be under-estimated when alcohols and volatiles form a substantial part of the dry matter.

Although 70% of the total ration was similar for all diets, the DM intake on both high moisture grass silages (MGS, FGS) was significantly lower, compared to WGS and WW. These findings agreed with the results found by Burgstaller & Huber (1984), Gordon (1980) and Zimmer & Wilkins (1984), when comparing wilted grass silage to high moisture grass silage diets. The tendency towards lower DM intake by group WW and the significantly lower DM intake on MGS and FGS as compared to WGS agreed with earlier findings of Lahr *et al.* (1983) and De Visser *et al.* (1987, 1990). They found a negative relationship between the dry matter content of total rations and DM intake, when DM content was below 40%. Total DM intake seemed to be related to total dry matter content (WW) as well as to dry matter content and fermentation end products present in grass silages (MGS, FGS). The first seemed more related to the capacity of the cow to consume large quantities of food in early lactation (bulk), while a combination of lower DM content and higher amounts of fermentation end products seem to reduce the DM intake more permanently.

The lower DM intake on cows fed MGS and FGS diets resulted in lower net energy (second method) and protein (dcp, DVE) intakes, which negatively affected milk yield and milk protein quantity (Table 5.7).

The animals fed MGS and FGS diets mobilized more energy reserves, which was reflected in a larger body weight loss (Table 5.7). During this period more long chain fatty acids were mobilized from adipose tissue. Probably, these extra long chain fatty acids were efficiently incorporated into milk fat by the mammary gland (Palmquist & Conrad, 1971; Vernon, 1988), compensating the lower energy intake. As a result milk fat content and productions did not differ significantly between

diets.
The lower rumen digestible OM intake (Table 5.5; Table 5.7), the increased mobilisation of body reserves (long chain fatty acids; Table 5.7) and the reduced protein intake (DVE) (Table 5.7) resulted in lower glucogenic/ketogenic and aminogenic/ketogenic nutrient ratios for cows fed high moisture silage diets (MGS, FGS), which probably resulted in a reduction of glucose and amino acids available for milk lactose and milk protein production (Vernon, 1988). This was reflected in a significantly lower milk production (lactose) as well as lower milk protein output. These results agreed with those of Broster & Thomas (1981), who also observed a lower milk protein content, when feeding below energy and protein requirements. The lower calculated DVE intakes on MGS and FGS diets agreed with the lower milk protein production and protein content and support the hypothesis of reduced microbial protein synthesis, which was reflected in higher concentrations of ammonia and branched chain fatty acids (BCFA) and valerate found in the rumen fluid of cannulated dairy cows fed the same diets (De Visser *et al.*, 1992). These higher concentrations also agreed with previous results, in which lower microbial protein synthesis was measured in relation to increased concentrations of ammonia, BCFA and valerate (De Visser *et al.*, 1987, 1991; Robinson *et al.*, 1987). However, our results disagree with those of Peoples & Gordon (1989), Steen & Gordon (1980), Gordon & Peoples (1986) and Zimmer & Wilkins (1984), who found similar milk protein outputs, when feeding wilted or high moisture grass silages. The discrepancy between our results and the literature mentioned, might be explained by differences in the fed supplement. In our experiment the grass silages were supplemented with by-products, partly consisting of moist ensiled products (Table 5.1). The supplements referred to in the literature consisted mainly of barley and soya bean meal. The amount of rumen fermentable carbohydrates, starch and sugars, was relatively low in our experiment (Table 5.3). However, when feeding supplements containing large quantities of barley a substantial amount of the carbohydrates became rapidly available for rumen fermentation, thus compensating for the loss of easily fermentable carbohydrates during the ensiling process of high moisture grass silages into fermentation end products. As a result a larger amount of nitrogen available as ammonia in the rumen after degradation of grass silage protein, might be incorporated into microbial protein (Van Straalen & Tamminga, 1990, Nocek & Russell, 1988). The new Dutch protein system (DVE, Van Straalen & Tamminga, 1991) proved to be more accurate in predicting protein balance of WGS and WW than was the dcp system (Table 5.7). However, it was less accurate for moist ensiled grass silage made with an additive. The equations for grass silage used in the Dutch DVE system were derived from wilted grass silage data (Van Straalen, pers. comm.), which might explain the difference found in this experiment.
Body weight changes on both the moist treatments (MGS, FGS) did not correspond with the energy balances calculated according to the Dutch net energy system (Van Es, 1978), using energy values for volatiles as 100% digestible grass silage

OM (first method). Yet when the energy values for individual volatile components (CVB Table, anonymous, 1991) were used to calculate net energy intake (second method) and net energy balance, they compared more favourably with the observed changes in body weight. The ensiling method influenced total dry matter- and energy intake, as well as milk production. The energy values of grass silages should be calculated using individual energy values for volatiles. The type of supplement used, should be optimized allowing for rumen fermentable carbohydrates to avoid negative influences on milk protein output.

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Chapter 6

Autumn-cut grass silage as roughage component in dairy cow rations. 2. Rumen degradation, fermentation and kinetics

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Abstract

A 4x4 Latin square experiment was performed to study the effects of dry matter content and/or the extent of fermentation in grass silages on the pattern of rumen fermentation and rumen kinetics. In a separate study two animals were used to measure the rate of degradation using the dacron bag technique. Four rumen cannulated dairy cows were used to measure rumen fermentation pattern, rumen kinetics were measured in three of these animals. The basal diets (70% of total DM) consisted of maize silage, moist ensiled beet pulp, moist ensiled maize gluten feed, moist ensiled brewers'grains and a concentrate mixture. The remainder of the diet (30% of total DM) consisted either of wilted grass silage (WGS), high moisture grass silage with molasses (MGS), high moisture silage with formic acid (FGS) or wilted grass silage with additional water (WW). All diets were fed as totally mixed rations (TMR). The pH of the rumen fluid was lower on the MGS and FGS diets. The concentrations of total VFA, acetic acid, ammonia and branched-chain fatty acids (BCFA) were highest on high moisture diets (MGS and FGS). The rates of clearance and digestion of the OM fractions were or showed tendencies towards being negatively influenced by both high moisture grass silages (MGS, FGS), but remained uneffected by DM content (WGS, WW). Degradability of the grass silages was influenced by fermentation in the silo (lower digestible fractions and higher soluble fractions), as were the rates of degradation (higher). Results of the degradability measured on the basal diet ingredients (maize silage, beet pulp, maize gluten feed, brewers' grains) were in agreement with literature published and showed a strong correlation between in vitro OM digestibility and the undigestible fraction.

Introduction

During the winter period grass silage is the most important roughage component in Dutch dairy diets. These diets are supplemented with by-product based concentrate mixtures to meet energy and protein requirements. The concentrates are fed dry, but occasionally are partly replaced by moist ensiled by-products. In general, the chemical composition of these moist concentrates is similar to the dried by-products (De Visser & Steg, 1988). However, during the ensiling process easily fermentable components, such as sugars and rapidly degradable starch and protein, are fermented by micro-organisms and are transformed into volatile fatty

acids, lactic acid and alcohols (De Visser & Tamminga, 1987; De Visser & Hindle, 1990). Likewise, the energy available in the roughage of dairy diets can easily be converted into fermentation end products with a concomitant reduction of easily fermentable carbohydrates. Highest concentrations of fermentation end products were found in high moisture silage as compared to wilted grass silage (Donaldson & Edwards, 1976; Murphy & Gleeson, 1984; De Visser & Hindle, 1992). Feeding such high moisture diets to dairy cows in early lactation may reduce milk protein output (De Visser & Tamminga, 1987; De Visser & Hindle, 1992). In a fermentation study, Robinson *et al.* (1986) found that lower amounts of microbial protein were synthesized in the rumen of dairy cows fed moist instead of dry by-products. In addition to a feeding trial comparing grass silages either wilted, or moist ensiled with additives, molasses or formic acid, (De Visser & Hindle, 1992), a rumen fermentation and kinetic study was performed. The aim was to investigate the influence of the moisture content and fermentation end products in silages on rumen fermentation pattern and kinetics as well as rumen degradability of various organic matter fractions.

Material and methods

Four dairy cows of the Dutch Friesian Black and White x Holstein breed were used in a 4x4 Latin square design. Three animals were fitted with a large rumen cannula (10 cm i.d. Bar-Diamond Inc., Parma, ID, USA); the fourth animal was equipped with a small rumen cannula (5 cm i.d. Eriks, Alkmaar, Netherlands). They were housed in a tie stall and offered a totally mixed ration (TMR), using a mixer-forage wagon to minimize selection of dietary intake. The rations fed were similar to those of an accompanying feeding trial (De Visser & Hindle, 1992). The basal diet which supplied 70% of total dry matter intake (DMI) consisted of maize silage (14% DMI), moist ensiled beet pulp (20% DMI), moist ensiled maize gluten feed (15% DMI), moist ensiled brewers'grains (10% DMI) and concentrates (11% DMI). The concentrate-mixture consisted of coconut expeller (40.5%), soya bean hulls (10%), linseed expeller (20%), soya bean meal solv. extr. (14.5%), tallow (3.3%), cane molasses (4%), calcium carbonate (1.7%), magnesium oxide (2%), sodium chloride (2%) and vitamins and minerals (2%). In addition to the basal diet four different grass silages were fed. Treatments consisted of wilted grass silage (WGS), moist grass silage with molasses (MGS; 40 kg per ton fresh grass), moist grass silage with formic acid (FGS; 6 litres per ton fresh grass) and wilted grass silage with added water (WW). This water was added during the preparation of the diet and was equal to the difference in water content between the wilted grass silage and the grass silage with molasses. All grass silages were harvested from the same pastures. Preparation and storage of the silages were as described by De Visser & Hindle (1992). The animals were fed twice daily. Forty percent of total DM was offered at 5.00 h and 60 percent at 14.00 h respectively. Feed intake level was

approximately 20 kg DM.
The design of the experiment is given in Table 6.1. Each experimental period lasted for 6 weeks. Four weeks were used for adaptation to the diet. During the 5th week rumen fermentation was studied, the 6th week was used to determine rumen kinetics.

Table 6.1 ***Experimental design: rumen fermentation and kinetic study***

Period	Cow numbers			
	744[1)]	829	843	1563
1	WGS	MGS	FGS	WW
2	MGS	FGS	WW	WGS
3	WW	WGS	MGS	FGS
4	FGS	WW	WGS	MGS

1) Cow fitted with a small cannula which was not used in the kinetic study
Treatments: WGS = wilted grass silage
MGS = moist grass silage with molasses
FGS = moist grass silage with formic acid
WW = wilted grass silage with extra water

During the last two weeks samples were taken from all diet ingredients and analysed for DM, ash, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), indigestible acid detergent fibre (IADF), sugars, starch and volatiles. The analytical methods used were as described by Robinson *et al.* (1986, 1987) and De Visser & Hindle (1990).

rumen fermentation
A period of 48 hours was taken to determine the fermentation pattern with 17 samples of the rumen fluid as described by De Visser *et al.* (1991). Samples were taken at 5.00, 7.00, 10.00, 14.00, 16.00, 19.00, 22.00, 2.00 and 5.00 h respectively. The samples were immediately analysed for pH, using a pH-meter (Philips 2000). Subsamples were taken, preserved and stored at -20°C for the analysis of volatile fatty acids (VFA), lactic acid (LA) and ammonia (NH_3-N) (Robinson *et al.*, 1986). The ratio between the non-glucogenic and glucogenic fatty acids (NGR) was calculated as described by Ørskov *et al.* (1975).
Statistical analyses were performed using cow, period and diets as explanatory variables with mean daily values (weighted for time intervals) and daily variations (expressed as standard deviation of daily values), using the statistical package Genstat (Alvey *et al.*, 1982).

rumen kinetics

Over a period of 36 hours the average daily rumen mass was calculated using manual evacuation of total rumen contents on three occasions (4.00, 10.00 and 20.00 h). Between evacuations animals had access to food and received at least one meal. The method of evacuation was as described by Robinson *et al.* (1987). During the evacuations a sample was taken of the total rumen mass, which was stored at -40°C, freeze dried and analysed for ash, N, NDF, ADF, ADL and IADF. The total VFA content (g) of the rumen was calculated using two methods. The first method estimated VFA by multiplying total rumen fluid measured by rumen evacuations by the concentration of VFA in the rumen fluid, collected immediately before rumen evacuation (total rumen VFA using fluid: TRVFAF). In the second method the concentration of VFA was estimated after an overnight extraction with water in a subsample (100 g) of evacuated rumen mass (H. Huisert & S.F. Spoelstra, 1987). The concentration analyzed was multiplied by total rumen mass (total rumen VFA using rumen mass: TRVFAM).

Clearance, passage and digestion of OM, N, NDF, ADF, lignin (ADL), digestible acid detergent fibre (DADF), hemicellulose (NDF-ADF) and cellulose (ADF-ADL) were calculated as:

- rate of clearance (k_c) = ((feed intake, kg/d)/(average rumen pool, kg))/24
- rate of passage (k_p) = ((IADF intake, kg/d)/(average rumen IADF pool, kg))/24
- rate of digestion (k_d) = k_c-k_p)

Because only 3 animals were fitted with a large rumen cannula, the design of the experiment was not completely orthogonal (Table 6.1). Analysis of variance was performed, using cow, period and diet as dependant variables for rumen kinetic parameters, using the statistical package Genstat (Alvey *et al.*, 1982). The values missing from the fourth animal were estimated as part of the statistical analysis.

rumen degradability

The degradability of all dietary ingredients was measured using the nylon bag technique. The method used was as described by Van Vuuren *et al.* (1989). The degradability was measured for OM, N and NDF, using two dairy cows fitted with a large rumen cannula (10 cm i.d. Bar-Diamond Inc., Parma, ID, USA). These cows were in the 6th month of lactation and were fed the intermediate diet of the rumen fermentation study (1/3 WGS + 1/3 MGS + 1/3 FGS), offered as TMR. Feeding procedures were similar to those of the rumen fermentation study. The animals received approximately 18 kg DM/day.

The incubation times were 0, 2, 4, 6, 12, 18, 24, 48 and 216 hours. The fractions were divided into a soluble fraction (S), an undigestible fraction (U) and a potentially digestible fraction (D = 100-S-U). The rate constant (k_d) of D was estimated by iteration (Robinson *et al*, 1987).

Table 6.2 ***The chemical composition of total diets (g/kg dry matter).***

	Diets			
	WGS	MGS	FGS	WW
Chemical composition total diet				
Dry matter (g/kg)	398	335	331	332
Ash	88	101	98	88
Nitrogen	28	28	28	28
Crude fat	38	38	39	38
Crude fibre	196	183	185	196
Neutral detergent fibre (NDF)	483	459	464	483
Acid detergent fibre (ADF)	228	210	215	228
Acid detergent lignin (ADL)	22	21	20	22
Indigestible acid detergent fibre (IADF)	36	32	31	36
Starch	66	66	66	66
Sugars	22	18	26	22
Vitro OM [1] (%)	77	77	77	77
Chemical composition grass silages				
Dry matter (g/kg)	456	246	233	
Ash	113	156	146	
Nitrogen	30	31	32	
Crude fat	39	42	46	
Crude fibre	268	222	229	
Neutral detergent fibre (NDF)	492	412	429	
Acid detergent fibre (ADF)	286	234	250	
Acid detergent lignin (ADL)	25	20	18	
Indigestible acid detergent fibre (IADF)	54	41	38	
Sugars	26	13	41	
Vitro OM (%)	73	73	73	
Volatile content grass silages				
Acetate	10	18	19	
Butyrate	0	0	2	
Lactate	36	118	26	
Ammonia	3	2	1	
Alcohols	2	7	7	

WGS = Wilted grass silage; MGS = Moist grass silage with molasses;
FGS = Moist grass silage with formic acid; WW = Wilted grass silage with extra water
1) *vitro* OM = digestibility measured *in vitro* (Tilley and Terry, 1963) as modified by IVVO (Van der Meer, 1980)

Results

Apart from the DM content, variations in chemical composition of the diets were limited; only minor differences were observed in ash and cell wall fractions (Table 6.2). The chemical composition of the basal diet ingredients, maize silage, pressed ensiled beet pulp, ensiled maize gluten feed, ensiled brewer's grains and the concentrate mixture are shown in Table 6.3. However, the chemical composition in the grass silages varied (Table 6.2).
Wilted grass silage was highest in DM and cell wall fractions compared to both high moisture silages. Grass silages also differed in volatile content. Especially, MGS silage contained large quantities of lactic acid (Table 6.2). Both high moisture silages (MGS, FGS) showed higher quantities of acetic acid and alcohols (Table 6.2).

Table 6.3 ***Chemical composition of basal diet ingredients (g/kg dry matter)***

	Maize silage	Beet pulp	Maize gluten feed	Brewers' grains	Concentrate mixture
Dry matter (g/kg)	269	213	401	229	898
Ash	57	86	61	53	139
Nitrogen	13	16	34	44	37
Crude fat	25	--	22	100	83
Crude fibre	227	196	103	161	127
Neutral detergent fibre (NDF)	453	530	435	614	360
Starch	259	--	168	22	17
Sugars	--	13	10	--	88
Vitro OM (%)	71	87	88	56	80

Beet pulp = moist ensiled pressed beet pulp
Maize gluten feed = moist ensiled maize gluten feed
Brewers'grains = moist ensiled brewers'grains

The results of the rumen fermentation study are shown in Table 6.4. Mean pH was lowest for both high moisture silage diets (MGS, FGS), whereas the mean concentration of total VFA was highest for these diets. The NGR tended to be lower for MGS and FGS reflecting changes in the major VFA's (acetic and propionic acid). The concentration of NH_3-N, branched-chain fatty acids and valerate (BCFA) was higher for high moisture diets (MGS, FGS). Lactic acid tended to be higher in cows fed MGS. Except for the NH_3-N concentrations, no significant effect on ranges in fermentation parameters were observed. The total amount of VFA present in the rumen was highest for TRVFAM, compared to TRVFAF (Figure 6.1).

Table 6.4 ***Rumen characteristics (pH, osmolality and concentrations of volatile fatty acids, lactic acid, ammonia and branched-chain fatty acids (mMol/l).***

Treatments	WGS	MGS	FGS	WW	SED
DM intake (kg/ day)	20.7	20.5	20.8	20.6	0.80
pH, mean	6.10[a]	5.98[b]	6.03[ab]	6.12[a]	0.03
pH, range	0.35	0.34	0.33	0.33	0.03
Osmolality, mean	0.321[a]	0.333[b]	0.328[b]	0.320[a]	0.001
Osmolality, range	0.03	0.03	0.03	0.02	0.002
Total VFA, mean	121[a]	129[b]	130[b]	120[a]	3.03
Total VFA, range	15.5	16.5	17.9	14.3	1.88
NGR [1], mean	4.64	4.44	4.50	4.63	0.16
NGR, range	0.63	0.54	0.58	0.62	0.05
Lactate, mean	2.74	3.19	2.29	1.79	0.63
Lactate, range	4.45	4.44	3.95	2.81	1.48
Ammonia, mean	9.27[ab]	10.41[b]	9.86[ab]	8.99[a]	0.37
Ammonia, range	7.26[a]	7.58[a]	7.25[a]	6.50[b]	0.25
Acetate, mean	79[b]	82[b]	84[b]	76[a]	1.80
Acetate, range	7.87	8.14	9.70	7.75	1.27
Propionate, mean	23	26	25	23	1.06
Propionate, range	5.00	5.16	5.30	4.63	0.29
Butyrate, mean	16	18	17	17	0.51
Butyrate, range	2.01	2.44	2.97	2.05	0.34
BCFA, mean	3.16[a]	3.83[b]	3.97[b]	3.33[a]	0.23
BCFA, range	0.44	0.46	0.43	0.41	0.03

1) NGR = non-glucogenic glucogenic ratio (Ørskov, 1975)
Figures with a different superscript are significantly different ($p < 0.05$)
SED = standard error of difference
mean = mean daily values (weighted for time intervals)
range = calculated as standard deviation of daily values
BCFA = branched-chain fatty acids (iso-butyrate, 2-and 3-methyl butyrate and valerate)

Rumen pool sizes of different OM components did not differ between diets, except for ADL and IADF (Table 6.5). ADL and IADF followed the same pattern and were lowest for both moist diets (MGS and FGS). The clearance rate of OM, NDF, ADF, DADF, hemicellulose and cellulose was lower or tended to be lower for the groups fed high moisture silage (MGS, FGS, Table 6.6). The rate of clearance was similar for both ADL and IADF fractions. Substractions, using the average rate of clearance of the IADF and ADL fractions as the rate of passage of particles, resulted in tendencies towards reduced rates of digestion of the OM, NDF, ADF, DADF, hemicellulose and cellulose fractions on high moisture diets (MGS, FGS; Table 6.6).

Table 6.5 ***Total dry matter intake (TDM) and average rumen pool sizes of dry matter (DM), organic matter (OM), nitrogen (N) and cell wall constituents***

Treatment	WGS	MGS	FGS	WW	SED
TDM intake (kg)	24.4	23.7	23.5	23.8	1.16
Body weight (kg)	610	595	588	612	
DM pool/kg bodyweight (g)	18.7	18.0	18.5	18.5	
Total rumen contents					
Non-dry matter (kg)	74.5	75.4	73.5	73.0	2.65
Dry matter (kg)	11.2	11.1	10.9	11.0	0.51
Total ingesta (kg)	85.9	86.5	84.4	84.0	3.16
Percentage DM	13.1	12.8	12.9	13.1	0.17
Rumen pool sizes					
OM (kg)	10.1	10.0	9.7	10.0	0.46
N (g)	394	372	383	398	10.32
NDF (kg)	5.9	5.6	5.4	5.7	0.32
ADF (kg)	3.2	3.1	2.8	3.2	0.21
ADL (kg)	0.56[a]	0.52[b]	0.47[c]	0.53[b]	0.005
IADF (kg)	0.96[a]	0.89[b]	0.73[c]	0.93[a]	0.05
DADF (kg)	2.3	2.3	2.1	2.3	0.21
Hemicellulose (kg)	2.7	2.3	2.5	2.7	0.21
Cellulose (kg)	2.6	2.6	2.5	2.7	0.27

Figures with a different superscript differ significantly ($p<0.05$)
SED = standard error of difference

Table 6.6 ***Turnover of organic matter (OM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), digestible acid detergent fibre (DADF), hemicellulose and cellulose calculated from rumen evacuation data (rates %/hour)***

Treatment	WGS	MGS	FGS	WW	SED
ADL rates					
$k_{PASSAGE}$	4.1	3.9	3.8	4.2	0.10
IADF rates					
$k_{PASSAGE}$	4.0	3.70	3.8	4.0	0.43
OM rates					
$k_{CLEARANCE}$	9.3	8.8	8.4	9.2	0.45
$k_{PASSAGE}$	4.0	3.7	3.8	4.0	0.43
$k_{DIGESTION}$	5.3[a]	5.1[a]	4.6[b]	5.2[a]	0.17
N rates					
$k_{CLEARANCE}$	7.5	7.3	6.7	7.9	0.44
$k_{DIGESTION}$	5.3[a]	3.6[b]	2.9[b]	5.2[a]	0.22
NDF rates					
$k_{CLEARANCE}$	8.1	7.4	7.2	8.2	0.56
$k_{DIGESTION}$	4.1	3.7	3.6	4.2	0.30
ADF rates					
$k_{CLEARANCE}$	7.5	6.4	6.5	7.3	0.65
$k_{DIGESTION}$	3.5	2.7	2.7	3.3	0.45
DADF rates					
$k_{CLEARANCE}$	8.3	7.1	7.2	8.2	0.55
$k_{DIGESTION}$	4.3	3.4	3.4	4.2	0.29
Hemicellulose rates					
$k_{CLEARANCE}$	8.6	8.2	8.1	8.6	0.34
$k_{DIGESTION}$	4.6	3.5	3.3	4.6	0.55
Cellulose rates					
$k_{CLEARANCE}$	7.9	6.8	7.1	8.1	0.70
$k_{DIGESTION}$	3.9	3.1	3.3	4.1	0.58

Figures with a different superscript are significantly different ($p<0.05$)
WGS = diet with wilted grass silage
MGS = diet with moist grass silage with molasses
FGS = diet with moist grass silage with formic acid
WW = diet with wilted grass silage and extra water

Table 6.7 ***Degradability characteristics for diet components using nylon bag incubations in dairy cows.***

Component	Fraction			
	S	U	D	k_d
Organic matter				
Wilted grass silage	27	13	60	4.85
Grass silage with molasses	30	11	59	5.64
Grass silage with formic acid	31	10	59	5.65
Maize silage	45	17	38	2.73
Beet pulp (moist ensiled)	10	4	85	6.55
Maize gluten feed (moist ensiled)	37	3	60	4.36
Brewers'grains (moist ensiled)	16	18	66	3.68
Concentrates	37	6	57	9.71
Nitrogen				
Wilted grass silage	54	10	36	6.25
Grass silage with molasses	52	10	38	7.95
Grass silage with formic acid	48	8	44	7.85
Maize silage	67	22	11	1.30
Beet pulp (moist ensiled)	15	77	8	6.10
Maize gluten feed (moist ensiled)	73	3	24	5.85
Brewers'grains (moist ensiled)	27	7	66	2.95
Concentrates	30	3	67	9.15
Neutral detergent fibre				
Wilted grass silage	0	18	82	4.28
Grass silage with molasses	0	14	86	4.85
Grass silage with formic acid	0	13	87	4.91
Maize silage	0	29	71	2.24
Beet pulp (moist ensiled)	0	6	94	5.95
Maize gluten feed (moist ensiled)	0	5	95	3.69
Brewers'grains (moist ensiled)	0	24	76	4.76
Concentrates	0	13	87	9.30

S = soluble fraction
U = undigestible fraction
D = potential digestible fraction
k_d = rate of degradation of the digestible fraction

The S, D and U fractions of the various dietary components observed in the *in situ* study are shown in Table 6.7. The basal-diet components showed large differences in the rate of degradation for the various OM fractions. Pressed beet pulp was

lowest in S and U fractions of the OM, N and NDF. Brewers'grains and maize silage showed the highest U fractions for OM and NDF, while the S fraction of N was highest in maize gluten feed. As a result the rate of degradation differed between basal diet ingredients. Degradation of the OM, N and NDF fractions was slower in WGS silage compared to MGS and FGS silage.

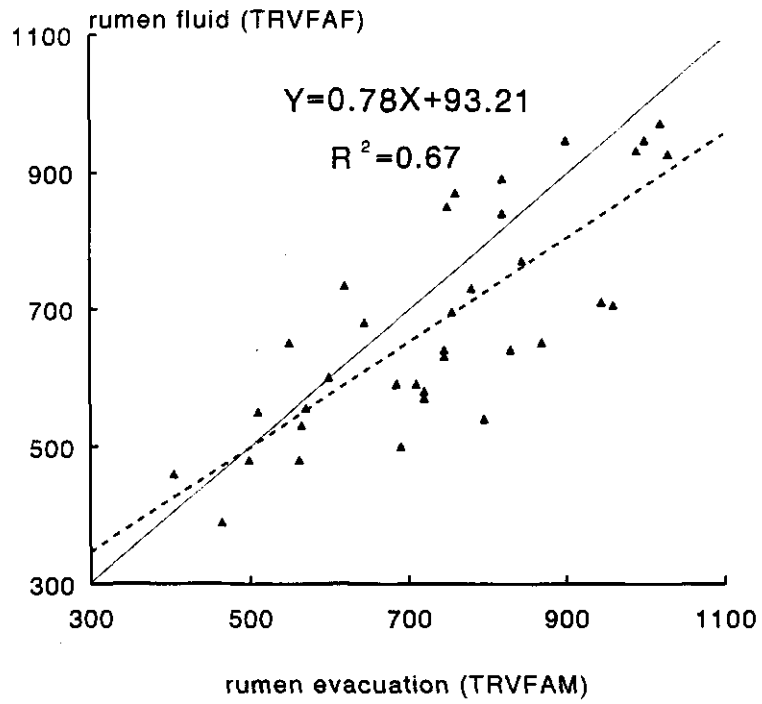

Figure 6.1 ***Calculation of rumen VFA (g) from rumen fluid (TRVFAF) or evacuation (TRVFAM) data***

Discussion

WGS silage showed the highest NDF values in comparison to the high moisture silages (MGS, FGS). This finding agreed with results from earlier laboratory studies performed by Van Vuuren *et al.* (1989). They concluded that the drying process itself positively influenced NDF levels; probably as a result of a Maillard reaction. The high moisture silages (MGS, FGS) were higher in acetic acid and alcohols than the WGS silage. MGS silage displayed the highest lactic acid content, which agreed with previous findings (Donaldson & Edwards, 1976; Murphy & Gleeson, 1984; De Visser & Hindle, 1992), as a result of differences in type and rate of fermenta-

tion during the ensiling process.
The differences in the soluble fractions between N and OM of the grass silages suggested differences in NH_3-N between silages, which was confirmed by the higher amount of NH_3-N found in WGS silage and the lowest value in FGS silage (Tables 6.2 and 6.7).
The results of the *in situ* study with the grass silages agreed with results presented by Bosch (1991). The results obtained with WGS silage compared favourably with the relationships that Bosch (1991) found between S-, U-fractions and the NDF content of grass silages. However, our findings for MGS and FGS silage did not compare as well as the others, due to the lower NDF contents measured in both of these high moisture silages.
The OM of the maize silage displayed a higher S-fraction and lower U-fraction compared to earlier findings published by De Visser *et al.* (1991), although the chemical composition of the maize silage appeared to be similar. Differences are probably attributable to the stage of maturity of the maize silage (De Visser, unpubl.). However, the degradation rate of OM fell within the range published. The OM fraction in pressed beet pulp had a lower S-fraction compared to earlier results published by De Visser *et al.* (1991). The U-fraction, however, was low and agreed with the results of De Visser *et al.* (1991) and corresponded well with the *in vitro* digestibility (Table 6.3). The lower S-fraction was compensated for by a rate of OM degradation that was higher than that observed earlier by De Visser *et al.* (1991). Results agreed favourably with data for dried beet pulp (Tamminga *et al.*, 1991; De Visser *et al.*, 1991). The degradation characteristics of moist maize gluten feed agreed with previous results (Firkins *et al.*, 1984; Steg, unpublished; Klop & De Visser, unpublished). The results displayed the highest U-fraction in moist brewers'grains, which was in agreement with previous findings (Tamminga *et al.*, 1991; Steg, unpublished). The relationship between the U-fraction and the *in vitro* digestibility of the OM (Table 6.3) seems to be poor, when compared to the results obtained with maize silage. Probably OM digestibility measured *in vitro* was underestimated due to the high fat content of the brewers' grains (Table 6.3). Differences in U-fraction of the OM of the dietary ingredients would appear to be strongly related to the U-fraction of the NDF of these feedstuffs, whereas the S-fraction appeared to be more strongly related to nitrogen and the non-structural carbohydrates (sugars, starch; Tables 6.2 and 6.7).
In situ results with the grass silages showed a more rapid degradation of the N as compared to energy sources (OM minus N; NDF; Table 6.7). The increased loss of N, due to amino acid fermentation was supported by increased concentrations of NH_3-N and BCFA measured in the rumen fluid on both high moisture diets (MGS and FGS), indicating reduced microbial protein synthesis, due to the discrepancy between the availability of N and energy. This was also confirmed by the pool size of N, which tended to be larger on WGS and WW (Table 6.4). Our results confirm previous findings when feeding diets differing in degradation rate between N and carbohydrates (barley *vs.* maize; beet pulp *vs.* maize bran; De Visser *et al.*, 1992);

feeding diets differing in the amount of energy available for microbial protein synthesis, due to higher indigestible fractions in combination with lower rates of degradation for the NDF fraction (beet pulp *vs* maize silage; De Visser *et al.*, 1991) and with diets in which energy sources are less efficiently utilized by rumen micro-organisms (fermentation end products *vs.* carbohydrates) due to fermentation of carbohydrates in the silo (Robinson *et al.*, 1987). These indications for reduced synthesis of microbial protein on high moisture silage diets (MGS, FGS) were confirmed by the results of the accompanying feeding trial (De Visser & Hindle, 1992), showing reduced milk protein output on both high moisture diets. Chamberlain *et al.* (1985), Chamberlain, Thomas & Quig (1986) and Rooke, Lee & Armstrong (1987) showed reduced amounts of microbial protein entering the small intestine, when feeding diets with an inbalance in ruminal N and available energy, which agreed with our results.

Reducing the DM content of a diet by adding water (WW) had no significant influence on rumen fermentation and rumen kinetic parameters (Table 6.4, 6.5, 6.6), when compared to WGS. However, high moisture silages in combination with increased fermentation in the silo (MGS and FGS) negatively influenced rumen fermentation and rumen kinetics.

During wilting the association of nitrogen/NDF complexes are probably responsible for the lower rate of degradation of N and NDF in WGS silage, as compared to MGS and FGS silage, which agrees with results observed by Van Vuuren *et al.* (1989).

On the one hand, the lower rumen pH and the higher concentrations of total VFA and lactic acid originated from higher concentrations of acetic acid (FGS) and lactic acid (MGS) fed with moist grass silages in combination with large amounts of moist ensiled by-products (Table 6.2). On the other hand the higher rates in degradation for MGS and FGS silage (Table 6.6) found in the *in situ* experiment (Table 6.7) will have initiated a further decrease in cellulolytic activity (Russell & Sniffen, 1984), a lower ruminal pH and a shift towards propionic acid production. Confirming the lower NGR values measured on high moisture diets (MGS, FGS, Table 6.4).

The decrease in cellulolytic activity is believed to be responsible for the reduced rates of clearance and digestion of the cell wall constituents on MGS and FGS diets (NDF, ADF, DADF, hemicellulose and cellulose, Table 6.6). These negative effects on rate of clearance of OM in cows fed high moisture diets was confirmed by the lower DM intake found in the accompanying feeding trial (De Visser & Hindle, 1992).

Total rumen content as related to bodyweight (BW) did not differ between diets and was approximately 18.5 g/kg BW. Our results were within the range found and reviewed by Bosch (1991). However, Bosch (1991) found lower values in silage cut in a more mature stage and found higher values in silages similar in quality to those studied here. The higher proportion of concentrates fed in our experiment compared to Bosch (1991) may have been responsible for the difference found,

because the rate of degradation and rate of passage of concentrates are higher than those of roughage components.
The rate of passage of particles, using $k_{PASSAGE}$ of IADF as a marker, was similar for all diets (Table 6.6) and agreed with earlier results published by Tamminga *et al.* (1989). The diets fed here and by Tamminga *et al.* (1989) were relatively high in IADF, due to the use of brewers'grains, which contains large quantities of IADF. Brewers'grains are considered to constitute to the small particle fraction of the rumen pool and may leave the rumen relatively sooner than IADF which originates from large particles such as maize silage or grass silages.
This hypothesis agrees with the findings of Poppi *et al.* (1981), who indicated a negative influence from smaller particles on the retention time in the rumen. Bosch (1991), Van Vuuren *et al.* (1992) and De Visser *et al.* (1992) showed lower rates of passage measured using IADF as a marker, but those diets consisted to a larger extent of grass silage (Bosch, 1991) or fresh grass (Van Vuuren, unpublished data) or included concentrates with a relatively low IADF content (De Visser *et al.*, 1992), resulting in minor effects on the retention time of IADF in the rumen.
Total clearance of the OM fraction (Table 6.6) in the rumen was higher than those found by Bosch (1991). However, in our experiment a relatively larger proportion of the total diets fed consisted of by-products and a concentrate mixture, which were mainly part of the small particle pool in the rumen, negatively influencing the retention time in the rumen (Poppi *et al.* (1981). The higher rate of degradation of the concentrate mixtures and by-product ingredients relative to the roughage fed by Bosch (1991) was another influencing factor.
Total VFA present in the rumen was underestimated, when using the common rumen fluid method (TRVFAF) instead of the total rumen mass method (TRVFAM; Figure 6.1). Differences between both methods are related to the concentration gradient which exists between rumen fluid and OM being fermented by micro-organisms. Differences between both methods are of importance in relation to absorption coefficients of various VFA's (Murphy, Baldwin & Koong, 1982).
Grass silages, varying in DM and fermentation end products, differ in degradation characteristics, which negatively influence rumen kinetics. Attention should be given to the chemical composition and degradation characteristics of supplemented concentrates consisting largely of by-products, in an attempt to avoid or minimize negative effects on DM intake, microbial protein synthesis and milk protein output.

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Chapter 7

Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations. 1. Feed intake and milk production.

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Abstract

A feeding trial was carried out with 64 multiparous dairy cows, in which the effects of type of carbohydrates in concentrate mixtures (starch versus cell wall constituents) and differences in rumen degradation (fast versus slow) on feed intake and milk production were studied. The experiment started immediately after parturition and lasted for 15 weeks. The basal diet which comprised 75 % of the total dry matter (DM) intake, consisted of wilted grass silage, maize silage and concentrates. The remaining part of the diet consisted of barley (B), maize (M), pressed ensiled beet pulp (P) or moist ensiled maize bran (MB). All diets were fed as totally mixed rations (TMR). Total intake of DM and net energy did not differ between diets, but differences were found in energy partition. There was a tendency for cows fed diet B to show increased body weight gain, while cows fed P mobilized more body reserves, compared to the other treatments.
Milk production did not differ between diets, but milk fat content was higher for diet P. Milk protein content was higher for diets B and M, compared to P and MB. The lower protein content of the milk of treatment P can be explained by a longer period of negative energy balance, while the lower milk protein in cows fed diet MB probably resulted from a reduced microbial protein synthesis.

Introduction

Dairy cows cannot achieve high yields if they are fed with roughages as the only feed. This is especially the case in early lactation, when the needs for energy are high, but roughage intake is limited. This limited capacity to consume roughage necessitates supplementation of dairy diets with concentrates to meet the cow's requirements. In concentrates as well as roughages over 60% of the organic matter consists of carbohydrates, which consequently are the most important energy source of a dairy cow.
Carbohydrates are a very heterogeneous group of complex chemical compounds. Van Soest (1982) divided them into constituents originating from plant cell walls or structural carbohydrates (hemicellulose, cellulose, pectins) and constituents originating from the cell contents or non-structural carbohydrates (starches, sugars). Properties of carbohydrates such as rumen solubility, rumen degradability or ruminal rate of degradation are to a large extent determined by their physical

structure (crystalline, amorphous) and encrustration or encapsulation with other compounds. Chesson & Forsberg (1988) reported a large variability in complexity of the chemical composition within different groups of carbohydrates.
Hemicellulose and cellulose are often encrusted by lignin, a complex polymer of phenolic acids, whereas starches are usually stored in granules surrounded by a layer of which the chemical composition and physical structure have not yet been completely elucidated.
Differences in type of carbohydrates and amount fed cause differences in rate of ruminal fermentation (Tamminga *et al.*, 1990), site of digestion in the digestive tract (Waldo, 1973; Sutton, 1985) and total digestion (De Visser & Steg, 1988). Also differences may occur in type of micro-organisms in the rumen (Russell & Sniffen, 1984) and pattern of fermentation end products (De Visser & De Groot, 1980; Sutton *et al.*, 1987; Tamminga *et al.*, 1990). Changes in fermentation end products may subsequently influence milk production and composition (De Visser & De Groot, 1980; Thomas *et al.*, 1986; Sutton *et al.*, 1987).
De Visser & De Groot (1980) demonstrated that feeding concentrates with a starch content increasing from 12 to 47% dramatically influenced rumen fermentation. Large quantities of starch caused high concentrations of short-chain, mainly volatile fatty acids, particularly propionic and lactic acid. This resulted in a severe drop in ruminal pH, which in turn caused rumen acidosis and off-feed. When concentrates containing over 35% of starch were fed, the number of animals suffering from rumen acidosis increased rapidly. As a result feed intake, milk production and milk fat content were negatively influenced. The starch sources used in those experiments were mixtures of tapioca, hominy feed, maize gluten feed and wheat.
Malestein *et al.* (1988), Tamminga *et al.* (1990) and Tamminga (pers. comm.) investigated differences in rumen fermentation characteristics between various types of starch in more detail using *in vitro*, *in sacco* and *in vivo* techniques. Differences in extent of degradation were found between *in vivo* (Tamminga *et al.*, 1990) and *in vitro* studies (Malestein *et al.*, 1988), but the ranking in rumen, degradability of starches of different origin was very similar. Cell wall constituents vary not only in the rate of rumen degradation, but also in the extent (degradability) because a varying proportion is not available for rumen degradation (Tamminga *et al.*, 1990).
To investigate the practical significance of differences in digestive behaviour of different carbohydrates, a feeding trial was performed with dairy cows in which two types of starch (barley and maize) and two types of cell wall constituents (beet pulp and maize bran) were compared. The objective of the study was to establish effects on feed intake, milk production and milk composition of differences in degradability of carbohydrates measured in the fermentation studies. The feeding experiment was complemented with a study with rumen cannulated dairy cows in which the effects on rumen fermentation pattern, *in vivo* rumen digestion and kinetics and degradation by means of nylon bag incubations were measured.

More detailed results of this experiment will be discussed in a subsequent paper, but when relevant, reference will be made to these results in this paper too.

Material and methods

The feeding trial was performed with 4 groups of 16 animals of the Dutch Black and White or Dutch Black and White x Holstein breed. All animals were in second or later lactation. The experiment started immediately after parturition and lasted for 15 weeks. The animals were fed equal before parturition according to the advice of the Central Bureau of Nutrition.
Based on previous milk production the animals were grouped into blocks of 4 animals, and within each block allocated at random to one of the four dietary treatments. The basal diet which supplied 75% of the total dry matter (DM) intake, consisted of wilted grass silage, maize silage and a concentrate mixture. In addition, 25% of the DM consisted of either barley (B), maize (M), pressed ensiled beet pulp (P) or moist ensiled maize bran (MB). The composition of the diets is shown in Table 7.1. The composition of the concentrate mixtures is given in Table 7.2. The diets were offered as a totally mixed ration (TMR) twice daily with 40% of the daily allowance at 5:00 h and 60% at 15:00 h. The animals were fed individually and refusals were recorded once daily (14:00 h).
Pressed beet pulp, maize bran and concentrates were sampled once a week and analysed for their dry matter (DM) content, whereas wilted grass silage and maize silage were sampled twice a week and also analysed for DM. The week samples of each ration component were subsampled over a monthly period and analysed for their content of ash, nitrogen (N), crude fat, crude fibre, neutral detergent fibre (NDF), sugars, starch and *in vitro* digestibility of the organic matter.

Table 7.1 ***Diet composition (g/kg^{-1} dry matter)***

Component	Barley (B)	Maize (M)	Pulp (P)	Maize bran (MB)
Wilted grass silage	250	250	250	250
Maize silage	250	250	250	250
Concentrate barley[1]	500	-	-	-
Concentrate maize[2]	-	500	-	-
Concentrate cell wall	-	-	250	250
Pressed ensiled beet pulp	-	-	250	-
Moist ensiled maize bran	-	-	-	250

[1] Concentrate barley contained 49% barley (see Table 7.2).
[2] Concentrate maize contained 48% maize (see Table 7.2).

Samples of wilted grass silage, maize silage, pressed beet pulp and moist maize bran were regularly analysed for volatiles, lactic acid and ammonia to enable corrections on dry matter content and energy value for losses of volatiles. The analytical methods used were as described by Robinson *et al.* (1986) and De Visser & Hindle (1990).
During the total experimental period, milk production was recorded daily.
Milk samples, taken during 8 consecutive milkings each week, were analysed for fat, protein and lactose (Melk controle station Noord-Nederland). Each week the animals were weighed on 8 consecutive times directly after milking. These data were used to calculate average weekly body weight and body weight changes. The average weekly milk yield, milk composition and body weight were used to calculate energy requirements.

Table 7.2 ***Ingredient composition of the concentrate mixtures (g/k^{-1} product)***

Component	Concentrate mixture		
	barley	maize	cell wall
Potato protein	40	40	80
Maize gluten	40	40	80
Coconut expeller	112	112	215
Linseed expeller	88	88	170
Soybean hulls	58	58	115
Barley	490	-	-
Maize	-	480	-
Animal fat	10	-	10
Soybean meal solv. extr.	140	159	290
Calcium carbonate	7	7	10
Calcium phosphate	3	4	6
Magnesium oxide	5	5	10
Premix (vitamins and minerals)	7	7	14

Experimental results were subjected to analysis of variance with the statistical package Genstat (Alvey *et al.*, 1982), using treatment effects within blocks as variables.

Results

The chemical composition and the energy and protein values of the wilted grass silage, maize silage, concentrate mixtures, pressed beet pulp, moist maize bran

Table 7.3 ***The chemical composition and feeding value of the wilted grass silage, maize silage, concentrate barley, concentrate maize, concentrate cell wall, pressed beet pulp, moist maize bran and the four TMR diets***

Ingredient	DM[1]	Ash	N	CFAT	CF	NDF	Sugars	Starch	d_{OM} *in vitro*	NEL	DCP
Grass silage	500	102	35	47	234	451	68	-	78	6.41	154
Maize silage	345	51	13	25	220	441	-	274	72	6.17	44
Beet pulp (pressed ensiled)	219	71	16	7	202	468	23	-	88	7.47	63
Maize bran (moist ensiled)	383	9	21	33	123	563	-	258	88	7.48	91
Conc. cell wall[2]	894	112	56	46	107	289	75	39	83	7.48	304
Conc. barley[3]	874	80	38	38	79	255	55	270	83	7.56	196
Conc. maize[4]	877	72	39	41	66	211	55	325	86	7.84	194
Diet barley	648	79	31	37	153	351	44	204	-	6.93	147
Diet maize	649	74	31	39	147	329	45	231	-	7.07	146
Diet beet pulp	489	84	30	31	191	412	42	78	-	6.88	141
Diet maize bran	530	69	31	38	171	436	36	143	-	6.89	148

[1] DM = dry matter (g kg^{-1}); Ash (g kg^{-1} DM); N = nitrogen (g kg^{-1} DM); CFAT = crude fat (g kg^{-1} DM); CF = crude fibre (g kg^{-1} DM); NDF = neutral detergent fibre (g kg^{-1} DM); Sugars (g kg^{-1} DM); Starch (g kg^{-1} DM); d_{OM} *in vitro* = digestibility of organic matter *in vitro* (%); NEL = net energy lactation (MJ kg^{-1} DM; Van Es, 1978); DCP = digestible crude protein (g kg^{-1} DM).

[2] Conc. cell wall = concentrate used in diets P and MB.

[3] Conc. barley = concentrate including 49% barley.

[4] Conc. maize = concentrate including 48% maize.

and the four TMR diets are shown in Table 7.3. The results of feed intake and milk production and composition are given in Table 7.4 and Figures 7.1 and 7.2. Summarized data of the rumen fermentation study are shown in Table 7.5.
Mean total dry matter intake was high (approximately 24 kg DM), but did not differ significantly between diets. However, cows fed diet P tended to have a lower DM intake, compared to the treatments B, M or MB. Due to the excellent quality of the wilted grass silage (Table 7.3) resulting in a high energy density and the high levels of intake, net energy intake was also high for all diets.

Table 7.4 ***Feed intake, milk production, milk composition and body weight changes of the cows during the experimental period of 15 weeks (means of 16 animals)***

	Barley (B)	Maize (M)	Pulp (P)	Maize bran (MB)	SED[4]
Feed intake					
Total dry matter intake (kg d^{-1})	24.7	24.4	23.7	24.3	0.56
Net energy intake (MJ d^{-1})	169	172	163	166	3.93
Digestible crude protein (g d^{-1})	3661	3549	3298	3586	90
Energy ratio[1] (%)	107[a]	106[a]	100[b]	102[ab]	2.4
DCP ratio[2] (%)	130[a]	124[a]	113[b]	124[a]	3.3
Milk production					
Milk (kg d^{-1})	37.1	38.2	37.7	38.1	1.27
PFCM[3] (kg d^{-1})	38.7	39.5	40.1	39.7	1.24
Fat (%)	4.34[a]	4.25[a]	4.54[b]	4.34[a]	0.08
Protein (%)	3.55[a]	3.53[a]	3.42[b]	3.41[b]	0.05
Lactose (%)	4.65	4.67	4.71	4.72	0.03
Fat (g d^{-1})	1589	1610	1700	1645	55
Protein (g d^{-1})	1298	1335	1280	1292	37
Lactose (g d^{-1})	1726	1782	1775	1797	62
Body weight					
Body weight change (kg)	+2	-8	-19	-11	10
Body weight (kg)	602	611	620	608	15

[1] Energy ratio = energy intake/energy requirements x 100.
[2] DCP ratio = digestible crude protein intake/requirements x 100.
[3] PFCM = protein and fat corrected milk.
[4] SED = standard error of difference.
Figures with a different superscript differ significantly ($P<0.05$).

Net energy intake did not differ significantly between diets, but intake of diet P was lowest, due to a somewhat lower DM intake (Table 7.4) and intake of diet M was highest due to the higher energy density (Table 7.3). Milk production was similar for all treatments, although small differences were found. These differences could be explained by differences in energy intake or changes in body weight (Table 7.4).

Milk fat content was significantly higher ($P < 0.05$) for diet P compared to diets B, M and MB (Table 7.4). Milk protein content of cows fed the diets M and B, which were relatively high in starch, were significantly higher ($P < 0.05$) than those after feeding diets P and MB (Table 7.4; Fig. 7.2). The milk protein content on treatment P showed a different pattern during the experiment compared to diet MB. During the first period of the experiment the milk protein content was similar for diets P and MB, while at the later stage of the trial milk protein content on treatment P showed an increase towards the higher values measured with diets B and M (Fig. 7.2). The lactose content of the milk was similar for all diets, although the values were lower for both starch diets. The production (g/d^{-1}) of milk fat, milk protein and lactose did not differ significantly between treatments (Table 7.4).

Energy ratio (intake as percentage of requirements) was significantly lower ($P < 0.05$) for treatment P than for B or M, but similar to MB (Table 7.4). Differences in the utilization of energy occurred between diet B and the other diets M, P and MB. Cows fed barley showed a reduced milk energy output (PFCM) and an increased body weight gain over the total experimental period, while cows on diet P showed the most negative energy balance because of a lower energy intake and a higher milk energy output, resulting in an increased mobilization of body reserves (Table 7.4; Fig. 7.1).

Table 7.5 ***Rumen fermentation characteristics after feeding concentrates containing carbohydrates of different type and origin***

	Barley (B)	Maize (M)	Pulp (P)	Maize bran (MB)	SED
pH	5.73	5.76	5.77	5.70	0.04
Total VFA ($mmol/l^{-1}$)[1]	142	142	145	146	3.62
NGR[2]	3.35	3.50	3.99	3.59	0.26
Acetic acid ($mmol/l^{-1}$)	86	85	92	90	2.75
Propionic acid ($mmol/l^{-1}$)	35	35	32	35	2.07
Ammonia ($mmol/l^{-1}$)	7.46	8.37	7.27	9.45	0.96
Iso butyrate ($mmol/l^{-1}$)	0.8	0.8	0.8	1.0	0.09

[1] VFA = volatile fatty acids.

[2] NGR = non-glucogenic/glucogenic ratio (Ørskov, 1975).

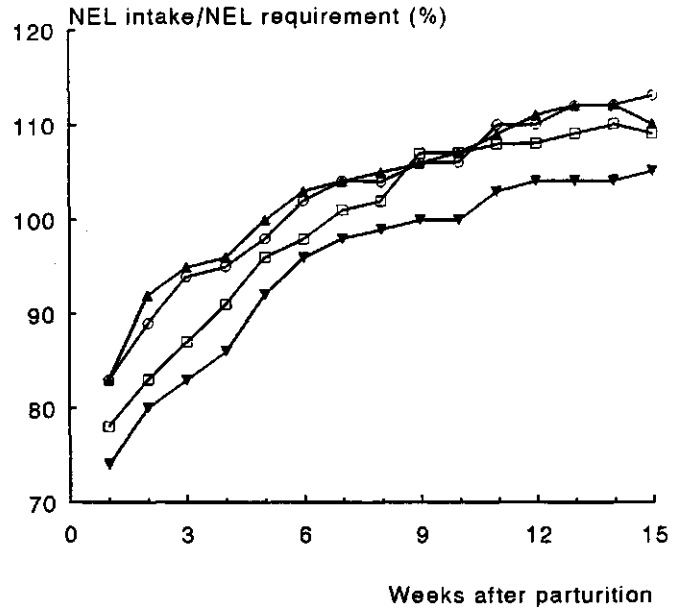

Figure 7.1 ***Time-course of net energy requirement ratio***
Barley, ▲-▲***; Maize,*** ○-○***; Pulp,*** ▼-▼***; Maize bran,*** □-□.

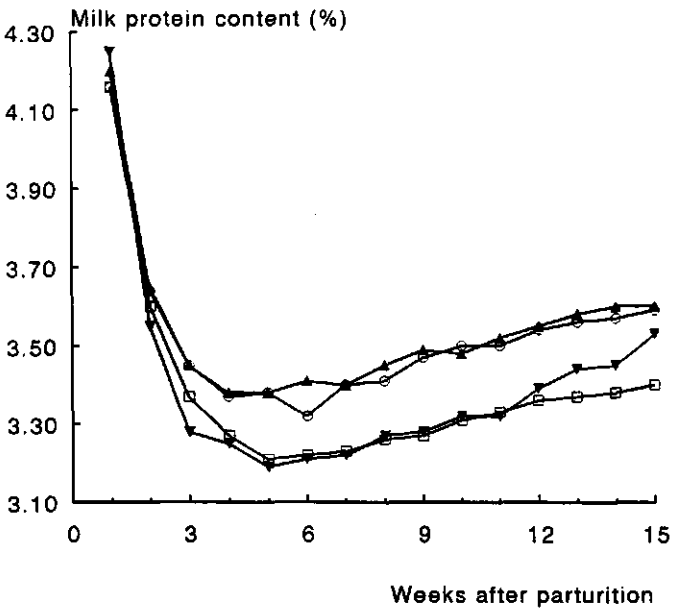

Figure 7.2 ***Time-course of protein content***
Barley, ▲-▲***; Maize,*** ○-○***; Pulp,*** ▼-▼***; Maize bran,*** □-□.

Discussion

High annual milk ouputs of dairy cows are only possible with high feed intakes. The first limitation in this respect is often the quality of the roughage, because it largely defines the energy density of the total diet. Energy density of the diets fed in this experiment was high with on average 6.9 MJ NE/kg^{-1} dry matter. Dry matter intake was also high and averaged 24.3 kg DM d^{-1} cow^{-1}. Because of differences in dry matter content of around 15% between diets P and MB on the one hand and diets B and M on the other (Table 7.3), a reduced intake for diets P and MB was expected as observed before in other experiments with silage based diets (Lahr *et al.*, 1983; De Visser & Hindle, 1990). Although the differences in intake reflected the expected pattern, they did not reach significance. Differences in feed intake were also expected because of expected differences in rumen fermentation pattern between diets. De Visser & De Groot (1980) observed a reduced feed intake with diets high in starch and sugars (>420 g kg^{-1}DM) compared to diets high in cell wall components. They explained these differences in feed intake by differences in pH, concentration of propionic acid and lactic acid of rumen fluid. When feeding diets high in starch, Malestein & Van 't Klooster (1986) observed a reduced roughage intake when maize starch was replaced by tapioca starch. Tamminga *et al.* (1990) and Tamminga (pers. comm.) studied rumen fermentation patterns after feeding diets high in tapioca, barley or maize. Rumen fermentation pattern after feeding maize differed from that after feeding barley or tapioca, which were very similar. Feeding barley or tapioca resulted in more propionic and lactic acid, a lower Nonglucogenic Glucogenic Ratio (NGR) and a slower feed intake. In the experiment reported here, differences in rumen fermentation pattern between diets were present but comparatively small (Table 7.5). This seems to contradict the earlier findings, but it should be realized that in the experiment of De Visser & De Groot (1980) considerably higher amounts of starch and sugars per kg DM concentrates were fed (>420 g kg^{-1} DM). Moreover, the amount of roughage fed in the experiments of De Visser & De Groot (1980) and of Tamminga (pers. comm.) was restricted to between 6 and 7.5 kg DM d^{-1}, while in this trial total DM intake of roughage was approximately 12 kg DM d^{-1}. Higher amounts of roughage are considered to influence positively buffering capacity, due to increased saliva production. A further reason why the differences in rumen fermentation pattern in this experiment were only small is the difference in method of feeding. In the experiments of De Visser & De Groot (1980) and Malestein & Van 't Klooster (1986) roughage and concentrates were fed separately, but in this experiment diets were fed as totally mixed rations. TMR usually result in a more stable rumen fermentation (Rohr & Schlunsen, 1986). Additional differences between the experiment reported here and previous experiments were the quality of the roughage, the level of milk production and the level of feed intake. Roughage quality in the experiment of Malestein & Van 't Klooster (1986) and of Tamminga (pers. comm.) was rather poor and roughage was fed to low producing dairy cows

at a medium level of feed intake. Low ruminal pH affects cellulolytic activity (Russell & Sniffen, 1984). Therefore it is probable, that the degradation of low quality roughage, which is high in cellulose and lignine, is more inhibited than that of high quality.
High levels of milk production cause a rapid clearance of VFA from the rumen and may prevent a severe drop in ruminal pH (Van der Walt, 1984).
Some researchers have reported higher milk productions with barley diets compared to pulp diets (Tremere *et al.*, 1968; Sutton *et al.*, 1987), while others reported lower milk yields (Castle *et al.*, 1981; Thomas *et al.*, 1986; Sutton *et al.*, 1987).
In the experiment reported here, energy intake and milk production did not differ between diets. However, partition of energy did differ, resulting in differences in body weight changes and in milk composition. Cows fed diet P had a higher fat content in their milk, compared to diets B, M. These findings agreed with the results of Sutton *et al.* (1987) and Thomas *et al.* (1986) comparing the effect of concentrates rich in starch with those rich in fibre. The protein content of the milk was significantly higher for diets B and M, compared to both rations which were high in cell wall constituents (P and MB). This agreed with the result of Thomas *et al.* (1986), but disagreed with the results of Sutton *et al.* (1987) and Castle *et al.* (1981).
The differences in energy partition can be explained by a number of phenomena, probably occurring simultaneously. The first explanation is a difference in rumen fermentation pattern. Rumen fermentation after feeding diet B, M and MB changed to a higher proportion of propionic acid in the volatile fatty acids (VFA) causing a lower NGR, as is shown in Table 7.5.
This high amount of propionic acid probably caused an increased concentration and/or activity of insulin (Sutton *et al.*, 1980; Van Beukelen, 1983) resulting in a change of fat secretion by the mammary gland towards fat deposition in adipose tissue (Vernon, 1988). The resulting lower milk fat contents for diets M and B confirmed earlier findings with comparable diets (De Visser & De Groot, 1980; Van Beukelen, 1983; Thomas *et al.*, 1986; Sutton *et al.*, 1987). In addition, the period the animals were in negative energy balance was longest for animals fed diet P (Fig. 7.1; Table 7.4). During negative energy balance, long-chain fatty acids are mobilized from adipose tissue and become available for fat synthesis in the mammary gland. During negative energy balance, a larger proportion of milk fatty acids originates from direct incorporation of these long-chain fatty acids into milk fat (Palmquist & Conrad, 1971); the latter fatty acids are very efficiently utilized by mammary gland tissue (Vernon, 1988).
The lower milk fat content of animals fed diet MB was unexpected, because diet MB contained a large amount of NDF, at least compared to diets B and M (Table 7.3). The findings agree however with the lower NGR values measured in the rumen fermentation study (Table 7.5). Maize bran has a high content of starch compared to beet pulp (Table 7.3). This starch is relatively rapidly fermentable in

the rumen, which may have influenced rumen fermentation pattern towards more propionic acid.

It is noteworthy that the milk fat content of treatment M was lower than that of the cows fed diet B, despite the fact that NGR was higher. This apparent discrepancy can be explained by a difference in ruminal behaviour of the starch in both diets. Barley starch is known to be degraded rapidly and almost completely whereas a significant proportion of maize starch usually escapes from fermentation (Waldo, 1973). Starch escaping rumen degradation may become available as glucose after intestinal absorption (Sutton, 1985; MacRae *et al.*, 1988). Barley will be rapidly and extensively degraded in the rumen resulting in a large quantity of the glucogenic precursor propionic acid, but also significant amounts of the lipogenic precursor acetic acid will be produced. As a result the amount of glucogenic precursors will be higher with the maize containing diet.

Both diets rich in cell wall constituents (P and MB) showed a lower milk protein content than diets B and M. Considering the milk protein content during the experiment (Fig. 7.2), a different pattern between diets P and MB was revealed. During the first part of the experiment both diets showed a reduced milk protein content, compared to diets B and M.

However, at the last weeks of the experiment the milk protein content after feeding diet P increased towards the values measured with diets B and M. At that time, energy intake of the animals fed diet P reached energy balance (Fig. 7.1). This observation agrees with the results of Broster & Thomas (1981), who found a positive relationship between energy balance and milk protein content.

Further differences between diets P, B and M on the one hand and diet MB on the other were the differences in the concentration of ammonia and iso-butyrate of the rumen fluid, which were increased with diet MB, compared to the other diets (Table 7.5), indicating a reduced microbial protein synthesis. These findings agree with the results of Miller (1982) and Robinson *et al.* (1987) and might have been responsible for the persistent lower milk protein content of diet MB, compared to the other diets, due to reduced intestinal protein supply.

In conclusion, the results of this study show that high milk yields can be achieved with high-quality diets. Milk composition however can be manipulated by changing the carbohydrate composition of the concentrate part of the diet. The effects of the different cell wall constituents need further research.

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Chapter 8

Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations. 2. Rumen degradation, fermentation and kinetics

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Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations
2. Rumen degradation, fermentation and kinetics

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Abstract

In an experiment executed as a 4x4 Latin square, the effects of carbohydrates in concentrate mixtures (starch versus cell wall constituents) and in rate of rumen degradation (rapid versus slow) on rumen fermentation and rumen kinetics were studied.
In a separate experiment three animals were used to measure the rate of degradation, using nylon bag incubations.
Four ruminally cannulated dairy cows were used to study rumen fermentation pattern, while rumen kinetics was measured in three of these animals. The basal diets, which comprised 75% of total dry matter, consisted of wilted grass silage, maize silage and concentrates. The remainder consisted of barley (B), maize (M), pressed ensiled beet pulp (P) or moist ensiled maize bran (MB). All diets were fed as totally mixed rations (TMR). The pH of the rumen fluid and the total concentration of volatile fatty acids (tVFA) did not differ among diets. The major volatile fatty acids did differ between diets and were expressed as the non-glucogenic glucogenic ratio (NGR), being lowest for diet B and highest for diet P. Differences in NGR could be explained by differences in the rate of degradation of starch and/or NDF, using nylon bag incubations. Concentrations of ammonia and the branched chain fatty acids (BCFA) were higher for diet MB and corresponded with the availability of nitrogen and energy for microbial protein synthesis, which was also reflected in the amount of bacterial protein in the rumen. Degradability was lower for maize starch compared to starch from barley. The rate of digestion measured with nylon bag incubations and from rumen evacuations were similar for OM and N, but the nylon bag showed a lower estimate for starch and NDF. The large particle fraction of mean total rumen contents mainly consisted of cell wall constituents and did not differ between diets.

Introduction

Dairy cows can only produce high milk yields if their intermediary metabolism is supplied with sufficient nutrients (glycogenic, ketogenic, aminogenic nutrients, minerals and vitamins). The limited capacity of the dairy cow to consume roughage to meet nutrient requirements necessitates that dairy diets be supplemented with concentrates, especially in early lactation. The inclusion of concentrates in diets

changes total dry matter intake, particle size distribution of the rumen content and often the chemical composition of the diet (Bosch, 1991). Changes in the chemical composition of diets is reflected in degradability of the organic matter, fermentation pattern and kinetics of particle digestion and passage in the rumen (Robinson *et al.*, 1987a, 1987b). Concentrate mixtures are composed of various ingredients and formulations are based on energy and protein values and the costs of individual ingredients. In addition, there is a need for further specification on the basis of the nutrients (eg. acetic acid, propionic acid) they will supply, because these nutrients determine the actual production of lactose, milk fat and milk protein.
De Visser *et al.* (1980, 1988) reported large differences in feed intake, milk production and milk composition when varying the type of carbohydrates (structural *vs.* non-structural) in concentrate mixtures. During these experiments an increase of the portion of rapidly fermentable carbohydrates (i.e. starch and sugars) reduced feed (energy) intake and as a result milk output; especially when feeding roughage and concentrates separately. Milk fat content was decreased, which agreed with the shift of rumen fermentation towards more propionic acid (De Visser *et al.*, 1980, 1988, 1991; Robinson *et al.*, 1986, 1987c, 1987d). Within the various types of carbohydrates, such as starch and cell wall constituents differences in rumen degradability and rate of degradation were found among ingredients (Tamminga *et al.*, 1990; Malestijn *et al.*, 1986, 1988; Van Vuuren *et al.*, 1990). However, these observations were made in non-producing or low-producing animals, fed twice daily at moderate levels of intake with roughage and concentrates fed separately. It was therefore decided to study the effect of structural and non-structural carbohydrates in concentrate supplements in high producing animals fed totally mixed rations (TMR) at high levels of intake. In the feeding trial, the effects on feed intake, milk production and milk composition of differences in carbohydrate source (barley, maize, beet pulp or maize bran) were studied (De Visser *et al.*, 1990b). Results of the feeding trial showed differences in milk fat and protein outputs among treatments. The experiment described in this paper complements the study reported previously, aiming to investigate how the different diets of the feeding trial affected rumen fermentation, rumen digestion (*in vivo* and *in sacco*) and digestion kinetics.

Material and methods

The experiment was carried out as a 4x4 Latin square arrangement of treatments with four Dutch Friesian dairy cows fitted with a rumen cannula. Three of the cows had a large rumen cannula (10 cm internal diameter, Bar Diamond Inc., Parma, ID, USA), while the fourth animal was fitted with a small cannula (5 cm internal diameter, Eriks, Alkmaar, NL). The experiment started approximately 3 weeks after parturition and lasted for 5 months.

Table 8.1 ***Diet ingredients (g kg^{-1} DM), chemical composition of the diets (g kg^{-1} DM) and the composition of the concentrate mixtures (g kg^{-1})***

	Barley	Maize	Pulp	Maize bran
Diets				
Wilted grass silage	250	250	250	250
Maize silage	250	250	250	250
Cell wall concentrate	-	-	250	250
Barley concentrate	500	-	-	-
Maize concentrate	-	500	-	-
Pressed ensiled beet pulp	-	-	250	-
Moist ensiled maize bran	-	-	-	250
Chemical composition of diets				
DM	552	555	385	456
Ash	78	74	80	69
N	31	31	30	31
NDF	367	345	438	448
ADF	180	173	216	197
Hemicellulose[1]	187	172	222	251
lADF	38	34	38	36
Starch	204	228	84	138
Sugars	44	45	42	36
Acetic acid	5	5	10	7
Lactic acid	15	15	30	22
Ammonia	3	3	3	4

Composition of concentrates

	Concentrate mixture		
	barley	maize	cell wall
Potato protein	40	40	80
Maize gluten	40	40	80
Coconut expeller	112	112	215
Linseed expeller	88	88	170
Soybean hulls	58	58	115
Barley	490	-	-
Maize	-	480	-
Soybean meal solv. extr.	140	159	290
Calcium carbonate	7	7	10
Calcium phosphate	3	4	6
Magnesium oxide	5	5	10
Premix (vitamins and minerals)	7	7	14

[1] hemicellulose is calculated as NDF-ADF.

Cows were tethered in tie-stalls and had free access to block salt and water. To prevent ingredient selection diets were offered as TMR prepared in a forage-mixer wagon.
The basal diet, which supplied 75% of the total dry matter (DM) intake, consisted of wilted grass silage (25%), maize silage (25%) and a concentrate mixture (25%). In addition, 25% of the DM consisted either of barley (B), maize (M), moist pressed ensiled beet pulp (P) or moist ensiled maize bran (MB).

Table 8.2 ***Experimental design (rumen fermentation and kinetics)***

Period	Cow numbers			
	744[1]	829[2]	843[2]	1536[2]
1	pulp	maize bran	barley	maize
2	maize bran	barley	maize	pulp
3	maize	pulp	maize bran	barley
4	barley	maize	pulp	maize bran

[1] animal only used in the fermentation study
[2] animals used in the fermentation- as well as the rumen kinetic study

Both dried ingredients (barley and maize) were included in the concentrate mixture and were ground. The composition of the diets and the concentrate mixtures are in Table 8.1. The animals were fed twice daily with 40% of the daily allowance at 5.00 h and 60% at 14.00 h. Animals were fed *ad libitum*, but refusals were restricted to a maximum of approximately 2% as fed. During the four experimental periods individual animals were offered the same amount of dry matter as was consumed by that cow during the first period. The design of the experiment is in Table 8.2. Each experimental period lasted for 5 weeks. The first 4 weeks were for adaptation to the diets, while during the fifth week of each period the fermentation pattern and rumen kinetics were measured.
During the experimental week samples were taken from all diet ingredients and analysed for dry matter (DM), ash, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), indigestible acid detergent fibre (IADF), sugar and starch. The analytical methods used were as described by Robinson *et al.* (1986) and De Visser & Hindle (1990a).

Rumen fermentation
During the experimental week the fermentation pattern was determined for 48 hours by taking 17 samples of the rumen fluid as described by De Visser *et al.* (1991). The samples were taken at 5.00, 7.00, 10.00, 14.00, 16.00, 19.00,

22.00, 2.00 and 5.00 hours, respectively, and immediately analysed for pH with a pH meter (Yokoyama). Subsamples were taken and stored for analysis of volatile fatty acids (VFA), lactic acid (HL) and ammonia (NH_3-N) as described by Robinson *et al.* (1986). The ratio between the non-glucogenic and glucogenic fatty acids (NGR) was calculated according to the method as described by Ørskov *et al.* (1975), except that lactic acid was considered as a glucogenic precursor.
The diurnal pattern of feed and water intake was recorded.
Analysis of variance using cow, period and diets as variables influencing rumen parameters was completed with mean daily values (weighted for time intervals) and daily variations (expressed as standard deviation of daily values), using the statistical package Genstat (Alvey *et al.*, 1982).

Rumen kinetics

The three dairy cows with the large rumen cannula were used for measuring rumen ingesta mass, composition and kinetics. Average rumen mass was measured by manual evacuation on three occasions (4.00 h, 10.00 h and 20.00 h), while the animals had access to feed. The method of evacuation was as described by Robinson *et al.* (1987b).
Two samples of total rumen mass were taken at each sampling time. The first was freeze dried and analysed for ash, N, NDF, ADF, IADF, starch and diaminopimelic acid (DAPA). The second was placed in a large nylon bag (25 x 40 cm) with a pore size of 2 mm and washed twice in a washing machine for 55 minutes with cold water, without washing powder and spin drying (wool wash programme). The residue after washing was removed from the bag, freeze dried and analysed for ash, N, NDF, ADF and IADF. Total rumen pool and pool of particles >2 mm (OM, NDF, ADF, IADF) were measured, while the pool of particles <2 mm was calculated by subtraction.
Rumen bacteria were isolated by differential centrifugation, freeze dried and analysed for N and DAPA, as described by Robinson *et al.* (1987a). By calculation, total rumen N-pool (NAN) was separated into a bacterial N and a residual N fraction.
Kinetics of rumen OM, N, starch, NDF and ADF clearance, passage and digestion were calculated as:

- rate of clearance (k_c) = ((feed intake, kg d^{-1})/(average rumen pool, kg))/24
- rate of passage (k_p) = ((IADF intake, kg d^{-1})/(average rumen IADF pool, kg))/24
- rate of digestion (k_d) = (k_c-k_p)

Kinetics of rumen OM, NDF and IADF of the large particle fraction were also calculated using the same equations. The clearance of the large particles was calculated with the assumption that the concentrate part of the diet (concentrate mixtures and by-products) only consisted of small particles (<2 mm). The NDF and IADF of the roughage components (i.e. grass silage and maize silage) were assumed to be part of the large particle fraction. As a result of these assumptions both large particle cell wall fractions (NDF, IADF) of the rumen contents can only

originate from the roughage components of the diet. Because of the number of animals with a large rumen cannula (3), the design of the experiment was not completely orthogonally balanced (Table 8.2). Analysis of variance was performed, using cow, period and diets as variables determining rumen kinetic parameters, using the statistical package Genstat (Alvey *et al.*, 1982). The missing values of the fourth animal were estimated as part of the statistical analysis.

Rumen degradability

After the rumen fermentation and kinetic study was completed the three animals with the large rumen cannulas were used to measure *in sacco* rumen OM degradation of all diet ingredients as described by Van Vuuren *et al.* (1989). Animals were fed the average of all four diets of the rumen fermentation study (Table 8.1) by mixing these diets in the forage mixer wagon. Results for the individual ingredients were used to calculate the rate of degradation of the OM, N, NDF and starch of total diets.

The times of incubation were 0, 2, 4, 8, 12, 24, 48, 72 and 336 hours. The OM fraction was divided into a soluble (S), undigestible (U) and a potentially digestible (D = 100-S-U) fraction. The rate constant (k_d) of D was estimated by iteration.

Results

Starch-rich diets (B, M) contained more starch and less NDF than both cell wall diets (P, MB) (Table 8.1). Diets were similar for N, ash, lADF and sugar contents. During the last experimental period of the rumen fermentation study animal 744 suffered from *tarsitis*, so feed intake was dramatically reduced. Therefore fermentation characteristics of that animal were omitted and were estimated during statistical analysis.

Rumen fermentation

During the rumen fermentation study the average intake of diets M and P tended to be lower than those of diets B and MB (Table 8.3).

Mean daily pH and concentrations of total VFA and butyrate were similar for all diets. The NGR was significantly higher as was the concentration of BCFA (branched chain fatty acids) lower for diet P. The concentration of acetic acid tended to be higher, while the concentration of propionic acid tended ($p<0.07$) to be lower at diet P, compared to the other diets (B, M and MB). The concentration of ammonia was significantly higher at diet MB. The concentration of BCFA was lowest for diet P, while diets M and MB were higher, compared to diet B.

Rumen kinetics

Total DM intakes were lower at diets M and P (Table 8.4). Although DM intake differed among diets, total ingesta, dry matter and non-dry matter contents did not

differ significantly between diets. DM percentage of total rumen mass tended to be lower for cows fed diet P.

Table 8.3 ***Rumen pH and concentrations of volatile fatty acids, lactic acid, ammonia (mMol/l^{-1}), between diets barley (B), maize (M), beet pulp (P) and maize bran (MB)***

	Barley	Maize	Pulp	Maize bran	SED
DM intake (kg/day^{-1})	24.0	23.1	22.9	24.2	0.7
pH, mean	5.73	5.76	5.77	5.70	0.037
pH, range	0.28	0.27	0.23	0.22	0.019
Total VFA, mean	142	142	145	146	3.62
Total VFA, range	14	13	13	11	1.14
NGR[1], mean	3.35^{a}	3.50^{a}	3.99^{b}	3.59^{a}	0.26
NGR, range	0.23	0.25	0.18	0.16	0.02
Lactate, mean	1.13	1.70	1.69	0.85	0.34
Lactate, range	1.43	2.66	3.16	1.16	0.86
Ammonia, mean	7.46^{a}	8.36^{ab}	7.27^{a}	9.45^{b}	0.96
Ammonia, range	4.45	4.71	4.09	4.07	0.32
Acetate, mean	86	85	92	90	2.75
Acetate, range	7^{a}	6^{a}	8^{b}	6^{a}	0.52
Propionate, mean	35	35	32	35	2.07
Propionate, range	5	4	4	3	0.59
Butyrate, mean	17	18	17	17	0.53
Butyrate, range	2.5	2.7	1.9	1.8	0.42
BCFA, mean	4.11^{a}	4.37^{b}	3.36^{c}	4.43^{b}	0.11
BCFA, range	0.15	0.21	0.15	0.21	0.05

[1] NGR = Non-glucogenic Glucogenic Ratio (Ørskov, 1975).
Figures with a different superscript are significantly different ($p < 0.05$).
SED = standard error of difference.
BCFA = branched chain fatty acids (iso-butyrate + 2-3 Methyl butyrate + valerate).
Mean = mean daily values (weighted for time intervals).
Range = calculated as standard deviation (sd) of daily values.

The rumen pool of the OM, total and bacterial N and the cell wall constituents (NDF, ADF, IADF, DADF) did not differ significantly between diets. The pool size of hemicellulose was highest for diet MB, as was starch for diet M. The volumes of the average daily rumen pool of cell wall constituents (NDF, ADF) were almost equal to cell wall constituent intakes, whereas that of starch, sugars and total-N were lower (Figure 8.1).

Table 8.4 ***Rumen kinetic study (3 cows). Total dry matter intake and the daily mean rumen pool sizes of dry matter, organic matter, nitrogen, starch and cell wall constituents***

	Barley	Maize	Pulp	Maize bran	SED
DM intake (kg)	22.7[a]	21.1[b]	21.1[b]	23.9[c]	0.6
Bodyweight (kg)	612	610	615	630	
DM pool/kg bodyweight (g)	19.9	18.9	20.0	20.5	
Total rumen contents					
Non-dry matter (kg)	67.2	64.0	72.6	72.4	3.7
Dry matter (kg)	12.2	11.5	12.3	12.9	0.9
Total ingesta (kg)	79.4	75.5	84.9	85.3	4.5
Percentage DM	15.3	15.2	14.4	15.1	0.5
Rumen pool sizes					
OM (kg)	11.2	10.6	11.2	11.9	0.8
NAN (g)	385	364	394	374	15.3
Bacterial N (g)	211	210	215	202	9.4
Starch (g)	424[a]	610[b]	374[a]	382[a]	28.8
NDF (kg)	6.9	6.4	6.7	7.5	0.4
ADF (kg)	3.8	3.6	3.7	4.0	0.4
IADF (kg)	1.2	1.1	1.2	1.2	0.1
DADF (kg)	2.6	2.5	2.5	2.8	0.2
Hemicellulose (kg)	3.1[a]	2.7[a]	3.0[a]	3.5[b]	0.1
Rumen pool sizes of large particles (> 2mm)					
OM (kg)	5.5	5.1	5.4	5.7	0.6
NDF (kg)	4.5	4.2	4.3	4.7	0.6
ADF (kg)	2.5	2.4	2.4	2.6	0.4
IADF (kg)	0.6	0.6	0.6	0.7	0.1

Figures with a different superscript are significantly different ($p < 0.05$)

Approximately 48% of total rumen OM content consisted of large particles (> 2 mm). Fractions (OM, NDF, ADF) were similar for all diets. The large particle fraction

Table 8.5 ***Turnover of the organic matter, neutral detergent fibre, acid detergent fibre, digestible acid detergent fibre and hemicellulose calculated from rumen evacuation data***

	Barley	Maize	Pulp	Maize bran	SED
OM rates (% h^{-1})					
$k_{CLEARANCE}$	8.04	7.83	7.54	8.00	0.15
$k_{PASSAGE}$ [1)]	2.96	2.75	3.04	3.00	0.12
$k_{DIGESTION}$	5.08	5.08	4.50	5.00	0.12
NDF rates (% h^{-1})					
$k_{CLEARANCE}$	5.08^{a}	4.79^{a}	6.25^{b}	6.17^{b}	0.12
$k_{DIGESTION}$	2.13^{a}	2.04^{a}	3.21^{b}	3.17^{b}	0.12
ADF rates (% h^{-1})					
$k_{CLEARANCE}$	4.50^{a}	4.29^{a}	5.42^{b}	5.04^{ab}	0.17
$k_{DIGESTION}$	1.54^{a}	1.54^{a}	2.38^{b}	2.04^{ab}	0.10
DADF rates (% h^{-1})					
$k_{CLEARANCE}$	5.21^{a}	5.00^{a}	6.50^{b}	5.96^{ab}	0.17
$k_{DIGESTION}$	2.29^{a}	2.25^{a}	3.45^{b}	2.96^{b}	0.11
Hemicellulose rates (% h^{-1})					
$k_{CLEARANCE}$	5.83^{a}	5.46^{a}	7.25^{b}	7.54^{b}	0.15
$k_{DIGESTION}$	2.88^{a}	2.71^{a}	4.21^{b}	4.54^{b}	0.24
Starch rates (% h^{-1})					
$k_{CLEARANCE}$	45.50^{a}	32.06^{b}	19.75^{c}	35.98^{b}	0.33
$k_{DIGESTION}$	42.54^{a}	30.11^{b}	16.71^{c}	32.98^{b}	0.37
N rates (% h^{-1}) total N content					
$k_{CLEARANCE}$	16.85	17.70	14.73	17.95	0.25
$k_{DIGESTION}$	13.89	14.95	11.69	14.95	0.37
N rates (% h^{-1}) residual N content					
$k_{CLEARANCE}$	7.62	7.49	6.69	8.25	0.23
$k_{DIGESTION}$	4.66	4.73	3.65	5.25	0.30
NDF rates of large particles (% h^{-1})					
$k_{CLEARANCE}$	4.75	4.87	4.33	4.67	0.13
lADF rates of large particles (% h^{-1})					
$k_{CLEARANCE}$	4.76	4.74	4.25	4.81	0.16

Figures with a different superscript are significantly different ($p<0.05$)

[1] $k_{PASSAGE}$ was calculated from $k_{CLEARANCE}$ of lADF ($k_C = k_P$)

Table 8.6 ***The soluble (S), potentially fermentable (D), undegradable fraction (U) (g/kg^{-1}) and rate of degradation (k_d)(% h^{-1}) of the organic matter, nitrogen, neutral detergent fibre and starch of the diets barley (B), maize (M), beet pulp (P) and maize bran (MB) and the starch and neutral detergent fibre of the diet ingredients barley, maize, pressed beet pulp and moist ensiled maize bran.***

	Diet			
	Barley	Maize	Pulp	Maize bran
Organic matter				
S fraction	341	295	243	279
D fraction	553	618	663	627
U fraction	101	87	93	102
K_d	5.42	4.79	4.44	3.99
Nitrogen				
S fraction	438	429	363	403
D fraction	485	500	546	514
U fraction	77	71	91	83
K_d	4.65	4.18	3.85	5.33
Neutral detergent fibre				
S fraction	13	13	13	23
D fraction	788	825	835	822
U fraction	199	162	152	155
K_d	3.69	3.76	4.09	3.21
Starch				
S fraction	582	373	674	604
D fraction	416	616	322	392
U fraction	2	1	4	4
K_d	21.33	9.03	12.28	13.35

	Ingredient			
	Barley	Maize	Pulp	Maize bran
Neutral detergent fibre				
S fraction	0	17	0	42
D fraction	678	936	942	891
U fraction	322	47	58	67
K_d	9.44	2.25	5.03	1.64
Starch				
S fraction	115	50	-	482
D fraction	885	949	-	515
U fraction	0	1	-	3
K_d	25.83	8.66	-	15.92

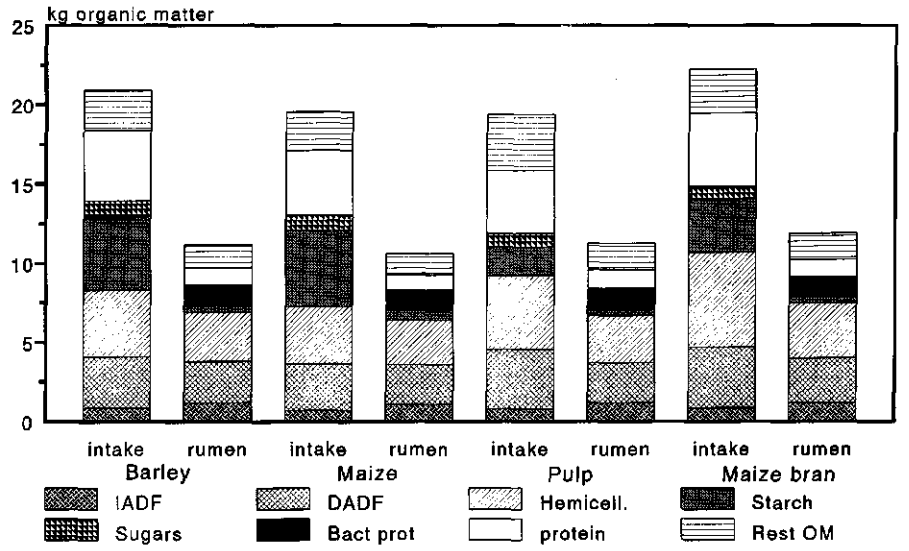

Figure 8.1 ***Composition of OM intake and rumen pool***

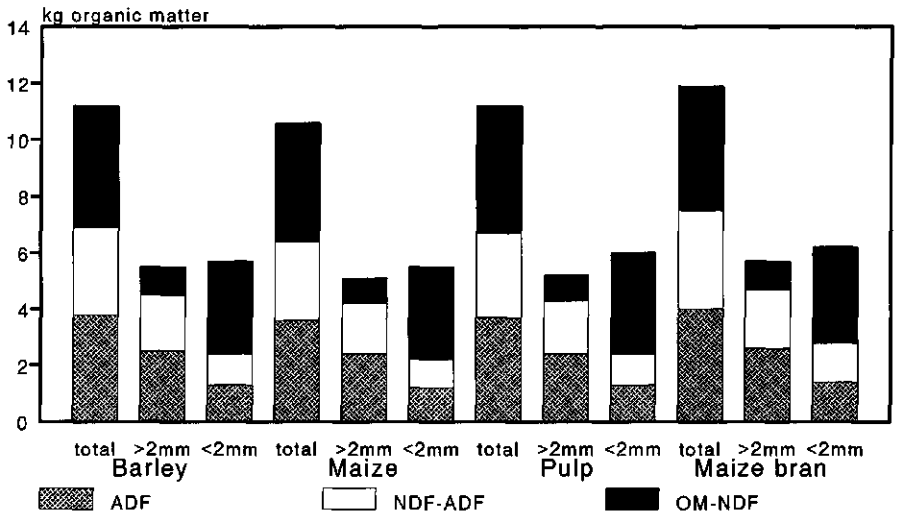

Figure 8.2 ***Partition of OM, hemicellulose and ADF of the rumen pool***

mainly consisted of cell wall constituents (approximately 80%), whereas the small particle fraction (<2mm) consisted to a large extent of non-cell wall constituent OM (Figure 8.2).
The rumen turnover of OM, NDF, ADF, DADF, hemicellulose, starch and protein is given in Table 8.5.
The rates of passage as calculated from the ratio between intakes (Table 8.1; Table 8.4) and pool sizes of the IADF-data of Table 8.4, were 2.96, 2.75, 3.04 and 3.00 % h^{-1} for diets B, M, P and MB, respectively.
The rate of digestion of the OM was similar for all diets, whereas that of NDF, ADF and hemicellulose was highest for both cell wall constituent diets (P, MB). That of starch was highest for cow's fed B and lowest for those fed P.

Rumen degradation
The results of calculated rumen degradation, using the nylon bag incubation technique, are given in Table 8.6. The rate of degradation of OM was highest for diet B and lowest for diet MB. The U fraction was similar for all diets, while the S fraction was highest for diet B and lowest for diet P. The rate of degradation of the NDF was lowest for diet MB, compared to diets B, M and P. The rate of degradation of starch was highest for diet B and lowest for diet M.

Discussion

Average pH was lower and average VFA higher than those found by Robinson *et al.* (1986), feeding comparable levels of starch in the concentrates (up to 320 g/kg^{-1} DM). The difference between minimum and maximum, expressed as range in Table 8.3, was lower in this experiment. Differences between the latter and this experiment were the lower feed intake (19.5 *versus* 22.5 kg DM), the time animals had access to feed and the type and quality of the roughage fed (poor quality hay *versus* wilted grass silage and maize silage), percentage of roughage (35 *vs.* 50% of total DM) and method of feeding (roughage and concentrates separately *vs.* TMR).
The pH of the rumen fluid and the concentration of total VFA was highly correlated ($r^2 = 0.92$) and fitted well to the equation derived by Tamminga & Van Vuuren (1988). In a later experiment (Tamminga, unpublished) it was discovered that rumen fluid contents increased sharply after feeding, resulting in dilution of the VFA and buffering the pH to decline. In addition, rate of absorption from the rumen increases with higher VFA concentrations (Dijkstra *et al.*, 1992). Hence the ruminant animal has a number of mechanisms which will prevent the average VFA concentration from rising above a maximum of around 150 mMol/l^{-1} (Robinson *et al.*, 1986, 1987b; Tamminga *et al.*, 1988; De Visser *et al.*, 1991) thereby preventing a pH drop to values that inhibit rate of degradation. In this experiment, feeding TMR may have slowed the rate of intake of rapidly degradable OM (starch,

sugars, soluble protein), enabling the animals to consume large amounts of feed without having too strong negative effects on rumen fermentation, which agrees with results of Rohr & Schlunsen (1986), who compared TMR against separate feeding of concentrates and roughage. Although the concentration of total VFA was similar for all diets, there was a difference in the ratio of the major volatile fatty acids and the calculated NGR values. The lower NGR value of diet B compared to diet M corresponded well with the higher rate of degradation of barley starch (Table 8.6; Tamminga *et al.*, 1990), its higher rate of digestion calculated from rumen evacuation data (Table 8.5) and nylon bag incubations (Table 8.6; Tamminga *et al.*, 1990; Malestijn *et al.*, 1988), which agrees with the lower rumen starch pool size (Table 8.4). As a result, a lower amount of starch probably escaped rumen fermentation, which agrees with the work of Waldo (1974) and Nocek & Tamminga (1991). The lowest NGR value of diet B corresponded with its high starch content and its higher measured k_d of the OM and starch. The highest NGR-value of diet P agreed with its higher NDF content and lower k_d of the OM and NDF (Table 8.1; Table 8.6). These findings are consistent with results of Sutton *et al.*, (1987) and Thomas *et al.* (1986), who fed starchy *versus* fibrous concentrates (barley, beet pulp).

It was expected for diet MB and diet P to have similar NGR values, because of the high NDF content (Table 8.1) and the low rate of degradation of the NDF (Table 8.6). However diet MB contained an higher amount of starch (Table 8.1), which was relatively rapidly degradable (Table 8.6), probably due to processing. This resulted in a tendency towards a lower average rumen pH and caused a shift in fermentation pattern towards propionic acid with a more starchy diet compared to a more fibrous one (Table 8.3).

The higher concentrations of ammonia and BCFA on diet MB probably were the result of an imbalance between the availability of N and energy for microbial protein synthesis. This is consistent with the very low k_d of the NDF and the higher k_d of the nitrogen of diet MB (Table 8.6), as well as the tendency towards a lower amount of rumen microbial protein (Table 8.4), which agrees with results reviewed by Nocek & Russell (1988). The accompanying feeding trial (De Visser *et al.*, 1990b) showed a lower milk protein content for cows fed diet MB, which is consistent with these ruminal data. Also diet M showed a tendency towards a higher concentration of ammonia in the rumen fluid. Starch escaping rumen fermentation results in a lower energy supply for microbes (Van Vuuren *et al.*, 1990), supplementing fresh grass with concentrates based on various carbohydrates varying in rate of degradation, as well as data reviewed by Nocek & Russell (1988). However, cows fed diet M did not show lower milk protein contents (De Visser *et al.*, 1990b). Our findings of tendencies towards reduced microbial protein synthesis agree with results of Robinson *et al.* (1987c), when feeding diets high in ensiled products with fermentation endproducts and thus a reduced amount of energy available for microbial growth. Robinson *et al.* (1987b) also found a tendency towards lower amounts of microbial protein in the rumen content when

decreasing starch in the diet. However, in this experiment this was not confirmed, by comparing diet P with diets M and B, probably as the result of the higher quality of the roughage used in this experiment. Total rumen content as related to bodyweight (BW) did not differ among diets and was approximately 20g/kg^{-1} BW, agreeing with results found and reviewed by Bosch (1991).

The highest rumen starch pool of diet M can be explained by the lower k_d of maize starch as compared to starch originating from barley or maize bran (Table 8.4; Table 8.6), which agrees with other observations found by Waldo (1973) and reviewed by Nocek & Tamminga (1991), comparing the digestion of starches in the gastrointestinal tract of dairy cows.

The similar pool size of the cell wall constituents among diets did not reflect the differences in NDF intake. A major part of NDF intake originated from the basal diet and was the same in all diets. Rumen degradation characteristics of NDF from beet pulp and maize bran, although different, were clearly not different enough to result in differences in rumen pool size.

The similar composition of the rumen pool of large particles (>2 mm, Table 8.4; Figure 8.2) are strongly related to the roughage part of the basal diet fed in all treatments (Table 8.1). The large particle pool consisted mainly of cell wall constituents (approx. 80%), because the other components were part of the more rapidly degradable or soluble fraction, which disappeared from the large particle fraction. Bosch (1991) showed similar data, when comparing grass silages in various stages of maturity.

The $k_{clearance}$ of NDF and IADF were similar for large particles (>2 mm, Table 8.5), suggesting that disappearance of NDF from the large particle pool was primarily the result of ruminating instead of degradation by micro-organisms, which confirms results of Bosch (1991), Moseley & Jones (1984), Smith *et al.* (1983) and Ulyatt *et al.* (1984).

The similar small particle pool (Figure 8.2) was unexpected, because large differences occurred in the composition of these fractions in the diets. Due to the time after feeding the rumen content was evacuated (on average approximately 5 hours after feeding) the more rapidly degradable components (sugars, starch, pectines, fermentation-endproducts) probably were fermented (Table 8.6) or had disappeared by passage.

The rate of passage of particles, calculated as $k_{clearance}$ of IADF was similar for all diets (Table 8.5) and agreed with the rate of passage found by Bosch (1991), De Visser *et al.* (1992) and Van Vuuren *et al.* (1992). However, the rate of passage of IADF probably underestimated the rate of passage of other organic components, such as starch.

The calculated rate of digestion of OM and N using the method of dacron bag incubations as well as rumen evacuation showed similar data. However, for starch and cell wall constituents the results disagree. The effects of the soluble fraction, rate of passage of particles and average time of rumen evacuation after feeding are probably responsible for the measured differences. Feeding diets varying in

carbohydrate composition and rate of degradation (starch and cell wall constituents) influence the rate of rumen fermentation, the amount of starch escaping rumen fermentation and the balance between energy and nitrogen available for rumen microbial growth. As a result rumen fermentation pattern was changed, which had its effect on milk performance.

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Chapter 9

General discussion

General discussion

Feed intake

Genetic potential

During recent decades successful breeding programmes have increased the production potential of our dairy cows considerably. Crossbreeding with Holsteins from the USA and Canada transformed the Dutch dairy cow from a dual purpose animal (milk and meat) into an animal selected mainly for milk production. These crossbreds are larger and it is probably by chance that they have inherited a relatively higher rumen volume:bodyweight ratio, resulting in a gastrointestinal tract with a larger capacity to digest organic matter.

Although, selection was concentrated on milk production and/or composition, these high producers displayed a simultaneous effect on the endocrine control of energy metabolism (Hart *et al.*, 1979). This gave these animals an increased capacity to mobilize and utilize bodyreserves more efficiently. The functioning mammary gland is one of the most highly differentiated and metabolically active tissues in the body (Davies & Bauman, 1974). During the first weeks of lactation, marked alterations in the general partitioning of nutrients and the animal's metabolism take place in an attempt to accomodate the increasing demands of the mammary gland (Vernon, 1988). Nutrient requirements of the mammary gland in high-yielding dairy cattle are of such a magnitude relative to total metabolism that the cow may be considered an appendage of its udder rather than the reverse (Brown, 1969). One of the major metabolic changes which is initiated at the onset of lactation occurs in adipose tissue; uptake of nutrients for synthesis of storage lipids is transformed towards mobilization instead (Metz & Van der Bergh, 1977). Another key nutrient is glucose and the maximally secreting mammary gland may require up to 80% of the total glucose turnover, approximately 18 Mol glucose, when producing 40 kg of milk daily (MacRae *et al.*, 1988). Rates of gluconeogenesis in the liver increase and presumably glycogen is mobilized, whereas other body tissues oxidize fatty acids rather than glucose as energy sources in an attempt to increase the availability of glucose for milk synthesis (Bennink *et al.*, 1972). At the same time protein reserves are used to meet the amino acid needs for milk protein and glucose synthesis in early lactation. Data concerning the capability of the dairy cow to mobilize protein reserves are contradictory. Bots *et al.* (1979) suggested that cows may mobilize more than 25% of their total body protein, whereas according to Gibb *et al.* (1992) approximately 7% of total body protein was mobilized. This would suggest that cows mobilize between 6 and 21 kg crude protein, the equivalent of what is required for the production of between 150 and 500 kg of milk.

The partitioning of nutrients during lactogenesis between the mammary gland and other body tissues is related to control mechanisms involving homeostasis

and homeorhesis (Bauman & Currie, 1980). Hart *et al.* (1975, 1978, 1979) observed that the concentration of growth hormone in blood was higher, whereas insuline was lower in a group of high-producing dairy cows, compared to low-producing crossbred animals. These results emphasize that these hormones are key endocrine factors that differ between high and low yielding cows, which are inherited differences in the partitioning of nutrients supporting lactation. However, the same magnitude of differences in growth hormone and insuline occur if high yielding animals are overfed or underfed to the same degree (Bauman *et al.*, 1979; Vasilatos & Wangsness, 1979).
The selection of animals for high milk production has also changed the genetics of the endocrine control mechanisms, providing the high yielding dairy cow with the possibility of supplying the mammary gland with sufficient nutrients even when nutrient uptake via the feed is limited. The control of mobilization of body reserves redirecting the nutrient supply towards the udder and maintenance gives the animal the opportunity to provide the mammary gland with an optimum of first limiting nutrients, such as glucose and amino acids.
Individual data of the 4 highest and 4 lowest producing animals from Chapters 3, 5 and 7 were used to calculate the difference in intake and milk performance over the first 2 weeks of lactation (Table 9.1).

Table 9.1 ***Comparison between DM intake and FPCM during the first 2 weeks of lactation for low (4) and high (4) producing animals on each treatment***

	Low	High	SED	Sign
DM intake (kg)	17.6	18.2	1.0	NS
FPCM (kg)	31.5	42.1	2.2	$p<0.05$
Energy deficit (MJ NE)	18.8	50.7	4.8	$p<0.05$

The results show, that low and high yielding animals did not differ significantly in DM intake immediately after parturition, whereas milk output varied significantly. This indicates that in early lactation differences in milk production are related more to variation in the capacity of the animals to mobilize energy and protein reserves, due to endocrine control mechanisms, than to feed (nutrient) intake. However, the effects over the whole of the lactation period are completely the opposite, because high yielding animals have to balance their higher energy and protein outputs with their intakes.
Recent studies with bovine somatotropin have shown that milk performance of high yielding dairy cows can be improved by changing the endocrine status of these animals from the moment of energy equilibrium onwards, re-establishing the same metabolic status as during the first weeks after parturition (Rypkema *et al.*, 1990; Oldenbroek *et al.*, 1991).

In conclusion: Animal performance during the first month of lactation is strongly related to nutrient supply to the mammary gland, especially the supply of first limiting nutrients and to the influence of the nutrient supply on endocrine regulation factors.

Fill

Feed intake is a constraining factor in high-level dairy performance (Bines, 1976). Milk yield is considered an important factor in controlling feed intake, due to its role in defining the physiological status of the animal (Vadiveloo & Holmes, 1979; Neal *et al.*, 1984; Bosch, 1991) and the extraction of nutrients from the metabolic pool by the excretion of milk (MacRae *et al.*, 1988).
Especially in early lactation cows are unable to increase DM intake (DMI) sufficiently to meet their nutrient requirements. Dairy cows at peak production may consume up to four times their maintenance requirements, but may have a production level of five times their maintenance requirements (Chapters 3, 5, 7). Factors associated with feeds are considerably important in the lactating cow; these include nutritional and physical characteristics of feed alongside gastrointestinal and metabolic factors (Baile & Della-Ferra, 1988). Carbohydrates, being the major component in dairy diets, have an important influence on these feed characteristics.
In this respect the physical capacity of the rumen to digest roughages and concentrates is an important factor. Garrige *et al.* (1986), Mertens (1987) and Bosch (1991) used "rumen fill" as a restricting factor on feed intake; NDF content, its particle size, its degradability and the passage rate of undegraded particles were the main factors involved. The DMI data of the feeding experiments (Chapters 3, 5 and 7) and the results of the accompanying fermentation and/or kinetic studies (Chapters 4, 6 and 8) were used to compare the measured DMI with that predicted with three different methods. The first method used was the equation of Mertens (1987), which assumes the ratio between Net Energy Requirement (NER) and Net Energy content per kg DM (NE) to be inversely related with the effect of rumen fill:

$$DMI = NER/NE$$

The second method was that of Vadiveloo & Holmes (1979), who estimated DMI using feed characteristics (percentage concentrate in the diet, C), rumen capacity (body weight, LW) and a production related factor (weeks after parturition, WL):

$$TDMI = -4.14 + 0.43 \times C + 0.015 \times LW - 0.095 \times WL + 4.04 \times \log WL$$

The third method used, was that of Bosch (1991), in which the variation in rate of clearance of the DM was estimated from fat and protein corrected milk

production/kg BW $^{0.75}$ (FPCMMW), NDF content of the roughage (NDFR), the percentage concentrate in the diet (C%) and body weight (LW):

TDMI = (265.9 + 1.39 x FPCMMW - 0.26 x NDFR + 2.11 x C% + 0.47 x LW) x 24

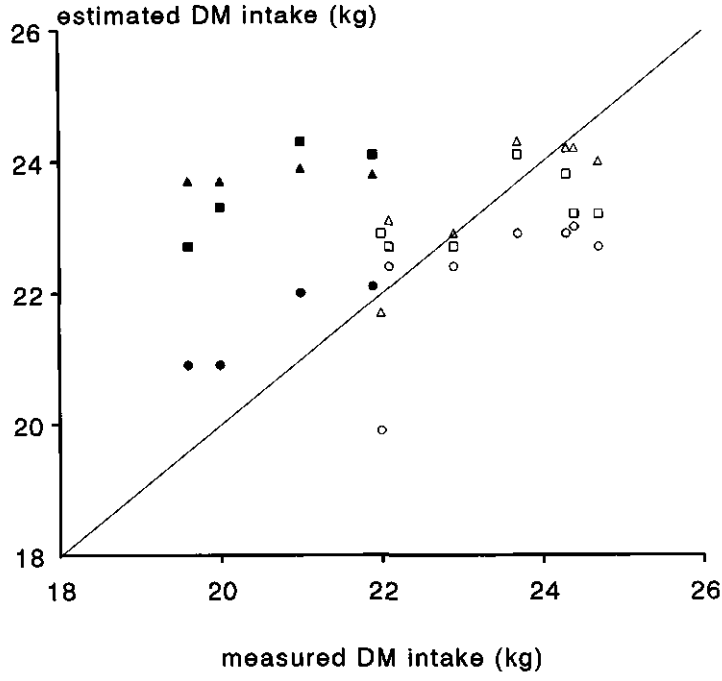

Figure 9.1 ***Measured and predicted TDMI, according to the equations of Mertens (1987), □-■; Vadiveloo & Holmes (1979), ○-●; Bosch (1991), △-▲. Diets: DM content <400 g/kg □, ○, △; DM content >400 g/kg ■, ●, ▲.***

In general, all equations overestimated DMI on moist diets (< 400g DM/kg). A poor relationship was established between the energy requirements and the energy content of the diets fed (Mertens, 1987), because of the limited variation in *in vitro* and *in vivo* digestibility and in the undegradable OM- or NDF-fractions. The results of Conrad, Pratt & Hibbs (1964), who found a negative relationship between the digestibility of the cell wall fraction of roughage and total DM intake, could not be confirmed. The differences measured in energy requirement / intake ratios (Tables 3.6, 5.7 and 7.4) did not vary much between treatments, because the animals were fed slightly above or slightly below their requirements.

The equation of Vadiveloo & Holmes (1979) gave reasonable estimates. Moist diets were slightly overestimated, whereas dry diets were underestimated. An exception was the diet with extra maize silage (Chapter 3) which was considerably outside the predicted range. This was probably due to the fact, that the maize silage was considered to be a roughage component, which made this diet different in concentrate:roughage ratio in comparison to the other diets tested. Estimates obtained with the equation of Bosch (1991) seemed to be accurate for diets with a DM content above 500g/kg. Diets with a DM content below 400g/kg were overestimated. The pressed beet pulp treatment in Chapter 3 and all moist diets of the experiment in Chapter 5 predicted higher DMI's than were actually measured. This was probably a result of the lower DMI measured during the first six weeks of lactation, which was included in the measured DMI data of the experiments. Lower DMI on high moisture diets was also observed by others in their experiments (Waldo; 1978; Steen & Gordon, 1980; Lahr *et al.*, 1983; Rohr & Thomas, 1984; Gordon & Peoples, 1986; Peoples & Gordon, 1989; De Visser & Tamminga, 1987). Firstly, lower DMI on moist diets might be explained by the larger bulk which must be eaten by the animals. Especially in early lactation this had a negative influence on DMI by at least 1 kg, as found in the experiment discussed in Chapter 5 (comparing WGS to WW), which was confirmed by Jarrige *et al.* (1986). The effects of DM content in TMR diets were significantly lower, when DM content remained below 400 g/kg, no further improvement of DMI was measured above a DM content of 600 g/kg (Tables 5.7; 7.4), which confirmed results of Lahr *et al.* (1983). Secondly, the effects might also be related to the higher amounts of fermentation end products (VFA, lactic acid; Murphy & Gleeson, 1984; Donaldson & Edwards, 1976; Derbyshire *et al.*, 1975; Zimmer & Wilkins, 1984) in the silage, replacing rapidly degradable carbohydrates (sugars, rumen degradable starch) and the increase in rate of degradability of the N-components. In this situation, N-components and rumen degradable carbohydrates are out of balance which in turn reduces the capacity of the rumen to digest slowly degradable NDF, due to a temporary lack of nitrogen (Van Straalen & Tamminga, 1991; Hvelplund & Madsen, 1985; Hoover, 1986). This agreed with the tendencies towards reductions in rumen cell wall constituents found by clearance (Table 6.6) and the concentrations of NH_3-N measured in the rumen fluid. The equation published by Bosch (1991) is based on data derived from experiments with various roughage components. The grass silages used, differed mainly in stage of maturity, but it did not include data from grass silages low in DM content or high in the amount of fermentation end products. The amount of concentrates fed was lower than in the diets of our studies. Especially in the high moisture diet experiment (Chapter 5), the concentrates fed were extremely low in easily fermentable carbohydrates and a large part of the concentrates was fed as ensiled by-products. This may have had a negative influence on the degradation of the cell wall fraction of these diets, which can explain the difference found between the results of the

experiment (Chapter 5) and the predicted values according to Bosch (1991). Addition of small amounts of rumen digestible starch and/or sugars may improve microbial activity under these circumstances, and as such may partly reduce the negative effects on roughage DMI (Tamminga, 1981; Chamberlain, Thomas & Quig, 1986; Rooke, Lee & Armstrong, 1987).

In conclusion: Equations developed to predict the intake of dairy cows should contain the dry matter content of the diet as an independant variable.

Rumen fermentation

The fermentation of cell wall constituents is positively related to a high cellulolytic activity of the microbial population. Increasing the proportion of starch-rich concentrates in dairy diets reduces the cellulolytic activity (Porter *et al.*, 1972; Taylor & Aston, 1976; Russell *et al.*, 1979; Hiltner & Dehority, 1983; Hoover, 1986), due to a shift towards amylolytic activity. This increase in amylolytic activity is the result of an increased supply of easily fermentable carbohydrates (sugars, rumen degradable starch) as substrate, which is rapidly fermented in the rumen. As a result concentrations of volatile fatty acids are increased and rumen pH is lowered (Tamminga & Van Vuuren, 1988). Therefore optimal conditions for ruminal digestion aimed at maximalization of DMI, have to provide a balance between a high level of cellulolytic activity in order to digest as much NDF (roughage, concentrates) as possible (Russell & Sniffen, 1984), and a high rate of digestion of rapidly degradable concentrate carbohydrates (sugars, rumen degradable starch), while taking care not to induce rumen acidosis (Giesecke, Bartelmus & Stangassinger, 1976; Counotte, 1981).

Increasing the amount of easily fermentable carbohydrates in concentrates lowered the rumen pH (Figure 2.1), which negatively influenced DMI (Table 2.6). De Visser & De Groot (1980) found that the dramatic reduction in intake following the feeding of high starch concentrates immediately after parturition, was the result of an increased number of animals suffering from rumen acidosis (3 *versus* >60%). Under less extreme circumstances Castle & Watson (1975), Meys (1985) and Thomas *et al.* (1986) found reduced DMI, when concentrates high in cell wall constituents (beet pulp, rice bran, dried roughages) were substituted with concentrates high in rapidly rumen degradable starch (barley, tapioca, wheat).

The negative effect of the starch content in concentrates on feed intake is also related to the total amount of concentrates fed. The results shown in Figure 2.2 indicate a decrease in the pH of the rumen fluid, where there is an increase in the amount fed of concentrates containing higher levels of rumen fermentable carbohydrates. Starch escaping rumen fermentation does not negatively influence cellulolytic activity of the rumen population.

Rumen fermentation does not only depend on energy supply, but is also related to the availability of N to the rumen microbes. In *in vitro* studies, using rumen

fluid from a dairy cow fed with hay only, Malestijn *et al.* (1981) showed that supplementing an easily fermentable starch (tapioca) or sugar source (citrus pulp) with high N containing by-product ingredients (soya bean meal solv. extr.), increased the concentration of lactic acid and reduced the rumen pH more dramatically than with tapioca alone.
Reduced intakes of moist ensiled grass silage compared to wilted grass silage as found in Chapter 3, may partly be the result of an imbalance between energy and nitrogen sources available to micro-organisms. Differences in N-degradability as reported in Table 6.7 seem to confirm this supported by the results of Hoover (1986).
The negative effect on DMI seems more pronounced with prefermented diets. A possible explanation for this observation is that the energy lost during fermentation in the silo is not compensated for by easily fermentable carbohydrates in the concentrates. This could explain why in the experiments of Gordon & Peoples (1989), Steen & Gordon (1980), Rohr & Thomas (1984) and Waldo (1978) less pronounced differences were found when comparing low and high moisture diets, because in these experiments the concentrates fed, were based mainly on barley, supplying easily fermentable carbohydrates to the rumen for microbial activity.

In conclusion: High moisture diets reduce feed intake in early lactation, because of the larger amount of bulk which must be eaten by the animals. A more permanent negative effect on feed intake remains during lactation, because of an imbalance between energy and protein available for microbial activity in the rumen, which can be compensated by adding small amounts of easily fermentable carbohydrates to the basal diets.

Feeding method
Animals fed *ad libitum* consume several relatively small meals as compared to animals fed restricted amounts of feed, who change their intake pattern into a small number of large meals (Chapter 4, Figure 4.1). Our results and those of Robinson *et al.* (1986) show a large influence of feeding level on diurnal pattern of the fermentation. Although, at high levels of intake more substrate is available to the microbial population, the fermentation pattern becomes more stable, because the amount of substrate enters the rumen in small portions throughout the day. The diurnal pattern on low intakes will be much more pronounced, which was shown by our results (Table 4.3) and those of Robinson *et al.* (1986).
When animals are fed a TMR *ad libitum* or roughage and concentrates separately at regular intervals (6 times daily), feed intake pattern changes in favour of more and smaller meals, reducing the diurnal variation, as found by Rohr & Schlunsen (1986) and Malestein *et al.* (1981).
If high and low levels of intake coincide with a high and low milk yield a stabili-

zing effect on rumen fermentation can also be expected from the rapid removal of fermentation end products at high milk production levels (Dijkstra *et al.*, 1993). Therefore the effect of intake level on the fermentation characteristics in the rumen is strongly related to the diurnal balance of various substrates available for rumen fermentation (Murphy *et al.*, 1982; Dijkstra *et al.*, 1992), the clearance of substrates and fermentation end products (Dijkstra *et al.*, 1993) and the use of nutrients by the mammary gland and other tissues (MacRae *et al.*, 1988).
When roughage and concentrates are offered separately, feed intake pattern of roughage depends on the amount and frequency at which concentrates are fed, as shown by the comparison between flat rate feeding and feeding to requirements. Restricted allowance to concentrates especially in early lactation increased roughage intake (Rypkema *et al.*, 1990; Ekern, 1972; Journet & Remond, 1976). However, DMI on a flat rate or TMR system depends on the level of concentrates offered to the animals, as was found by Østergaard (1979), comparing different levels of concentrates on a flat rate system.

In conclusion: Feeding *ad libitum* and feeding TMR diets results in a shift in intake pattern towards more and smaller meals reducing the diurnal pattern of rumen fermentation, which stabilizes rumen fermentation.

Fermentation and digestion

Because carbohydrates are the main chemical component in ruminant feedstuffs. They are the most important substrate for rumen fermentation and digestion in the intestines and will therefore provide the dairy cow with substantial amounts of nutrients for milk synthesis. In nature, the occurrence of carbohydrates is strongly related to their functional properties (Aman & Graham, 1990), which can be classified as primary energy carrier, intermediary energy source, energy store, energy transport medium and a structural element. These functional properties are reflected in the type of carbohydrate (sugars, starch, cell wall constituents) and define its chemical structure (Aman & Graham, 1990), its type of bonding between chains of saccharides (Boon, 1989; Meier & Reid, 1982; Van Soest, 1982), solubility (Aman & Graham, 1990; Nocek & Tamminga, 1991) and degradability (Nocek & Tamminga, 1991; Casper & Schingoethe, 1989; Herrera-Saldena & Huber, 1989; McCarthy *et al.*, 1989; Malestein *et al.*, 1981). The diets used in this study (Chapters 2, 4, 6 and 8) were designed to attempt to create variation in carbohydrate composition (sugars, starch and cell wall constituents) and to examine any effect on solubility, indigestibility and degradability (Chapters 4, 6, 8). The supply of nutrients from polysaccharides (starch, cell wall constituents) depends on enzymatic hydrolysis releasing their component monosaccharides. Ruminants, as well as

other mammals and birds are assumed to be capable of degrading starch in the small intestine through the action of their endogenous enzymes (Chesson, 1990). Starch becomes available as glucose, which can be used after absorption for metabolism.

In contrast, the hydrolysis of structural polysaccharides from plants is wholly dependent on the enzyme systems of the microbes in the intestinal tract. The degradation of these carbohydrates also depends on other components (amino acids and nitrogen) needed to meet the nutrient requirements of the microbes. As a result the host animal is supplied with microbial fermentation products. In ruminants part of the starch is also hydrolysed by the microbes and becomes available as microbial fermentation products (e.g. microbial protein and volatile fatty acids (VFA)). The amount of starch escaping rumen fermentation depends on the rate of degradation and the rate of passage of particles through the rumen (Nocek and Tamminga, 1991). Harmon (1992) questioned if the hydrolytic capacity of the small intestine of ruminants was capable of digesting all starch entering the small intestine, because no relationship was found between the increase in starch and carbohydrase content in the small intestine. Residence time and surface area exposure may also limit starch digestion in the small intestine (Owens *et al.*, 1986).

Estimations were made, using the results from Chapters 5, 6, 7 and 8, of the amount of nutrients available after fermentation or digestion by means of the rumen fermentation model of Dijkstra *et al.* (1992), which are shown in Table 9.2. The rates of passage of fluid from the rumen, if not measured during the experiments (Chapters 6 and 8), were calculated using the equation of Owens & Goetsch (1986). Starch entering the small intestine was assumed to be completely digested in the small intestine and absorbed as glucose.

On the "starch-rich diets" (B and M) relatively low levels of starch escaped rumen fermentation and amounts entering the small intestine were below the maximum capacity mentioned by Harmon (1992). Due to the differences between both starch diets (rapidly and slowly degradable) measured *in situ* large differences in starch entering the small intestine are expected (Table 8.6) between both diets. However, on the one hand the high level of DMI may have increased the amount of starch escaping rumen fermentation with the rapidly degradable starch source (barley), whereas on the other hand the rumen passage rate of the slowly degradable starch source (maize) may have been slower than assumed when estimating starch degradability in the rumen with a passage rate of 6%/h. The assumption of a single passage rate irrespective of feed type is open to criticism, but *in vivo* measurements for each individual ingredient are not available. Another complication in the tabulation of starch degradability in feedstuffs involves the influence of treatment during the production process and, in particular, treatment during the production of compound feeds; grinding, exposure to high temperatures and pressure.

Table 9.2 ***Estimation of available nutrients of the diets of Chapters 5 and 6 (WGS, MGS, FGS, WW) and chapters 7 and 8 (B, M, P, MB) produced during rumen fermentation and intestinal digestion (VFA, glucose (Mol/day), estimated DVE intake using the model or in situ data (g/day), the rumen fermentable OM/microbial N ratio (RFOM/N), lipids (feed and microbial origin), digested LCFA and glycerol (g/day) and the deposition and reposition of body fat (LCFA, glycerol) and protein (DVE)***

Nutrients from feed intake

					Estimated DVE				Digestibility			
diet	ac	prop	but	glucose	model	*in situ*	RFOM/N	lipids	%	LCFA	glycerol	experiment
WGS	62	20	13	6	1645	1712	16.5	1173	80	798	176	5, 6
MGS	55	19	12	6	1552	1480	17.9	1044	80	739	120	5, 6
FGS	56	19	11	6	1594	1546	16.9	1094	80	744	126	5, 6
WW	59	20	13	6	1678	1642	16.5	1131	80	801	130	5, 6
B	68	28	13	9	2288	2391	16.2	1416	80	1002	163	7, 8
M	69	28	15	10	2236	2452	16.5	1457	80	1031	168	7, 8
P	74	26	14	7	2228	2460	16.4	1264	80	952	145	7, 8
MB	70	27	13	8	2239	2551	16.7	1446	80	1024	166	7, 8

Nutrients from body reserves

	Deposition				Reposition			
treatment	fat	LCFA	glycerol	DVE	fat	LCFA	glycerol	DVE
WGS	434	384	50	98	-	-	-	-
MGS	690	610	80	155	-	-	-	-
FGS	628	555	73	141	-	-	-	-
WW	578	512	66	130	-	-	-	-
B	106	93	13	24	202	179	23	57
M	140	123	17	31	194	172	22	55
P	322	285	37	72	22	71	9	22
MB	225	199	26	51	51	135	17	43

The volatile fatty acid ratio was calculated using the predicted production of total VFA in the model of Dijkstra *et al.* (1992) and the concentration measured in the fermentation studies (Chapters 6 and 8). Neal *et al.* (1992) displayed inaccuracies in the predictions of the production of propionic (overestimated) and butyric acid (underestimated), which were probably due to the limited *in vivo* data from high-yielding dairy cows used by Murphy *et al.* (1982, 1988) to derive the equation used in Dijkstra's model (Dijkstra *et al.* (1992).
Differences in total amounts of VFA, glucose and DVE observed among treatments in Table 9.2 correspond with differences in DMI and net energy intakes (Chapters 5 and 7). However, DMI and net energy intake (VEM) did not explain the variation in the pattern of available nutrients between treatments for milk production.

In conclusion: Differences in degradability occur between and within carbohydrates (starch, cell wall constituents). This variation in rate of degradation influences the fermentation pattern, due to changes in cellulolytic and amylolytic activity of the rumen microbes and the site of digestion (rumen, small intestine). This influences the nutrients available for maintenance and milk production.

Type of carbohydrate
In the experiments described in Chapters 5, 6, 7 and 8, the diets varied in cell wall constituents and starch content. The diets considered in Chapter 5 (WGS, MGS, FGS, WW) and those of Chapter 7 (P and MB) were based on cell wall constituents (cellulose and hemicellulose) and showed higher acetic acid concentration in relation to propionic acid than those of the starch-rich diets in Chapter 7 (B and M). This corresponds favourably with the results reviewed by Murphy *et al.* (1982), who attempted to predict VFA proportions produced from various carbohydrate sources (sugars, starch, cellulose, hemicellulose) and between roughage and concentrate ingredients. In general, the results displayed in Tables 6.3 and 8.4 corresponded with the VFA pattern described in literature, being higher in acetic acid concentration, when feeding diets rich in cell wall constituents (beet pulp, rice bran, etc.) than on rumen degradable starch-rich diets (barley) (Thomas *et al.*, 1984; Trémère *et al.*, 1968; Lees *et al.*, 1982; Sutton *et al.*, 1984; Thomas *et al.*, 1984; Valk & Hobbelink, 1992; Van Vuuren *et al.*, 1993).

In conclusion: The type of carbohydrate (starch, cell wall constituents) influences the relative proportions of VFA.

Rate of degradation
The treatments examined in Chapters 3, 5 and 7 showed variation in the rates of degradation between carbohydrates (NDF, starch; Table 8.6) as well as within carbohydrate sources (Tables 4.4, 6.7 and 8.6). The observed differen-

ces correspond with results found by Tamminga (1989) and reviewed by Nocek & Tamminga (1991). Large differences were found in the *in situ* degradability of starch fed with diets M and B (Chapter 7 and 8; Table 8.6). The differences in degradation of starch measured *in situ*, were not supported by the predictions of the amount of starch escaping rumen fermentation by use of the model of Dijkstra *et al.* (1992). However, the data in Table 9.2 agree favourably with the relatively small variation in VFA concentrations of the rumen fluid (Table 8.3) between starch sources as reported in Chapter 8.
However, the relatively small difference in the amount of starch escaping rumen fermentation, predicted by the model (Dijkstra *et al.*, 1992), agreed with the relatively small differences observed in starch rumen poolsizes in kinetic studies between starchy diets (M and B) and among starch-rich and NDF-rich diets (B, M and P, MB; Table 8.4). However, results of Waldo (1973), Malestein & Van 't Klooster (1986), Casper & Schingoethe (1989), Herrera-Saldena & Huber (1989) and McCarthy *et al.* (1989) indicated much larger differences in the potential amount of starch escaping rumen fermentation. Apparently, results are influenced by particle size and their reduction. In the diets considered in Chapter 5 (WGS, MGS, FGS, WW) and some of the diets in Chapter 7 (P, MB), starch originated mainly from maize silage which was crushed at harvest, whereas both high starch diets (B, M) were obtained by inclusion of ground barley or maize in the concentrates. Thus particle size of the starch in both starch-rich diets (B, M) was smallest, increasing the rate and extent of fermentation, which could have reduced the rate of passage of these particles from the rumen (Nocek & Kohn, 1987; Ehle, Murphy & Clark, 1982; Ewing & Johnson, 1987; Ewing *et al.*, 1986). However, when feeding at high levels of intake the rumen contains a thicker layer of a structural mass, which might have worked as a filter for smaller particles, thereby reducing the rate of passage of these particles.

In conclusion: The variation in *in situ* rate of starch degradation among different sources is considerable. However, the amount of starch escaping rumen fermentation depends on many more variables, than *in situ* degradation.

Fermentation pattern
The differences in fermentation pattern estimated with the model (Dijkstra *et al.*, 1992) agreed with the results of the fermentation studies (Tables 6.4 and 8.3). As stated previously differences result from differences in carbohydrate composition (NDF, starch; Tables 6.2 and 8.1). However, similar diets in both experiments (WGS and P) showed large differences in VFA ratio (Tables 6.3 and 8.4). This can be partly explained by differences in the sugar and starch content (Tables 6.2 and 7.3) and by the difference in digestibility (Tables 6.2 and 7.3), especially for grass silage. Dry matter intake of WGS was also lower than that of P (21.9 *versus* 23.7 kg/d), which increased the rumen pool sizes, probably

increased the DM/fluid ratio (Tamminga, unpublished), increased VFA concentrations and reduced rumen pH, which negatively influenced the cellulolytic activity, initiating a shift towards higher propionic acid ratio's. Robinson *et al.* (1986) observed similar results, when increasing daily DMI from 6 to 24 kg.

In conclusion: The rumen fermentation pattern is influenced by the type of carbohydrate fed, yet other factors must be considered as important variables; feed intake level, roughage/concentrate ratio, particle size and the passage rate of particles and fluid.

Balance between rumen degradable crude protein and carbohydrates

The results of the fermentation (Tables 4.3, 6.4, 8.3) and the degradability studies (Tables 4.4, 6.7, 8.6) indicate reduced microbial protein synthesis (increased concentrations of rumen ammonia and BCFA). This can be attributed to higher undegradable fractions (Table 4.4), an imbalance between the rate of degradation of crude protein (cp) and energy sources (Table 8.6), or replacement of carbohydrates by fermentation end products during preservation in the silo (Table 6.7). These indications are corroborated by the results of Chamberlain *et al.* (1985), Rooke *et al.* (1987) and Hoover (1986) who found increased amounts of microbial protein entering the small intestine, when optimizing N and energy availability in the rumen. Sinclair *et al.* (1993) also showed that the synchronization of the rate at which energy and N are released from the diet positively influenced microbial protein synthesis, when accounting for the reflux of urea with saliva.

The ratio between rumen fermented OM and microbial N, estimated by the model of Dijkstra *et al.* (1992), is an additional indication of differences between treatments. The range of the estimated values agreed with those published by Demeyer & Tamminga (1987) (Table 9.2). The ratio between rumen available N and rumen available carbohydrates fell within the range considered conducive to optimal microbial growths (4.6 to 3.9) (Czerkawski, 1986).

Prediction of the duodenal protein supply, using the model of Dijkstra *et al.* (1992), appeared comparable to the predicted amount of protein available for maintenance and/or milk production (DVE), using the Dutch protein system (CVB, 1991b). In general, the DVE values were higher than the supply estimated by the model (Table 9.2), except for diets MGS and FGS. However, higher model estimates for the diets MGS and FGS agree with the results of the feeding trial (Chapter 5). This indicates an underestimation for the DVE values of moist grass silages, when DVE is calculated using the equations for grass silages (CVB, 1991b).

In conclusion: Microbial protein synthesized in the rumen is strongly related to the availability of fermentable OM and cp in the rumen and is therefore related

to the degradation characteristics of all ingredients and their chemical composition.

Lipids

All diets used, contained moderate amounts of lipids varying between 25 and 39 g/kg DM (Tables 4.1, 6.2 and 7.3). According to the ingredient composition of the diets the fatty acid composition was very similar for all treatments (CVB, 1991). The total amount of unsaturated fatty acids, originating mainly from soya bean meal, linseed expeller or palm kernel expeller, remained limited, since in all experiments relatively small amounts of these ingredients were included in the concentrates fed (CVB, 1991). Most of the unsaturated fatty acids will have been hydrogenated during their residence in the rumen (Storry, 1985; Palmquist & Jenkins, 1980). Lipids fed, were mainly triglycerides, which are hydrolized and separated into glycerol and fatty acids by the rumen microbes. Glycerol represented 11.5% of total fat, according to the average fatty acid composition of feed fat (CVB, 1991). Fatty acids are not used by micro-organisms in the rumen as an energy source and the outflow from the rumen is related to the fluid phase (Dijkstra *et al.*, 1992). Some of the lipids entering the small intestine are synthesized by microbes in the rumen and were included as lipids estimated to be available for digestion (Table 9.2).
Glycerol originating from feed lipids was assumed not to enter the small intestine, but to be used by the rumen micro-organisms as an energy source (hexose-pool) (Dijkstra *et al.*, 1992). Glycerol from fat of microbial origin enters the small intestine in limited amounts.

In conclusion: Limited amounts of fat were offered, which were assumed to be completely hydrolized. Unsaturated fatty acids were assumed completely hydrogenated in the rumen. Fatty acids are not an important energy source for microbial activity.

Absorption and partitioning of nutrients

Nutrients available after fermentation and digestion in the gastro intestinal tract have to be absorbed before they can be utilized by the animal. One of the major groups of nutrients is formed by the VFA's, these are absorbed in the reticulo-rumen/omasum and abomasum. Absorption is assumed to progress as a simple diffusion (Hogan, 1961; Dijkstra *et al.*, 1993) through the rumen wall and the rate is represented by a function of individual VFA concentration, modified sigmoidally by rumen fluid pH. However, it should be recognized that the rate of absorption of VFA increases with chain length, this was neglected by Murphy (1984). The amount of VFA escaping the rumen by passage in the fluid, influenced the rate and probably also the extent to which VFA is absorbed, due

to the lower pH in the abomasum compared to the rumen, increasing the rate of diffusion (Dijkstra *et al.*, 1993). As a result the concentrations of VFA in the rumen will be reduced, positively influencing cellulolytic activity in the rumen. This hypothesis is confirmed by the increase in the rate of passage of fluid found by Robinson *et al.* (1986), when DMI was increased and a shift in the absorption of VFA towards postruminal sites was observed (Tamminga & Van Vuuren, 1988). Casse *et al.* (1993a,b) showed an increase in propionic acid flux in portal drained viscera (PDV), when infusing propionic acid in the rumen of dairy cows. However, the amount of VFA absorbed from both rumen and abomasum has not yet been studied in relation to diet composition, feed intake level and stage of lactation. Therefore, there is room for improvement in predictions of VFA available to dairy cows for maintenance and milk production.
Another major nutrient is glucose, this becomes available after digestion of escaped starch in the intestine (Nocek & Tamminga, 1991). The amount of starch entering the intestine might exceed the capacity of the small intestine to hydrolyse the starch (Harmon, 1992) either because of a limited enzymatic capacity or a limited residence time. Huntington & Reynolds (1986) showed increased glucose absorption (65%), when infusing glucose in the abomasum of heifers and a cow. However, infusing starch increased absorption in the heifers (35%) and too a lesser degree in the cow (8%). Kreikemeier *et al.* (1991) reported similar results. Effects found, were lower than expected, but could have been influenced by the type of animal used (dry or growing animals) instead of high-yielding dairy cows, the latter having a greater demand for glucose, necessary for lactose production. Under these circumstances, oxidation of glucose in the intestinal wall and liver will be reduced (Vernon, 1988).
Amino acids are another group of important nutrients absorbed from the intestine. Absorption of amino acids is a complex mechanism, which uses several carrier systems (Larson & Jorgensen, 1974; Mepham, 1982, 1988; Webb, 1984). Absorbed amino acids are used as precursors for protein synthesis in the mammary gland and/or protein needed for maintenance (Oldham, 1984; Emmans & Fisher, 1986). However, glycogenic amino acids are also used as glucose precursor in gluconeogenesis (MacRae *et al.*, 1988). Especially in early lactation, when animals are fed below their energy requirements, the dairy cow seems to give preference to glucose, probably as a result of hormonal regulation (insulin), reducing the use of glucose for oxidation (Vernon, 1988). Effects are related to the stage of lactation as shown in experiments with increased protein supply from optimal rumen microbial protein synthesis on herbage (Meys, 1985; Valk *et al.*, 1992)(Table 9.3).
It should be noted, that many effects of changes in nutrient supply in relation to absorption have been studied by means of infusion of simple nutrients or substitution of ingredients without any consideration of the basal diet fed, hormonal control mechanisms, type of animal and stage of lactation. This makes it very difficult to use the information in predictions for milk performan-

ces (Thomas & Martin, 1988).

Table 9.3 ***Milk performance on herbage diets balanced for N:carbohydrate composition by adding carbohydrates (starch and NDF)***

Stage of lactation	Meys late		Valk et al. early	
diet	grass	grass/maize	grass	grass/maize
DM intake (kg)	12.9	14.4	18.6	18.7
Milk (kg)	22.0	22.8	26.9	29.7
Milk protein (g/kg)	32.9	34.2	31.4	31.1
Milk protein yield (g)	683	779	845	923
Milk protein per KVEM[1)]	93.6	95.0	66.5	75.1
Milk lactose yield (g)	1012	1049	1237	1366
Milk lactose per KVEM[1)]	138.8	127.9	97.4	111.1

After Meys, 1985; Valk *et al.*, 1992
1) after correction for maintenance

Deposition and reposition of nutrient reserves

During the early stage of lactation the intake of high-yielding dairy cows does not meet their requirements. As shown previously (Table 9.1) high-yielding dairy cows can only produce large quantities of milk solids, when they are able to mobilize body reserves. The reserves of the major nutrients (glucose, amino acids and long-chain fatty acids) differ. Glucose reserves are assumed to be very limited (Vernon, 1988). An increase in supply of glucose to the mammary gland is achieved by increasing the size of the liver (Butler-Hogg *et al.*, 1985), an increase in the activity of the liver (Vernon *et al.*, 1987) and an increase in the supply of glucogenic precursors (Baird *et al.*, 1980).
Dairy cows can mobilize large quantities of long-chain fatty acids (LCFA) from adipose tissue. During early lactation lipogenesis is decreased due to low or no activity of acetyl CoA carboxylase (Metz & Van der Berg, 1977; McNamara & Hillers, 1986). The mobilization of reserves during peak demand and the partitioning of nutrients between milk production and replenishment of body reserves is extremely complex, and is influenced by the level and composition of the diet (Cowan *et al.*, 1980; Ørskov *et al.*, 1983), as well as by the milk production potential of the individual cow (Moe *et al.*, 1971; Garnsworthy, 1988). The amount of fat mobilized by the animals in the feeding experiments considered in Chapters 5 and 7 were estimated, using the difference between energy intake and energy requirements during the experimental period, according to the Dutch energy system (Van Es, 1972). The overall fat deposition and reposition was

estimated for the average experimental period (Table 9.2; Figure 9.2) (Van Es, 1972; Flatt *et al.*, 1972) and divided into LCFA and glycerol (Bondi, 1987). The total amount of fat mobilized agreed with estimated changes in body weight as mentioned in Chapters 5 and 7 and confirmed the values measured by Gibb *et al.* (1992). The animals used in the experiment considered in chapter 7 showed a positive energy balance from 7 to 10 weeks onwards and gained weight during the later stage of the experiment (Table 9.2; Figure 9.2).

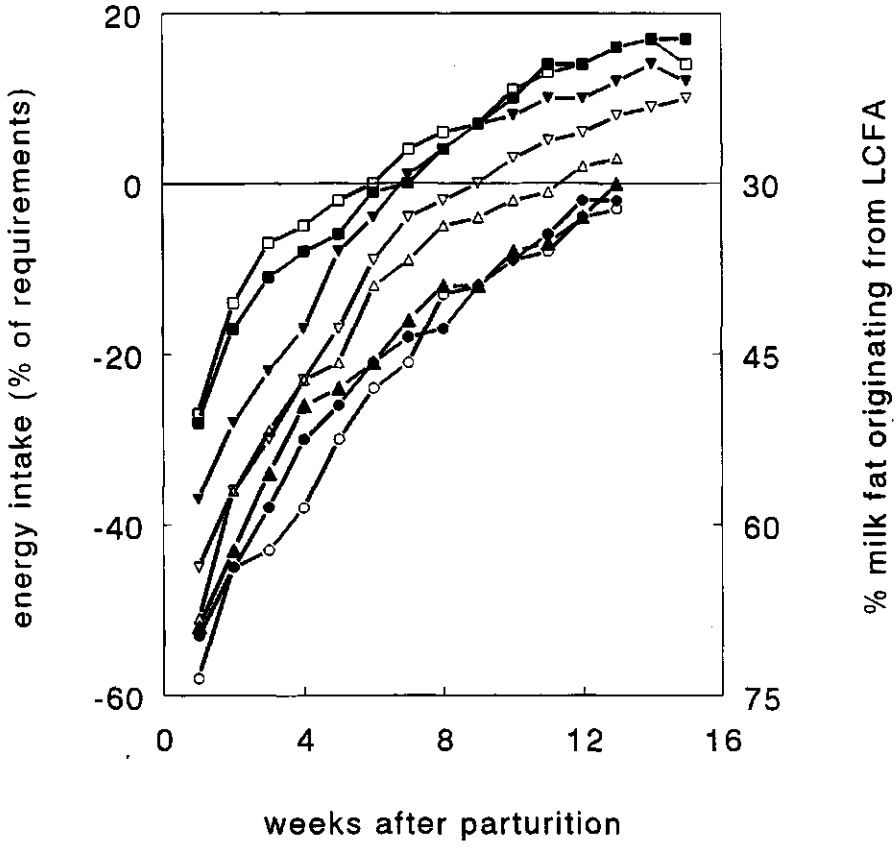

Figure 9.2 ***Energy balance during the experimental period and the percentage of milk fat synthesized from LCFA. Treatment: WGS,*** ▵-▵***; MGS,*** ○-○***; FGS,*** ●-●***; WW,*** ▲-▲***; B,*** □-□***; M,*** ■-■***; P,*** ▽-▽***; MB,*** ▼-▼.

The deposition and reposition of protein was estimated, according to the DVE system, assuming a mobilization of 45 g DVE per 1000 VEM negative energy balance and a requirement of 57g DVE for every 1000 VEM in a positive energy balance (CVB, 1991b). The amount of protein mobilized was different between both experiments (Table 9.2; Figure 9.2), due to the differences in the energy balance among treatments (CVB, 1991b). The estimated values were within the range which was assumed possible according to the results found by Botts *et al.* (1979) and Gibb *et al.* (1992), being 7% of total body protein.

In conclusion: In early lactation dairy cows mobilize large quantities of body reserves, due to hormonal drive which stimulates milk production to the absolute maximum.

Milk performance in relation to supply of nutrients to the mammary gland

High levels of milk production from dairy cows are impossible without sufficient nutrient supply to the mammary gland. Currently, energy and protein intake of animals are being used as indicators of nutrient supply (ARC, 1984; NRC, 1988; CVB, 1991, 1991b). However, the accuracy of prediction of milk yield or milk composition using these energy systems is limited (MacRae *et al.*, 1988). In order to improve the accuracy of prediction, information is required concerning supply to the mammary gland of nutrients available for biosynthesis of each milk constituent (Oldham & Sutton, 1979; Larson & Smith, 1974; Mepham, 1982; Mepham, 1988; MacRae *et al.*, 1988; Vernon, 1988; Madsen, 1983; Bergman, 1983; Giesecke, 1983; Bondi, 1982). As shown previously, dietary changes and energy and protein balance influence the availability of major precursors (Table 9.2), which may influence milk quantity and composition.

Precursors for milk components

Lipids in milk are mainly triglycerides (Depeters & Cant, 1992). A large quantity is obtained from plasma triglycerides, which originate either from dietary fats (Storry, 1985) or from mobilized energy reserves (adipose tissue; Madsen, 1983; Bondi, 1982; Vernon, 1988). The remainder of the milk fat is synthesized *de novo* in the mammary gland from ketogenic precursors (acetate and beta hydroxybutyrate), which are provided by rumen carbohydrate fermentation (Baldwin & Smith, 1983; Danfoer, 1991; Baldwin *et al.*, 1987a; Baldwin *et al.*, 1987b; Baldwin *et al.*, 1987; MacRay *et al.*, 1988). The results of the feeding experiments discussed in Chapters 5 and 7 were used to estimate the ratio of milk fat from *de novo* synthesis to LCFA origin. The amount of milk fat originating from LCFA was estimated according to Palmquist & Conrad (1971), who suggested that in positive energy balance 30 percent of the milk fat originates from LCFA and 70 percent from *de novo* synthesis. However, in negative

energy balance a larger quantity of the milk fat will originate from LCFA. Palmquist & Conrad (1971) assumed, that during the first week of lactation 70% of the milk fat came from LCFA's. Figure 9.2 shows the energy balances during the experiments (Chapters 5 and 7) and the percentage of milk fat, which was provided by LCFA. The fatty acid composition of milk fat measured during the experiment in Chapter 7 (Table 9.4) confirmed the results of Palmquist & Conrad (1971). The results measured during the positive energy balance (week 12) agreed with those published by Mepham (1973).

Table 9.4 ***Fatty acid composition of milk fat in relation to stage of lactation (Mol% of total)***

	Week after parturition				
Fatty acids	1	6	12	SED	Sign
C4.0 - C14.0	21.5	34.7	38.9	5.0	$p<0.05$
C16.0	39.3	36.3	40.2	6.0	NS
C18.0 - C22.0	39.2	29.0	20.9	3.4	$p<0.05$

De Visser, unpublished data

Using the negative energy balance (Figure 9.2), average milk fat production of the feeding experiments considered in Chapters 5 and 7, was divided into a fraction originating from LCFA's and *de novo* synthesis (Table 9.5). The glycerol required for the triglyceride production was assumed to originate from the mobilization of body reserves (Table 9.2), while the rest of the glycerol was synthesized from glucose (MacRay *et al.*, 1988).

Table 9.5 ***Milk output as nutrients***

Treatment	Lactose	Fat	Fatcor[1)]	*De novo*	LCFA	Glycerol	Protein
WGS	1680	1640	41	839	621	180	1152
MGS	1581	1570	48	708	696	167	1077
FGS	1592	1572	46	735	667	169	1080
WW	1682	1664	45	793	691	179	1138
B	1726	1589	33	923	483	182	1298
M	1782	1610	34	921	505	183	1335
P	1775	1700	38	913	596	190	1280
MB	1797	1645	36	912	546	186	1292

1) percentage fat originated from LCFA

Milk lactose is synthesized from glucose and UDP-galactose, both are derived from glucose either directly or via gluconeogenesis of amino acids or propionic acid (MacRay *et al.*, 1988).
Milk proteins are mainly synthesized in the mammary gland from the pool of free amino acids (DePeters & Cant, 1992). The remainder was supplied by preformed blood proteins (DePeters & Cant, 1992). The amino acids were provided by the microbial protein synthesized in the rumen, rumen undegradable protein and mobilization of protein reserves (Table 9.2), as described in the DVE system (CVB, 1991b).

In conclusion: Milk composition is related to energy balance and the availability of substrate originating from fermentation and digestion in the digestive tract.

Milk fat
Feeding large quantities of concentrates, containing rapidly rumen degradable starch instead of rumen degradable NDF, increases amylolytic activity in the rumen, inducing a higher concentration of propionic acid in the rumen fluid (Thomas *et al.*, 1986; Trémère *et al.*, 1968; Lees, Garnsworthy & Oldham, 1982; Sutton *et al.*, 1984; Thomas *et al.*, 1984; De Visser *et al.*, 1990; Valk & Hobbelink, 1992). Cows fed these rumen degradable starch diets showed decreased levels in content and yield of milk fat, compared to NDF-rich diets, which agreed with the results discussed in Chapters 5 and 7. Westerhuis & De Visser (1974) and Van Beukelen (1983) feeding flaked maize, containing very rapidly rumen degradable starch, found dramatic decreases in acetic:propionic acid ratios in the rumen, which resulted in extremely low milk fat contents (<20 g/kg milk) and yields. Casse *et al.* (1993a,b) reported a similar decrease in milk fat content in dairy cows, when propionic acid was infused into the blood of portal drained viscera (PDV). Total net flux of propionate was increased in PDV, as was the splanchnic flux of glucose, initiating an increase in glucogenic precursors available to the mammary gland.
Feeding large quantities of concentrates, containing slowly, as opposed to rapidly, rumen degradable starch had a positive influence on rumen fermentation, because a substantial part of the starch escaped rumen fermentation, reducing the supply of rumen degradable OM. Malestein & Van 't Klooster (1986); Waldo (1973); Sutton (1985); Casper & Schingoethe (1989); Herrera-Saldena *et al.* (1989) and McCarthy *et al.* (1989) fed diets, which differed in the starch degradation rate. The milk fat content was lowest on slowly degradable starch diets, as compared to rapidly degradable starch diets, which agreed with the results found in the experiment considered in Chapter 7. Measurements of the rumen fermentation characteristics, showed higher pH's, reduced concentrations of total VFA and higher acetic:propionic acid ratios; all indications of reduced amylolytic activity in the rumen of cows fed slowly degradable starch. However, milk fat content on these diets indicated an increase in supply of

glucogenic precursors to the mammary gland.

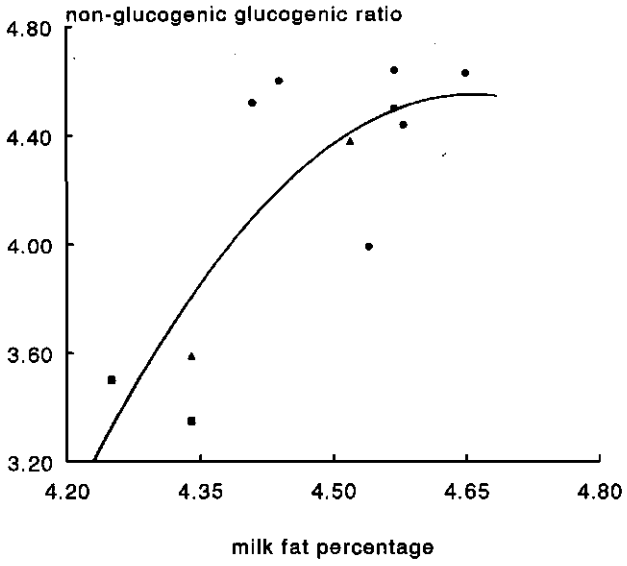

Figure 9.3 ***Relationship among Nonglucogenic-Glucogenic Ratio and milk fat percentage; Starch content of total diet: > 200 g/kg DM, ▪; < 200 > 100 g/kg DM, ▲; < 100 g/kg DM, ●.***

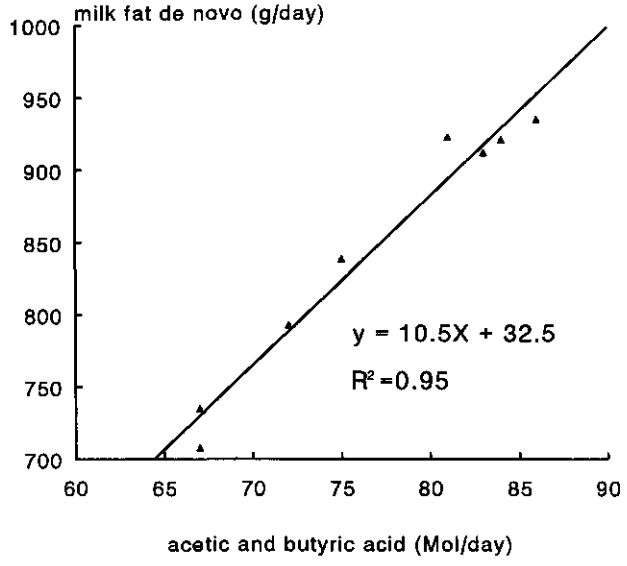

Figure 9.4 ***Relationship between predicted acetic and butyric acid production and de novo milk fat production.***

When comparing the glucogenic-nonglucogenic ratio (NGR; Ørskov, 1975) as measured in the experiments described in Chapters 4, 6 and 8 with the measured milk fat content of the experiments discussed in Chapters 3, 5 and 7 a non linear relationship was observed (Figure 9.3).
However, prediction of milk fat content on the basis of NGR values were poor. After correction for the percentage LCFA a linear relationship was found between the supply of rumen ketogenic precursors (acetic acid and butyric acid; Table 9.2) and *de novo* milk fat production (Table 9.5; Figure 9.4).
Differences found in milk fat synthesis between treatments were thus in line with the estimated amounts of nutrients supplied.

Milk protein and lactose

Although milk protein is synthesized from aminogenic precursors (amino acids), carbohydrates are also related to milk protein output. Firstly, carbohydrates are involved in microbial protein synthesis in the rumen and secondly, a limited supply of propionic acid or glucose during a negative energy balance, stimulates the use of amino acids as glucose precursor (gluconeogenesis), which may reduce the availability of amino acids for milk protein synthesis. Milk protein output appears difficult to manipulate. However, during the lactational cycle several feeding conditions can reduce milk protein output. Protein content of milk rapidly declines after parturition. Lowest values are often measured at the peak of lactation. Broster & Thomas (1981) showed a negative relationship between energy intake and milk protein output. Under such circumstances a lower supply of N and carbohydrates reduce total microbial protein synthesis in the rumen and availability of by-pass protein. Both conditions reduce the supply of aminogenic precursors to the mammary gland.
Rumen degraded starch and NDF from concentrates had similar effects on milk protein content and yield, when equal amounts of rumen degraded OM were supplied to the rumen microbes (Thomas *et al.*, 1986; Sutton *et al.*, 1984). However, in their experiments comparisons were made between barley starch and beet pulp NDF. The results given in Chapter 7, show a lower milk protein content and yield when comparing rapidly degradable starch (barley) and NDF (maize bran). This could be explained by differences in degradation characteristics between barley and maize bran. In this respect the solubility (S) of protein and carbohydrates, as well as the rate of degradation of the ruminal digestible fraction (k_d) have to be taken into account. Results of Chamberlain *et al.* (1986) and Rooke *et al.* (1987) showed an increase in microbial protein synthesis in the rumen, when the rate of degradation of the protein and the carbohydrates was in balance. Under these more balanced conditions the supply of aminogenic nutrients to the mammary gland may be improved.
Feeding more slowly degradable starch decreased the supply of energy available to the rumen microbes, which may result in lower microbial protein synthesis. However, in our experiment (Chapter 7) and those of Waldo (1973) milk protein

content and yield were not affected. The increased supply of glucose to the small intestine, when feeding rumen undegradable starch, may have reduced the oxidation of amino acids by the intestinal wall, which would compensate the loss of microbial protein synthesis in the rumen. Huntington (personal communications) found tendencies towards higher net fluxes in PDV of α-amino-N, when infusing starch into the small intestine of steers. Although, not yet confirmed by experiments with high yielding dairy cows in early lactation, the extra glucose available at the wall of the small intestine may increase the supply of amino acids to the mammary gland at low energy intakes.

Feeding moist diets (roughage and/or by-products) often decreases milk protein yield and content (De Visser & Tamminga, 1987; Chapter 5), attributed to a reduction in energy available for microbial protein synthesis (carbohydrates *versus* fermentation end products) and an increase in the extent of protein degradation in ensiled products. However, results from Gordon & Peoples (1986), Peoples & Gordon, (1989), Steen & Gordon (1980) and Zimmer & Wilkins (1984) comparing wilted grass silage to moist grass silage, showed no reduction in milk protein yield and content. However, these experiments were performed with concentrate supplements based on barley, which may have compensated for the loss of rapidly fermentable carbohydrates in the grass silages.

The lactose content of milk is only slightly influenced by nutritional factors. Immediately after parturition the content of milk lactose was low (2.3; De Visser, unpublished data; Balch, 1972) but increased very rapidly during the first 2 weeks of lactation and afterwards remained fairly constant. The low values in early lactation suggests that the lactose content is related to the osmotic pressure of milk, because during that period the animals produce high amounts of minerals in milk (Balch, 1972).

Relationships between nutrient supply and milk performance

The results of the energy and protein intake, deposition and reposition (Table 9.2) and milk performance (Table 9.5) were used to estimate the availability of ketogenic, glycogenic and aminogenic precursors in relation to the output of these substrates in milk. All intakes, deposition, reposition and milk performances were recalculated into metabolizable energy (ME), according to values published by Van Es (1972) and Armstrong (1972). The results of these calculations are shown in Table 9.6 and Figure 9.5.

After correction for maintenance (approximately 34.5 MJ) the overall efficiency for milk production fell between 62 and 68%. The percentage of ketogenic precursors were highest on the diets presented in Chapters 5 (WGS, MGS, FGS and WW) and 7 (P and MB), as a result of relatively higher productions of acetic and butyric acid during rumen fermentation (Dijkstra *et al.*, 1992) and mobilizati-

on of body fat.The percentage of ketogenic precursors on both starch rich diets (Chapter 7; B and M) was lowest as a result of a shift towards a higher production of propionic acid (Table 9.2). The percentage of ketogenic precursors was even higher for the animals in the experiment described in Chapter 5 (WGS, MGS, FGS, WW) due to the higher amount of precursors provided by the deposition of energy reserves (LCFA; Table 9.2). Ketogenic output in milk was not affected, but the origin of milk fat, differed between treatments, which was expressed in the ratio between LCFA and *de novo* synthesis (Table 9.5). Efficiency of transfer of ketogenic energy is lower than with glycogenic and aminogenic energy. This would seem to suggest that the maintenance requirements were preferably fulfilled by ketogenic energy, probably because utilization of glycogenic and aminogenic nutrients shows a high priority for synthesis of milk components.

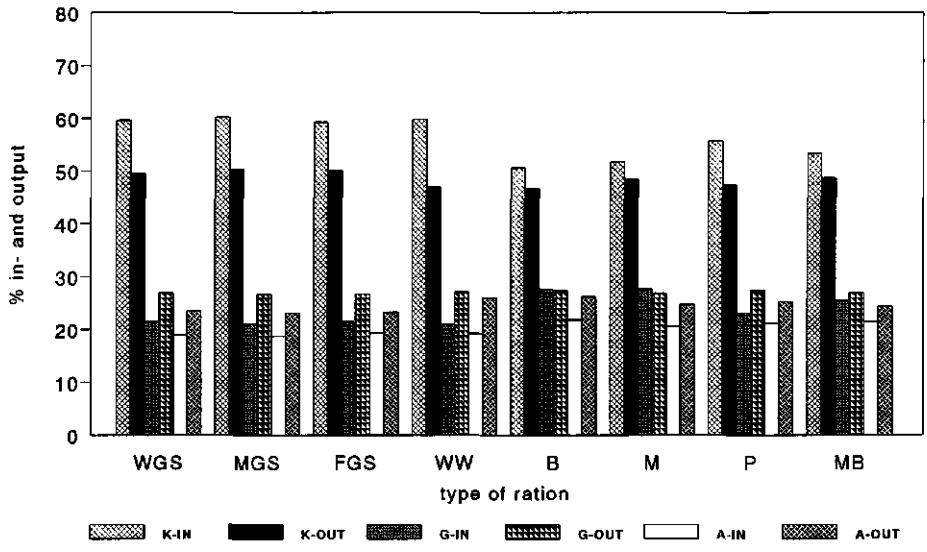

Figure 9.5 ***Distribution of ketogenic, aminogenic and glucogenic nutrients available for maintenance and milk production (ketogenic, K; glucogenic, G; aminogenic, A; input, IN; output, OUT)***

Table 9.6 Input and output energy distribution into ketogenic (keto), glycogenic (glyco) and aminogenic (amino) nutrients estimated as metabolizable energy (ME, MJ/d) and efficiency factors

	Input				Output				Efficiency of transfer			
treatment	keto	glyco	amino	total	keto	glyco	amino	total	keto	glyco	amino	total
WGS	131.1	47.6	41.8	220.5	58.4	31.6	27.6	117.6	44.2	66.4	66.1	53.1
MGS	131.7	46.1	41.0	218.8	56.2	29.7	25.8	111.7	42.7	64.5	63.1	51.1
FGS	128.1	46.1	41.6	215.8	56.1	29.9	25.9	111.9	43.8	65.0	62.2	51.9
WW	135.4	47.6	43.4	226.4	59.4	31.6	27.3	118.3	43.9	66.4	62.9	52.3
B	125.3	68.4	54.1	247.8	56.2	32.4	31.2	119.8	44.9	47.4	57.6	48.4
M	133.3	71.2	53.1	257.6	57.0	33.4	32.0	122.4	42.8	46.9	60.4	47.6
P	143.7	59.7	54.7	257.9	60.4	33.4	30.7	124.4	42.0	56.0	56.2	48.3
MB	134.4	64.0	53.9	252.3	58.3	33.7	31.0	123.0	43.4	52.7	57.5	48.8

	Nutrient intake ratio			Nutrient output ratio				
treatment	keto	glyco	amino	keto	glyco	amino	PR/ME[1)]	Milk protein efficiency[2)]
WGS	59.6	21.5	18.9	49.6	26.9	23.5	12.5	0.70
MGS	60.2	21.1	18.7	50.3	26.6	23.1	11.8	0.67
FGS	59.3	21.4	19.3	50.1	26.7	23.2	12.0	0.66
WW	59.8	21.0	19.2	46.9	27.1	26.0	12.1	0.67
B	50.6	27.6	21.8	46.6	27.3	26.2	12.6	0.60
M	51.8	27.6	20.6	48.5	26.8	24.7	12.4	0.63
P	55.7	23.1	21.2	47.4	27.4	25.2	11.9	0.59
MB	53.3	25.4	21.4	48.7	26.9	24.4	12.3	0.60
mean				48.7	26.9	24.4		
s				1.78	0.23	1.29		
vc				3.7	0.9	5.3		

1) protein output as percentage of total ME intake
2) efficiency of milk protein production after correction for maintenance

The glycogenic input differed between treatments (Table 9.6; Figure 9.5) and as was shown by a shift towards increased amounts of propionic acid produced in the rumen (Table 9.2) and/or increased levels of glucose production, due to increased amounts of starch escaping degradation in the rumen (Table 9.2). Glycogenic output levels showed little variation between treatments (Table 9.6), illustrated by the low coefficient of variation (Table 9.6). This phenomenon suggests that the dairy cow in early lactation prefers to produce lactose instead of other solids. However, when glycogenic energy is in short supply (WGS, MGS, FGS and WW) the efficiency of transfer is high (64-66%). Under these conditions aminogenic energy accounts for a relatively small proportion of the output (24%), despite a high efficiency of transfer; negatively influencing milk protein production.
Therefore milk protein output will be the result of the availability of substrate from rumen fermentation (microbial protein), digestion (by-pass protein) and mobilization of protein reserves and the use of amino acids in early lactation for gluconeogenesis. The positive influence on milk yield and milk protein content observed in early lactation, when increasing the DVE intake above requirements are probably related to this phenomenon.
A large proportion (26%) of the glycogenic energy input (B, M, P and MB) results not only in a low efficiency for glycogenic energy, but also of total energy. This is probably related to the influence of glucose on endocrine control mechanisms, which reduces the mobilization of body reserves in early lactation and initiates a shift towards repositioning of energy reserves instead of milk solids at a later stage in lactation.

In conclusion: Milk production and composition are related to the production of substrates during fermentation and digestion in the digestive tract. During early lactation the intermediary metabolism influences the composition of milk due to preference given to the production of lactose instead of milk protein. During this period in lactation, feeding should be aimed at influencing the availability of glucose/propionic acid in an attempt to reduce the loss of amino acids via gluconeogenesis.

General conclusion

Carbohydrates are important ingredients in dairy diets. Nutritionists use empirical methods to divide carbohydrates into various fractions (sugars, starch and cell wall constituents). Modern methods can quantify the chemical composition and physical structures and identify the types of bonding involved. However, these methods are not yet available for practical use. Carbohydrates vary in complexity, due to chemical composition and rumen degradation and intestinal digestion. Carbohydrates may be ranked in accordance with these characteris-

tics, which in turn may alter rumen fermentation, intestinal digestion and the supply of nutrients to the mammary gland. Carbohydrates are strongly related to protein availability, because they are very important as energy sources for microbial protein synthesis.

The supply of nutrients for maintenance and milk production can be altered by changes in the composition and/or degradability of these carbohydrates in the diet. Such changes can be predicted by the use of sophisticated mechanistic models. The differences in the supply of nutrients to the mammary gland affect the composition of the milk. An increase in glycogenic precursors instead of ketogenic precursors available for *de novo* synthesis reduces milk fat production. During the period of negative energy balance, this phenomenon is overruled by an increase in the synthesis of milk fat from LCFA, temporarily available from deposition of energy reserves. Cows in early lactation give preference to the supply of glycogenic precursors for milk lactose production, using amino acids for gluconeogenesis, above an increase in milk protein output. As a consequence maintenance requirements are primarily met by ketogenic nutrients.

More information concerning the effects of carbohydrate composition of the basal diets and concentrates will provide farmers with the opportunity to balance their home-grown basal diets more effectively, which in turn may reduce the total amount of concentrates fed. Also the amount of minerals which are inefficiently utilized by the animals, when there is an imbalance in supply and demand of nutrients to the mammary gland, will provide a source of increased environmental pollution.

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Summary

Summary

Food for human consumption originates directly from plants, after processing, or indirectly by conversion of plant materials into food of animal origin through livestock. An important example of food of animal origin are dairy products such as milk, cheese, butter, yoghurt, etc.
During the last decades milk production from Dutch dairy herds has increased considerably. This increase in production, yield and content, was the result of a combination of improvements in genetic potential, due to breeding and progress made in nutrition. Within the area of nutrition better quality roughage and increased usage of concentrates were at the root of this progress.
Initially the concentrates were based mainly on grains and oil cakes. These feedstuffs showed slight variation in energy and protein value. Large quantities of by-products, varying in origin, are produced as a consequence of food production. Without alternative use, these by-products would be wasted and as such provide an additional source of environmental pollution. Fortunately, many by-products of food production have considerable value as feedstuffs and are used in ruminant nutrition.
For the profitable utilization of by-products in dairy cow feeding a precise evaluation of their feeding value is essential, because of the high degree of variation involved. Product classification according to the processing methods is a help, but considerable emphasis should be given to chemical composition and predictive methods of evaluation using this or alternative sources of information. Some of these by-products are produced using high moisture techniques. These products sometimes become available after artificially drying or are delivered with a high moisture content and have to be ensiled.
One of the chemical components, which varies the most in by-products is the carbohydrate fraction. Sometimes, some of the carbohydrates (starch, sugars) are removed during processing, increasing the concentration of cell wall constituents, ash, fat and protein content in the remaining product. By selecting and combining various by-product ingredients, large variations in the carbohydrate composition of concentrates can be achieved, even when feeding isocaloric diets. Although these concentrates are similar in their energy value, the fermentation pattern in the rumen may differ widely. If the products contain large quantities of starch such concentrates may even cause rumen acidosis, because of their rapid fermentation and accumulation of lactic acid in the rumen. By replacing starch with highly digestible cell wall constituents the pattern changes in favour of more acetic acid instead of propionic and/or lactic acid. These changes in fermentation pattern had a positive influence on total DM intake, which resulted in an increase in milk yield (Chapter 2).

In a subsequent study, comprising a feeding trial (Chapter 3) and fermentation study (Chapter 4) an investigation was made of the influence of the dry matter

content of some concentrates (dried *versus* pressed ensiled beet pulp) and replacements of some concentrates with extra roughage (maize silage). Total DM intake was highest on the dried beet pulp diet. Milk production did not differ between treatments, as with fat yield and content. Milk protein yield and content tended to be lowest for the group fed maize silage. The rumen fermentation study showed similar concentrations of major VFA's on all diets. The concentration of branched-chain fatty acids and ammonia were highest for the cows fed maize silage, indicating reduced microbial protein synthesis, which confirmed the tendency towards lower milk protein yield measured in the feeding trial. The degradation characteristics of the OM showed the lowest rate with maize silage. The undigestible fraction (U) was highest with maize silage. The results of both experiments demonstrated the importance of the balance between energy and protein availability for rumen fermentation.
Higher levels of intake caused an increase in the concentration of volatiles and reduced rumen fluid pH. Feed intake level influenced the pattern of consumption, which changed from an intake pattern of two large meals at low intake level, towards several smaller meals at the high level of intake. Although concentrations were highest at the high intake level, diurnal variation was highest on the low level of intake, due to the meal size.

Grass silage can be fed as wilted or wet ensiled material. In the latter case some carbohydrates will be replaced by fermentation end products during the ensiling process, reducing the amount of easily fermentable carbohydrates and increasing the soluble fraction and rate of N degradation (Chapter 6). In a feeding trial (Chapter 5), in which a comparison was made between the DM content and the amount of fermentation end products, reduced DM intakes were found on diets low in DM content as well as those high in fermentation end products. The effects of DM content as such were minor, compared to those of the combination of low DM content and increased amounts of fermentation end products. Reduction in DM intake, due to a low DM content, was restricted to the first 6 weeks of lactation, whereas the combination effect remained throughout the experiment. Total DM intake was lowest for high moisture diets, which was reflected in lower energy and protein intakes. Milk production was lowest on high moisture diets, reflecting the lower energy intake. Milk fat content and yield were not affected, partly because of the increased mobilization of body fat during the period of negative energy balance. Milk protein content and yield were lowest on high moisture diets. The fermentation and kinetic studies (Chapter 6) showed reduced pH and increased concentrations of total VFA, acetic acid, ammonia and BCFA on high moisture diets. The rates of clearance of the OM fraction were significant or showed a tendency towards lower values for high moisture diets, which agreed with the lower total DM intake found in the feeding trial. These results were negatively influenced by the type of concentrates fed in both experiments, a very low amount of easily fermentable

carbohydrates were fed (ensiled by-products low in starch and sugars). Animals fed these diets were unable to balance the availability of nitrogen and energy for microbial protein synthesis. This resulted in a lower yield of milk protein and a reduction in milk protein content.

An attempt was made to compare the effects on feed intake and milk performance of the type of carbohydrate (starch *versus* cell wall constituents) and rate of degradation (rapidly *versus* slowly). This was performed in a feeding trial (Chapter 7) accompanied by fermentation and kinetic studies (Chapter 8). Starch reduced milk fat content, which in the case of rapidly fermentable starch can be explained by a decrease in the ratio of non-glucogenic to glucogenic volatile fatty acids (NGR) found in the rumen. The shift from fermentation in the rumen towards digestion in the small intestine, increase in by-pass starch, partly eliminated the lower NGR in the rumen, but the total amount of glycogenic precursors was found to be higher in animals fed by-pass starch. Milk fat content was highest from animals fed high levels of cell wall constituents. Milk protein content was highest on both starch diets, while the diets rich in cell wall constituents displayed the lowest values. Rumen fermentation characteristics showed an increase in the concentration of ammonia and BCFA and a tendency towards lower amounts of microbial protein in the rumen, which confirmed the results of the feeding trial. Differences occurred between both cell wall diets, because one of these diets did not have an optimal balance between cell wall and nitrogen degradation rates.

In the general discussion (Chapter 9) an attempt is made to predict feed intake, milk yield (lactose), milk fat and milk protein production. Different models were used to predict the total DM intake as measured in the feeding trials. Relationships were poor, when predicting DM intake using the energy requirements and the energy density per kg DM. Relationships were improved when body weight, stage of lactation and percentage concentrate in the diet were included as independant variables. Results were much improved, when DM intake was predicted by means of equations derived from rumen kinetic parameters. However, all predictions were poor for diets with a dry matter content below 35 percent. This was due to the lower intake measured during the first weeks of lactation, and to the fact that these types of diets were not included in the data set, from which the equations were derived. An attempt was made to estimate the production of nutrients using a rumen fermentation model. Differences in predicted nutrient supply occurred between the diets fed in the different experiments described in the various chapters. The major differences were found between starch-rich diets and diets rich in cell wall constituents in the amount of glycogenic precursors (propionate and glucose) and from the total level of feed intake, which increased the amount of propionic acid produced in the rumen. Relationships between rumen fermentation parameters (NGR) and

milk fat content were adequate within experiments. However, relationships between experiments were poor. Large between treatment differences were found in the amount of ketogenic precursors available for milk fat production from mobilization of body reserves. Estimation of the amount of milk fat produced from rumen ketogenic nutrients (acetic and butyric acid) by *de novo* synthesis and the production of ketogenic nutrients in the rumen predicted by the model showed a good relationship. Differences between total milk fat production and *de novo* synthesis could be explained by the availability of long-chain fatty acids (LCFA) available to the animal directly from the feed or from mobilization of body reserves. Milk protein was related to the amount of DVE available for milk production. The DVE intake between treatments was related to the microbial protein synthesis in the rumen and could be explained by the balance between nitrogen and energy sources. Milk lactose was related to the availability of glycogenic precursors and showed a very low variation between treatments indicating that the high yielding dairy cow in early lactation is probably hormonally geared towards the production of milk lactose, which might even be preferred above the production of milk protein, due to gluconeogenesis. Although the results of the feeding trials could be explained afterwards from the total energy intake, milk production and milk composition and the deposition and/or repositioning of body reserves, prediction of milk yield and composition was impossible. However, when including the chemical composition of the diets, its rate of degradation, the kinetics of the dietary ingredients and the extent of deposition and reposition, it became possible to predict milk production and composition.

In conclusion it can be stated that milk composition could be explained reasonably well from the estimated supply of individual nutrients. However, it should be realised that more information is required concerning the partitioning of nutrients to the various tissues in the animal, and the relationships involved with fermentation and digestion before evaluation based on net energy (VEM) and protein absorbed from the small intestine (DVE) can be replaced by more accurate predictive methods for practical use.

Samenvatting

Samenvatting

Het voedselpakket van de mens bestaat uit het eten van plantaardig materiaal als zodanig, na procesmatige verwerking, of na vervoedering via dieren. Een belangrijk voorbeeld van het laatste zijn zuivelprodukten (melk, kaas, boter, yoghurt, etc.).
In de afgelopen decennia is de melkproduktie van de Nederlandse veestapel aanzienlijk gestegen. De stijging in melkproduktie en gehalten, was het resultaat van een verbetering van het genetisch potentieel, als gevolg van fokkerij en van vooruitgang als gevolg van een betere voeding. Op het gebied van de voeding werd met name de kwaliteit van het ruwvoer aanzienlijk verbeterd en nam het gebruik van krachtvoer toe. Ook de krachtvoersamenstelling was en is aan veranderingen onderhevig. Het krachtvoer bestond aanvankelijk vooral uit granen en koeken afkomstig van de olieverwerkende industrie. De variatie in de voederwaarde en chemische samenstelling was gering. Als restprodukt van de humane voedingsmiddelenindustrie worden aanzienlijke hoeveelheden bijprodukten geproduceerd. Zonder alternatief gebruik zouden deze middelen verloren gaan, hetgeen zou kunnen leiden tot een ernstige milieuverontreiniging. Gelukkig hebben vele bijprodukten een aanzienlijke waarde als veevoer, waardoor ze gebruikt worden in de herkauwervoeding. Het adequaat gebruiken van deze voedermiddelen kan alleen wanneer de voederwaarde goed kan worden voorspeld. Het indelen van deze voedermiddelen in kwaliteitsklassen is dan ook noodzakelijk. Een indeling op basis van het produktieproces is een mogelijkheid, maar veel aandacht moet worden besteed aan het bepalen van de voederwaarde op basis van chemische en andere kenmerken. Ten dele komen de bijprodukten als een droog produkt op de markt, maar als gevolg van een aantal "natte" processen worden de produkten ook in natte, veelal geënsileerde, vorm gebruikt.
De koolhydraten vormen chemisch de belangrijkste groep van bestanddelen. Veelal wordt een deel van deze koolhydraten verwijderd tijdens de procesmatige verwerking (zetmeel, suikers), waarna de overige componenten, zoals as, celwandbestanddelen, vet en eiwit, relatief toenemen. Als gevolg van deze processen ontstaan bijprodukten , die een aanzienlijke variatie in koolhydraatsamenstelling hebben. Met behulp van deze bijprodukten is het mogelijk een grote variatie in de koolhydraatsamenstelling van het voer aan te brengen bij eenzelfde energetische waarde van het voer. Verschuivingen in de samenstelling van zetmeel naar celwandbestanddelen veroorzaakt een verandering van de fermentatie in de pens, hetgeen in extreme gevallen zelfs kan leiden tot het optreden van pensacidose. Een vermindering van het gehalte aan zetmeel leidde tot een verhoging van de drogestof-opname en een verbetering van de melkproduktie (Hoofdstuk 2).

In een volgend onderzoek, bestaande uit een voederproef (Hoofstuk 3) en een

fermentatiestudie (Hoofdstuk 4) werd de invloed bestudeerd van het drogestofgehalte van krachtvoercomponenten (droge pulp *versus* perspulp) en het verstrekken van extra ruwvoer (snijmais) als alternatief. De drogestofopname was het hoogst op het rantsoen met droge pulp. De melkproduktie verschilde niet tussen de behandelingen. Ook het vetgehalte en de vetproduktie verschilden niet wezenlijk van elkaar, mede als gevolg van een grotere mobilisatie van vetreserves op het perspulprantsoen. De melkeiwit-produktie en het gehalte tendeerden tot lagere waarden voor de met snijmais gevoerde groep. De fermentatiestudie toonde aan, dat er geen verschillen waren in de belangrijkste vluchtige vetzuren. De concentratie aan ammoniak en vertakte vetzuren was wezenlijk hoger op het rantsoen met snijmais, hetgeen wijst op een lagere microbiële eiwitproduktie, wat goed overeenkomt met de tendens van een lagere melkeiwitproduktie. Ook de verschillen in de afbraakkarakteristieken geven aan, dat snijmais een lagere afbraak kende, waardoor er minder microbieel eiwit kon worden gevormd.
Het effect van het voederniveau op fermentatie kenmerken gaf aan, dat de concentraties aan vluchtige vetzuren toenamen bij een hogere drogestof opname. Het opnamepatroon veranderde ook, namelijk bij een laag voederniveau werd het voer in twee grote maaltijden opgenomen, terwijl bij het hoge voederniveau een meer gespreide opname plaatsvond. Als gevolg hiervan was de variatie in fermentatiekenmerken gedurende een etmaal geringer op het hoge voederniveau.

Grassilage kan worden gewonnen na een voordroogproces op het land of direct na maaien met een conserveringsmiddel worden ingekuild. Tijdens de laatste methode wordt een deel van de koolhydraten omgezet in vluchtige vetzuren, alcoholen en melkzuur. Als gevolg hiervan worden de oplosbare fraktie en afbraaksnelheid van N vergroot. In een voederproef (Hoofdstuk 5) en een fermentatie- en kinetiek studie (Hoofdstuk 6), werden het effect van het drogestofgehalte en het aandeel fermentatieprodukten onderzocht. De drogestofopname was lager op de nattere rantsoenen, waarbij met name het effect van het drogestofgehalte zich beperkte tot circa 6 weken, terwijl de kombinatie van een laag drogestofgehalte en veel fermentatieprodukten een meer permanent karakter had. De lagere drogestofopname leidde tot een lagere energie- en eiwit opname, hetgeen een evenredige verlaging van de melkproduktie tot gevolg had. Het vetgehalte van de melk was niet wezenlijk verschillend tussen de groepen. Het eiwitgehalte en de eiwitproduktie waren lager op de natte rantsoenen. De fermentatie en de kinetiekstudie (Hoofdstuk 6) gaven een lagere pH en een verhoging van de concentraties aan vluchtige vetzuren, ammoniak en vertakte vetzuren te zien op de natte rantsoenen. De verdwijning van organische stof uit de pens was lager op de natte rantsoenen, hetgeen goed overeenkwam met de gevonden lagere totale drogestofopname in de voederproef. De resultaten van de voederproef werden nog versterkt, omdat het krachtvoer vrijwel uitsluitend

uit natte ingekuilde bijprodukten bestond, waardoor het aandeel gemakkelijk verteerbare koolhydraten zeer gering was en de microbiële eiwitsynthese in gevaar kwam. Het gevolg hiervan was een verlaging van het eiwitgehalte en de eiwitproduktie op de natte rantsoenen.

In een voederproef (Hoofdstuk 7) en een begeleidende fermentatie en kinetiek studie (Hoofdstuk 8) werd vervolgens een vergelijking gemaakt tussen het type koolhydraat (zetmeel *versus* celwandbestanddelen) en de mate van snelheid van afbraak (snel *versus* langzaam) op de gevolgen voor de voeropname, melkproduktie en melksamenstelling. Zetmeel verlaagde het melkvetgehalte, hetgeen werd bevestigd door de verhouding van de vluchtige vetzuren (NGR). Een verschuiving van de vertering van zetmeel van de pens naar het darmkanaal had een verlaging van het aandeel vluchtige vetzuren tot gevolg, maar de totale hoeveelheid glycogene nutriënten was hoger op het meer bestendige rantsoen. De dieren die rantsoenen kregen met meer celwandbestanddelen hadden een hoger vetgehalte in de melk. Het melkeiwitgehalte was het hoogst op zetmeelrijke rantsoenen. De resultaten van het fermentatieonderzoek gaven lagere concentraties aan vertakte vetzuren en ammoniak te zien op de zetmeelrijke rantsoenen. De verschillen tussen beide celwandrijke rantsoenen konden worden verklaard uit de verschillen tussen de afbraakkarakteristieken van de energie leverende componenten en de stikstof uit het voer, waardoor er minder microbieel eiwit kon worden gevormd.

In een totaal overzicht (Hoofdstuk 9) is getracht de voeropname, de melkproduktie en de melksamenstelling te schatten. Verschillende modellen zijn gebruikt om de voederopname te bepalen. De relatie tussen de energiebehoefte en de energie inhoud per kilogram drogestof bleek een slechte voorspeller van de opname te zijn. Indien relaties werden onderzocht, waarbij het lichaamsgewicht, het lactatiestadium en het percentage krachtvoer in het rantsoen werden betrokken kon de voeropname redelijk worden voorspeld. Een model, dat was afgeleid van penskinetische waarnemingen, gaf de beste voorspelling van de voeropname. Alle modellen gaven een overschatting van de voeropname te zien bij zeer natte rantsoenen (<35% drogestof). Dit was mede het gevolg van het feit, dat de voeropname moest worden voorspeld van dieren gedurende de eerste 3 maanden van de lactatie, maar ook vanwege het feit, dat de modellen waren afgeleid van een dataset waarin rantsoenen met dergelijke lage drogestofgehaltes niet voorkwamen. Met behulp van een dynamisch pensmodel is getracht de beschikbaarheid van nutriënten en de produktie van melk en haar samenstelling te schatten. Grote verschillen werden gevonden tussen de produktie van glycogene precursors (propionaat en glucose) tussen zetmeelrijke en celwandrijke rantsoenen en verschillen als gevolg van de totale drogestofopname. De relaties tussen de verhouding van de vluchtige vetzuren (NGR) en het melkvetgehalte waren binnen een proef goed, maar tussen proeven werden de

relaties zeer matig. Grote verschillen traden op tussen de verschillende proeven in de melkvetproduktie afkomstig van vluchtige vetzuren uit de pens (*de novo* synthese) of van voedingsvetten en/of mobilisatie van vetreserves. De hoeveelheid vet afkomstig van *de novo* synthese kon goed worden voorspeld op basis van de produktie van vluchtige vetzuren in de pens. Het melkeiwitgehalte was gerelateerd aan de DVE beschikbaarheid en relateerde goed aan de hoeveelheid microbieel eiwit dat op de verschillende rantsoenen kon worden geproduceerd. Melklactose was gerelateerd aan de beschikbaarheid van glycogene nutriënten en vertoonde een lage variatie tussen de behandelingen. Sterke aanwijzingen werden gevonden dat de produktie van lactose in het begin van de lactatie, waarschijnlijk onder hormonale druk gestimuleerd wordt, waardoor eventueel zelfs eiwit wordt gebruikt als glucosebron ten koste van een hogere melkeiwitproduktie.

De produktie resultaten kwamen na afloop goed overeen met de energiebalans zoals die volgens het VEM-systeem op basis van voeropname, mobilisatie van reserves en de melkproduktie en -samenstelling kon worden berekend. Vooraf kon echter met behulp van dit systeem de melkproduktie niet worden voorspeld. Wanneer echter de chemische samenstelling van de rantsoenen, hun afbraakkarakteristieken en de kinetiek van deeltjes werden opgenomen, kon de melkproduktie beter worden voorspeld.

Concluderend kan gesteld worden, dat door het schatten van het aanbod van de diverse nutriënten de melksamenstelling achteraf redelijk goed verklaard kon worden.

Men moet zich echter realiseren, dat aanzienlijk meer kennis nodig is omtrent de verdeling van nutriënten over de verschillende weefsels en hun relatie tot fermentatie en vertering, voordat het huidige systeem, dat gebaseerd is op netto energie, zonder onderscheid in soort energie, enerzijds en darmverteerbaar eiwit anderzijds, kan worden vervangen door een accuraat praktisch systeem gebaseerd op individuele nutriënten.

Curriculum vitae

Henk de Visser werd op 1 februari 1947 geboren te Rotterdam. Via de LLS, MLS en avondlyceum, behaalde hij in 1971 het diploma van de HLS te Dordrecht.
Op 1 augustus 1971 trad hij in dienst van het Instituut voor Veevoedingsonderzoek "Hoorn" te Hoorn als onderzoeksassistent, speciaal belast met het onderzoek met melkkoeien. Per 1 januari 1977 vond een verhuizing plaats van Hoorn naar Lelystad. In de loop der jaren verschoof de opgedragen taak zich meer en meer in de richting van zelfstandig onderzoeker. Het onderzoeksterrein bevond zich op het gebied van de voeding en gezondheid van melkvee, wat uiteindelijk resulteerde in dit proefschrift.
Sinds 1 januari 1992 vond een bevordering plaats tot wetenschappelijk onderzoeker bij het IVVO-DLO.