Forages from intensively managed and semi-natural grasslands in the diet of dairy cows

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Abstract

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This thesis focuses on the nutritional value of grass from intensively managed as well as semi-natural grasslands in diets fed to dairy cows. Aims were to explain why performance of dairy cows, fed intensively managed grass, is lower than expected based on their calculated energy intake, and to obtain knowledge on the nutritional value of forages from semi-natural grasslands if fed to dairy cows.

In order to understand the reason for the overestimation of the performance of dairy cows, several feeding and respiration trials were collected. From the feeding trials it was concluded that there was a discrepancy between energy input in grass and concentrates and energy output in milk and maintenance. This was due to the composition of grass and diet, but also due to higher maintenance requirements on grass-based diets than currently assumed. In the respiration trials it was observed that maintenance requirements for dairy cows on grass-based diets should be increased by 10%. This increase was attributed to nitrogen excretion and energy required for digestion.

The digestibility of forages from semi-natural grasslands is often low, due to a delayed harvesting date, and thus an advanced stage of maturity. However, though the (*in situ* and *in vitro*) degradation rate of mature grasses was low, the degradation rate of some dicotyledonous species appeared to be high. Also the intake of silage from semi-natural grasslands, with high proportions of dicotyledonous species was observed to be relatively high, especially when compared to the intake of silages from semi-natural grasslands mainly consisting of grasses in an advanced stage of maturity. The milk production of diets containing large proportions of semi-natural silages was low compared to diets containing mostly intensively managed grass. *In vivo* digestibility of forages from semi-natural grasslands was approximately similar to the *in vitro* digestibility. Digestibility could not be estimated based on chemical composition. Rumen fermentation of semi-natural forages did not deviate from expectations based on the chemical composition of the diet. However, a study of the rumen kinetics showed that diets containing large proportions of dicotyledonous species had a higher intake rate and passage rate than expected, and diets containing large proportions of mature grasses had a faster particle size reduction than expected.

Also on forages from semi-natural grasslands, maintenance requirements should be increased, due to required energy for nitrogen excretion and for chewing and rumination. For intensively managed grass as well as for forages from semi-natural grasslands, a correction of the energy value of grass, by correcting for the surplus of protein per kg grass, is suggested, together with an increase of maintenance requirements by 10% for digestion and rumination. Including forages from semi-natural grasslands in diets of dairy cows is possible, especially if the forages are fed in small amounts. Replacement of intensively managed grass by forages from semi-natural grasslands until a maximum of 30% seems to offer best possibilities.

Keywords: Intensively managed grass, semi-natural grasslands, forage species, dairy cows, *in vivo* digestibility, feed degradation, energy metabolism, milk production, ruminant nutrition, rumen fermentation, rumen kinetics, voluntary intake, feed evaluation

Woord vooraf

Het is dan toch zo ver gekomen… Na bijna vier en half jaar van literatuur lezen, berekeningen, proeven uitvoeren, besprekingen, en schrijven en herschrijven van publicaties, is het boekje af. Zo'n boekje kan je niet maken zonder de hulp van anderen, die ik langs deze weg wil bedanken.

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Contents

Chapter 1

General introduction

General introduction

Grass and dairy cows

High production levels in dairy cows are achieved by a high intrinsic productivity of the cows (after long-term and strong selection for productivity) and by an intensive feeding system. In temperate regions, the latter is usually based on concentrates, maize silage and grass. Fresh or ensiled grass is one of the main components of the diet, in summer as well as in winter. To increase the productivity of the grasslands, they are heavily fertilized with nitrogen. Furthermore, because of the high growth rate of the heavily fertilized grasslands, the forage is harvested early and thus in a young stage of maturity. The stem to leaf ratio is then low, the cell wall and lignin concentrations are low and the proportion of easily digestible cell content is high (Beever *et al*., 2000). This results in a high digestibility and a high protein concentration, and thus in a high quality of the forage.

Besides forages from heavily fertilized grasslands, also forages from semi-natural grasslands are used for ruminants (Tallowin & Jefferson, 1999), and, more specifically, for dairy cows (Korevaar & Van der Wel, 1997). In this thesis, semi-natural grasslands are defined as grasslands for which farmers have agreed to adjust their management in order to allow birds or plants to reproduce (management agreements), or grasslands bought and managed by non-governmental nature conservation organizations in order to maintain ecologically vulnerable grasslands. On semi-natural grasslands cutting date is usually delayed and fertilization is restricted. Due to the delay in harvesting, cell wall and lignin concentrations are usually high, and concentrations of cell contents are low (Beever *et al*., 2000), resulting in a low digestibility and a low protein concentration, and thus in a low quality of the forage.

Quality assessment of forages

The quality of forages is partly reflected in the energy value. Within the VEM-system (in Dutch: Voedereenheid voor Melkproductie) (Van Es, 1978) this is expressed in Net Energy for Lactation (NE_L). Also energy requirements of the animals, e.g. for milk and maintenance, are expressed in NE_L.

The NE_L (in kJ kg^{-1}) of feeds is estimated according to the following equation:

$$NE_L = 0.6 * (1+0.004 * (q - 57) * 0.9752 * ME) \qquad (1)$$

in which

q is the ratio (in percentage) metabolizable energy (ME, in kJ kg^{-1}) to gross energy (GE, in kJ kg^{-1}), which indicates the quality of the feed,

0.9752 is a correction for the feeding level of cows (2.38 for cows with a daily milk production of 15 kg); the digestibility depression is 1.8 % per unit of feeding level above 1, and

ME is metabolizable energy in kJ kg^{-1} (Van Es, 1978).

For most feedstuffs the ME is calculated based on the concentration of digestible nutrients, according to the following equation:

$$ME = 15.9 * DCP + 37.7 * DCFAT + 13.8 * DCFI + 14.6 * DNFE - 0.63 * SU \qquad (2)$$

where
DCP is digestible crude protein,
DCFAT is digestible crude fat,
DCFI is digestible crude fibre,
DNFE is digestible nitrogen free extract
and all values are in g kg^{-1}.
The constants reflect the energy concentration of each of the nutrients (kJ g^{-1}). The inclusion of sugars (SU) is only necessary if the sugar concentration is higher than 80 g kg^{-1}.

For forages simplified equations have been developed, which are based on the weighted average of the regression coefficients of Equation 2. For grass the following two formulae are used, depending on the digestible crude protein concentration of the grass:

$$ME = 15.1 * \text{digestible organic matter (DOM)}; DOM / DCP > 7. \qquad (3)$$

$$ME = 14.2 * DOM + 5.9 * DCP; DOM / DCP < 7. \qquad (4)$$

Values of DOM and DCP are in g kg^{-1}. The positive impact of DCP on ME expressed in the last formula was attributed to a positive relation between the fat concentration and the CP concentration of grass.

Problem statement

It is questionable whether the simplified ME equations (Equations 2 and 3) are valid for high quality grass, with high digestibility and high protein concentrations, or for low quality grass, with low digestibility and high cell wall concentrations. It should therefore be investigated whether:

1. Extrapolation of the ME formulae to high quality grass is possible, and if this is not the case, why the extrapolation is not valid.
2. The simplified ME formulae can be extrapolated to low quality grass, i.e. grass with high cell wall concentrations and a low digestibility, often with a heterogenous botanical composition.

The direct motive to investigate the first problem came from literature. Experiments with stable-fed, high-yielding dairy cows have shown that animals fed on high quality grass do not reach their expected output (Valk *et al.*, 2000). The expected output was calculated based on energy intake.

The motive to investigate the low quality range of forages is not merely scientific. In the last few decades, the Dutch government has stimulated extensive management of grasslands by arranging management agreements with farmers (Korevaar, 1986). Furthermore, non-governmental nature conservation organizations have bought and still buy grasslands for nature conservation purposes. Semi-natural grasslands are often characterized by a diversity of forage species, grasses as well as dicotyledonous species, in different stages of maturity and the grasslands will be grazed by ruminants or mown and then fed to ruminants. If more is known about the nutritive value of forages from semi-natural grasslands, farmers may be less reluctant to use such forages for their dairy cows, which will have a positive effect on the increase of the area of semi-natural grasslands. It is therefore important to assess whether application of the existing ME formula to this type of grass is possible. Furthermore, it is important to investigate if forages from semi-natural grasslands can successfully be integrated in the diets of dairy cows, as the specific agronomic characteristics of those forages will also affect nutritional characteristics, such as intake, rate of degradation and fermentation. Also those characteristics need to be studied.

Objectives and approach

In this thesis two topics will be investigated:

1. The energy value of intensively managed grass and the reason why the predicted milk production is not realized on this intensively managed grass;
2. The evaluation of the nutritive value of low-quality grass and the integration of this grass in diets for dairy cows.

Concerning the first topic, the objective was to obtain a better understanding of factors determining energy concentration of forages and energy utilization of forages by dairy cows. This was done by a desk study and an analysis of existing data. To identify problems occurring on intensively managed grass, results of performance trials with grass-fed dairy cows were collected. Energy in- and outputs were compared and a number of variables were tested to identify possible causes of the overestimation of animal performance. Those variables were characteristics of the animals, the total diet or the grass component of the diet.

Causes of an overestimation of milk production of dairy cows on grass-based diets may include an overestimation of the energy concentration of grass, an underestimation of the requirements for milk or maintenance, or an overestimation of the efficiency of energy utilization. To test if the overestimated performance of lactating dairy cows is caused by energy metabolism on grass, i.e. a lower efficiency of ME utilization or higher maintenance requirements than presently assumed, results of respiration trials with fresh grass were collected. The experiments were analysed for the relationship between ME input and NE output.

Knowledge about the nutritive value of forages from semi-natural grasslands is scarce (Tallowin & Jefferson, 1999). Therefore, concerning the second topic, the objective was to get a clear understanding of characteristics determining the nutritive value of forages from semi-natural grasslands, and how those forages behave in comparison to grass from intensively managed grasslands. For that purpose understanding of digestibility (*in vitro* and *in vivo*), degradability (*in situ* and *in vitro*), intake, rumen fermentation characteristics, rumen kinetics and animal performance had to be increased. To reach this objective, several steps were taken. First the digestibility, degradability, possible intake and energy value of semi-natural grasslands were studied in a desk study. Subsequently, three different types of grass silage, one from an intensively managed grassland, one from a grassland with management agreements, and one from a grassland managed by a non-governmental organization were purchased. Several experiments were carried out with those three silages. First degradation characteristics of the three silages were determined *in situ* (Ørskov & McDonald, 1979). However, also some *in vitro* methods have become quite promising to estimate degradation of several feedstuffs (Cone *et al.*, 1996; 1997). The gas production technique is easier to perform than *in situ* techniques, because fewer animals are needed and circumstances are easier to standardize than in the *in situ* techniques. Therefore, to estimate degradation and rate of degradation, *in vitro* and *in situ* methods were also used to estimate the nutritive value of the different feeds. Subsequently, a performance trial with dairy cows was carried out to have an indication about possible performance on diets in which forages from semi-natural grasslands were included.

From the equations to estimate the ME concentrations of forages it is obvious that digestibility is important for the estimation of the energy value of a feed. Therefore, in animal trials it was investigated if the *in vivo* digestibility is estimated accurately based on *in vitro* methods (Tilley & Terry, 1963) as well as on regression equations to estimate digestible organic matter based on chemical composition of the forage (CVB, 2001). *Lolium perenne* may react differently in the rumen than other grass species. The intake of forages from semi-natural grasslands may be lower and determined by other factors than the intake of grass from grasslands mainly consisting of *L. perenne*. Therefore, in this trial also voluntary intake was measured, as this is expected to be lower on forages from semi-natural grasslands than on forages from intensively managed grasslands.

Intake depends on rumen capacity and degradation rate, whereas production level of the animal depends on the nutrients available in the rumen. Rumen kinetics and rumen fermentation will give insight into those characteristics. As milk production is underestimated for diets with high percentages of intensively managed grass, a certain proportion of low quality forage could have a positive impact on milk production, due to the higher amount of fibre, which might stimulate cellulolytic microbes and movement of the digesta. Therefore, in an experiment with rumen cannulated dairy cows, samples were taken of the rumen contents of dairy cows on diets consisting of concentrates, intensively managed grass and / or semi-natural forages.

Outline of this thesis

The thesis is divided into two parts. The first part concerns only research on intensively managed, high-quality grass and the prediction of milk production; the second part concerns semi-natural, low-quality grass and its characterization, compared to intensively managed grass.

Part I consists of the Chapters 2 and 3. In Chapter 2, an attemption was made to indicate possible causes of the overestimation of milk production by comparing energy in- and outputs in grass-fed dairy cows and to test several variables. In Chapter 3, maintenance requirements and efficiency of energy utilization were calculated, based on the analysis of three respiration trials with grass-fed dairy cows.

Part II consists of the Chapters 4, 5, 6, 7 and 8. In Chapter 4, a review is given on the problems concerning the estimation of the digestibility or the nutritive value of forages from semi-natural grasslands. In Chapter 5, the degradability of the three purchased silages (from an intensively managed grassland, and two semi-natural grasslands: a grassland with management agreements and a grassland managed by a non-governmental organization) is measured *in vitro* with the gas production technique and *in situ* with the nylon bag technique. In Chapter 6, the potential milk production of dairy cows is studied when silages from semi-natural grasslands are integrated in mixed rations including concentrates, maize silage, and intensively managed grass silage. In Chapter 7, an experiment is reported in which the *in vivo* digestibility of diets based on concentrates and intensively managed silage and / or one of the two types of silages from the two semi-natural grasslands is studied and compared with their *in vitro* digestibility and chemical composition. Furthermore, in Chapter 7 also the voluntary intake of silages from two semi-natural grasslands is reported, and compared to the voluntary intake of *L. perenne*. Results of intake and differences in rumen contents and in rumen degradation and fermentation characteristics are reported in Chapter 8.

Finally, the General discussion (Chapter 9) focuses on the estimation of the nutritive value of grass from intensively as well as extensively managed grasslands. Estimation methods for digestible organic matter and metabolizable energy are further discussed, as well as energy requirements in diets based on grass. It is tried to identify positive and negative factors of semi-natural grasslands, and to provide a method to validate energy content of grass from these grasslands and to assess when to use it. Finally, an effort is made to adapt the formulae used for grass for high as well as for low quality grasses.

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Part I

The feed evaluation of intensively managed grass

Chapter 2

Energy evaluation of fresh grass in the diets of lactating dairy cows

Bruinenberg, M.H., Zom, R.L.G. & Valk, H. (2003) Energy evaluation of fresh grass in the diets of lactating dairy cows. Netherlands Journal of Agricultural Science, in press.

Energy evaluation of fresh grass in the diets of lactating dairy cows

Abstract
The discrepancy between the estimated feeding value of fresh grass and the output per kg grass in milk and maintenance was studied by evaluating 12 experiments with grass-fed dairy cows. Intake and milk production per day were measured. The percentage of grass in the diets varied between 40 and 90. Per treatment a number of factors, relating to composition of the grass, characteristics of the animals, and composition of the total diet were calculated. The correlation between the measured discrepancy and these factors were assessed by regression analysis. The digestible organic matter in the grass (DOM), intake of grass, intestinal digestible protein in the total diet, percentage of milk protein and body weight gain correlated well with the discrepancy. It was concluded that energy input from grass and energy output in production are significantly different (P < 0.05). With diets with 80-90% grass, high DOM increases discrepancy. Furthermore, the maintenance requirements of lactating dairy cows fed grass-based diets are probably higher than the currently used values, due to energy requirements in the gastro-intestinal tract and to nitrogen excretion.

***Keywords*:** Ruminant nutrition, feed evaluation, grass-based diets

Introduction

In temperate regions, fresh grass is one of the main components in the diet for dairy cows. Due to high rates of nitrogen fertilizer, this grass (mainly *Lolium perenne*) is usually highly digestible and contains large amounts of protein and low amounts of cell walls. These characteristics suggest a high quality of the grass, and grass of such a composition is expected to enable high production performance of lactating cows. However, when feeding fresh grass with a high quality to dairy cows, Valk *et al*. (2000) observed that cows produced less milk than was predicted from their net energy intake. Also for ensiled grass this problem has been described: Thomas & Gill (1988) concluded that cattle, offered diets that contained high proportions of grass silage, had a low efficiency of energy utilization. Ergo, for cows on diets based on fresh or ensiled grass, discrepancies occur between the estimated intake of net energy and the actual net energy output through milk. For an efficient utilization of the nutrients in grass, an accurate estimate of the feeding value of grass is required. It is therefore important to find out why grass-fed dairy cows do not reach their expected output.

The feeding value of grass is expressed as net energy for lactation (NE_L; Van Es, 1978), which is estimated based on the relationship between NE_L and chemical characteristics of the feed as observed in respiration trials (Van der Honing *et al*., 1977). The discrepancy between the expected and the actual energy output observed by Valk *et al*. (2000) can be caused by an underestimation of the energy requirements for maintenance and production on forage-based diets or by an overestimation of the energy content of the grass. Since the introduction of the feed unit for dairy cows (VEM) system in the Netherlands in 1977 (Van der Honing *et al*., 1977), cow performance and grassland management have changed. Nowadays, cows produce more milk and consume more energy and dry matter (DM) than in the past. Previous research suggested an increase in maintenance requirements of 10% with dairy cows fed on grass compared to the present calculation rules (Bruinenberg *et al*., 2002).

The aim of the present study was to evaluate results of performance experiments with dairy cows fed fresh grass in order to find possible causes of the discrepancy and to formulate possible corrections to improve the feed evaluation system. Three hypotheses were considered.

1) The feed evaluation formula for grass is incorrect in all cases, and a new formula has to be developed to replace the present formula.

2) The present formula calculates the potential energy value, which in some cases is not fully utilized, depending on factors such as an unbalanced nutrient supply. In this case a correction factor should be added to the present formula.

3) The requirements of dairy cows in grass-based diets are higher than previously assumed, as suggested by Bruinenberg *et al*. (2002). The equation for the estimation of energy requirements should then be changed.

In this study, data of twelve feeding trials with lactating dairy cows on grass-based diets were collected. Differences between the potential milk production, based on dry matter intake and the calculated energy value of the ingested feed, and the actual milk output were calculated. An attempt was made to explain the discrepancy between the potential and true milk energy output, relating it to variables such as nutrient concentration of grass, diet composition and production level.

Material and methods

Experimental details

Data from twelve experiments with stall-fed, multiparous lactating dairy cows were used. In these experiments feed intake, composition of the diet, milk production and live weight of the animals were measured. In all experiments the cows were fed fresh grass (mainly *Lolium perenne*) *ad libitum*, with the percentage of grass in the dry matter (DM) in the diet varying between 40 and 90 (Table 1). Nine experiments were carried out at ID-Lelystad (Experiments 1-9) and three experiments were carried out at the Research Institute for Animal Husbandry in Lelystad (Experiments 10-12). In Table 1 the experiments are summarized and in Table 2 a list of abbreviations is given.

Experiment 5, 10, 11 and 12 are not published. Therefore, some additional information is given about the treatments. Experiment 5 had five treatments: 1. Grass + concentrates, 2. Grass + dried sugar beet pulp, 3. Grass + ensiled pressed sugar beet pulp, 4. Grass + maize cob silage without husks, 5. Grass + maize cob silage with husks (H. Valk, unpublished data).

In Experiment 10 and 11, cows were divided into three treatment groups: treatments were grasses from different plots, fertilized with 300 and 150 kg N ha^{-1} yr^{-1}. Part of the grass fertilized with 150 kg N ha^{-1} yr^{-1} grass was cut at the same time (after 20-40 days of growth; R.L.G. Zom, unpublished data) and part was cut at the same DM yield as the grass fertilized with the 300 kg N ha^{-1} yr^{-1} (1500-2000 kg ha^{-1}; R.L.G. Zom, unpublished data).

Table 1. Details of the experiments.

Exp. no.	Year	No. of treatments	% grass	FPCM[1] (kg d^{-1})	Duration (weeks)	No. of animals per treatment[2]	Treatments[3] in exp.	Reference
1	1987	4	40-80	29	6	8	A,B	Valk, 1994
2	1988	4	40-60	28	6	9	B,C,D	Valk, 1994
3	1989	4	65	31	6	9	B,C,D,E	Valk *et al*., 1990; Van Vuuren *et al*., 1993
4	1989	5	65-90	22	6	7	A,C,D	Van Vuuren *et al*., 1993
5	1990	5	65-90	24	6	7	A,C,D	Valk, unpublished
6	1991	3	85-90	24	8	12	A	Valk *et al*., 2000
7	1992	3	85-90	23	8	9	A	Valk *et al*., 2000
8	1992	3	85-90	24	6	9	A	Valk *et al*., 2000
9	1993	3	85-90	24	6	9	A	Valk *et al*., 2000
10	1992	3	85-90	25	6	7	A	R.L.G. Zom, unpublished
11	1993	3	85-90	24	6	7	A	R.L.G. Zom, unpublished
12	1993	2	85-90	25	4	7	A	R.L.G. Zom, unpublished

[1] FPCM = fat and protein corrected milk.

[2] The number of animals in this table may differ from the number of animals in the literature references. The differences are caused by the exclusion of heifers from the calculations in this study.

[3] A: 80-90% grass; B: grass supplemented with maize silage and concentrates; C: grass supplemented with beetpulp concentrates; D: grass supplemented with maize concentrates; E: grass supplemented with beetpulp and maize concentrates.

Table 2. Abbreviations and units used in this chapter.

Abbreviation	Variable	Unit
BW	Body weight	kg
ASH	Crude ash	g kg^{-1} DM
CF	Crude fibre	g kg^{-1} DM
CFAT	Crude fat	g kg^{-1} DM
CP	Crude protein	g kg^{-1} DM
DCP	Digestible crude protein	g kg^{-1} DM
DM	Dry matter	-
DMI	Dry matter intake	kg
DMI_{grass}	Dry matter intake of grass	kg
DMI_{supp}	Dry matter intake of supplements	kg
DOM	Digestible organic matter	g kg^{-1} DM
DVE	Digestible protein in the intestine, amount of DVE in the grass	g kg^{-1} DM
DVE diet	Average DVE in the total diet, per kg DM	g kg^{-1} DM
FCM	Fat corrected milk	kg
FL	Feeding level	-
FPCM	Fat and protein corrected milk	kg
GE	Gross energy	kJ
% grass	Percentage of grass in the total diet	%
GPCM	Grass intake (DM) per kg fat and protein corrected milk	g kg^{-1}
GPMW	Grass intake (DM) per kg metabolic weight	g kg^{-1}
k_l	Efficiency of ME utilization for lactation	%
ME	Metabolizable energy	kJ
MF	Percentage of fat in milk	%
ML	Percentage of lactose in milk	%
MP	Percentage of protein in milk	%
N	Nitrogen	-
$NE_{L,grass}$	Net energy in the grass	kJ
$NE_{L,required}$	Calculated NE requirements per day of the dairy cow	kJ
$NE_{L,supp}$	Mean net energy per kg DM of supplement	kJ
NFE	Nitrogen-free extract	g kg^{-1} DM
OEB	Undegradable protein balance in the rumen, OEB in grass	g kg^{-1} DM
OEB diet	Average OEB in the total diet	g kg^{-1} DM
OM	Organic matter	g kg^{-1} DM
%OMD	Digestibility of the organic matter	%
$Output_{grass}$	Calculated output in NE_L per kg grass	kJ
q	Metabolizability of the gross energy	%
SU	Sugars	g kg^{-1} DM
VEM	Feed unit for dairy cows	-

In Experiment 12, two treatments were carried out: grass fertilized with 300 kg N ha^{-1} yr^{-1} and grass fertilized with 150 kg N ha^{-1} yr^{-1}, both cut at a same DM yield (1500-2000 kg DM^{-1}; R.L.G. Zom, unpublished data).

Variables measured

The following grass-related variables were assessed and used: analysed concentrations (in g kg^{-1} DM) of crude ash (ASH), nitrogen (N), crude fibre (CF), and sugars (SU). Because crude fat (CFAT) in forages is seldom measured, for gross energy (GE) calculations we assumed a fat concentration of 40 g per kg DM in all forages. Nitrogen-free extract (NFE) was calculated by subtracting ASH, CP, CF and CFAT from 1000 g DM, and organic matter (OM) was calculated by subtracting ASH from 1000 g DM. Also the digestibility of OM (%OMD) was assessed, according to the method of Tilley & Terry (1963). In concentrates the same analyses were carried out.

Digestible organic matter (DOM) was calculated from OM and %OMD. Digestible crude protein (DCP) was calculated using standard calculation rules (CVB, 2000a). Digestible protein in the intestine (DVE), and the rumen undegradable protein balance (OEB) were calculated according to Tamminga *et al*. (1994). The net energy (NE_L) value of grass ($NE_{L,grass}$) was calculated from the gross energy (GE, in kJ), the metabolizable energy (ME, in kJ) and the metabolizability of the feed (100*ME/GE = q), according to the calculation rules (Van der Honing & Alderman, 1988; Van Es, 1978; CVB, 2000a). The net energy for lactation (NE_L, kJ) was calculated using the following equation:

$$NE_L = (0.6 * (1+0.004 * (q - 57)) * 0.9752 * ME) \qquad (1)$$

where

q is 100 * metabolizable energy / gross energy,

ME is metabolizable energy (kJ), being for grass: 14.2 * DOM + 5.9 * DCP

For all cows the DM intake of grass (DMI_{grass}) and supplements (DMI_{supp}) were measured. The percentage of grass in the diet (%grass) was calculated from the DMI_{grass} and DMI_{supp}.

In Experiments 1-9, body weight of the animals (BW) was measured twice a day, after milking, and in Experiments 10-12, body weight was measured (on three subsequent days on the same time of the day) in three weeks: one at the start, one in the middle, and one at the end of the experiments. In all experiments body weight change (BW change) of the animals was calculated by subtracting the weight at the onset of the experiments from the weight at the end of the experiments. Furthermore, for each cow the milk production (kg per day) was measured, together with the concentrations of milk fat (MF), milk protein (MP), and lactose (ML). From these parameters the fat and protein corrected milk production (FPCM) was calculated, according to CVB (2000b). Other animal variables that were measured, included the feeding level (FL= energy intake / maintenance requirements), the amount of grass consumed per kg metabolic weight (GPMW) and the amount of grass consumed per kg corrected milk (GPCM). In Table 3, the minimum, maximum and average values of the grass, the diet and the animal variables are given.

Table 3. Minimum (min.), mean and maximum (max.) values of the calculated variables.

		80-90% grass			40-65% grass		
Variable[1]	Units	Min.	Mean	Max.	Min.	Mean	Max.
Grass factors							
ASH	g kg DM^{-1}	87	104	131	99	131	116
CP	g kg DM^{-1}	134	195	281	175	221	281
CF	g kg DM^{-1}	194	219	245	194	219	232
NFE	g kg DM^{-1}	354	442	509	354	415	492
SU	g kg DM^{-1}	63	125	177	63	94	146
%OMD	%	74	79	84	77	79	81
DOM	g kg DM^{-1}	651	712	746	669	700	729
DCP	g kg DM^{-1}	93	151	241	132	178	241
GE	MJ	17.7	18.2	18.5	18.1	18.4	18.7
q	-	56	61	64	59	60	63
NE	MJ	5.8	6.5	7.1	6.3	6.5	6.9
DVE	g kg DM^{-1}	78	93	106	93	96	105
OEB	g kg DM^{-1}	-9	40	110	13	58	110
Diet factors							
DMI_{grass}	kg DM	14.6	16.3	18.1	7.2	11.3	13.5
DMI_{supp}	kg DM	1.7	2.2	3.5	5.3	7.9	12.0
DMI_{total}	kg DM	17.2	18.5	19.9	17.1	19.2	21.4
% grass	%	80.9	87.9	91.3	38.7	59.0	69.2
Feeding level	-	2.9	3.4	3.7	3.1	3.6	4.2
$NE_{L,supp}$	MJ	7.0	7.2	7.3	6.1	7.1	8.1
DVE diet	g kg DM^{-1}	82	94	105	57	88	99
OEB diet	g kg DM^{-1}	-7	35	87	-11	23	42
Animals factors							
Milk	kg	18.8	22.8	27.0	20.2	25.6	31.9
MF	%	4.2	4.5	4.7	3.7	4.4	4.9
MP	%	3.1	3.4	3.7	3.0	3.4	3.6
ML	%	4.2	4.4	4.5	4.3	4.4	4.5
FPCM	kg	20.4	24.0	28.3	21.8	26.8	31.6
BW	kg	579	623	656	574	612	643
BW change	kg	-29	6	35	-31	0	22

For abbreviations see Table 2.

The discrepancy between the net energy and the net energy output

The daily energy requirement ($NE_{L,required}$, kJ) was calculated, using the following equation (Van Es, 1978; CVB, 2000b):

$$NE_{L,required} = 6.9 * \{(42.4*BW^{0.75} + 442*CM) * [1+(CM-15)*0.00165]\} \qquad (2)$$

where

BW is body weight (kg)

CM is fat and protein corrected milk (kg per day)

It is assumed that the estimated energy content of supplements per kg DM ($NE_{L,supp}$) was correct. This value was calculated according to the formulae as used in the NE_L system, based on the chemical composition (Van Es, 1978). The energy output in kJ per kg DM of grass ($Output_{grass}$) was calculated using the following equation:

$$Output_{grass} = (NE_{L,required} - DMI_{supp} * NE_{L,supp}) / DMI_{grass} \qquad (3)$$

where

$NE_{L,required}$ is the daily energy requirements (kJ)

DMI_{supp} is the DM intake of supplements (kg)

$NE_{L,supp}$ is the estimated energy content of the supplements (kJ kg^{-1})

DMI_{grass} is the DM intake of grass per day (kg)

Subsequently the discrepancy between $NE_{L,grass}$ and $Output_{grass}$ was calculated in absolute and relative terms.

$$\text{Discrepancy} = NE_{L,grass} - Output_{grass} \qquad (4)$$

$$\text{\% Discrepancy} = 100 * (\text{discrepancy}/ NE_{L,grass}) \qquad (5)$$

where

$NE_{L,grass}$ is the estimated energy content of grass (kJ kg^{-1})

Thus, % discrepancy is the energy balance expressed per kg consumed grass. A positive discrepancy indicates an overestimation of the $NE_{L,grass}$ compared to animal performance expressed as $Output_{grass}$.

Statistical analysis

Firstly, the averages per treatment in each experiment were calculated. Subsequently, the average $NE_{L,grass}$ and the average $Output_{grass}$ were compared for each treatment and differences between $NE_{L,grass}$ and $Output_{grass}$ were tested for significance with the Student's t-test.

Next, the data were divided into two different groups:

- the diets with 80 to 90% grass (n=23),
- the diets with 40-65% grass (n=19).

For each group the percentage of variance of the discrepancy that can be declared by each of the different variables was calculated by linear regression analysis. Significance of the analysis was tested with the *F*-test.

Results

$NE_{L,grass}$ versus $Output_{grass}$

A variety of treatments was tested in the selected experiments, covering a broad range in diet composition, feeding level, and milk production. As a result the data set contained a large variation in % discrepancy and variables that were correlated with the % discrepancy (Table 3). Discrepancy varied between –15.8 and 23.7%, with a mean of 9.9%.

In Figure 1, the $NE_{L,grass}$ and the $Output_{grass}$ per kg grass are plotted. Different symbols are used for the 80-90% grass diets and for the 40-65% grass diets. Most diets overestimated the energy input, i.e., the energy output through milk production was lower than expected based on their estimated energy intake. Only some of the diets with maize silage underestimated the energy input compared with the output. Using the Student's *t*-test, the NE_L input was significantly higher than the NE_L output ($P < 0.01$).

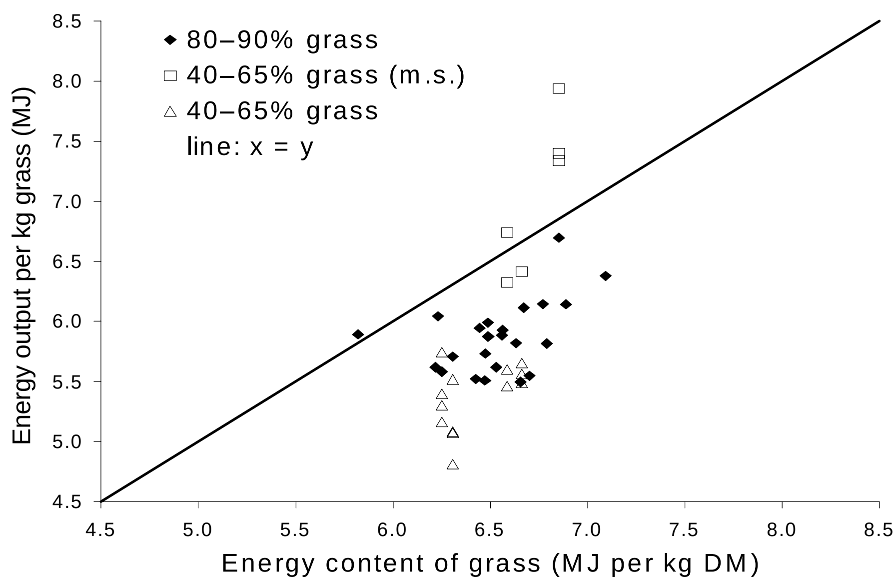

Figure 1. Relationship between energy input and energy output. The line x = y indicates an ideal situation in which energy input is equal to energy output. The further a data point deviates from the line, the higher the discrepancy. 40-65% grass (m.s.) = the treatments with maize silage.

Correlations with discrepancy

In Table 4, the correlations (R^2) between the discrepancy and the factors are shown for the two groups.

Table 4. The percentage of the variance (R^2) of the discrepancy accounted for by the variables, by using the diets with 80-90% grass and the diets with 40-65% grass.

	80-90% grass (n=23)	40-65% grass (n=19)
Ash	36.3** (-)	-
CP	1.4	21.1* (-)
CF	15.8* (-)	51.5*** (+)[1]
NFE	20.8* (+)	-
SU	22.4* (+)	-
%OMD	13.6* (+)	26.9* (-)
DOM	31.8** (+)	3.4
ME	12.0	37.6** (-)
GE	-	-
q	12.5	59.8*** (-)
DVE	-	46.9*** (-)
OEB	4.4	16.0
DMI_{grass}	47.1*** (+)	76.3*** (+)
DMI_{supp}	12.3	70.6*** (-)
DMI_{total}	29.5** (+)	-
% grass	23.5* (+)	77.6*** (+)
FL	16.1* (+)	-
$NE_{L,supp}$	-	74.2*** (+)
DVE diet	-	77.5*** (+)
OEB diet	2.1	5.0
Milk	-	5.6
MF	-	8.8
MP	14.4* (+)	64.4*** (+)
FPCM	-	15.3
BW	8.3	9.1
BW change	31.9** (+)	55.9*** (+)
GPCM	35.0** (+)	77.6*** (+)
GPMW	34.4** (+)	70.9*** (+)

For abbreviations and units see Table 2.

*: P <0.05; **: P < 0.01; ***: P < 0.001

(-): reduction of discrepancy; (+): increase of discrepancy

[1]: Residuals resulting from the regression analysis were not random.

Diets with 80-90% grass. In the diets with 80-90% grass, the most important grass-related variable with a positive relationship with the %discrepancy was DOM (P < 0.01). Discrepancy was negatively correlated with CF (P < 0.05) and ASH (P < 0.01). The most important diet-related variables with a positive relationship with the %discrepancy were DMI_{total} (P < 0.01), DMI_{grass}(P < 0.001), and the most important animal-related variable was BW change (P < 0.01).

Diets with 40-65% grass. The correlations of the %discrepancy with grass related variables seemed to have an opposite effect compared to the diets with 80-90% grass: CF had a positive effect (P < 0.001) and %OMD a negative effect (P < 0.05). The %discrepancy was also negatively correlated to q (P < 0.001), ME (P < 0.01) and DVE (P < 0.001). Of the diet variables the %discrepancy was positively correlated (P < 0.001) with DMI_{grass}, %grass, $NE_{L,supp}$ and DVE diet and negatively correlated (P < 0.001) with DMI_{supp}. Of the animal-related variables the %discrepancy was positively correlated (P < 0.001) with milk protein, BW change and DVEdiet. Correlations were strong in this analysis, which was probably due to the presence of outliers (three of the diets with maize silage, all of Experiment 1, showing a negative discrepancy). Without those outliers, correlations were less strong, or disappeared completely.

Discussion

The $NE_{L,grass}$ was overestimated compared with the $Output_{grass}$. This discrepancy indicates that on grass-based diets farmers overestimate the expected milk yield of their cows. It is thus desirable to have insight in the causes of the discrepancy, because it could indicate how to produce grass of a composition that enables more efficient milk production, how to adjust the diet, or just to take lower milk productions into account if certain factors cannot be manipulated. To test the hypotheses mentioned earlier (see introduction), the observed discrepancies are discussed in relation to grass variables, diet variables and animal variables, respectively.

Correlation of discrepancy with grass variables; Hypothesis 1

If the first hypothesis (the feed evaluation of grass is incorrect in all cases) is true, a positive correlation between discrepancy and grass factors would be expected. However, correlations between discrepancy and grass factors were not always evident. In both analyses some significant correlations were observed, but they were often contradictory. With the 80-90% grass diets the DOM had the highest positive correlation (i.e. higher chance of overestimation of milk production) and the ASH the highest negative correlation. Those factors were also mutually correlated (R^2 = 37.2%, P < 0.01, in 80-90% grass group), which is caused by the relation of ash with OM. A high OM concentration will probably result in a high DOM. As grass with high DOM coincides with relatively low concentrations of CF (R^2 = 20.1%, P < 0.05, in the 80-90% grass group), altered rumen fermentation may occur, resulting in negative effects on ruminal digestive efficiency (Mertens, 1997; Ferris *et al.*, 2000). Furthermore, in grass, digestible CP is a big part of total DOM, and although correlations between discrepancy and digestible CP were not observed, probably a surplus of nitrogen did occur. A relative deficiency of available energy reduces growth of microbes and may increase lysis of microbial cells, resulting in a decreased quantity and efficiency of passage of microbial protein to the small intestine (Clark *et al*., 1992). Energy is then used for maintenance rather than for growth. A mixture of forage and concentrates (with a concentrate content of 30-70%) would result in a more efficient microbial growth than either forage or concentrate alone, because of optimization of availability of fermented substrate and increased rate of passage of digesta from the rumen (Clark *et al*., 1992). Therefore, in the 40-65% grass diets, the imbalance was decreased because of the variation between feeds, as the composition of the other feeds in the diet varied.

Within the 40-65% grass diets, a high quality of the grass (high ME, q, CP and DVE, and a low CF, most of which are mutually correlated) decreased the discrepancy, which is the opposite as what happened in the 80-90% grass group. This could be attributed to the more balanced situation in the rumen, as suggested earlier, or to interactions between grass and supplements. It could also indicate that the energy value of grass is wrongly calculated, which was observed only in the 80-90% grass diets, and not in the 40-65% grass diets. As correlations between the discrepancy and grass factors did not hold when the outliers in the 40-65% grass group were excluded, the negative effect on discrepancy of the high quality grass was probably due to specific effects of feeding maize silage. Reasons for those effects of maize silage in the diet will be discussed in the next paragraph.

Correlation of discrepancy with diet variables; Hypothesis 2

If the second hypothesis (the calculated energy value gives the potential energy value of the grass, but due to certain factors as diet composition, this value does not always show) would be valid, positive correlations between discrepancy and diet factors would be expected. Such correlations were indeed found.

In the 80-90% grass diets, the discrepancy is positively correlated with the DMI_{total}. This was expected because with higher intake more energy would be required within the gastrointestinal tract to support the contractions of the rumen and intestine. Higher levels of nutrition are related to a higher blood flow and oxygen consumption in the viscera (Burrin *et al*., 1989). However, the oxygen consumption per g of liver tissue remained equal between sheep fed restricted on maintenance level and sheep fed *ad libitum*. It is therefore suggested that level of feed intake did not affect tissue metabolic activity (Burrin *et al*., 1989), which explains why an effect of DMI_{total} was not observed in the 40-65% group. In the 80-90% grass analysis the DMI_{total} was positively correlated with DMI_{grass} (R^2 = 70.3%, $P < 0.05$). High DMI_{grass} and high %grass resulted in a higher discrepancy in both analyses. Some literature suggests higher energy requirements when cows are fed with forage based diets. A diet rich in forages will probably result in a larger digestive tract, which might increase maintenance costs for the accompanying higher oxygen consumption attributable to a larger digestive tract (Agnew *et al*., 1998). Experiments with beef cattle fed 75% alfalfa or 75% concentrates also showed that the efficiency for gain is more efficient with the concentrate diet than with the alfalfa diet, due to intense metabolic activity in gut and liver with forage based diets (Reynolds *et al*., 1991).

The discrepancy was negatively correlated to DMI_{supp} in both situations, although it was most prominent for the 40-65% grass group. The reason that this correlation is lower in the 80-90% grass group is probably because the variation in percentage of grass (and thus supplements) in the diet is less in that group. The percentage of grass varied between 80.9 and 91.3, with an average of 87.9, but the median was 89.5%, so 50% of the data set had a percentage of grass that was higher than 89.5%. The residuals of the analysis with DMI_{supp} in the 80-90% grass group were not random. In the 40-65% grass group, the variation was higher and more random. However, the reduction in discrepancy with high DMI_{supp} was probably due to the positive effect of maize silage. Although cows with the maize silage diets were in the same stage of lactation as the cows with the other diets, in four diets with maize silage the energy content of the grass was underestimated compared to the output. In diets with maize silage, high quality grass reduced the discrepancy, whereas in the 80-90% grass group, high quality grass increased the discrepancy. This is probably due to interaction between grass and maize silage. The positive effects of maize silage (negative effect on discrepancy) may be attributed to a positive effect of slowly degradable starch on milk yield (Nocek & Tamminga, 1991), the equalization of the degradation of energy and protein in the rumen (Tamminga, 1992) and thus a more efficient production of microbial protein (Clark *et al*., 1992) or improved utilization of protein and energy (Moran & Stockdale, 1992).

Another possibility in this respect is that the estimated feeding value of the supplements was not correct. This is confirmed by the high positive correlation of the discrepancy with $NE_{L,supp}$. This correlation remains significant even when the three outliers (with their negative discrepancy) were excluded from calculations (R^2=67.5%, $P < 0.001$; not shown). The reason why no correlations were observed in the 80-90% grass group, was that the variation of supplements was low, varying between 7.0 and 7.2 MJ kg DM^{-1}.

The discrepancy was positively correlated to DVE diet in both cases, but the correlation was not significant in the 80-90% grass group. If DVE diet is high, a surplus of N will probably occur. Surplus N has to be converted to urea in the liver and it has to be excreted in the kidneys. Both processes require energy. According to Valk (1994), 221 g more nitrogen in the 80-90% grass diets (compared to a diet with a grass-maize silage mixture) requires an energy equivalent of 1.64 kg FCM for excretion. Some of the urea is recycled to the rumen in the saliva, increasing the internal pool of N. This turnover of N with a high rate of ureogenesis in the liver might therefore require more energy than expected from theoretical calculations. However, the fact that ketoacids that result from deaminated amino acids are used as energy sources in the Krebs cycle (Satter *et al*., 1998) partly compensates for the energy costs for N excretion.

Kirkpatrick *et al*. (1997) found a (non-significant) lower N retention in diets for beef cattle, based on grass silage only, compared to low-silage diets or high silage diets with concentrates, even if the N retention was calculated as a proportion of total N intake. Diets with a high amount of grass silage are associated with high urinary energy losses, due to increased N excretion from inefficient utilization of N in ensiled grass (Thomas & Gill, 1988).

Supplementation with barley reduced the energy losses in urine (Thomas & Gill, 1988; Kelly & Thomas, 1978) and thus increased the efficiency of energy utilization.

Because of the positive correlations found between discrepancy and diet variables, and because results reported in the literature (e.g. Agnew *et al*., 1998; Reynolds *et al*., 1991) to confirm the hypothesis, we may conclude that with high amounts of grass the chance for a discrepancy between expected and actual production may increase. A well-balanced diet could probably improve the efficiency of energy utilization in dairy cows.

Correlation of discrepancy with animal factors; Hypothesis 3

If the third hypothesis (higher energy requirements for maintenance or lactation) is valid, positive correlations with animal characteristics would be expected.

Energy requirements for cows are calculated from milk production and maintenance requirements for metabolic body weight (Equation 2). An increase in maintenance requirements of dairy cows on grass-based diets might be possible (Patle & Mudgal, 1977; Unsworth *et al*., 1994; Yan *et al*., 1997; Bruinenberg *et al*., 2002). However, BW hardly influenced the discrepancy, although the chance on discrepancy was in both cases higher with a higher BW. An increase in maintenance requirements of the cows in the present study by 10% decreased the discrepancy, but it remained significant. However, BW gain was positively correlated with discrepancy in all analyses. For multiparous cows, BW gain is usually not included in the regular calculation for requirements, even though body weight gain is quite usual after the peak period of lactation. Perhaps relatively more energy is used for gain on grass diets than on other diets. Because it was not certain that an increase in body weight is fat, and not water or feed in the rumen, BW change was not considered reliable enough to correct the total energy requirements for BW change. Still, some calculations (not shown) indicated that the remaining part of the discrepancy would disappear if such a correction could be made.

Probably also pregnancy of the cows could have some effect in this respect. An energy bonus for pregnancy is usually given starting at six months of pregnancy (Van Es, 1978; CVB, 2000b). This state of pregnancy was not reached in any of the experiments, and therefore no extra energy for pregnancy was added in this study.

Furthermore, there was a highly positive correlation between discrepancy and milk protein. Animals with an energy shortage usually show this by lowering protein in milk (Blaxter, 1962). Therefore, it was clear that there was no energy shortage in case of discrepancy, but that an energy surplus would be more likely. This energy surplus was, however, not used for the production of milk.

Possibilities for adaptation of the energy evaluation of grass

The correlations of the discrepancy with the grass variables, such as CP, CF, DOM, OEB or NFE, were low. It was expected that the surplus of N, which is expressed in the OEB, would influence the overestimation, but correlations were not significant. Because discrepancy was positively correlated with DOM in the 80-90% grass group, it may be concluded that with high DOM, and thus low CF, in the grass chances on discrepancy increase. The significant correlation of discrepancy with DOM disappears in the 40-65% grass group and %OMD even becomes negatively correlated. Those effects may be attributed to too low amounts of grass in those diets to observe a significant effect of chemical composition or it may be attributed to an influence of the composition of the total diet on the discrepancy. It would therefore also be likely to support the second hypothesis, namely that grass is not fully utilized in some situations. This would be caused by the high amounts of grass in the diet or by the balance of the diet. The second hypothesis can be combined with part of the third hypothesis: higher maintenance requirements for dairy cows on grass-based diets. In a previous study, an increase of 10% was suggested (Bruinenberg *et al*., 2002). This could be caused by (i) increased energy requirements in the gastro-intestinal tract for movement of the digesta and (ii) the imbalance of this type of diet, resulting in a reduction of ruminal microbial efficiency and increased energy costs for nitrogen excretion. Furthermore, some extra requirements for weight gain in the second half of the lactation may be expected.

Conclusions

The estimated energy input on grass-based diets and the energy output in milk and maintenance are not similar. Especially the proportion of grass in the diet affected this discrepancy, probably due to either an imbalance of nutrient supply or to the higher maintenance requirements on a grass-based diet. Furthermore, in diets with more than 80% grass, high DOM in the grass increased discrepancy.

In diets with more than 35% supplements, the estimation of the feeding value of grasses does not seem to be wrong. However, an adaptation in the calculation of energy requirements for maintenance, is probably necessary. This adaptation could be an increase of 10% for maintenance requirements for lactating dairy cows on diets of which the main component is grass.

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Chapter 3

Energy metabolism of dairy cows fed on grass

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Energy metabolism of dairy cows fed on grass

Abstract

Production performance of grass-fed dairy cows is often lower than expected from the estimated energy supply. To explain the overestimation of the energy content of grass for dairy cows, data from energy balance trials from three different laboratories (Wageningen, Lelystad and Hillsborough) were collected. The trials in Wageningen and Lelystad were carried out in the 1970s and those in Hillsborough in the 1990s. Regression analyses were carried out with the complete data set as well as per laboratory to identify differences per laboratory. Average net maintenance requirements per $kg^{3/4}$ (NE_m) were 0.573 MJ, whereas the efficiency of metabolizable energy utilization for lactation (k_l) was 0.777. When NE_m was fixed at the presently used value of 0.293 MJ/$kg^{3/4}$, k_l was 0.60. Between laboratories NE_m varied between 0.294 (Lelystad) and 0.786 MJ/$kg^{3/4}$ (Hillsborough), whereas k_l varied between 0.57 (Lelystad) and 0.84 (Hillsborough). For Wageningen and Hillsborough NE_m was high, whilst k_l was high as well. With the intercept fixed at 0.293 MJ/$kg^{3/4}$, efficiency varied between 0.53 (Hillsborough) and 0.62 (Wageningen). The k_l and NE_m were interrelated. Based on these data we surmise maintenance requirements for grass fed dairy cows to be 10% higher than presently assumed, with no change in k_l.

Keywords: Dairy cattle, nutrition, energy utilization, grass-based diet

Introduction

In temperate regions fresh grass is an important feed for dairy cows. When produced in intensively managed systems, such as the systems used in Western Europe, grass has a high feeding value. This is reflected in high concentrations of metabolizable or net energy (ME or NE) and crude protein (CP) and low concentrations of fibre. Despite this high quality, production performance of dairy cows on grass based diets is often lower than expected from the estimated energy content of the grass and the measured intake (Valk *et al.*, 2000). Assuming that intake is measured correctly, a reduced milk output suggests a low efficiency of energy utilization, whilst valuable nutrients, such as CP, are not fully utilized, resulting in excessive nitrogen (N) excretion in urine. This is undesirable from both environmental and economic points of view.

The reasons behind this unsatisfactory performance of dairy cows on fresh grass are not clear. Possible reasons are an overestimation of the energy value or the energy intake of grass, an imbalance of the consumed diet, e.g. deficiency of glycogenic or aminogenic nutrients, excessive ureogenesis, or an effect of the diet on endocrine control influencing the repartitioning of nutrients.

This paper focuses on the possible overestimation of the NE content of grass. Such an overestimation may be due to a lower efficiency of ME utilization for milk (k_l) than originally estimated or higher energy requirements for lactation required for maintenance (ME_m or NE_m).

The present energy evaluation systems were developed on the basis of experimental data, obtained in the 1970s or earlier (Van Es, 1978; Van der Honing & Alderman, 1988). Since then grassland management and feeding strategies may have changed, but also genetic merit of the animals has increased (e.g. higher body weight of the cows, higher milk production). All these may have changed k_l or ME_m as well as NE_m. In energy balance studies, k_l and ME_m are interrelated and should always be interpreted in combination to each other. This is a consequence of linear regression analyses of the data. Extrapolation beyond the range of measurements is not valid. At similar ME_m levels, Ferris *et al*. (1999) observed a positive relationship between genetic merit and k_l. However, differences in milk yield as such do not alter the efficiency of energy utilization, which is assumed to be 0.60 with ME_m of 0.49 MJ/kg$^{3/4}$ (Van der Honing, pers. comm.), according to the present Dutch energy system.

Higher maintenance requirements on forage-based diets could result from higher energy requirements of the gastro-intestinal tract. Oxygen consumption in the intestines has been shown to increase on diets rich in forage (Reynolds *et al*., 1991). With diets consisting of mostly roughage, supplemented with concentrates, Patle & Mudgal (1977) found a k_l of 0.66, with a ME_m of 0.574 MJ/kg$^{3/4}$, which values are much higher than used in the present Dutch energy system. Unsworth *et al*. (1994) suggested a k_l of 0.56 with an assumed ME_m of 0.64 MJ/kg$^{3/4}$ for cows on forage-based diets comparable to the diets used by Pattle & Mudgal (1977). However, Van Es (1975) suggested a k_l of 0.60, with ME requirements for maintenance of 0.49 MJ/kg$^{3/4}$, independent of diet composition.

Besides diet effects also the effect of being in lactation may affect maintenance requirements. Lactating animals have bigger internal organs, e.g. heart, lungs, stomach and intestines, and a higher feed consumption than non-lactating animals. Smith & Baldwin (1974) suggested that maintenance requirements for lactating cows are about 10% higher than for non-lactating cows. It is uncertain whether animals fed on a diet with grass only have a different k_l or ME_m than presently used in the Dutch energy system. In this study, energy metabolism experiments with dairy cows on fresh grass were extracted from various databases and analysed for the relationship between ME intake and NE output.

Material and methods

Database

For this study we used a database constructed on the basis of 96 data sets derived from energy balance (indirect calorimetry) experiments with individual lactating cows fed fresh grass and carried out in three different laboratories. Sixty-three of these data sets were acquired from trials carried out in Wageningen, the Netherlands in the late 1970s (Van Es & Van der Honing, 1976) and 20 of the data sets came from experiments carried out in Lelystad, the Netherlands in the 1970s (Van der Honing, unpublished). The remaining 13 data sets came from experiments carried out in Hillsborough, Northern-Ireland in the 1990s (Cushnahan, 1993). Only data sets from cows fed a grass diet with less than 10% of dry matter from concentrates were used.

In the energy balance trials several parameters concerning the energy metabolism of cows were assessed. Data collected included gross energy intake (GE_{food}), energy in milk (RE_{lac}), faeces (GE_{feces}), urine (GE_{urine}), methane (GE_{gas}), and heat production (Q). Based on these collected data, the ratio between metabolizable energy (ME) and gross energy (GE) was calculated to define the quality (metabolizability, q = 100*ME/GE) of the feed. Metabolizable energy intake (MEI) was calculated as: MEI = $GE_{food} - (GE_{feces} + GE_{urine} + GE_{gas})$.

MEI can be converted to net energy intake (NEI) by multiplying the MEI with the k_l. In most systems k_l is assumed to be 0.59-0.64 (Van der Honing & Alderman, 1988). For practical use k_l is also used for ME_m, because the changes in efficiencies of utilization of MEI are similar for milk and maintenance at an increasing value of the metabolizability of the diet. However in dry cows k_m is somewhat higher, about 0.7 (Van Es, 1972; 1978; AFRC, 1995). Most cows are not in energy balance and may gain or lose some body weight. This is shown by the retained energy (RE_g), which is calculated according to Equation 1.

$$RE_g = MEI - (RE_{lac} + Q) \tag{1}$$

in units of MJ day^{-1}.

Net energy intake (NEI) was assumed to be divided into maintenance, milk and retention (Equation 2).

$$NEI = NE_m + RE_{lac} + \text{pos } RE_g \text{ (or } 0.8 * \text{neg } RE_g) \tag{2}$$

where NE_m is net energy requirements for maintenance (kJ) per day, RE_{lac} is net energy requirements for milk (kJ) per day and RE_g is energy in body weight gain (pos RE_g; kJ) or loss (neg RE_g; kJ). Efficiency for mobilization of body tissue for milk production is assumed to be 0.8 instead of 0.6 (Van Es, 1975), because of absence of metabolic losses in digestion and the low energy costs of converting lipids from body stores into milk.

In the comparison of NEI in feed and NE output in milk, it is necessary to adjust RE_{lac} for energy retention, because otherwise the energy recovered in milk is overestimated with tissue mobilization and underestimated with tissue gain. Adjusted milk energy (NE_{adj}; kJ) was defined as NE production corrected for RE:

$$NE_{adj} = RE_{lac} + \text{pos } RE_g \text{ (or } 0.8 * \text{neg } RE_g) \tag{3}$$

Statistical methods

To estimate the NE_m and k_l for dairy cattle, regression analyses were carried out with Genstat (Genstat 5 Committee, 1993). In the analysis NE_{adj} / kg metabolic weight ($kg^{3/4} = BM^{3/4}$) was the y-variable, and MEI/ $kg^{3/4}$ the x-variable. The model used was according to Equation 4.

$$y = ax - b \tag{4}$$

where y equals $NE_{adj}/kg^{3/4}$, a equals the regression coefficient which is equal to k_l, x equals $MEI/kg^{3/4}$, and b equals $NE_m/kg^{3/4}$.

The data were analysed using the entire database as well as per laboratory, because differences occurred between laboratories. However, it is difficult to separate energy used as ME_m and for milk production (ME_{lac}). Heat production (Q) includes heat losses caused by converting ME into milk and body tissue and all heat produced by maintenance processes and during activity of the animals. From the linear regression analysis it is clear that ME_m and k_l are interrelated and that the size of the regression coefficient is of large influence on the size of the intercept. Therefore three approaches were used to analyse the data. In the first approach, regression analyses were carried out with variable regression coefficient (k_l) and free intercept (NE_m) to get the best fit to the data. An increase in k_l coincided with an increase in NE_m. To reduce the effect of this interrelation a second approach was carried out. In the regression analysis the intercept was fixed on the NE_m used in the VEM system, 0.293 MJ/ $kg^{3/4}$ (Van Es, 1975; CVB, 2000). The third approach was to compare the predicted NE-values from the equation in the Dutch VEM system [k_l being 0.6 * (1 + 0.004 * (q − 57)) and NE_m = 0.293MJ/$kg^{3/4}$] with the measured NE_{adj} values in the trials. A student's *t*-test was carried out to test if the differences between the predicted NEI and the net output were significant.

Results

Range of variation in database

The database comprised data sets from 96 energy balance experiments at three different laboratories. Whereas all cows were lactating, milk yield varied substantially from 8.6 to 28.2 kg per day (Table 1). Intake of GE varied from 188 to 359 MJ d^{-1} and MEI varied from 116 to 238 MJ d^{-1}. The percentage of dry matter from forage in the diet varied from 91% to 100% and the digestibility of energy of the diet varied from 70% to 82% (Table 1).

Ranges of the energy and production data differed substantially between laboratories (Table 2). Milk yield was highest in Lelystad and lowest in Hillsborough. Intake of ME was lowest in Lelystad and highest in Hillsborough. Average RE as body tissue was 20 MJ d^{-1} in Wageningen, − 16 MJ d^{-1} in Lelystad and 1 MJ d^{-1} in Hillsborough.

The average percentage of dry matter from grass in the total diet was 94% in Wageningen, 93% in Lelystad and 100% in Hillsborough.

Efficiency of utilization of ME

The overall relationship between MEI and NE_{adj} from the first approach was described by the equation

$$NE_{adj} = -0.573\ (\pm 0.05) + 0.777\ (\pm 0.03) * MEI$$

(Figure 1, dotted line; Table 3, Equation (1)). This equation assumes a k_l of 0.777, with an assumed NE_m of 0.573 MJ/$kg^{3/4}$. With a free intercept in the regression equations, the k_l and NE_m were highly variable between laboratories. For Wageningen the k_l was 0.791 (±0.03) and the NE_m 0.572 (±0.04) MJ/$kg^{3/4}$, for Lelystad the k_l was 0.579 (±0.05) and the NE_m 0.294

(±0.07) MJ/kg$^{3/4}$. For Hillsborough the k_l was 0.842 (±0.07) and the NE_m 0.786 was (±0.12) MJ/kg$^{3/4}$ (Table 3, Equations (2)-(4)). For Lelystad, the regression coefficient and the intercept were almost similar to the values of 0.6 and 0.293 MJ/kg$^{3/4}$, respectively, which is presently used in feed evaluation for feeds with q = 57 (Vermorel & Coulon, 1998; Van der Honing & Alderman, 1988). In contrast, the coefficients from the Wageningen and Hillsborough data were significantly higher ($P < 0.05$) than the values commonly used. Especially in Wageningen and Hillsborough, the relatively high k_l coincided with relatively high NE_m requirements, of 0.572 (±0.04) and 0.786 (±0.12) MJ/kg$^{3/4}$, respectively. With a free intercept NE_m was higher in this analysis with the complete database than in the presently used system, but the k_l was also higher. Furthermore, NE_m of Wageningen and Hillsborough was higher than that of Lelystad, but also here k_l was higher with higher NE_m. Due to this compensation, the ME requirements for cows producing 30 kg of milk per day were relatively similar for the presently used system, the complete database and the different databases (Figure 3).

Table 1. Characteristics of grass and animals and energy metabolism of grass-fed dairy cows. Average vales of 96 respiration experiments. S.D. = standard deviation.

	Mean	S.D.	Minimum	Maximum
Grass quality				
Digestible organic matter (g kg^{-1} DM)	719	24.5	638	781
Digestible crude protein (g kg^{-1} DM)	166	38.4	81	233
% of grass in diet	94	2.4	91	100
Animal characteristics				
Milk yield (kg day^{-1})	18.2	4.6	8.6	28.2
Days in lactation[a]	147	65.1	24	306
Liveweight (kg)	521	37.3	455	607
Grass intake (kg DM d^{-1})	13.4	2.2	6.7	20.8
Intake of digestible organic matter (kg d^{-1})	10.1	1.4	7.1	13.7
Intake of digestible crude protein (kg d^{-1})	2.4	0.5	1.2	3.8
Energy (MJ d^{-1})				
Intake of gross energy	266	36.6	188	359
Energy in faeces	66	10.9	45	97
Energy in urine	15	2.7	8	21
Energy in methane	16	2.7	9	22
Heat production	101	10.7	80	138
Energy in milk	57	13.6	29	87
Retained energy	10	22.9	-49	57
Intake of metabolizable energy	169	26.5	116	238
Digestible energy / gross energy	0.75	0.025	0.70	0.82
Metabolizable energy / gross energy	0.63	0.029	0.57	0.70

[a] Average of days in lactation only from Wageningen and Lelystad. These data were not available from Hillsborough.

With the second approach, where NE_m was fixed at 0.293 MJ/kg$^{3/4}$, a value used in various systems (Van Es, 1978; Van der Honing & Alderman, 1988; Vermorel & Coulon, 1998), the overall k_l was calculated as 0.60, with an RSD of the equation of 0.084 (Figure 1, solid line; Table 3, Equation (5)). In the analyses per laboratory, k_l varied between 0.532, RSD of the equation of 0.11, for Hillsborough and 0.621, with an RSD of 0.061, for Wageningen (Table 3, Equation (8) and (6), respectively).

Table 2. Characteristics of grass and animals and energy metabolism of grass fed dairy cows as estimated in three different laboratories. S.D. = standard deviation.

	Laboratory					
	Wageningen		Lelystad		Hillsborough [1)]	
Number of measurements	63		20		13	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Grass quality						
Digestible organic matter (g kg^{-1} DM)	723	20.9	708	34.6	717	18.6
Digestible crude protein (g kg^{-1} DM)	172	35.3	173	37.8	126	29.6
% of grass in diet	93.8	0.8	92.9	0.99	100.0	0
Animal characteristics						
Milk yield (kg d^{-1})	17.8	4.8	21.1	4.5	17.1	2.7
Days in lactation	155	64.7	109	51.6	*	*
Live weight (kg)	518	36.9	516	28.9	546	43.7
Grass intake (kg DM d^{-1})	13.6	1.7	11.8	1.6	14.7	3.3
Intake of digestible organic matter (kg d^{-1})	10.5	1.2	9.0	1.3	11.1	1.9
Intake of digestible crude protein (kg d^{-1})	2.5	0.5	2.1	0.5	1.9	0.6
Energy, MJ d^{-1}						
Intake of gross energy	275	31.8	231	29.6	275	37.3
Energy in faeces	69	10.0	55	7.4	68	9.1
Energy in urine	16	2.6	15	1.7	12	2.6
Energy in methane	16	1.5	13	2.7	20	2.0
Heat production	99	7.5	99	9.1	118	10.7
Energy in milk	55	13.9	65	13.3	56	8.6
Retained energy	20	16.5	−16	14.2	1	25.7
Intake of metabolizable energy	174	23.3	147	22.1	175	32.0
Digestible energy/ gross energy	0.75	2.2	0.76	2.7	0.75	3.5
Metabolizable energy/ gross energy	0.63	2.8	0.64	2.5	0.64	3.7

[1)] In the Hillsborough trials digestible organic matter and digestible crude protein were estimated from estimation formulae (CVB, 1998).

* = not known.

As a consequence, ME_m differed between laboratories resulting from the differences in k_l. The ME_m was highest for Hillsborough and lowest for Wageningen.

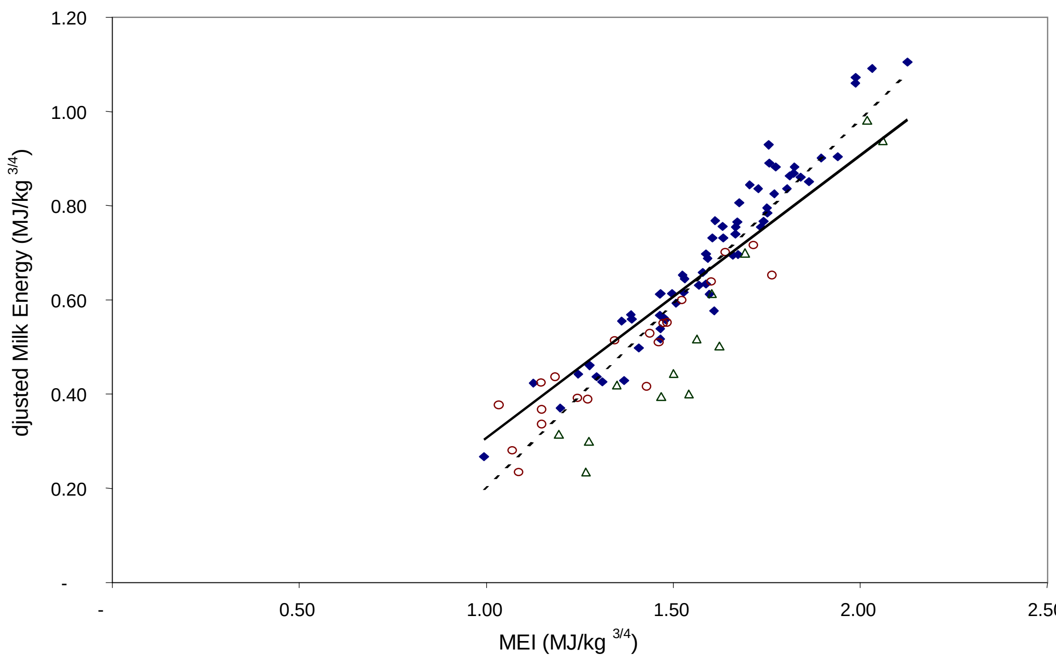

Figure 1. The relationship between the MEI/ $kg^{3/4}$ and the Adjusted milk output/ $kg^{3/4}$. The dotted line is the line with the free intercept, and the solid line is the line with the fixed intercept [(♦) Wageningen, (○) Lelystad, (Δ) Hillsborough].

Table 3. Linear relationships between net energy output in milk (Y, MJ/day/$W^{3/4}$), adjusted for retained energy, and intake of metabolisable energy (X, MJ/day/$W^{3/4}$) in dairy cows fed solely on grass diets. Y = aX + b. Relationships were estimated either with a free intercept (Equations 1 to 4) or with the intercept fixed at –0.293 MJ/day/$W^{3/4}$ (Equations 5 to 8). Relationships were estimated for the complete database as well as per laboratory. R.S.D. = residual standard deviation.

Equation	Database	N	Intercept	Efficiency (a)	NE_m (b)	R.S.D.	R^2
(1)	Complete	99	Free	0.777	−0.573	0.0714	0.879
(2)	Wageningen	63	Free	0.791	−0.572	0.0473	0.934
(3)	Lelystad	20	Free	0.569	−0.294	0.0523	0.863
(4)	Hillsborough	13	Free	0.842	−0.786	0.0673	0.923
(5)	Complete	99	Fixed	0.600	−0.293	0.0841	0.833
(6)	Wageningen	63	Fixed	0.621	−0.293	0.0610	0.863
(7)	Lelystad	20	Fixed	0.568	−0.293	0.0523	0.863
(8)	Hillsborough	13	Fixed	0.532	−0.29	0.0110	0.795
Van Es, 1978; CVB, 2000				0.59 to 0.65[1]	−0.293		

[1]The efficiency was calculated by $k_l = 0.6* (1 + 0.004 * (q-57))$ (CVB, 1999) or $k_l = 0.35 * q_m + 0.420$ (AFRC, 1995).

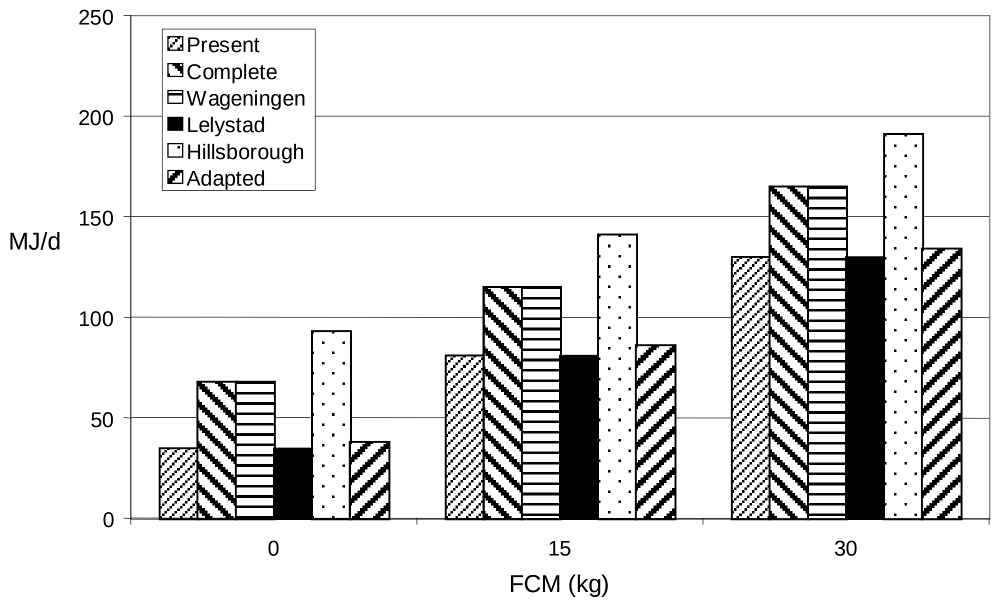

Figure 2. Net energy requirements (MJ d^{-1}) for maintenance and production of 0, 15 and 30 kg of fat corrected milk, using the calculation rules for the VEM-system (present), the regression equations for the complete database (complete), the regression equations for the three laboratories (Wageningen, Lelystad, Hillsborough) and the adapted regression equation with increased maintenance requirements (adapted; $y = 0.6x - 0.322$). Approach 1 is shown in 'complete', 'Wageningen', 'Lelystad' and Hillsborough'; Approach 3 is shown in 'present' and 'adapted'.

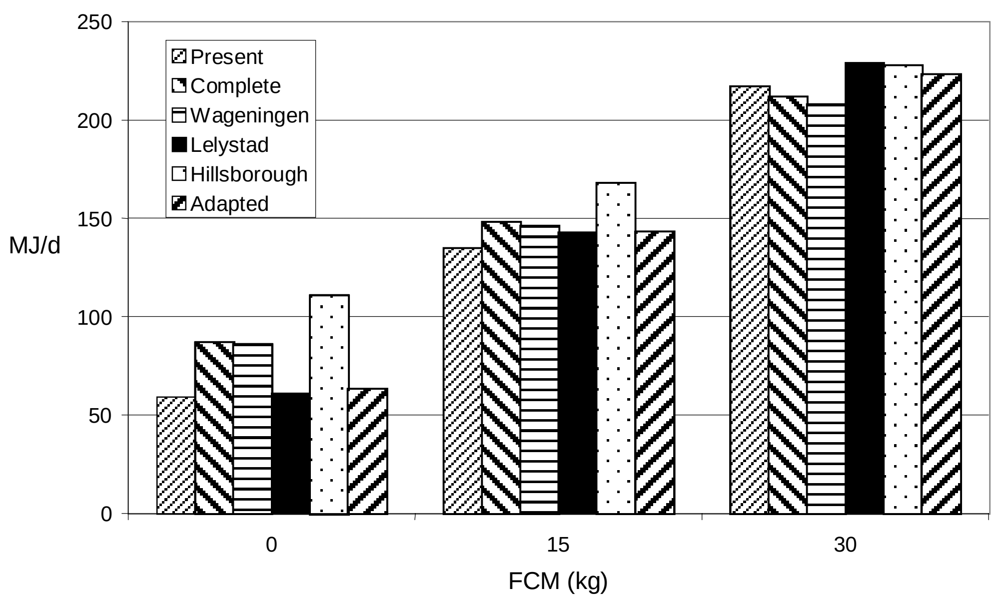

Figure 3. Metabolizable energy requirements (MJ d^{-1}) for maintenance and production of 0, 15 and 30 kg of fat corrected milk, using the calculation rules for the VEM-system (present), the regression equations for the complete database (complete), the regression equations for the three laboratories (Wageningen, Lelystad, Hillsborough) and the adapted regression equation with increased maintenance requirements (adapted; $y = 0.6x - 0.322$). Approach 1 is shown in 'complete', 'Wageningen', 'Lelystad' and Hillsborough'; Approach 3 is shown in 'present' and 'adapted'.

A comparison between predicted and measured NE-values according to the third approach is presented in Figure 4, where the difference between predicted and measured NE is plotted against MEI, both expressed per kg$^{3/4}$. These differences appeared to be significant ($P < 0.05$) for the complete database.

Discussion

To estimate the NE_m and k_l, we used energy balance data and carried out linear regression with the data, using a free intercept. The validity of this database will be discussed. The linear regression analysis approach has the disadvantage that NE_m and k_l are interrelated. To reduce this problem, we made use of two alternative approaches as described before. Results will be discussed. After predictions from the present Dutch VEM-system comparisons were made with measured NE-values. We discuss how differences can be minimized.

Database of energy balance results

The average milk production in the database was 18.2 kg per cow, with a maximum value of 28.2 kg. Compared with production levels of high merit cows nowadays, this production is low. However, if no concentrates are supplemented, grazing dairy cows consume approximately 120-130 g DM/kg$^{3/4}$ of body weight per day. Such an intake meets the requirements for a daily milk production of approximately 22-23 kg milk (Meijs, 1981). Even in high-producing dairy cows, milk productions of 30 kg or more on total grass diets are rare (Meijs, 1981; Kolver & Muller, 1998).

Although experiments in Wageningen and Lelystad were carried out more than 20 years ago, milk yield was in the range that may be expected on grass-based diets. The data from Hillsborough were acquired more recently (1991). It is unlikely that in this study changes in ME_m or k_l can be attributed to changes in the genetic potential of the animals, because milk yield was as low as in Wageningen early 1970s. No major differences could be identified on the sampling and analyses of feed, faeces, urine and gas exchange measurements between laboratories. Although the circumstances of carrying out the energy balance trials between laboratories are not supposed to be quite different, this could not be confirmed.

Energy requirements for lactating dairy cows

Net energy requirements for dairy cows can be divided into requirements for maintenance, milk production, weight gain and pregnancy (Van Es, 1975). The focus in this study was on efficiency of ME utilization for milk production and on energy requirements for maintenance. The gross energy of fat corrected milk is well defined and documented with a low standard deviation. Therefore, the requirements of NE for fat corrected milk production were not considered as a possibility for adaptation, because no data were found to doubt the NE requirements for fat corrected milk production. This requirement is equal to the gross energy of 3.05 MJ of a kg FCM (CVB, 2000; Crovetto & Van der Honing, 1984).

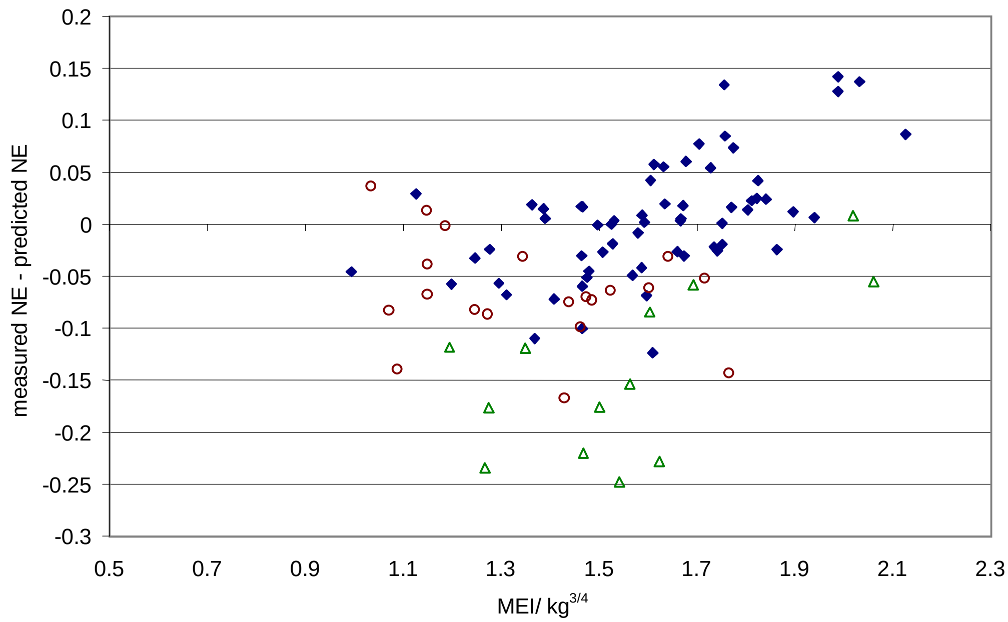

Figure 4. Difference between measured net energy (NE), from the equation in the VEM-system, and predicted NE (MJ/kg$^{3/4}$) as related to MEI. [(♦ Wageningen, (○) Lelystad, (Δ) Hillsborough].

Energy utilization depends on the final product in milk or gain (Van Es *et al*., 1978). In this study the values used for the efficiency for lactation (k_l) and the efficiency for maintenance (k_m) are equal, increasing slightly with q, according to Van Es (1975). This is in contrast with the AFRC (1995) system that used a higher k_m over k_l. Also Van Es suggested a higher k_m for non-lactating cows from literature studies (Van Es, 1972). But a higher k_m will result in a higher value for NE_m, which is calculated from the amount of ME_m. If the NE_m-value is adapted to a change in k_m in a feed evaluation system the standards will remain appropriate. In addition, Van Es (1978) argued that use of a combined efficiency for both maintenance and lactation in high yielding dairy cows is allowed, because milk production requires much more energy than maintenance and the effect of q is small and similar on k_m and k_l. Blaxter (1961) showed no significant difference in k_l between diets ranging from poor quality forage to all concentrate diets.

The efficiency of utilization of ME normally varies between 0.59 and 0.64, depending on the q of the diet (AFRC, 1995; Van Es, 1978), whilst NE_m varies between 0.29 and 0.30 MJ/kg$^{3/4}$, with no modifications for grass fed cows (Vermorel & Coulon, 1998; Van der Honing & Alderman, 1988). Trigg *et al*. (1982) have reported lower values of k_l of 0.56 and 0.50 with an intercept of 0.321 and 0.230, respectively with lactating cows in early (spring) and midlaction (autumn) in New Zealand on fresh grass in calorimetric chambers.

Using the first method, with a free intercept in the analysis, the k_l is above instead of below 0.6, with a markedly higher NE_m than used in the recommendation of current systems. Higher k_l does not seem to agree with the disappointing performance observed in production trials (Valk *et al*., 2000), which suggests a reduced efficiency. Depression in efficiency is usually associated with imbalances in nutrient supply and in ratio of supplies of energy and protein (Thomas & Chamberlain, 1990; Waghorn & Barry, 1987). Van Es (1975) found that excess protein in the diet slightly decreased the efficiency of utilization of ME. Furthermore, feed evaluation systems assume rather well balanced nutrient composition in the diet. The data analysed here are on diets of 90 to 100% grass, and some of them were relatively unbalanced with regard to energy to protein ratio.

Higher requirements for maintenance than recommended in energy systems (0.29- 0.30 MJ/kg$^{3/4}$) may occur, due to increased oxygen consumption in the gastrointestinal tract with forage-based diets, together with a higher heat production in the intestine to digest the feed (Reynolds *et al*., 1991). These higher requirements could be due to the movement of fibrous digesta, or to specific end products from forage diets (Thomas & Chamberlain, 1990). Furthermore, cows on fibre-rich diets such as grass, but also on other diets with high fibre contents, spend more time on chewing and rumination than cows on diets including less fibre (Holmes, 1980). This would have increased energy costs of maintenance. In practic, grazing and walking will increase costs of maintenance even more.

Another factor increasing NE_m would be the excess of nitrogen in the diet. Ortigues & Doreau (1995) mentioned that changes in hepatic metabolism with level of intake will be greater in grass based diets than in concentrate based diets. High concentrations of protein can be found in grass, and surpluses of nitrogen have to be excreted. The synthesis and excretion of urea in sheep requires 21.8 kJ per g excreted N (Martin & Blaxter, 1965). Heat production for urea synthesis was 20% higher than was expected on biochemical calculations, which was attributed to recycling of urea in the gastro-intestinal tract. This percentage for recycling seems rather low, and could be much higher in dairy cows fed protein rich grass. Besides the actual excretion, there might be an increased demand for energy to support maintenance and turnover of the liver and kidneys. In sheep on poor quality grass, Ferrell *et al*. (1999) found that net oxygen uptake by the liver was greater if the diets were supplemented with both energy and nitrogen than with energy only. This suggests higher metabolism in the liver on diets with high nitrogen or protein content. In this study, the average amount of surplus of DCP is 860 g. Theoretically, the excretion of an excess of 100 g DCP costs 150 kJ NE, although Tyrell *et al*. (1970) report a nearly double cost of energy in urea of 289 kJ NE per 100 g excess. Per kg$^{3/4}$, 22.8 kJ will then be required for the excretion of nitrogen. Adding 22.8 kJ to 0.293 MJ results in maintenance requirements of 0.316 MJ/kg$^{3/4}$.

The condition of the cows may also play a role in increased maintenance requirements. Lean cows may have higher maintenance requirements than fat cows, because of the higher rate of turnover of protein compared to body fat (Kirkland & Gordon, 1999). No abnormal conditions were found in this study.

In the analyses, the NE_m and k_l were varying per laboratory. Also in literature data on k_l are variable. Yan *et al*. (1997) suggested a k_l of 0.65 and a NE_m of 0.435 MJ/kg$^{3/4}$on grass silage-based diets, whereas Unsworth *et al*. (1994) reported a k_l of 0.56 and a NE_m of 0.358 MJ/kg$^{3/4}$. Moe *et al*. (1970) reported a k_l of 0.644 with a NE_m of 0.328 MJ/kg$^{3/4}$ for cows on grass silage diets. Patle & Mudgal (1977) found a k_l of 0.66 in late and a k_l of 0.64 in early lactation for crossbred cows (Brown Swiss x Sahiwal) on forage based diets. The NE_m was 0.379 MJ/kg$^{3/4}$. According to Patle & Mudgal (1977), these values are similar to those used in Holstein-Friesian cows in the same period.

Some workers have tried to establish differences in k_l and ME_m between different breeds. Münger (1991) compared the k_l and the ME_m of Jerseys, Simmentals and Holstein Friesians and concluded that there is no significant difference between breeds. The average k_l of these three breeds was 0.602 and the average ME_m was 0.51 MJ/kg$^{3/4}$ (Münger, 1991). The ME_m in that study is comparable with the ME_m in the Dutch situation.

From the reviewed literature (Yan *et al.*, 1997; Unsworth *et al.*, 1994; Moe *et al.*, 1970; Patle & Mudgal, 1977) it may be concluded that the k_l is around or just above 0.60. A value of 0.78, being the mean k_l in our database, seems very high, particularly because the diets used were not well balanced. The latter will have consequences for the utilization of energy (Van Es, 1975). Moreover, the maximal biochemical efficiency of milk components synthesis in ruminants from propionate, acetate, amino acids, etc., would be less than 0.80. Measured efficiencies from a wide range of observations all over the world show an average of 0.60, as shown by Van der Honing & Alderman (1988), which is comparable to what has been observed with sows. For that reason the high k_l of 0.78 is unrealistic. Probably also the limited number of cows measured in our database as well as the restricted variation in dietary formulation is a reason to be careful with the interpretation of the results.

Possibilities for adaptation to performance in practice

The VEM system is based on a k_l and NE_m of respectively 0.60 and 0.293 MJ/kg$^{3/4}$, which are lower than the values from the analyses in this study with the first method. The relationships observed suggest that the present systems underestimate the total requirements of NE (Figure 1). Requirements of ME in the present system seem underestimated only at low production levels, because with higher production levels, the higher k_l in our model can compensate for the higher NE_m. So although maintenance requirements are higher in the model, milk production requires less energy because of the higher efficiency of utilization of ME. However, there is no physiological explanation for such a high k_l, but increased maintenance costs have more basis (excess N-excretion, work of digestion, etc.).

Using the second method, with NE_m fixed on 0.293 MJ/kg$^{3/4}$, k_l is rather variable between laboratories and the overall k_l is lower than the value normally used. With NE_m increased by 10% and fixed on 0.322 MJ/kg$^{3/4}$, k_l seems to be more appropriate.

Figure 2 and 3 show the implications of an adoption of the regression formulae from the equations with free intercept in Table 3 (Equations (1)-(4)). In the NE (Figure 2) the difference between the present system and the developed regression formulae remains constant with 0, 15 and 30 kg of milk. If requirements are expressed in ME (Figure 3) instead of NE, the difference depends on the level of production (Figure 3). If no milk is produced, the requirements in the ME are much higher for the equation with the free intercept (Approach 1) than for the present system (Approach 3). With a milk production of 30 kg FPCM, the ME requirements are a fraction higher with the released intercept. However, milk productions of over 30 kg FPCM on diets consisting of 90% grass or more will be rare (Kolver & Muller, 1998). In this study the highest milk production was about 28 kg milk per day (Table 1).

From Figure 4 (Approach 3) it can be seen that, for most data, the predicted NEI is higher than the measured output, especially at lower MEI. This observation corresponds with results of feeding trials using forage diets. An option to correct the energy requirements for dairy cows to results of feeding trials would be to increase the NE_m by 10%. As a result the average difference between predicted and measured NEI became insignificant if NE_m was fixed at 0.322 MJ/kg$^{3/4}$.

The increase of maintenance requirement seems appropriate, because a substantial part of it could be explained by the energy required for urea as a result of excess of N supply. It also takes into account a part of the effect of factors such as pregnancy, exercise, etc., which to some extent will increase the amount of energy required for maintenance as suggested by Moe *et al.* (1970; 1972) and Moe (1981). For grazing lactating cattle it is therefore recommended to increase the NE_m to 0.322 MJ/kg$^{3/4}$ to improve the prediction of performance.

Conclusions

The energy balance data in our study have limitations to evaluate k_l and NE_m. Linear regression analyses may lead to confusing values for k_l and NE_m, mainly due to the interrelation between the two parameters and the extrapolation far beyond the range of data.

The results from Hillsborough deviate substantially from the other results and show less NE output than predicted from the Dutch VEM-equation, although also in most data of Lelystad and some of Wageningen an under-prediction of the milk output occurs. A relation to differences in maintenance requirements is unlikely.

Although the significant differences between NE output and predicted NEI can be attributed partly to the energy costs for the metabolism and excretion of excess N, also other factors are involved. One factor not included in our measurements is the activity for walking and grazing, because animals were inside the calorimetric chamber. By assuming a 10% higher NE_m than the present value of 0.293 MJ/kg$^{3/4}$, and maintaining k_l to the value used in the VEM system (0.6) it was observed that the differences between NE output and NEI became insignificant. It is therefore recommended for grazing lactating cattle to increase the NE_m to 0.322 MJ/kg$^{3/4}$ to improve the prediction of performance.

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Part II

The nutritional value of forages from semi-natural grasslands

Chapter 4

Factors affecting digestibility of temperate forages from semi-natural grasslands

Bruinenberg, M.H., Valk, H., Korevaar, H. & Struik, P.C. (2002) Factors affecting digestibility of temperate forages from semi-natural grasslands. Grass and Forage Science 57, 292-301.

Factors affecting digestibility of temperate forages from semi-natural grasslands

Abstract

To indicate possibilities for the use of forages from semi-natural grasslands in ruminant production systems, a literature study was carried out to describe the nutritive value of those forages. In species rich grasslands, the digestibility of forages is usually lower than the digestibility of forages produced by grasslands used for intensive production. There is also more variation within a species rich sward, because of different stages of maturity of the individual species and because of likely variations in digestibility among forage species independent of stage. Moreover, the presence of dicotyledonous species may have a positive or negative effect on digestibility. In forages from semi-natural grasslands, the relationship between chemical composition and digestibility differs from that of *Lolium perenne*, which is often used as research standard. Therefore, predictions of the digestibility of species rich forage can be inaccurate. The relationship between *in vivo* and *in vitro* digestibility may also be different from the relationship that is common for *L. perenne*. In some cases, the *in vivo* digestibility is higher than the *in vitro* value, as calibrations are based on *L. perenne.* Therefore, the quality of forages from semi-natural grasslands might be higher than predicted, and there is scope for practical use of this kind of forage in ruminant nutrition systems.

Keywords: Digestibility, forage species, semi-natural grassland, stage of maturity

Introduction

Grasslands used for intensive production are usually abundantly fertilized with nitrogen and phosphorus to increase the production and quality of forages. This practice is incompatible with the objective of creating or maintaining species rich vegetation (Peeters & Janssens, 1998). However, in recent years, the interest in botanically diverse, semi-natural grasslands in the Netherlands has increased. The government subsidizes farmers to support grassland management that stimulates the nesting of meadow birds and non-governmental nature conservation organizations buy and manage grasslands to maintain species rich vegetation (Korevaar, 1986).

In the Netherlands, most forages from these semi-natural grasslands are used on intensive dairy farms (Korevaar & Van der Wel, 1997). Throughout Europe, these forages are also used by sheep and beef farmers. There may, however, be reluctance to use such forages, because there are no adequate estimates of their feeding value (Korevaar, 1986; Tallowin & Jefferson, 1999). A lower digestibility and a lower intake are expected (Korevaar, 1986) and, therefore, a reduced performance cannot be excluded when feeding these forages. It is necessary to evaluate the nutritive value of forages from such grasslands. Prediction of the nutritive value of forages includes methods to estimate digestibility, which, combined with the voluntary intake, will consequently give the potential for milk of meat production on these forages.

In this context, semi-natural grasslands are defined as all communities of native grasses and dicotyledonous herbs, with a few, if any, woody species, that have been largely created by agricultural practices, not involving the regular use of inorganic fertilizers, herbicides or cultivation (Crofts & Jefferson, 1994). In this chapter, we use the term semi-natural grassland for species rich grasslands that are maintained for nature conservation purposes. These include grasslands in nature conservation areas, as well as grasslands with prescribed management, as agreed upon by the farmers and some governmental or non-governmental organization (management agreements). Different types of semi-natural grasslands can be distinguished, depending on the type of soil, nutritional status of the soil, water availability and management (IKC Natuurbeheer, 1997; Blackstock *et al*., 1999). Management varies, depending on the purpose of the grassland. The objective for some grasslands is to conserve the grass or herb species and thus prevent their extinction, whereas other grasslands are intended to maintain certain vegetation types, thereby achieving maximal diversity in the vegetation or maintaining populations of meadow birds (Korevaar, 1986).

In this chapter, possible problems concerning the integration of forages from semi-natural grasslands on farms are discussed. The main focus is on aspects concerned with the nutritive value of the feeds, such as current methods of estimating the nutritive value of grass, plant characteristics, ontogeny, and the relationship between plant characteristics, ontogeny and digestibility. Also factors affecting intake of some temperate grass and legume species are discussed. Based on this analysis, options will be proposed for the inclusion of forages harvested from semi-natural grasslands into the feed rations for cattle and sheep.

The review of Tallowin & Jefferson (1999) should be referred to for information about the mineral content of forages in semi-natural grassland.

Prediction of the nutritive value

In the Netherlands, the nutritive value of forage, mainly consisting of grass, is estimated as the metabolizable energy (ME) concentration according to Equations (1) and (2) (Van Es, 1978).

$$\text{ME concentration (kJ kg}^{-1}\text{ DM)} = 14.2 * \text{DOM} + 5.9 * \text{DCP (if DOM/ DCP} < 7) \quad (1)$$

$$\text{ME concentration (kJ kg}^{-1}\text{ DM)} = 15.1 * \text{DOM (if DOM/ DCP} > 7) \quad (2)$$

in which DOM is digestible organic matter in the dry matter (g kg^{-1} DM) and DCP is digestible crude protein concentration (g kg^{-1} DM). Also in other countries, the ME value of forages is usually calculated based on DOM or digestible nutrients, although there are small differences in the equations used among systems (Van der Honing & Alderman, 1988). This results in some differences regarding the ME content of the forages among the different systems.

The estimate for DOM is based on the digestibility of the organic matter (OMD), which is measured in sheep fed on maintenance level (feed intake level 1). The ME concentration can be transformed into a net energy concentration (NE) (Van Es, 1978). Thus, OMD is an important aspect in the estimation of the feeding value. Because of the costs and the labour involved, *in vitro* methods (e.g. Tilley & Terry, 1963) were developed to estimate *in vivo* digestibility (e.g. Steg *et al.*, 1990). However, for routine analysis those methods are not sufficiently efficient and, therefore, linear regression models based on crude fibre and ash concentrations, DM content and days after 1 April (CVB, 1999) were developed, as well as near infrared reflectance spectroscopy calibration curves (NIRS; e.g. Park *et al.*, 1998). However, mainly grass dominated by *Lolium perenne* was used in most experimental trials and models to predict OMD, and ME and NE concentrations. Therefore, it is likely that the feeding value of forages from botanically diverse grasslands is not correctly estimated by the regression method as well as by Tilley and Terry's technique (Tilley & Terry, 1963), and as a consequence by NIRS also. Korevaar (1986) found a higher ME concentration when the linear regression model, based on chemical composition to predict DOM concentration, was compared to the *in vitro* method for forages from semi-natural grasslands (with a maximal overestimation of 1150 kJ kg^{-1} DM for *Agrostis capillaris* and *Agrostis stolonifera*). Forage species in botanically diverse grasslands differ from *L. perenne* with regard to their relationship between chemical composition and digestibility, even when compared at the same stage of development (Åman & Lindgren, 1983; Korevaar, 1986). In addition, species from semi-natural grasslands vary in their ontogeny (e.g. changes in proportions of leaves or stem during ageing) compared to *L. perenne,* and ontogeny has a dramatic effect on quality, both in grass species and in herbs (Osbourn, 1980; Wind & Elzebroek, 1989; Bosch *et al*., 1992). It would probably be possible to develop adequate regression formulae, if those are based on the same specific community of forages (e.g. Daccord, 1988). Scehovic (1991) developed a correction formula for estimating the OMD of forages from Swiss semi-natural grasslands using a linear regression model. This model is based on chemical composition, including quite different components, such as lignin and cellulose, from those determined in the Weende analysis. It would be worthwhile to compare the accuracy of the Scehovic-model with a model based on the Weende components using forages from semi-natural grasslands.

The NIRS method can accurately be used to determine the digestibility of forages (Marten *et al*., 1988; Duru, 1997), as long as the appropriate calibration curves are used. Using fresh grass silage, Park *et al*. (1998) predicted the *in vivo* digestibility with a considerable accuracy (R^2 of 0.79-0.85). However, no information about the composition of the silage was given.

When Armstrong *et al*. (1989) compared *in vivo* and *in vitro* digestibility (in sheep) of some species of semi-natural grasslands (e.g. *Molinia caerulea*), they observed higher levels for the *in vivo* digestibility. This can probably be explained by the fact that the rumen fluid used for the determination of *in vitro* digestibility method was obtained from donor sheep fed high-quality chopped dried perennial ryegrass. It is likely that the microbial population in the rumen fluid of those sheep is not adjusted to ferment low-quality forage, as it is in the *in vivo* situation. Cone *et al*. (1999), using grass dominated by *L. perenne* and cut at different stages of growth, also suggested that adaptation of the microbial population in the rumen for perennial ryegrass at the different stages of growth occurred. In contrast to the observations of Amstrong *et al*. (1989), Korevaar (1986) found higher *in vitro* than *in vivo* digestibility values (in sheep) for grass from botanically diverse grasslands, but Korevaar used rumen fluid from sheep that were offered the experimental grasses. These higher *in vitro* digestibility values might have occurred because the *in vitro* digestibility technique gives the potential digestibility after 48 h, whereas the *in vivo* digestibility is not a constant value, because of variation between animals caused by factors such as retention time in the rumen. It can be expected that in routine laboratory assays the *in vitro* digestibility of forages from semi-natural grasslands will often be lower than the *in vivo* digestibility due to the standardized dietary circumstances (high-quality hay and concentrates), resulting in an underestimation of the energy value. However, using rumen fluid from animals on high-quality forage may also have positive effects on *in vitro* digestibility, because of the higher nutrient supply in the rumen, which will increase the amount of microbes per ml rumen fluid compared to rumen fluid of animals on low-quality forages. This could compensate for the lack of adaptation to the diet, possibly resulting in a relatively high *in vitro* digestibility compared to the *in vivo* digestibility, and thus in a overestimation of the energy value *in vitro* compared to the *in vivo* situation.

For the dicotyledonous species *Taraxacum officinale* and *Rumex obtusifolius*, Derrick *et al*. (1993) reported comparable values for the *in vitro* and *in vivo* digestibility, without any information being given about the diet of the donor animals. This comparability was not general: with *Plantago lanceolata* the *in vivo* digestibility was lower than the *in vitro* digestibility. In grasslands used for intensive production dominated by *L. perenne*, there is more uniformity in plant characteristics and the relationships between *in vivo* and *in vitro* digestibility are therefore less ambiguous.

Species rich grasslands are mixtures in more than one way; they are mixtures of species, stages of maturity and plant tissues. When harvested on the same date species may vary in digestibility because of variation in stage of maturity (Wind & Elzebroek, 1989; CPRO-DLO, 1995), differences in proportions of tissues (Wilson, 1993) and chemical composition (Akin *et al*., 1990; Osbourn, 1980; Korevaar, 1986). Species at different stages of maturity vary in digestibility because of differences in proportions of tissues (Terry & Tilley, 1964; Wilson, 1994) and chemical composition (Korevaar, 1986; Wilson, 1994; Wilson & Hatfield, 1997). The tissues vary in chemical composition (Wilson, 1993) and three-dimensional structure (Wilson, 1993; Wilson & Mertens, 1995) and thus in the rumen in the intensity of surface contact between the microorganisms and the cell wall (Wilson & Mertens, 1995). In the next paragraphs these aspects of digestibility will be discussed in detail.

Plant characteristics and digestibility

In Western Europe, *L. perenne* is the forage species that is mostly used for experiments with ruminants (mainly sheep), which assess digestibility, intake or other aspects of quality. However, chemical composition, digestibility and intake vary with forage species (e.g. Frame, 1990; Mtengeti *et al*., 1996; Wilman *et al*., 1996). Table 1 shows the variation in OMD values (*in vitro*) within and between different grass and herb species observed by different research workers. The data in Table 1 should only be compared within an experiment and not between experiments, because between experiments other crucial factors affecting feeding value, such as weather, stage of maturity and season, may have varied. It is not intended that Table 1 gives a full overview. Other relevant references are Deinum & Dirven (1975), Åman & Lindgren (1983), Behaeghe & Carlier (1974), Frame *et al*. (1993) and Nielsen & Søegaard (2000).

In most studies mentioned in Table 1, *L. perenne* had the highest OMD, except in Experiment K2, where *Ranunculus repens* and *R. acetosa* showed higher OMD values. The forages in this experiment were harvested in August and therefore the high OMD values for the dicotyledonous species can be explained by the fact that they maintain their high digestibility longer than grasses (Peeters & Janssens, 1998). Derrick *et al*. (1993) also state that the potential digestibility of some dicotyledonous species is high. The grasses *Poa*

Table 1. The *in vitro* organic matter digestibility (OMD) of several grassland species.

	References									
	K1	K2	K3	K4	F1	F2	A1	A2	A3	B
Lolium perenne	0.80	0.75	0.82	0.71	0.80	0.73	0.81	0.68	0.61	
Poa pratensis	0.71	0.69			0.71	0.61				0.67
Poa trivialis	0.74		0.78	0.65						0.66
Agrostis stolonifera	0.70	0.67	0.77	0.69	0.71	0.63				0.71
Agrostis capillaris	0.70	0.67			0.70	0.58				
Elymus repens	0.74	0.69								0.73
Holcus lanatus	0.74	0.69	0.77	0.65	0.76	0.68				0.66
Festuca rubra					0.72	0.62				
Alopecurus geniculatus			0.77	0.69						0.76
Anthoxanthum odoratum					0.75	0.64				
Nardus stricta							0.63	0.53		
Molinea caerulea							0.62	0.47		
Trifolium repens							0.79	0.62		
Ranunculus repens	0.79	0.79								0.81
Rumex acetosa	0.63	0.78								0.55

K1: Korevaar (1986) Species harvested in May (first cut)
K2: Korevaar (1986) Species harvested in August (second cut)
K3: Korevaar & Van der Wel (1997) Species harvested in May
K4: Korevaar & Van der Wel (1997) Species harvested in June
F1: Frame (1991) Average of several cuts and fertilization levels of year 1 and 2
F2: Frame (1991) Average of several cuts and fertilization levels of year 4
A1: Armstrong *et al*. (1989) Species harvested in June, first growth; species dominant-grassland
A2: Armstrong *et al*. (1989) Species harvested in August, first growth; species dominant-grassland
A3: Armstrong *et al*. (1989) Species harvested in October, first growth; species dominant-grassland
B: Buske (personal communication), average of seasons in one year.

In the publication of Armstrong *et al*. samples of species-dominant grasslands are used. Samples are therefore not pure.

pratensis, *Poa trivialis*, *Elymus repens*, *Holcus lanatus* and the legume *Trifolium repens* had digestibility values that were sufficiently high to make them adequate forages for dairy cows (Table 1), which have the highest nutritional demands of farmed ruminants.

Attempts have been made to classify grasses into high-, moderate- and low-quality grasses, based on grassland productivity or nutritional value (e.g. Korevaar, 1986; Armstrong *et al.*, 1989). However, the separation into three categories of forage quality is arbitrary and depends mainly on the definition used in the references. Discrepancies between references can be explained by factors such as stage of maturity. *H. lanatus* and *E. repens* have a high digestibility in the vegetative stage, but digestibility decreases rapidly during maturation (Korevaar, 1986). Also *Phleum pratense* and *P. pratensis* seem to react differently on maturation. The stage of maturity has a more negative effect on the chemical composition of *P. pratense* than on the chemical composition of *P. pratensis* (Hole, 1985).

The digestibility of forages may also be affected by forage conservation. The reduction in digestibility during ensiling differs between species: for *P. pratense* this reduction is about 0.10 and for *L. perenne* or *Festuca arundinacea* about 0.05 (Demarquilly & Jarrige, 1971). Wilson & Collins (1980) concluded that *P. pratense* is not suitable to ensile, which could be due to a too slow release of water-soluble carbohydrates to achieve a quick fermentation. The sugar content of the fresh *P. pratense* is indeed low (Wilson & Collins, 1980). Furthermore, *P. pratense* develops a higher proportion of cell walls than *L. perenne* and *Festuca pratensis* at more mature growth stages (Osbourn, 1980). This high proportion of cell walls probably also affects the rate of release of water-soluble carbohydrates during ensiling. Also Hole (1985) observed a low feeding value of ensiled *P. pratense* which was even lower than that of *P. pratensis.* In fresh material Korevaar (1986) observed the opposite: a higher feeding value of *P. pratense* compared to *P. pratensis*.

The relationships between neutral-detergent fibre (NDF) concentration and digestibility or lignin concentration and digestibility vary between species, but especially between grasses and dicotyledonous species. With regard to the relationship between NDF concentration and digestibility, dicotyledonous species contain high amounts of pectins (Wilson, 1994) that are not determined in the NDF method. Pectins are almost completely digested in the rumen (Mertens, 1993; Tamminga, 1993), so digestibility will not be influenced negatively. Although the determined NDF concentration of some dicotyledonous species (e.g. *T. repens*) is markedly lower than for grasses (Wilman & Riley, 1993), no differences in digestibility are observed between such species and *L. perenne*. The NDF concentration in grass is more digestible than that in *T. repens*, which can be explained by a higher lignin concentration in leguminous species compared with that in grasses (Osbourn, 1980; Wilson, 1994). Another difference between grasses and dicotyledonous species is that the vascular bundles are arranged differently. Dicotyledonous species have a reticulate venation, whereas grasses have a parallell system of vascular bundles running to the full length of the leaf (Wilson, 1985). Those differences in venation may lead to less vascular tissue per unit volume and to shorter length of veins in dicotyledonous species (Wilson, 1985), resulting in an easier degradation of the dicotyledonous species. Forages from semi-natural grasslands, with a high proportion of dicotyledonous species, may therefore have a relatively high feeding value, especially if selection by animals is allowed.

In some cases a low digestibility of a forage is not only caused by the chemical composition of the cell wall, but also by anti-nutritional factors such as tannins or silica (Rezvani Moghaddam & Wilman, 1998). In many dicotyledonous species secondary metabolites are found which have an inhibitory effect on the digestibility (Scehovic, 1988; 1995; 1997). However, in small amounts (below 0.30 or 0.40), the occurrence of dicotyledonous species in the forage mixture can be beneficial to forage quality (Scehovic, 2000).

Digestibility and stage of maturity

One of the problems of using forage from botanically diverse grasslands, is that at a given harvesting date the stages of maturity vary amongst different grass species. Even within a grass plant, differences in digestibility occur due to variable age of leaves (Groot & Neuteboom, 1997) or differences in age of tillers (Deinum & Dirven, 1971; Van Loo, 1993). The timing of inflorescence emergence is also highly variable amongst different grass or legume species and between cultivars within a species. In the Netherlands, some grass species already head in May (e.g. *Lolium multiflorum*) or even in April (e.g. *A. pratensis*) whereas others do not head until July or August (e.g. *Molinea caerula*) (Wind & Elzebroek, 1989; Elzebroek *et al.*, 1991). In Table 2, the dates of stem elongation, which is the precursor to flowering, are given for several temperate grass species in the Netherlands.

With increasing stage of maturity the proportion of cell wall components of the grass (cellulose, hemicellulose and lignin) increases, while the proportion of cell contents decreases (Osbourn, 1980; Bosch *et al.*, 1992). The digestibility of the stem is already lower than the digestibility of the leaf before the plant reaches an advanced stage of maturity, but also declines faster over time, and with increasing maturity the ratio of stem to leaf increases (Terry & Tilley, 1964). Because of the changes in cell wall content and the stem:leaf ratio with increasing maturity, the digestibility of grasses is highest in the vegetative stage (Terry & Tilley, 1964; Groot, 1999). The rate of decline in digestibility with increasing maturity depends on temperature (Deinum *et al.*, 1981; Struik *et al.*, 1985; Wilson *et al.*, 1991) and species (Hole, 1985; Korevaar, 1986). As previously mentioned, *H. lanatus* and *E. repens* have a high digestibility in the vegetative stage, but their digestibility rapidly declines during maturation (Korevaar, 1986). A reason for this rapid decline in digestibility was not given, but it would probably have been caused by stem formation. The decline in digestibility is lower in other grass species, such as *P. pratensis* and *A. stolonifera* (Korevaar, 1986).

In Table 3, the change in proportions of leaf, leaf sheath and stem during spring, and the digestibility of these plant parts are shown for *Dactylis glomerata*, *L. perenne*, *P. pratensis* and *F. arundinacea*. Dead leaves and inflorescences are not included in this table. In consequence, the sum of the different plant parts is often considerably lower than 1.0. Table 3 clearly shows the changes in proportions of the different organs and the decrease in digestibility over time. Such changes will also apply to other grass species (Terry & Tilley,

Table 2. Date of beginning of stem elongation and start of flowering per species in the Netherlands (Wind & Elzebroek, 1989; CPRO-DLO, 1995).

Grass species	Stem elongation [1]	Start of flowering[1]
Agrostis capillaris	First half of June	June
Agrostis stolonifera	First half of June	June
Alopecurus geniculatus	-	May
Alopecurus pratensis	April	April
Anthoxanthum odoratum	-	May
Bromus hordeaceus	-	May
Dactylis glomerata	Second half of May	-
Elymus repens	-	June
Festuca arundinacea	Second half of May	June
Festuca pratensis	End of May	June
Festuca rubra	End of April/ Beginning of May	
Holcus lanatus	-	May
Lolium perenne	June	June
Lolium multiflorum	End of May	-
Phleum pratense	After the first week of June	End of June
Poa annua	-	All year
Poa pratensis	First half of May	-
Poa trivialis	Halfway May	-

[1] These are the dates of stem elongation or the start of flowering (anthesis).

For some cultivated species (e.g. *L. perenne*) more variation in date of stem elongation does occur than described here. Elongation dates in this table are therefore just global rules.

1964). The OMD values of temperate grasses can decrease from about 0.80 well before flowering to only 0.60 for mature grasses (Armstrong *et al*., 1986), and probably digestibility can also be considerably higher or lower than the figures mentioned in that study. For animal production purposes it is therefore preferable to harvest the forage on semi-natural grasslands before most grass species start their stem elongation. However, in some of the ecologically vulnerable grasslands it is not permitted to harvest the grass before mid-June, to enable the grasses and meadow birds to reproduce. Especially for lactating dairy cows it is then preferred to use the grasslands with forage species, which show the least effect of maturation on digestibility, and with some late-flowering species, such as *P. pratense* or *A. stolonifera* (Table 2). For all grasslands, irrespective of specific differences in maturation or flowering date, late cutting will result in reduced digestibility. Early flowering species may have formed new, vegetative tillers in the end of spring, but after a first cut in the beginning of June, some other, later-flowering species will probably start their stem elongation (e.g. *M. caerulea, P. pratense, E. repens*; Table 2). So throughout summer digestibility, of forages from species rich grasslands will remain low (below 0.65).

Table 3. Proportion of plant fractions and dry matter digestibility (DMD) of fractions of four grass species (Terry & Tilley, 1964).

	Date of first cutting	Leaf blade		Leaf sheath		Stem	
		proportion	DMD	proportion	DMD	proportion	DMD
Dactylis	23 April	0.67	0.79	0.21	0.81	0	- [a]
glomerata	29 May	0.27	0.70	0.19	0.65	0.29	0.65
	2 July	0.20	0.66	0.13	0.58	0.35	0.44
Lolium	27 April	0.70	0.83	0.25	0.87	0.05	- [b]
perenne	19 May	0.31	0.82	0.16	0.77	0.34	0.75
	11 June	0.11	0.79	0.09	0.68	0.42	0.64
Phleum	5 May	0.85	0.83	0.12	0.86	0	-
pratense	26 May	0.50	0.79	0.24	0.70	0.22	0.85
	20 June	0.20	0.77	0.22	0.59	0.38	0.65
Festuca	19 April	0.56	0.84	0.33	0.78	0.11	0.86
arundinacea	10 May	0.40	0.80	0.24	0.65	0.36	0.76
	1 June	0.20	0.76	0.18	0.63	0.63	0.64

[a] In the first period no stem was harvested. However, two weeks later the digestibility of the stem was 0.86.

[b] Digestibility was not measured. However, two weeks later the digestibility of the stem was 0.85.

Not mentioned in this table are dead plant parts and inflorescence. Together these parts can, especially later in the season, be as high as 0.40. In the calculations in Table 4, those parts were included.

In Table 4 three harvesting periods are compared: at the end of April, before most species start their stem elongation; at the end of May, when some of the grasses have started stem elongation, and at the end of June, when most grasses have started their stem elongation, and have already decreased in digestibility. The overall digestibility of the forage mixture may decrease by over 0.20 (absolute) in these two months, which is probably a combined effect of progress in time (higher temperature) and the lower digestibility of diverse species, as already discussed. This lower digestibility is also expressed by a difference in rate of decline of digestibility between grass species (Table 4). The fraction 0.20 decrease in digestibility is in accordance with Tallowin & Jefferson (1999), who also concluded that grass species in semi-natural grasslands in mid-summer can have an *in vitro* digestibility up to over 0.20 below the *in vitro* digestibility of first cut grass of intensively managed grasslands.

Table 4. Overall digestibility of a grassland with fraction 0.40 *Lolium perenne*, 0.30 *Dactylis glomerata*, 0.15 *Phleum pratense* and 0.15 *Trifolium repens*, with three cutting dates (all a first cut). Dry matter digestibility (DMD) based on Table 3 (grasses), Wilman & Rezvani Moghaddam (1998; *Trifolium repens*) and Hacker & Minson (1981; *Trifolium repens*).

	Lolium perenne	*Dactylis glomerata*	*Phleum pratense*	*Trifolium repens*	Overall DMD
End of April	0.84	0.78	0.82	0.80	0.81
End of May	0.78	0.67	0.78	0.78	0.75
End of June	0.68	0.54	0.67	0.75	0.65

Although the percentage of grasses over time would vary, in this table we assumed a constant species composition in the sward over time.

Digestibility and breakdown of forages in relation to voluntary intake

Besides digestibility and NE concentration of a feed, voluntary intake also determines the nutritive value. However, there are no clear relationships described to predict the voluntary intake of forages from botanically diverse grasslands. Voluntary intake is often related to dry-matter digestibility, structural carbohydrate content and breakdown capacity in the rumen (Thomson *et al*., 1985; Armstrong *et al*., 1986; Derrick *et al*., 1993). Armstrong *et al*. (1986) observed only small differences in the relationship between digestibility and voluntary intake in different types of grassland, although 80% of the variation in intake between grasses could be explained by a common regression on digestibility (Armstrong *et al*., 1986). Intake of forages from semi-natural grasslands is found to be lower than intake from ryegrass and clover swards, mainly attributed to differences in digestibility (Armstrong *et al*., 1986). In general, the intake of legumes is higher than that of grasses, which can be attributed to higher crude protein concentration, lower cell wall content, faster particle size reduction in the rumen and faster rate of organic matter removal from the rumen (Meijs, 1981; Ulyatt, 1981; Thomson *et al*., 1985; Wilman *et al*., 1997). Also with some dicotyledonous species (e.g. *Spergula arvensis*) high voluntary intake can be observed, despite a high NDF concentration (Wilman *et al*., 1997). This might be due to the fact that tissues of dicot species are easier to break down in the rumen than those of grasses (Wilson, 1994; Wilman *et al*., 1997; Thomson *et al*., 1985; Derrick *et al*., 1993).

Animal performance on botanically diverse forage

The possibilities for using forages from semi-natural grasslands for ruminants seem low, because of the low digestibility of these forages. Based on the OMD value of forage species in Table 1 and the Equations 1 or 2, the ME concentration was estimated, assuming a fixed ash concentration of 100 g kg^{-1} DM. In August, grasses of semi-natural grasslands had ME concentrations of between 6.4 (*M. caerulea*) and 9.4 (*H. lanatus*) MJ kg^{-1} DM. A decrease in the ME concentration of the consumed herbage will rapidly result in a decline in milk output or growth. Assuming a dairy cow of 600 kg with a daily requirement of 59 MJ ME for maintenance and 5.1 MJ ME kg^{-1} of milk (Van Es, 1978), a daily intake of 17 kg DM of either *L. perenne*, *H. lanatus* / *Agrostis* spp. or *Nardus stricta*, which have predicted ME concentrations of 10.5, 9.5 and 8.5 MJ kg^{-1} DM, respectively, enables a daily milk production of 23.5, 20.2 or 16.8 kg, respectively. In this prediction possible deficiencies in minerals or protein shortages are not taken into account. Thus, the output in terms of milk could be reduced by up to 6.7 kg, when low quality grasses are fed. This reduction may be higher if intake is also reduced, which will probably occur with low digestible forages such as *N. stricta*.

For beef cattle and sheep a similar decline in ME intake will occur. Beef cattle weighing 300 kg and consuming 6 kg DM of forage, may reach a growth rate of approximately 1050 g d^{-1} (on *L. perenne*), 850 g d^{-1} (on *H. lanatus* / *Agrostis* spp.) or 650 g d^{-1} (on *N. stricta*) (based on Van Es, 1978; CVB, 2000). Feeding forages from semi-natural grasslands will thus increase the period required to finish the animals. For sheep in late pregnancy, the consumption of *L. perenne* and *H. lanatus* / *Agrostis* spp. will be sufficient to meet the ME requirements, but in the beginning of the lactation period, requirements are difficult to meet with all three forages, especially for ewes with more than one lamb. For ewes with twin lambs, only in the third month of lactation will the ME requirements be met on the *N. stricta* forage. Therefore, it would be better to feed forage of low quality, such as *N. stricta*, only after the end of lactation. Low quality forage can also be fed to fattening lambs, although growth rate will be reduced. For a lamb of 20 kg with a *N. stricta* intake of 0.8 kg DM, maximal growth rate is approximately 160 g d^{-1}. With *L. perenne* and *H. lanatus* / *Agrostis* spp. growth rates of about 220 and 190 g d^{-1} can be reached (based on CVB, 2000).

Because of the reduced output, farmers will probably hesitate to use such forages from semi-natural grasslands for their animals. However, also in literature there seems to be potential for the use low quality forages, like using them in sheep production systems (Osoro *et al*., 2000) or to rear dairy heifers (Fisher & Roberts, 1993). Finally, even feeding the material to lactating dairy cows is possible. Korevaar & Van der Wel (1997) carried out an experiment with low-producing cows (< 15 kg milk), feeding silage from swards dominated by high or by moderate digestible grass species. Although DM intake for the silage of moderately digestible grass species was 1.5 kg DM lower and the estimated NE content of the moderately digestible grass species 0.75 MJ kg^{-1} DM lower, they observed no significant differences in milk production between the groups. This could be attributed to an underestimation of the *in vivo* digestibility, because of adaptation of the microbes in the rumen to the diet, as discussed earlier, or to benefits of nutrient supply by forages from semi-natural grasslands for rumen function, as suggested by Tallowin & Jefferson (1999). As a consequence, when feeding botanical diverse forages, milk production or growth rate might not fall as quickly as expected based on the predicted nutritive value. The effect of including those forages in the ration of a ruminant might therefore be smaller than expected from predicted nutritive values. This aspect of feeding forage from semi-natural grasslands requires further investigation.

Concluding remarks

Uncertainties about the nutritive values of forages from semi-natural grasslands are due to various causes. Methods of feed evaluation for forages are mainly based on *L. perenne*, and not on forages from botanically diverse grasslands. The digestibility and feeding value of those forages are often lower than the digestibility of *L. perenne*, even at the same stage of maturity or with the same chemical composition. Moreover, because time of harvesting is often later in the season to meet conservation objectives, forages are mostly in a more advanced stage of maturity, which will also have a negative effect on digestibility. However, the relationship between the *in vitro* and the *in vivo* digestibility is often different for forages from semi-natural grasslands than for *L. perenne*; in some cases the *in vivo* digestibility is higher than the *in vitro* digestibility for forages from semi-natural grasslands. Also the occurrence of dicotyledonous species in semi-natural grasslands may have a positive influence on digestibility. If the actual digestibility and feed intake of forages from semi-natural grasslands are higher than presently predicted, farmers could be less reluctant to use such forages in the ration of their animals. Feeding trials are needed to quantify the possibilities.

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Chapter 5

Estimation of rumen degradability of forages from semi-natural grasslands, using nylon bag and gas production techniques

Bruinenberg, M.H., Van Gelder, A.H., González Pérez, P., Hindle, V.A. & Cone, J.W. (2003) Estimation of rumen degradability of forages from semi-natural grasslands, using nylon bag and gas production techniques. Submitted to Animal Science.

Estimation of rumen degradability of forages from semi-natural grasslands, using nylon bag and gas production techniques

Abstract

To obtain insight into ruminal digestion of forages from semi-natural grasslands, degradation characteristics and kinetics of three different forage silages in the rumen of lactating dairy cows were estimated *in vitro*, using the gas production technique (GPT), and *in situ*, using the nylon bag technique. Silages originated from an intensively managed grassland (IM), species poor grassland (SPP) and a species rich grassland (SPR). Some individual species, originating from SPP and SPR, were used to estimate their degradability with GPT, in order to obtain insight into the differences between the main species occurring on SPP and SPR. All used samples were also analysed for the *in vitro* organic matter digestibility (method of Tilley and Terry). Estimation of the *in situ* degradability was achieved by nylon bag incubation in the rumen of three dairy cows in two different periods. Rate of organic matter degradation was highest in IM (4.93 and 4.54% h^{-1}), intermediate in SPR (3.50 and 4.11% h^{-1}), and lowest in the SPP (2.62 and 2.72% h^{-1}). Also the rates of degradation for protein and neutral detergent fibre were higher in IM. The undegradable fraction was the same for SPP and SPR. For the silages, highest cell wall fermentation was observed in IM and lowest in SPP, although statistically SPP and SPR did not differ significantly. Of the individual species from the species poor and species rich grasslands *Lolium perenne* and *Dactylis glomerata* showed the highest cell wall degradability, whereas *Lathyrus pratensis* and *Anthriscus sylvestris* both had a low cell wall degradability. In conclusion, based on degradation characteristics *in situ* as well as *in vitro*, SPR seems to have more potential for inclusion in the ration of dairy cows than SPP.

Keywords: *in situ* degradation, gas production technique, forages, grass, silages

Introduction

Forage from semi-natural grasslands differs from forage from intensively managed grasslands: the former is often harvested later and may include many different forage species, including dicotyledonous species and a diversity of grass species. This makes it difficult to estimate the nutritional value of the forage from the semi-natural grasslands, and reduces the likelihood of integrating it into the ration of dairy cows (Bruinenberg *et al*., 2002).

Dicotyledonous species and the diversity of grass species in an advanced stage of reproductive development, occurring on the semi-natural grasslands are less readily digested than young, intensively managed grass (Bruinenberg *et al*., 2002), and dicotyledonous species may be better digestible than grass species mown on the same date (Duru, 1997). Dicotyledonous species have also been shown to have a faster *in situ* degradation rate in sheep than grasses of the same age (Lopez *et al*., 1991). For an efficient integration of forages from semi-natural grasslands in the ration of dairy cows, further information on rumen degradation characteristics of less common forage species is needed.

This chapter describes the rumen degradation characteristics of forages from lowland semi-natural grasslands, using the *in vitro* gas production technique and the *in situ* nylon bag technique. To investigate the differences among plant species, three different types of forage silages and some fresh individual species were tested. The silages originated from an intensively managed grassland (IM), a species rich grassland containing grasses and dicotyledonous species (SPR) and a species poor grassland containing mainly grasses (SPP) and the individual species originated from SPR and SPP. As the degradation rate has been shown to be higher in dicotyledonous species than in grasses of the same age (Lopez *et al*., 1991), degradation rates were expected to be higher for dicotyledonous species and for the mixture containing dicotyledonous species than for grass harvested at approximately the same date. Furthermore, because it was expected that the rumen microbial population would adapt to the diet (Grubb & Dehority, 1975), influence of the diet on degradation characteristics were expected, and therefore degradation was measured with (donor) dairy cows on different diets. Degradation characteristics of the silages were analysed both *in vitro* with the gas production technique (GPT) and *in situ* using the nylon bag technique. Degradation characteristics of the individual species were only performed *in vitro*.

Material and methods

Forage samples

Forage silages originated from three different grasslands. The first, IM, was a sward used for intensive production, consisting mainly of *Lolium perenne*, harvested in May 2000, the second, SPP, a semi-natural grassland, managed to stimulate nesting of birds (96% grasses; 4% herbs), harvested in June 2000 and the third, SPR, a semi-natural grassland with a high proportion of herbs managed by a non-governmental organization for nature conservation (53% grasses; 11% legumes; 36% herbs), harvested in June 2000. Further details concerning the botanical composition and management of the grasslands have been described by Bruinenberg *et al*. (2003).

Each silage was ensiled in individual bales of 400-500 kg prewilted material. Samples were taken from several bales for use in the *in situ* technique, which was carried out in two different periods, each period with samples from different bales. The samples were cut manually with a paper guillotine to a length of about 1 cm and an equivalent of approximately 5 g dry matter (DM) was weighed in polyamide bags (19x10 cm, pore size 41 μm, porosity 30%, Nybolt, Switzerland). Filled bags were stored at –20 °C until required for incubation or analysis. During weighing, subsamples were taken, dried at 70 °C and ground over a 1-mm screen for chemical analysis.

Samples analysed for GPT were taken from the same silage samples as those incubated in the rumen in the first period of the *in situ* technique. Furthermore, 15 dried samples of individual species and dried samples from the fresh (not ensiled) forages from SPP and SPR were incubated in GPT. The individual species analysed were the main species found on SPP and SPR (Bruinenberg *et al*., 2003). Five grass species from SPP were analysed (*Lolium perenne*, *Alopecurus geniculatus*, *Poa trivialis*, *Agrostis stolonifera* and *Holcus lanatus*).

From SPR four grass species (*Lolium perenne*, *Dactylis glomerata*, *Arrhenaterum eliatus* and *Alopecurus pratensis*), five dicotyledonous species (*Cirsium arvense*, *Galium mollugo*, *Ranunculus acris*, *Crepis biennis* and *Anthriscus sylvestris*) and one leguminous species (*Lathyrus pratensis*) were analysed. The samples of the species used to measure gas production characteristics were analysed for DM, ash and *in vitro* digestibility of organic matter (OMD).

Nylon bag incubation

In situ incubations were carried out in the rumen of three lactating dairy cows fitted with a large rumen cannula (ID 100 mm, Bar Diamond Inc., Parma, Idaho, USA). The incubations were performed twice, in two different periods, with each cow offered one of the three rations: Ration 100IM: 4.5 kg DM concentrates and 15 kg DM intensively managed grass silage per day; Ration 60SPP: 4.5 kg DM concentrates, 6 kg DM intensively managed grass silage and 9 kg DM species poor grass silage per day; Ration 60SPR: 4.5 kg DM concentrates, 6 kg DM intensively managed grass silage and 9 kg DM species rich forage silage per day. The diet per cow changed between the two periods, to prevent cow effects to be attributed to the diets.

One hour before incubation, nylon bags were taken from the freezer and each bag was attached to a polypropylene block weighing approximately 750-800 g, which was attached to the cannula by a 75 cm nylon cord. Incubations were performed with 24 bags per feed in each cow. The bags were incubated for 3 (3 bags), 6 (3 bags), 12 (6 bags), 24 (3 bags), 48 (4 bags) or 264 (5 bags) hours. Furthermore three bags per forage were washed without incubation to measure the fraction that disappears during washing in a washing machine (W). The residue after 264-hour incubation was regarded as the undegradable fraction (U).

After removal from the rumen, the nylon bags with incubation residues were rinsed with tap water to remove excess material on the outer surface and then stored in ice water to stop microbial activity. Subsequently, the bags were washed in a washing machine with cold tap water for 30 minutes, oven dried at 70 °C for at least 24 hours and weighed. The residues were pooled per feed, animal and incubation time, ground over a 1-mm screen and analysed for DM, ash, N and neutral detergent fibre (NDF).

Gas production technique

Gas production analysis, with the dried samples of fresh and ensiled forage and the individual species samples, was performed in two periods as described by Cone *et al*. (1996). During each period incubations were performed for 72 hours in two series, on different days, and each sample was incubated in duplicate. Rumen fluid required for the incubations was obtained from a lactating dairy cow fitted with a rumen cannula. The ration of the cow changed between the periods, but the rations were similar as two of the rations offered to the cows with the *in situ* experiment: the cow was offered 100IM in the first and 60SPP in the second period.

Gas production curves were fitted with a three-phasic-model, as described by Cone *et al.* (1996) and Groot *et al*. (1996). The cumulative gas production profiles were mathematically divided into three subcurves as described by Cone *et al*. (1997); 1: fermentation of soluble, readily degradable components; 2: fermentation of non-soluble components of the sample, in grass mainly the cell wall fraction; 3: gas production due to microbial turnover. The model is described by:

$$\text{ml gas} = a_1/(1+(b_1/t)^{c1}) + a_2/(1+(b_2/t)^{c2}) + a_3/(1+(b_3/t)^{c3})$$

where a_n is the maximum gas production in ml per g organic matter (OM), b_n is the time (h) needed to reach 50% of the maximum gas production; c_n is a constant determining the shape of the curve; and t is incubation time in hours. From the parameters b_n and c_n the fractional rates of digestion (R) can be calculated (Groot *et al*., 1996), and the maximum R (R_M) is reached when the size of the microbial population no longer limits fermentation of the forage. The time after the start of the incubation at which R_M occurs (t_{RM}) characterizes the growth of the microorganisms and colonization of the feed component (Groot *et al*., 1996).

In a second experiment with the GPT the three silages were incubated in rumen fluid obtained from three different cows on each of the three described rations (100IM, 60SPP and 60SPR) to determine the influence of the ration on rumen microbial activity. The donor cows were the cows used for the *in situ* technique in the second period. Interaction was calculated with the *F*-test.

Chemical analysis and in vitro digestibility

The DM concentration of the oven-dried samples was determined after 4 h at 103 °C and ash was determined after 3 h at 550 °C. The N concentration was determined using the Dumas method (Merz, 1979) and crude protein (CP) was calculated as 6.25 * N-Dumas. The NDF and lignin concentrations were determined according to Robertson & Van Soest (1981) and sugar was measured as described by Van Vuuren *et al*. (1993). The OMD was determined as described by Tilley & Terry (1963) and energy contents were calculated according to CVB (2001).

Calculation methods in situ

The degradation rate (k_d, % h^{-1}) of the insoluble but potentially degradable fraction (D, calculated as 100−W−U) was calculated according to the first order model of Robinson *et al*. (1986) including U, D and k_d, e.g. residue at time t = $D * e^{(-kd)\,(t)} + U$. Data were calculated for OM, CP and NDF.

Statistical analysis

Results of the different estimates of the three forages were statistically analysed with ANOVA (Genstat 5 Committee, 1993), using the model: $Y_{ij} = u + C_i + F_j + E_{ij}$ (C= cow, F = forage, E = standard error). Also the occurrence of interaction between samples and ration was tested with ANOVA (Genstat 5 Committee, 1993). Treatment means were compared with a Student's *t*-test.

Results

Degradation characteristics of the three types of forage silage

In vitro OM digestibility was lower for SPP and SPR than for IM (Table 1). The low OMD of SPP coincided with a high NDF and lignin concentration compared to IM. Due to the later stage of harvesting, the CP concentration was lower in SPP and SPR than in IM. The IM silage displayed a higher D_{OM}, D_{CP} and degradable NDF fraction (D_{NDF}) and those fractions also degraded faster than in SPP and SPR, although differences in degradability between IM and SPR in the second period were not significant (Table 2; $P > 0.05$). The U_{OM}, U_{CP} and U_{NDF} were significantly lower in IM than in the other silages.

The silages of the two semi-natural grasslands also differed from each other; k_{dOM} was higher in SPR ($P < 0.05$), although not significantly in the first period, but D_{OM} did not differ between SPP and SPR ($P > 0.05$). In SPR in the second period the W_{OM} seemed to be higher than in SPP. However, the W fractions could not be analysed for significance. Furthermore, k_{dCP} was also higher in SPR ($P < 0.05$) and the D_{CP} did not differ significantly between SPP and SPR ($P > 0.05$), except that D_{CP} in the second period of SPR was significantly higher compared to SPP and SPR in the first period ($P < 0.05$). SPP had a significantly higher D_{NDF} than SPR, but k_{dNDF} did not differ significantly between SPP and SPR ($P > 0.05$). The U_{NDF} was significantly lower in SPP than in SPR ($P < 0.05$).

Gas production and digestibility of the silages

Figures 1 and 2 display clearly the difference among the three silages in cumulative gas production and in gas production rate during the first 40 hours after the start of the incubation. In IM the OMD, gas production of the gradually degradable phase (A2), the gas production after 72 hours (GP72), and the maximal rate of degradation (R_M) were higher than in the two semi-natural silages ($P < 0.05$; Table 3). However, the amount of gas produced in the first (soluble) phase (A1) was similar for IM and SPR and the time taken to reach R_M (t_{RM}), was shorter for SPR than for IM.

Table 1. *In vitro* organic matter digestibility (OMD, %), energy content (MJ kg^{-1}), dry matter content (g kg^{-1} silage) and chemical composition (g kg^{-1} DM) of intensively managed grass (IM), species poor grass (SPP) and species rich grass (SPR) in the first (1) and second (2) period.

	IM		SPP		SPR	
	period 1	period 2	period 1	period 2	period 1	period 2
OMD	74.6	72.6	53.9	57.1	54.1	52.0
NE (MJ kg^{-1})	6.0	5.8	4.3	4.6	4.4	4.3
DM	571	610	716	751	589	548
Ash	116	120	117	101	101	92
OM	884	880	883	899	899	908
CP	180	177	125	131	99	99
NDF	470	463	553	547	489	475
lignin	14.6	13.0	40.8	35.7	57.0	54.3
sugar	31.1	40.0	44.5	59.5	42.4	24.3

Table 2. *In situ* degradation characteristics of intensively managed grass (IM), species poor grass (SPP) and species rich grass (SPR) in the first (1) and second (2) period. s.e.d = standard error of difference.

	IM		SPP		SPR		s.e.d.
	period 1	period 2	period 1	period 2	period 1	period 2	
Organic matter							
W	21.1	21.1	15.4	15.9	20.3	17.9	*
U	9.7[b]	10.5[b]	30.4[a]	28.0[a]	28.6[a]	29.8[a]	1.44
D[1]	69.9[a]	66.9[a]	52.9[bc]	55.2[b]	49.9[c]	52.7[bc]	1.44
k_d	4.9[a]	4.5[a]	2.6[c]	2.7[c]	3.5[bc]	4.1[ab]	0.42
Crude protein							
W	41.5	41.1	32.4	32.7	35.7	27.5	*
U	9.6[b]	11.6[b]	37.9[a]	32.8[a]	35.4[a]	32.6[a]	3.31
D[1]	46.8[a]	44.6[a]	26.4[b]	32.6[b]	30.6[b]	42.3[a]	2.94
k_d	6.4[a]	5.2[ab]	1.5[c]	1.3[c]	3.7[b]	4.2[b]	0.80
NDF							
U	8.8[e]	9.4[e]	28.9[c]	26.9[d]	35.5[b]	38.3[a]	0.82
D[1]	91.9[a]	88.5[b]	66.6[c]	66.7[c]	57.5[d]	57.4[d]	1.22
k_d	4.6[a]	4.1[a]	2.7[b]	2.9[b]	2.7[b]	3.1[ab]	0.46

W = soluble fraction (%), U = undegradable fraction (%), D = degradable fraction (%), calculated as 100 – U (– W), k_d = rate of degradation (% h^{-1}).

[1] The sum of W, U and D do not add up to 100, because of statistical calculations.

[a,b,c,d,e] Different superscripts in row depicts significant differences ($P < 0.05$).

* The s.e.d. could not be calculated.

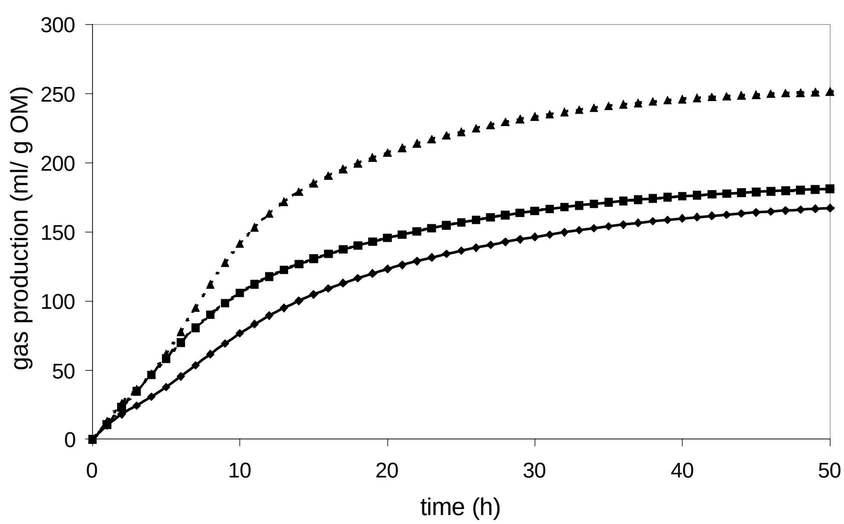

Figure 1. Cumulative gas production of the three silages (♦ = species poor forage silage, ■ = species rich forage silage, ▲= intensively managed grass silage).

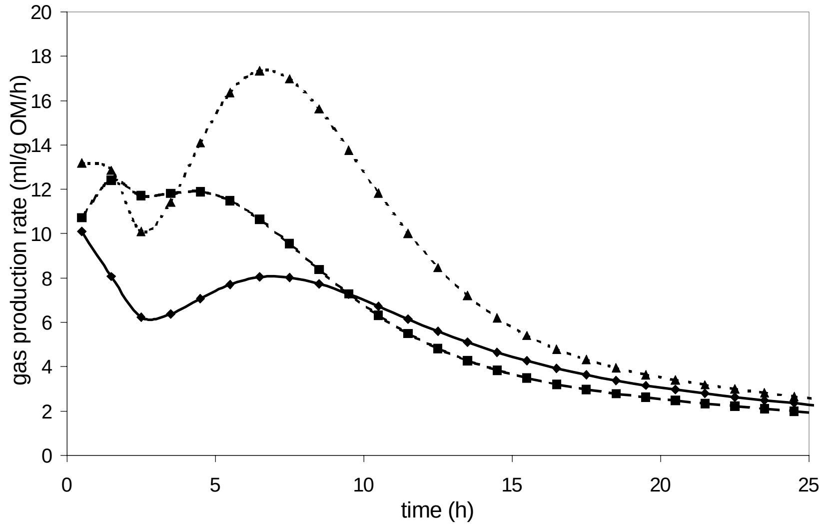

Figure 2. Gas production rate of the three silages (♦ = species poor forage silage, ■ = species rich forage silage, ▲= intensively managed grass silage).

In SPR, A1 and R_M were significantly higher than in SPP, and B2 and tR were significantly lower (P < 0.05). However, OMD, A2 and GP72 did not differ significantly between the two semi-natural silages.

Gas production of the individual species

In Figures 3, 4 and 5, the cumulative gas production, the cumulative gas production rate and the gas production rate of the second phase are shown for six different species, including three grasses, two dicots and the legume. Of the individual grass species, *Lolium perenne* had the highest and *Holcus lanatus* and *Alopecurus pratensis* had the lowest OMD, A2 and GP72. Of the dicot species, *Cirsium arvense* had the highest OMD but A2 and GP72 were equal for *Cirsium arvense* and *Ranunuclus acris* (Table 3). *Anthriscus sylvestris* and *Lathyrus pratensis* had the lowest OMD and GP72, but *Anthriscus sylvestris* and *Ranunculus acris* had the highest R_M. The herbs seemed to break down more rapidly than the grasses. They had a lower B2 and t_{RM} than the grasses and some of the dicots also had a high R_M (e.g. *Ranunculus acris, Crepis biennis* and *Anthriscus sylvestris*). The only legume analysed, *Lathyrus pratensis*, had the lowest OMD, and of the other, non-leguminous, dicots, the highest B2 and the lowest R_M.

Influence of ration

Significant interactions between ration and sample were observed for A2, B2 and R_M (Table 4), and a trend for interaction was observed for GP72. No significant interactions were observed for A1 and t_{RM}. Gas production on 100IM was initially rapid, whereas SPP started slowly. Maximum rate of degradation was higher with 100IM for the IM samples and with 60SPR for the SPP and SPR samples.

Table 3. *In vitro* organic matter digestibility and gas production characteristics of 15 forage species and some mixed samples (SPP = species poor grassland, SPR = species rich grassland, IM = intensively managed grassland). s.e.d. = standard error of difference.

		OMD	A1	A2	B2	GP72	R_M	t_{RM}
SPP fresh		62.6	42	109	9.4	195	13.7	10.9
SPP ensiled		53.9	25	99	9.9	173	12.7	11.5
SPR fresh		60.9	51	107	7.9	196	14.6	8.7
SPR ensiled		54.1	36	110	7.7	183	15.3	8.6
IM ensiled		74.6	36	171	8.6	253	18.4	10.7
species poor grassland								
Lolium perenne	G	74.0	59	153	8.9	257	15.8	10.8
Alopecurus geniculatus	G	67.0	66	116	8.9	243	12.8	9.9
Poa trivialis	G	64.9	60	121	9.8	245	11.6	10.7
Agrostis stolonifera	G	64.4	43	102	10.0	191	12.0	11.3
Holcus lanatus	G	61.7	45	109	10.1	205	11.6	11.3
species rich grassland								
Lolium perenne	G	66.4	66	124	8.8	233	13.9	10.1
Dactylis glomerata	G	65.1	55	150	7.7	254	19.4	9.6
Arrhenatherum eliatus	G	64.5	65	114	9.1	230	11.7	9.4
Alopecurus pratensis	G	59.6	43	108	10.5	213	11.0	11.7
Cirsium arvense	D	70.5	63	138	6.8	224	17.1	7.7
Galium mollugo	D	68.5	61	135	7.5	218	15.7	8.5
Ranunculus acris	D	61.4	55	142	6.8	229	20.1	8.1
Crepis biennis	D	60.9	48	126	6.9	200	19.8	8.3
Anthriscus sylvestris	D	55.6	54	95	6.3	169	20.1	7.3
Lathyrus pratensis	L	54.9	43	104	8.3	176	12.7	8.6
s.e.d.		*	5.1	9.3	0.21	16.0	0.63	0.24

OMD = Digestibility of organic matter *in vitro* (%), A1= gas production of the soluble phase (ml g^{-1} OM, A2= gas production of the non-soluble phase (ml g^{-1} OM), B2= time taken to reach half of the gas production of A2 (h), GP72 = gas production after 72 hours (ml g^{-1} OM), R_M = maximal relative rate of degradation of the non-soluble phase (ml g^{-1} OM h^{-1}), t_{RM} is time at which maximal rate of degradation is achieved (h).

G = grass species, D = dicotyledonous species, L = leguminous species

* means that the s.e.d. could not be calculated.

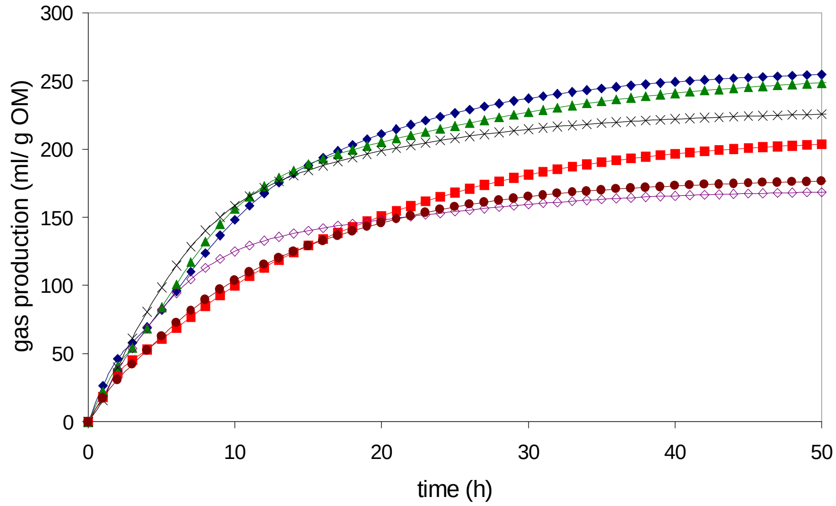

Figure 3. Cumulative gas production rate of six forage species (♦ = *L. perenne*, ■ = *H. lanatus*, ▲ = *D. glomerata*, x = *C. arvense*, ◊ = *A. sylvestris*, ● = *L. pratensis*).

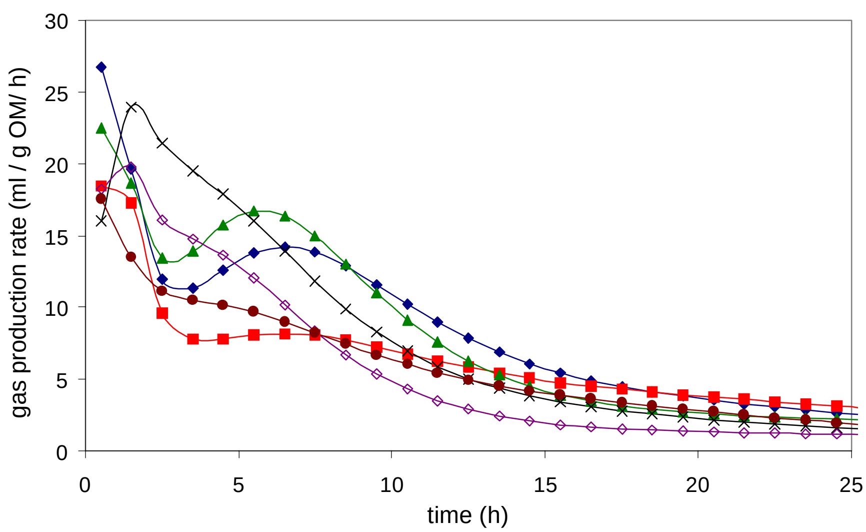

Figure 4. Gas production rate of six forage species (♦ = *L. perenne*, ■ = *H. lanatus*, ▲ = *D. glomerata*, x = *C. arvense*, ◊ = *A. sylvestris*, ● = *L. pratensis*).

Discussion

To characterize the three forages clearly, each forage is discussed in a separate paragraph. Firstly, the rumen degradation characteristics of IM will be discussed, subsequently the characteristics of SPP will be discussed, in comparison with IM, and after that the degradation characteristics of SPR will be discussed, in comparison with IM and SPP. Finally some attention will be given to the feed evaluation characteristics and the influence of the ration on degradation of the forages.

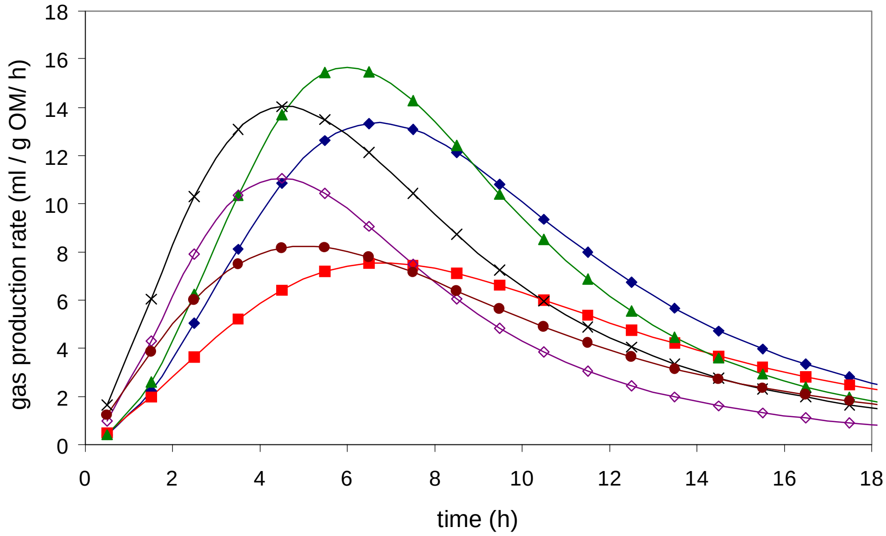

Figure 5. Gas production rate of the insoluble phase of six forage species (♦ = *L. perenne*, ■ = *H. lanatus*, ▲ = *D. glomerata*, x = *C. arvense*, ◊ = *A. sylvestris*, ● = *L. pratensis*).

Table 4. Gas production characteristics of species poor grass silage (SPP), species rich grass silage (SPR), intensively managed grass silage (IM) on three different rations, based on (1) IM and concentrates (100IM); (2) IM, SPP and concentrates (60SPP); and (3) IM, SPR and concentrates (60SPR).

	Ration	A1	A2	B2	GP72	R_M	t_{RM}
IM	100IM	39	152	7.8	254	18.9	9.6
IM	60SPP	33	185	8.9	256	17.7	11.3
IM	60SPR	25	182	9.2	246	16.1	11.4
SPP	100IM	42	102	8.7	192	12.9	9.5
SPP	60SPP	30	115	10.3	211	11.9	11.8
SPP	60SPR	20	86	9.8	167	13.6	11.7
SPR	100IM	43	89	6.6	166	15.3	6.7
SPR	60SPP	35	118	7.9	188	15.9	9.2
SPR	60SPR	31	113	7.3	188	16.2	8.3
s.e.d.		3.9	9.8	0.2	12.2	0.9	0.3
P value[1]		0.38	0.04	0.01	0.09	0.03	0.13

A1= gas production of the soluble phase (ml g^{-1} OM), A2= gas production of the non-soluble phase (ml g^{-1} OM), B2= time taken to reach half of the gas production of A2 (h), GP72 = gas production after 72 hours (ml g^{-1} OM), R_M = maximum relative rate of degradation of the non-soluble phase (ml g^{-1} OM h^{-1}), t_{RM} = time at which maximal rate of degradation is achieved (h).

[1] *P* is calculated with the *F*-test, $P < 0.05$ indicates interaction between sample and ration.

Intensively managed grass

The high D and k_d found for IM *in situ* were caused by the fact that the grass was harvested in an immature stage. The low concentration of undegradable fibre in immature grass resulted in the low U fractions of OM, CP and NDF and the high D fractions. The characteristics of IM are also more or less similar to other grass silage data from literature (e.g. Bosch *et al*., 1992, Van Vuuren *et al*., 1989; Valk *et al*., 1996). The IM silage is therefore thought to provide a good reference value for comparison with the forage silages from the semi-natural grasslands.

Species poor grass

As expected, *in situ* degradation characteristics of SPP were inferior to IM, because the forage was harvested later than IM (Bruinenberg *et al*., 2003) and most of the grasses were already in a mature stage, had already elongated their stems and produced inflorescences. This resulted in a high concentration of cell wall material, as shown by the high NDF content in comparison to IM (Table 1). Considering the advanced stage of maturity, the lignin content of SPP was also expected to be high compared to IM. This was confirmed by chemical analysis (Table 1). Furthermore, SPP consisted mainly of grass species that are assumed to be inferior to *Lolium perenne*, such as *Holcus lanatus* and *Agrostis stolonifera* (Bruinenberg *et al*., 2001; 2002; 2003). This was also confirmed *in vitro*, with the GPT, as relatively low degradation rates of OM were found for SPP silage. This is shown by relatively low values for R_M and B2 (Table 3). As expected, the low degradation rate of SPP silage was also found in relatively low degradation rates of the individual species obtained from SPP. Based on the OMD and the gas production characteristics, *Lolium perenne* was the best species, as expected, as it is known to be a highly digestible species (Bruinenberg *et al*., 2002). However, *Lolium perenne* only comprised 6% of the total botanical composition of SPP (Bruinenberg *et al*., 2003). The other grass species in SPP displayed low gas production rates, and low total gas productions, especially *Holcus lanatus*, which was the most frequently found species in SPP (35% of the botanical composition; Bruinenberg *et al*., 2003). *Holcus lanatus* is highly digestible in the vegetative stage, but digestibility declines rapidly during maturation (Korevaar, 1986). As in SPP *Holcus lanatus* already had produced inflorescences at the time of the harvest, the stage of maturity could be limiting for degradability. The digestibility of other grass species in SPP was consistent with observations of Korevaar & Van der Wel (1997). The slow fermentation could be caused by the structure of the grasses: because of their parallel venation (Wilson, 1985) and high NDF concentration (Table 1) microbes will probably have difficulties to penetrate grass tissues.

The results of the *in situ* degradability as well as of the *in vitro* gas production test indicate that the SPP forage is difficult to degrade in the rumen of dairy cows, which is probably due to the high cell wall content. High NDF, combined with a low degradation rate of NDF, is probably responsible for a reduction in feed intake *in vivo* (Forbes, 1995; Bruinenberg *et al*., 2003), because cows have a limited rumen content (De Visser *et al*., 1998). As the energy value of SPP was also low (Table 1), total energy intake would be limited, affecting milk production. Therefore, this type of forage should not be included in the ration of lactating dairy cows at too high proportions.

Species rich grass

The higher k_{dOM} and k_{dCP} observed in SPR compared to SPP was in agreement with Lopez *et al*. (1991), who found a higher rumen degradation rate of weeds compared to grasses of the same age. The higher k_{dOM} can be explained by the higher ratio between cell contents and cell walls in the SPR forage. However, k_{dNDF} was similar for SPP and SPR, and D_{NDF} of SPR lower than that of SPP. As SPR consisted for 45% of legumes and other dicotyledonous species (dicots), differences in degradation characteristics of the two forages could be explained by differences in degradability between grasses and dicots. Species rich forage silage contained more lignin than SPP, decreasing degradability. A higher lignin content in weeds combined with a lower cell wall content was also observed by Lopez *et al*. (1991). The rate of gas production observed *in vitro* also showed a rapid breakdown of OM in SPR, compared to SPP. The R_M of SPR was higher than that of SPP, but somewhat lower than that of IM, probably because IM only contained highly degradable grasses, whereas the grasses in SPR were mostly in an advanced stage of maturity, and therefore less readily degradable. The GP72 and A2 of SPR were low and comparable to that of SPP. This was expected as the D_{OM} and OMD were also comparable between SPR and SPP, even though D_{NDF} was lower in SPR. The GPT data of the individual species showed clearly that the high R_M of SPR could indeed be attributed to the presence of dicots in SPR, because the highest R_M was reached with the dicots *Anthriscus sylvestris* and *Ranunculus acris*. Although the R_M and the k_{dOM} were higher in SPR than in SPP, OMD was similar or even lower in SPR. The low OMD in SPR is probably due to the high lignin content (Table 1), whereas the difference in degradation rate between SPP and SPR indicates differences in accessibility of the cell contents, or differences in rate of degradation of cell wall material. The rapid breakdown of dicots was probably caused by several factors. The first factor could be that dicots have a reticulate venation, which might lead to less vascular tissue per unit volume. Furthermore, because of the junctions between cells, the fibre is more easily degraded into small particles than the veins in leaves of grasses, which have a parallel girder system of vascular bundles running through the full length of the leaves (Wilson, 1985). A second reason for the rapid breakdown of dicots might be related to the fact that the distribution of lignin in the stem from legumes is different from that in grasses. The lignin in the mature stem is mainly concentrated in xylem (Wilson & Hatfield, 1997), which is therefore highly indigestible. Pith and cortex are unlignified and therefore rapidly digestible explaining the high rate of stem digestion in legumes. In grasses lignin is distributed in most cell types (Wilson, 1993) and thus rate of digestion is affected in all cell types. No literature was found about distribution of lignin in other dicots, but it is assumed that these are probably more comparable with legumes than with grasses. A third reason for the higher k_{dOM} of SPR compared to SPP could be the higher amount of pectins in legume dicots than in grasses (Wilson, 1994). Pectins are (almost) completely degradable in the rumen (Tamminga, 1993). A high amount of pectins in SPR would be in agreement with the high content of OM components other than NDF and CP (rest components = OM − NDF − CP), which were 311 (SPR1) to 334 g (SPR2) in SPR. In IM and SPP rest components, including crude fat, soluble carbohydrates and organic acids, were between 205 and 240 g. The high A1 of SPR confirms this hypothesis.

The different results of degradation rate and digestibility indicate that an analysis of OMD alone is not sufficient to characterize forages from semi-natural grasslands, and that such an analysis should be complemented with the *in situ* technique or GPT.

The relatively high rate of degradation, *in vitro* (OM) as well as *in situ* (OM, CP), of SPR suggests that the intake of SPR could be higher than that of SPP. The similar k_{dNDF} of SPR and SPP might suggest the opposite, but the proportion of NDF was lower in SPR than in SPP (Table 1). A disadvantage of SPR *in vivo* could be that dicots are known to contain certain anti-nutritional factors, such as the presence of thorns (*Cirsium arvense*), a low palatability (fresh *Rumex obtusifolius*; Derrick *et al*., 1993), or the presence of secondary metabolites, which might inhibit enzymatic or microbial activity in the rumen (Scehovic, 1995). However, in earlier research, intake of a mixed ration consisting of SPR, IM, maize silage and concentrates (ratio SPR:IM = 6:4) was observed to be equal to intake of a mixed ration consisting of IM, maize silage and concentrates (Bruinenberg *et al*., 2003). Thus in that experiment, anti-nutritional factors did not cause a reduction in intake.

Influence of the ration of donor animals

Effects of the ration of the donor animals were expected, because the microbial population in the rumen will adapt to available nutrients (Grubb & Dehority, 1975). Indeed, interactions between ration of the donor animal and samples were observed, while testing the individual grass species as well as with the second test with the three different cows on different rations. In the second test, only one animal per ration was used, and therefore it remained uncertain if the observed effect was a result of ration or animal. No interactions were observed for GP72 and A2, but the B2 was higher on 60SPP. This was not expected, because microbial population on that ration should be adapted to it, and therefore able to ‘attack’ earlier. However, because the energy content of this ration was lower, the number of microbes per ml rumen fluid was probably lower than in IM, which could reduce the rate of fermentation. Total gas production was in SPP when the donor animal consumed 60SPP.

Conclusions

Considering the degradation characteristics *in situ* as well as *in vitro*, species rich silage seems to have more potential for use on dairy farms than species poor silage. It became clear that OMD alone is insufficient for estimation of the nutritional value for such kind of forages because of differences in rate of degradability.

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Chapter 6

Effects on dairy cow performance of offering silages produced on semi-natural grasslands

Bruinenberg, M.H., Geerts, R.H.E.M., Struik, P.C. & Valk, H. (2003) Effects on dairy cow performance of offering silages produced on semi-natural grasslands. Submitted to Grass and Forage Science.

Effects on dairy cow performance of offering silages produced on semi-natural grasslands

Abstract

To study the effects of including forages from semi-natural grasslands in the diet of dairy cows, from 31 July to 8 October 2000 a feeding trial was performed with dairy cows in mid-lactation. Intensively managed grass silage in the ration was replaced by 0 (100IM), 20 (20SPP), 40 (40SPP) or 60% (60SPP) silage from species poor grassland or by 60% silage from species rich grassland (60SPR). On a dry matter basis, the total mixed ration contained 63% grass silage, 18% maize silage and 19% concentrates. Concentrates were either low or high in protein to prevent protein shortages. Cows received extra concentrates, added to the concentrates included in the mixed ration. Treatment 60SPP decreased voluntary intake by 1.4 kg DM. Uncorrected milk production was equal for all rations, but milk fat yield was lower in 60SPP and milk protein yield was lower in 60SPR than in the other treatments. There were no significant differences in fat corrected milk production between 100IM and 20SPP. For other treatments the fat and protein corrected milk productions were significantly lower. All cows gained body weight, but there were no significant differences in the amount of gain. In conclusion, if used in low quantities, silage from semi-natural grasslands can be used in the diet of lactating dairy cows without a reduction in production.

Keywords: Semi-natural grasslands, feeding value, dairy cows, forage

Introduction

In Western Europe, most grasslands are intensively managed and heavily fertilized with nitrogen. However, in order to protect plant diversity within grasslands, a number of governments and environmental organizations encourage the development and maintenance of semi-natural, species rich grasslands. In the Netherlands, most grasslands for which management agreements for nature conservation are in place, are managed and used by dairy farmers (Korevaar & Van der Wel, 1997). As the feeding value of forages from these semi-natural grasslands tend to be lower than for forages from intensively managed grasslands, milk yields from cows offered the former are likely to be reduced (Bruinenberg *et al.*, 2002). Although financially compensated for economic losses because of lower production, farmers are often reluctant to use forages from these semi-natural grasslands, as there is insufficient information about their use in rations of lactating dairy cows. Consequently, the number of management agreements with farmers will be limited (e.g. Tallowin & Jefferson, 1999). However, if more is known about the feeding value of forages from semi-natural grasslands, and the possibilities to include this material in diets for dairy cows, organizations that manage such grasslands will be able to increase the exploitation of such grasslands.

The main difficulty associated with including forages from semi-natural grasslands in the diet of dairy cows, is that their feeding value is not easily quantified. There are a number of reasons for this. Firstly, chemical composition and nutrient availability vary amongst plant species and are difficult to predict (e.g. Korevaar, 1986; Frame, 1990; Bruinenberg *et al.*, 2002). Secondly, the botanical composition of forages from semi-natural grasslands varies over time (De Vries & De Boer, 1959), because of differences between species in date of heading and reproduction. Thirdly, there are different types of semi-natural grasslands: some grasslands are managed to maintain large populations of birds (habitat conservation), others are managed to conserve certain plant species or vegetation types or to achieve maximal species or genotypic diversity in the grassland (Korevaar, 1986).

Research on the use of forages from semi-natural grasslands in the diet of lactating dairy cows is limited (e.g. Korevaar & Van der Wel, 1997). Thus, the objective of the present study was to examine the effects on feed intake and milk yield arising from different inclusion rates of forages from semi-natural grasslands in the diet of lactating dairy cows offered grass of a high quality from intensively managed grassland. The hypothesis was that the decrease in milk yield would be smaller than calculated based on the reduction of energy intake, due to more effective degradation of organic matter from the highly digestible grass as a result of reduced rumen degradation and passage rate of diets in which forages from semi-natural grasslands are included.

This study involved silage produced from two types of semi-natural grasslands: silage from a species poor grassland, dominated by grasses (SPP), and from a species rich grassland, with a mixture of grasses and herbs (SPR).

Material and methods

Forages

The study involved three different grass silages, with each silage being produced from different grasslands within the Netherlands:

1) Intensively managed grassland silage (IM): produced from the primary harvest of an intensively managed pasture (a monoculture of *Lolium perenne*) at ID-Lelystad, Lelystad (52°5’ N, 5°5’ E). In order to achieve a high quality of the forage, this pasture was harvested on 5 May 2000, from a sward growing on a clay soil, which received 112 kg N ha^{-1} on 22 March 2000.
2) Species poor grass silage (SPP): produced from a species poor wet grassland, which was dominated by grasses (*Holcus lanatus*, *Agrostis stolonifera*, *Alopecurus pratensis* and *Poa trivialis*), comparable with a MG13 community (Rodwell, 1993) or a sub-community of *Molinio Arrhenatheretea* (Schaminée *et al.*, 1996). This pasture was managed to encourage nesting of birds, and was fertilized on 10 March 2000 with 20 m^3 cattle slurry ha^{-1}. It was situated in Spijkerboor (52°5’ N, 5°0’ E), on a peat soil. In order to allow birds to complete nesting, harvesting of this grassland was not allowed before 7 June. The herbage was harvested on 7 June 2000.
3) Species rich grass silage (SPR): produced from a species rich grassland, composing a mixture of grasses and herbs, comparable with a MG1 community (Rodwell, 1993) or an *Arrhenatheretum eliatus* community (Schaminée *et al.*, 1996). The pasture was part

of a nature reserve and had not been fertilized since approximately 1980. It was situated in Amerongen (52°0’ N, 5°5’ E) on a riverbank of clay. In order to maintain biological diversity, harvesting of the grasslands was not allowed before 15 June. The herbage was harvested on 21 June 2000.

All herbages were wilted (maximum wilting period < 72 h) to a dry matter (DM) concentration of 600-750 g kg^{-1} and ensiled in bales.

Before harvesting, the species poor grassland and the species rich grassland were sampled to assess the botanical composition. The method of sampling and analysis of the air-dry weight were carried out as described by De Vries (1937) and De Vries & De Boer (1959). The intensively managed pasture was not analysed for botanical composition. This pasture was sown on 25 September 1998 with two varieties of *Lolium perenne* (50% Pagode and 50% Cambridge).

Cows and treatments

Thirty mid lactation multiparous dairy cows (days calved 183 ± 14 d; lactation number 2.5 ± 0.4) were blocked (5 cows per block) according to pre-experimental calving date, milk yield (36.3 kg d^{-1} ± 1.8) and milk composition (fat concentration 4.0% ± 0.3; protein concentration 3.4% ± 0.1). Within each block, cows were randomly allocated to one of five experimental treatments. The experiment started on 31 July 2000 and lasted 10 weeks, including a two-week adaptation period. Cows were housed in a free-range barn and offered a total mixed ration via roughage intake control stations (RIC; Insentec, Marknesse, the Netherlands). The RIC station recorded intake per cow per day. Intake was restricted, but not all cows reached the maximum intake. Throughout the day, the cows could visit the RIC station as often as they wanted, but when maximum intake of the mixed ration of a cow was achieved, access to the mixed ration was denied. On a DM basis, the mixed ration contained 63% grass silage, 18% maize silage and 19% concentrates. The ration was mixed daily in the morning and fed out on an average allowance of 19.7 kg DM per cow per day in two meals per day: one directly after mixing and one in the afternoon. Before the morning feeding, the feed residues of the previous day were removed.

Within the mixed ration, IM was partly replaced by SPP with replacement percentages of 0 (100IM), 20 (20SPP), 40 (40SPP) or 60 (60SPP), or by SPR with a replacement percentage of 60 (60SPR). In addition to the mixed ration, cows received 0.43 kg DM concentrates per day in the milking parlour. Some cows also received extra concentrates in concentrate boxes, because it was calculated that cows with higher milk production levels would not be able to maintain their production if the mixed ration was not supplemented with extra concentrates. The amount of extra concentrates offered, thus, depended on energy requirements, as calculated from requirements for milk and maintenance, of the cows in treatment group 100IM. Within blocks the amount of concentrates was equal for all cows, and the concentrates fed in the concentrate boxes were similar to the concentrates fed in the mixed ration. Calculations indicated that on the treatments 40SPP, 60SPP and 60SPR a protein deficiency could occur if concentrates with regular protein concentrations are used. To prevent such a protein deficiency in those treatments, cows in the treatments 40SPP, 60SPP and 60SPR received concentrates with 195 g true protein digested in the intestine

(DVE) or 302 g CP per kg DM, in contrast to cows in the treatments 100IM and 20SPP. Concentrates that were offered in those rations contained 147 g DVE or 236 g CP per kg DM. The composition of the two concentrates was kept as similar as possible (Table 1).

Measurements

Cows were milked twice daily (at 6:00 h and 15:00 h) and weighed after milking, with milk yield and liveweight being recorded automatically. Each week the average milk production per cow per day and the average weight per cow were calculated. In week 3-10, milk samples were taken on two consecutive milkings per week for fat, protein and lactose analysis, which were determined by infrared analysis (Stichting Melkcontrolestation Nederland, Zutphen).

During each of the weeks 3-10, grabbed samples of each of the grass silages offered were taken on five days each week before ration preparation. Daily samples were subsequently bulked for each 5 day period. The maize silage offered was sampled twice during the study (week 4 and 7), while a single sample of concentrates offered was taken during week 7. Maize silage and concentrates were each produced in one big bunch, and therefore, chemical composition in the feeds was assumed to be consistent over the weeks. All samples were stored at –18 °C until analysis.

Table 1. Composition of the concentrates in g kg^{-1} (in the fresh product).

Primary product	147 DVE	195 DVE
Toasted lupins	74	108
Brazilian soya beans extracted	50	84
Rape seed, extracted	107	122
Condensed molasse solubles	55	53
Premix minerals / vitamins	8	8
Chalk	9	4
Salt	1	1
Magnesium oxide	1	-
Citrus pulp	100	100
Coconut expeller	75	75
Maize gluten meal	100	100
Palm kernel expeller	200	10
Sugar beet pulp	50	50
Linseed extracted	100	100
Soya beans, extracted	10	125
Molasses, beet	60	60

DVE = true protein digested in small intestine (Tamminga *et al*., 1994).

Grass silage samples were oven-dried at 70 °C and analysed for DM, crude ash, Kjeldahl N, crude fibre (CF), neutral detergent fibre (NDF) and sugars (SU), according to the methods described by Van Vuuren *et al*. (1993). *In vitro* organic matter digestibility (OMD) was determined according to the method of Tilley and Terry, as modified by Van der Meer (1986). Ammonia (NH_3) was determined by a modified Berthelot method (Robinson *et al*., 1986). The CF, NDF and SU and NH_3 concentrations of the grass silages were only determined in the weeks 3, 6 and 9.

Samples of maize silage and concentrates were analysed for DM, crude ash, Kjeldahl N, CF, NDF, *in vitro* digestibility and starch. Maize silage was also analysed for NH_3. Methods of analysis were similar as for the grass silage.

Energy requirements of the dairy cows and the energy concentrations of the different feeds were calculated as net energy for lactation (NE_L, Van Es, 1978; CVB, 2001a). The protein requirements and concentrations were calculated as DVE and degraded protein balance in the rumen (OEB), according to Tamminga *et al*. (1994). The structure indices (SI) were calculated according to CVB (2001a; 2001b), e.g. SI = 0.0065 * NDF – 0.20 for grass silage.

Statistical analyses

The intake and production characteristics of the cows were analysed in a completely randomized block design and were subjected to analysis of variance. For intake the model $Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$ was used, in which μ is mean, α_i is effect of block i, β_j is effect of treatment j and e_{ij} is variation within a block. For milk production characteristics and animal weight the model: $Y_{ij} = \mu + \alpha_i + \beta_j + \gamma x_{ij} + e_{ij}$ was used, in which γx_{ij} is the covariate between blocks. The covariate was based on measurements recorded during a 14 day period prior to the start of the experiments. Statistical analysis was carried out using Genstat (Genstat 5 Committee, 1993). Treatment means were compared by Student's *t*-test.

Results

Grassland composition

Visual assessment suggested the intensively managed grassland to consist mainly of *Lolium perenne*. Within SPP, 12 species of grasses, one species of legumes and five species of herbs were identified with these representing 95.9, 0.03 and 4.05% of the (air dry basis) weight analysis, respectively (Table 2). *Holcus lanatus* dominated in SPP. Within SPR, 15 species of grasses, five species of legumes and 22 species of herbs were identified, representing 53.1, 10.5 and 36.3% of the (air dry basis) weight analysis, respectively (Table 2).

Chemical composition and nutritive value

Compared to IM, the crude protein (CP) concentration of forage from SPP was lower, whereas the NDF concentration was higher (Table 3). Furthermore, this material had a higher sugar concentration than the other forages (> 80 g kg^{-1}). The NE_L was lower than that from IM.

Compared to IM, the CP concentration of the forage from SPR was lower and therefore also DVE and OEB were lower in this treatment, whereas the NDF concentration was higher (not significant), although lower than in the forage from SPP (not significant). The NE_L of the forage from SPR was lower than the energy values of the forage from SPP or IM.

Table 2. The botanical composition of the species poor grassland (SPP) and the species rich grassland (SPR).

Species	proportion in dry weight		Species	proportion in dry weight	
	SPP	SPR		SPP	SPR
Grasses			Other herbs		
Agrostis stolonifera	0.123	0.033	*Achillea millefolium*	-	0.033
Alopecurus geniculatus	0.133	-	*Anthiscus sylvestris* †	-	0.041
Alopecurus pratensis	-	0.038	*Cardamine pratensis*	-	0.003
Anthoxanthum odoratum	0.002	0.006	*Centaurea jacea*	-	0.028
Arrhenatherum elatius	-	0.132	*Cerastium fontanum*	0.002	0.005
Avenula pubescens	-	0.001	*Cirsium arvense*	-	0.036
Bromus hordeaceus	0.031	0.029	*Crepis biennis*	-	0.038
Dactylis glomerata	-	0.036	*Galium mollugo*	-	0.039
Elymus repens	0.028	0.029	*Geranium* spp.	-	0.000
Festuca pratensis	0.005	0.003	*Glechoma hederacea*	-	0.001
Festuca rubra	-	0.031	*Heracleum sphondylium* †	-	0.031
Glyceria fluitans	0.012	-	*Leucanthemum vulgare*	-	0.000
Holcus lanatus	0.355	0.020	*Ornithogalum umbellatum*	-	0.001
Lolium perenne	0.059	0.041	*Pimpinella major*	-	0.004
Poa annua	0.005	-	*Plantago lanceolata*	-	0.034
Poa pratensis	-	0.000	*Prunella vulgaris*	-	0.000
Poa trivialis	0.139	0.018	*Ranunculus acris*	0.002	0.039
Trisetum flavescens	-	0.007	*Ranunculus repens*	0.032	0.002
Undetermined rest	0.068	0.107	*Rhinanthus angustifolius*	-	0.007
Total	0.959	0.531	*Rumex acetosa*	0.004	0.002
			Stellaria media	0.000	-
Legumes			*Tanacetum vulgare*	-	0.008
Lathyrus pratensis	-	0.049	*Taraxacum officinale*	-	0.011
Trifolium dubium	-	0.006	Total	0.041	0.363
Trifolium pratense	-	0.029			
Trifolium repens	0.000	0.017			
Vicia cracca	-	0.004			
Total	0.000	0.105			

† These species have heavy plants with a high dry weight, and could therefore account for a too high proportion of the total weight. This may mislead the outcome.

Table 3. Mean chemical composition and digestibility of the feedstuffs offered.

	g kg^{-1} product	g kg^{-1} dry matter					-	%	MJ kg^{-1} DM	g kg^{-1} DM	
	dry matter	ash	crude protein	sugars	starch	NDF	structure index†	organic matter digestibility	net energy for lactation‡	DVE§	OEB§
Intensively managed grass silage	601^{b}	107^{a}	186^{a}	58.5^{b}	-	513^{b}	3.14^{b}	75.0^{a}	6.0^{a}	83.3^{a}	35.5^{a}
Species poor grass silage	723^{a}	95^{b}	126^{b}	90.7^{a}	-	575^{a}	3.53^{a}	59.1^{b}	4.5^{b}	51.2^{b}	1.7^{b}
Species rich grass silage	589^{b}	98^{b}	101^{c}	58.0^{b}	-	541^{ab}	3.32^{ab}	56.1^{c}	4.2^{c}	33.4^{c}	-5.9^{c}
s.e.d.	11.8	3.5	2.5	8.4		15.9	0.103	0.89	0.07	1.645	2.3
Maize silage*	382	41	69	-	333	337	1.33	77.5	6.9	48	-34
Concentrates low DVE**	883.4	93	236	113	58.1	343	0.27	82.0	7.3	147	30.1
Concentrates high DVE**	873.6	91	302	129	69.7	264	0.22	84.8	7.4	195	51.5
Concentrates milking parlour **	892.2	92	178	140	53.9	-	0.26	-	7.3	117	0

- is not determined

† Structure index according to CVB (2001a; 2001b)

‡ NE_L = Net Energy for Lactation ((MJ kg^{-1}, Van Es, 1978)

§ DVE = true protein digested in the small intestine (g kg^{-1}) and OEB = degraded protein balance in the rumen (g kg^{-1}) (Tamminga *et al*., 1994)

* Chemical composition based on two samples

** Chemical composition based on a single sample

Feed intake

Replacement of the IM by forages from semi-natural grasslands did not reduce total dry matter intake (DMI), except for 60SPP (Table 4). Consequently, the NE_L and DVE intake of 60SPP were also significantly lower, but DVE intake was similar between 60SPP and 60SPR. Although DMI in 60SPR was equal with intake levels observed for 100IM, 20SPP and 40SPP, DVE intake was lower in 60SPR than in 100IM and 40SPP. The OEB intake was lowest in 60SPR. The low protein concentration of the species rich forage caused this effect. The NDF intake per kg DM was lowest in 60SPR and the structure index was lowest in 100IM and in 60SPR (Table 4).

Animal performance

Treatments had no significant effect on milk yield (Table 5), but milk composition and fat and protein yields were different amongst treatments. Consequently, also differences in fat and protein corrected milk (FPCM) were found.

Table 4. Treatment effects on nutrient intakes. Cows were offered only intensively managed grass silage (100IM) or diets in which part of the intensively managed grass was replaced by 20 (20SPP), 40 (40SPP), or 60% (60SPP) species poor forage silage or by 60% species rich forage silage (60SPR). s.e.d. = standard error of difference.

	100IM	20SPP	40SPP	60SPP	60SPR	s.e.d.
Mixed ration (kg DM)	19.0[a]	19.0[a]	18.8[a]	17.6[b]	19.0[a]	0.45
Intensively managed (kg DM)	11.8	9.3	6.9	4.2	4.7	-
Species poor (kg DM)	0	2.6	5.0	6.9	0	-
Species rich (kg DM)	0	0	0	0	7.0	-
Maize silage (kg DM)	3.4	3.4	3.3	3.1	3.5	-
Concentrates (kg DM)	3.7	3.7	3.5	3.3	3.7	-
Concentrates in boxes (kg DM)	2.0	2.0	2.0	2.0	2.0	-
Concentrates milking parlour (kg DM)	0.43	0.43	0.42	0.43	0.43	-
Total intake (kg DM)	21.4[a]	21.4[a]	21.2[a]	19.9[b]	21.3[a]	0.45
NE_L (MJ d^{-1} cow^{-1}) †	140[a]	136[ab]	131[bc]	120[d]	128[c]	2.6
DVE (g d^{-1} cow^{-1}) ‡	1884[ab]	1806[bc]	1939[a]	1769[c]	1771[c]	40
OEB (g d^{-1} cow^{-1}) ‡	462[a]	379[c]	401[b]	306[d]	264[e]	9.8
Crude protein (g kg^{-1} DM) §	180[b]	173[d]	183[a]	177[c]	170[e]	1.2
NDF g kg^{-1} DM §	435[b]	444[a]	431[c]	438[b]	423[d]	1.2
Structure index *	2.022[a]	2.082[b]	2.098[c]	2.135[d]	2.030[a]	0.006

[a,b,c,d,e]: Means in the same row with different superscripts differ significantly ($P < 0.05$)

† NE_L = net energy for lactation (Van Es, 1978)

‡ DVE = true protein digested in small intestine and OEB = Degraded protein balance in the rumen (Tamminga *et al.*, 1994)

§ crude protein and neutral detergent fibre (NDF); Average of the total diet, including concentrates in the boxes and in the stable.

* Structure index according to CVB (2001a; 2001b).

Table 5. Treatment effects on milk output and weight changes. Cows were offered only intensively managed grass silage (100IM) or diets in which part of the intensively managed grass was replaced by 20 (20SPP), 40 (40SPP), or 60% (60SPP) species poor forage silage or by 60% species rich forage silage (60SPR). s.e.d. = standard error of difference.

	100IM	20SPP	40SPP	60SPP	60SPR	s.e.d.
Milk (kg d^{-1}) †	26.8	26.7	25.6	25.7	25.3	0.92
Fat (%)†	4.56^{a}	4.54^{a}	4.43^{a}	4.07^{b}	4.43^{a}	0.11
Protein (%) †	3.47^{a}	3.51^{a}	3.46^{a}	3.49^{a}	3.37^{b}	0.04
Milk fat yield (kg d^{-1}) †	1.24^{a}	1.21^{ab}	1.12^{bc}	1.04^{d}	1.09^{cd}	0.04
Milk protein yield (kg d^{-1}) †	0.93^{a}	0.93^{a}	0.89^{ab}	0.89^{ab}	0.84^{b}	0.03
FPCM (kg d^{-1}) †	29.0^{a}	28.6^{ab}	26.9^{bc}	26.1^{c}	26.2^{c}	0.88
Mean body weight (kg)	611	602	631	604	622	31.0
Weight gain (kg in 8 weeks)	41	27	25	37	35	16.1

† Characteristics were corrected for covariate, FPCM = fat and protein corrected milk

^{a,b,c,d}: Means in the same row with different superscripts differ significantly (P < 0.05)

Milk production dropped during the experiment. In the first weeks, especially for 40SPP, 60SPP and 60SPR, milk production declined rapidly but this decline seemed less in 100IM and 20SPP (Figure 1). Milk fat and milk protein concentrations increased during the trial. There was a trend for a quicker decline in FPCM production in 60SPP, compared to the other treatments, but this was not significant. In all treatments animals gained weight during the experiment.

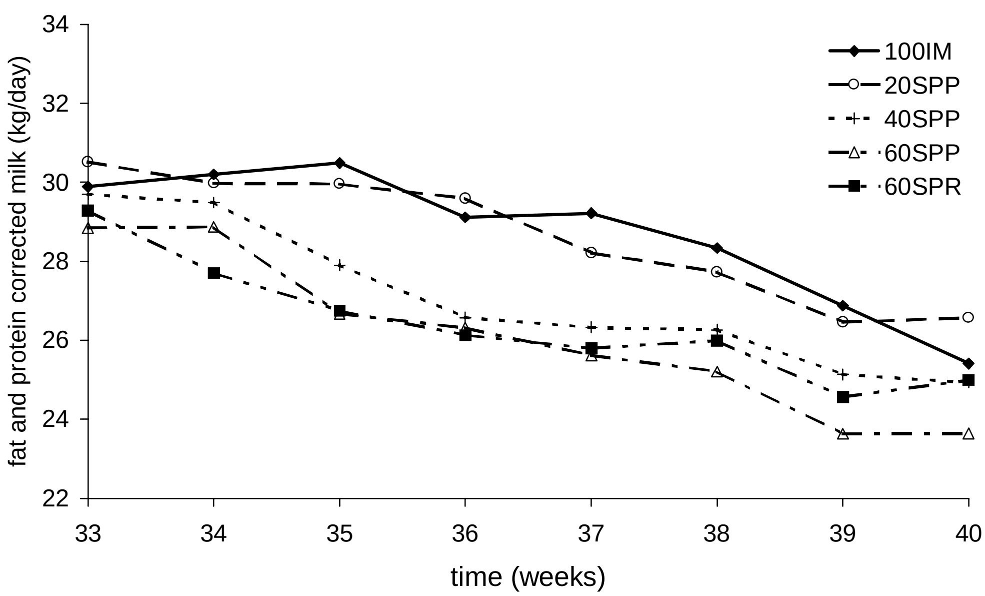

Figure 1. The fat and protein corrected milk yield over the weeks. Treatments were 100IM (ration consisting of intensively managed grass silage (IM), maize silage and concentrates), 20SPP, 40SPP, 60SPP (respectively 20, 40 and 60% the IM replaced by species poor grass silage) and 60SPR (60% of the IM replaced by species rich grass silage).

Discussion

Botanical and chemical composition

In general, forages from pastures managed to encourage nesting of birds are expected to have a higher feeding value than forages from natural grasslands with a botanical diversity objective, because of differences in demands of management, such as date of harvesting and possibilities of fertilization. Indeed, the NE_L was higher in the forage from SPP. However, the forage from SPR used in this trial consisted partly of dicots and legumes, which may have a positive influence on ingestion and degradation rate (Wilman *et al*., 1997; Thomson *et al*., 1985) and, therefore, on intake and performance of cows.

Although harvested earlier, silage produced from SPP grassland had a higher NDF concentration than silage produced from SPR grassland, perhaps a reflection of botanical differences between the two grassland types. Species poor forage contained mainly mature grass species with high NDF concentrations in contrast to SPR, which contained more herbs with lower NDF concentrations. However, the OMD of SPP was still higher than that of SPR. Herbs have higher lignin concentrations in the cell walls than grasses (Beever *et al*., 2000), so, therefore, the NDF of SPP is probably better digestible than that of SPR.

The CP concentrations of forages from semi-natural grasslands were low. This is in agreement with Tallowin & Jefferson (1999), taking harvesting date into account. Not using inorganic fertilizer combined with the late harvesting date results in the low CP concentrations. Although the proportion of legumes was higher in SPR than in SPP, the average CP concentration was lower in SPR. This can be attributed to the fact that the CP concentration of grasses and herbs occurring in SPR may be low compared to the CP concentration of grasses occurring in SPP, due to the later harvesting date or to the fertilization on SPP. The low CP concentration of the forages from semi-natural grasslands makes supplementation of the diet with protein-rich concentrates necessary, especially if the semi-natural forages are fed in high amounts.

In this study, only two types of forages from semi-natural grasslands were used, which were harvested at a specific time and at a specific location. Each of the grasslands had a specific botanical composition, and, therefore, the question could be raised if those grasslands are representative for semi-natural grasslands. However, it is believed that the characteristics of the forages in this study at least give an indication about the possibility to include forages from semi-natural grasslands in diets of dairy cows.

Forage composition and voluntary intake

Intake of 60SPR was significantly higher than that of 60SPP, although the replacement percentage was approximately similar. In 60SPP, cows ingested on average 6.9 kg of semi-natural grass silage (39% of the mixed ration), whereas in 60SPR, cows ingested 7.0 kg of semi-natural grass silage (37% of the mixed ration). The lower proportion of semi-natural grass in 60SPR could have caused the difference in intake between the treatments. However, since intake of 60SPR was fast, and the interest for the diet shown by the cows was high, it is believed that the higher intake of this diet was not due to the somewhat lower proportion in the diet, but to other factors. This is confirmed by the fact that the inclusion of SPR did not result in a decline in DM intake, compared to 100IM, whereas the inclusion of SPP led to a decrease in intake. The higher intake of 60SPR, compared to 60SPP can be attributed to the higher amount of legumes and other herbs in SPR. Some dicots have a high palatability and in general dicots are more easily disrupted in the rumen than grasses (Wilman *et al*., 1997; Thomson *et al*., 1985; Derrick *et al*., 1993), influencing intake and digestion positively (Wilman *et al*., 1997; Derrick *et al*., 1993). So, even with high NDF concentrations high intakes can be observed for some dicots (e.g. Wilman *et al*., 1997). In general legumes have higher intakes than grasses, which is attributed to lower cell wall concentrations, faster particle size reduction, a faster rate of OM removal from the rumen, and higher protein concentrations (Meijs, 1981; Ulyatt, 1981; Thomson *et al*., 1985; Wilman *et al*., 1997). Some herbs (e.g. *Cirsium arvense* and *Rumex acetosa*) may have a negative effect on intake (e.g. Derrick *et al*., 1993) but because of the low occurrence of these species in SPR, no effect on dry matter intake was found.

The low dry matter intake of 60SPP could partly be explained by the frequency of *Holcus lanatus* (35% of DM). This grass species declines rapidly in digestibility during maturation (Korevaar, 1986). In general, the effect of maturation on degradability is higher in grasses than in herbs (Peeters & Janssens, 1998). The proportion of grasses was higher in SPP (95%) than in SPR (50%).

Conrad *et al*. (1964) suggested a positive relationship between OMD and voluntary intake (VI), although this relationship disappears above an OMD of 70%. In our trials, overall OMD for all treatments was higher than 70% and therefore, according to Conrad *et al*. (1964) no effect of OMD on voluntary intake would be expected. This is in agreement with our results obtained for 100IM and 60SPR from which cows consumed the same amount of DM, with a large difference in OMD (77.3 versus 70.8%). Forbes (1995) suggested that NDF degradation is a better predictor for intake than digestibility, but NDF degradation was not measured in this experiment.

The DMI of 100IM and 60SPR was equal. This was not expected, because of differences in cell wall concentrations and in cell wall composition. It can be speculated that the high palatability of SPR is responsible for the relatively high DMI of 60SPR. The expected differences in the degradation rate and particle size reduction between IM and SPR did not result in differences in dry matter intake.

The replacement of IM by SPP did not result in a linear decrease in DMI. Only 60% replacement reduced DMI significantly compared to the other treatments. The reduction in feed intake observed for 60SPP is probably related to the capacity of the rumen (De Visser *et al*., 1998). Cows probably reduced intake because they could not increase their rumen content any further.

Production characteristics

Although a significant reduction in NE_L intake between 100IM and most of the other treatments was observed (Table 4), milk yield did not differ between treatments (Table 5). The lowest NE_L intake on 60SPP did not correspond to the lowest milk yield. From these observations, it might be concluded that cows with the highest NE_L (100IM and 20SPP) used the surplus energy for growth. However, body weight gain within the 8 weeks period of the experiment was not different between treatments (Table 5). So, it is surmised that energy utilization of the diet in terms of producing milk yield was increased by replacing highly digestible by poorly digestible grass. Milk yield expressed per 100 MJ NE_L intake was 19.1, 19.6, 19.5, 21.4 and 20.5 kg for the treatments 100IM, 20SPP, 40SPP, 60SPP and 60SPR, respectively. The differences between treatments could be due to the imbalance between energy and protein in the rumen, which can result in relatively higher utilization of energy for maintenance (Clark *et al*., 1992) and thus in a decreased efficiency of energy utilization.

The effect of a somewhat lower milk yield (40SPP, 60SPP and 60SPR), milk fat (especially for 60SPP) and milk protein (especially for 60SPR) concentration resulted in a significantly lower FPCM production on these mentioned treatments compared to 100IM and 20SPP. The lower milk fat concentration with treatment 60SPP was unexpected. For example, replacing highly digestible forage by a poorly digestible forage normally results in a higher milk fat concentration (Conrad *et al*., 1964; Miller, 1979). The fall in milk fat concentration cannot be attributed to a deficiency in structural material the SI being 2.1, whereas the SI requirement is >1.12 for cows producing 26 kg of milk (CVB, 2001a). While the composition of long-chain fatty acids in the ration may influence milk fat concentration, the differences in composition of fatty acids were relatively small (Fievez *et al*., 2002). The decline in milk fat concentration on 60SPP could therefore not be explained by the results of our experiment.

Milk protein declined on 60SPR compared to the other treatments but this reduction was small (maximum difference 0.14%) and the level was relatively high for all treatments. The latter is attributed to the fact that cows were in an advanced stage of lactation during this trial. The relatively low milk protein concentration on 60SPR could have been an indication of energy deficiency. However, NE_L intake on 60SPP was significantly lower than on 60SPR (Table 5) but milk protein was not reduced. Because protein (DVE) and OEB were offered in sufficient amounts for all treatments, it might not be expected that these parameters influenced milk yield or milk composition.

The decline in milk production and fat concentration, and thus of FPCM production, of the higher replacement percentages compared to the diets with 0% and 20% replacement indicates that if the replacement of intensively managed grass with forages from semi-natural grasslands is too high (over 40%), FPCM production will decrease. With these high replacement percentages, the diet will also have to be supplemented with protein-rich concentrates to maintain animal performance. Therefore, lower replacement percentages offer more possibilities for the inclusion in a dairy cow's diet.

Practical implications and conclusion

From these results it can be concluded that a replacement until 40% of intensively managed grass silage by semi-natural grass silage in a total mixed diets containing 55% grass, had no influence on milk performance of high yielding dairy cows. A higher replacement influenced milk yield and composition negatively. Although both semi-natural grassland silages used in this trial were poorly digestible, the effects of including these forages in diets for dairy cows on feed intake and milk performance were different. However, the overall conclusion is that there is scope to include forages from semi-natural grasslands into diets for dairy cows. This could have a positive impact on maintaining or increasing the flora and fauna in the landscape area.

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Chapter 7

The voluntary intake and *in vivo* digestibility of forages from semi-natural grasslands in dairy cows

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The voluntary intake and *in vivo* digestibility of forages from semi-natural grasslands in dairy cows

Abstract

To measure the *in vivo* digestibility of forages from semi-natural grasslands and to compare it with the digestibility of intensively managed forages, two experiments were performed with dairy cows. In Experiment 1, nine lactating animals were offered one of three different silages, daily supplemented with 4 kg dry matter (DM) of concentrates. Silages originated from an extensively managed species poor grassland (SPP) or a species rich grassland (SPR) and an intensively managed grassland (IM). Intake was measured and faeces were collected during 72 hours. In Experiment 2, four animals were offered IM or IM partly replaced by SPP (20% (20SPP) or 60% (60SPP) replaced) or SPR (60% replaced; 60SPR). Furthermore each cow consumed 4.5 kg DM concentrates day^{-1}. Intake was measured and faeces and urine were collected during 48 hours. Both experiments were carried out according to a Latin square design. In each period the silage and the faeces were sampled and analysed for chemical composition (organic matter, OM; crude protein, CP; neutral detergent fibre, NDF; and gross energy; GE) and *in vitro* digestibility. The urine was analysed for nitrogen to calculate the nitrogen balance. The intake was lowest on the diets with SPP ($P < 0.05$), but intake of diets based on SPR was not significantly lower than the intake of IM diets. In Experiment 1, *in vivo* digestibility of OM, CP, NDF and GE were highest for IM and lowest for SPR ($P < 0.05$). Also in Experiment 2, *in vivo* digestibility was highest for IM ($P < 0.05$) but no significant differences in digestibility were observed between 60SPP and 60SPR. In both experiments, the *in vivo* digestibility was approximately similar to the *in vitro* digestibility. In 60SPR, relatively more nitrogen was excreted in the faeces than in the other treatments and relatively less excreted in the urine ($P < 0.05$). In conclusion, although digestibility and CP concentration were lower in SPR than in SPP ($P < 0.05$), intake was higher on SPR ($P < 0.05$). Intake of digestible organic matter seemed to be higher for SPR than for SPP. Therefore, there seems to be more scope for SPR than for SPP.

Keywords: Dairy cows, *in vivo* digestibility, semi-natural grasslands, voluntary intake

Introduction

In temperate regions, most grasslands used to produce roughage for dairy cows are intensively managed, i.e. the grasslands are mainly monocultures of *Lolium perenne*, they are fertilized heavily and harvest takes place in an early stage of maturity. In the last decades, the interest for other, semi-natural grasslands, with high biodiversity has increased. Semi-natural grasslands have a botanical composition with more diverse, indigenous species, fertilization is restricted, and often also the first date of harvesting is delayed until the reproduction season of certain plant or bird species is finished (Korevaar, 1986). The different management and the variety of forage species in semi-natural grasslands complicate the estimation of the nutritional value and intake of the forages. The *in vivo* digestibility partly indicates the nutritional value, and can be predicted by chemical composition, *in vitro* digestibility or near infrared reflectance spectroscopy. However, those methods are mainly based on calibration data from *in vivo* trials with sheep fed *L. perenne* at maintenance level (e.g. Van Es, 1978; Steg *et al*., 1990). Unless suitable *in vivo* standards are used to estimate the *in vivo* digestibility from the *in vitro* digestibility over a large range of digestibilities, this indirect method does not seem appropriate to estimate the *in vivo* digestibility of forages from semi-natural grasslands in lactating dairy cows. Compared to intensively managed grass, the *in vivo* digestibility of forages from semi-natural grasslands is lower (e.g. Tallowin & Jefferson, 1999), because a different genetic make-up and late harvesting will cause high concentrations of lignified cell walls in the semi-natural forages (Bruinenberg *et al*., 2002). Furthermore, due to the relatively low digestibility, intake of those forages is also expected to be reduced (Korevaar & Van der Wel, 1997). Some research on *in vivo* digestibility and on intake of forages from semi-natural grasslands has been reported in literature, but these trials were mainly carried out with sheep (Armstrong *et al*., 1986; 1989; Derrick *et al*., 1993). As sheep are fed at a lower feeding level than lactating dairy cows, it is difficult to extrapolate results obtained with sheep to dairy cows. However, because in the Netherlands most forages from semi-natural grasslands are fed to dairy cattle (Korevaar & Van der Wel, 1997), it is important to evaluate the nutritive value and the potential intake of the forages for dairy cows. Therefore, in this study both the *in vivo* digestibility and the voluntary intake (VI) of silages from two types of semi-natural grasslands were measured in lactating dairy cows. The *in vivo* digestibility was compared with the *in vitro* digestibility (standardized for sheep digestibility) and the chemical composition of the silages. In order to compare the results from semi-natural grass with production grass, also an intensively managed grassland, containing mainly *L. perenne*, was included in the experiment. In a second experiment, the *L. perenne* silage was used as basal diet, and replaced in different proportions by the silages from the semi-natural grasslands. Semi-natural grass in the diet is expected to increase rumen retention time, stimulate rumination and reduce the nitrogen surplus in the rumen. This could result in an improved *in vivo* digestibility and nitrogen utilization, especially if the semi-natural grass is fed in combination with intensively managed grass. Therefore, in addition to the *in vivo* digestibility, also the results of a nitrogen balance trial with different proportions of semi-natural silage are reported. Furthermore, a comparison between the measured *in vivo* digestibility in Experiment 1 (separately feeding of the different silages) and Experiment 2 (feeding intensively managed and semi-natural silage in a mixture) could indicate if digestibility of the different forages is additive.

Material and methods

Experiment 1: animals, experimental design and treatments

In Experiment 1, nine multiparous lactating dairy cows were used, with an average daily milk production of 27.6 (22.9-31.0) kg milk at the start of the experiment. The experiment was designed as a Latin square with three treatments, three periods and three cows per treatment. Each period lasted four weeks with the first two weeks for adaptation to the diet, the third week for measuring voluntary intake and the fourth week for assessing total tract digestibility at a restricted DM intake. Daily rations of the cows consisted of grass silage, supplemented with 4 kg protein-rich concentrates and 0.4 kg additional concentrates offered in the milking parlour. Three silages were tested: silage from an intensively managed grassland (IM; cut 5 May 2000), silage from a species poor grassland (SPP; cut 7 June 2000) and silage from a species rich grassland (SPR; cut 21 June 2000). Silages were prewilted and ensiled in big bales. More information about the botanical composition of the silages can be found in Bruinenberg *et al.* (2003a). Silage was offered *ad libitum* in the first three weeks of the experiment, but in the fourth week, silage was fed restricted, to 12.5 kg DM d^{-1}, to prevent differences in digestibility caused by differences in DM intake. The proportion of silage in the diet varied between 70 and 73%, depending on silage intake.

The silages were weighed and sampled before feeding and in the weeks 3 and 4 of each period feed refusals were weighed and sampled daily. To measure voluntary intake, feed intake was measured during 7 days (Saturday to Friday), and to measure digestibility, feed intake was measured during 72 hours (Monday to Thursday). The faeces were collected quantitatively during 72 hours (Tuesday to Friday) and weighed and proportionally sampled daily. Faecal samples were stored at –18 °C until analysis. Samples of the three days were combined before analysis. The protein-rich concentrates and the concentrates fed in the milking parlour were both sampled once during the experiment. Samples were stored at –18 °C until analysis.

Experiment 2: animals, experimental design and treatments

In Experiment 2, four animals were used, with an average milk production of about 26.6 (23.4-32.4) kg per day at the start of the experiment. The experiment was designed as a Latin square with four treatments and four periods. It was part of another experiment, in which also fermentation characteristics were measured (Bruinenberg *et al.*, 2003b). Each period lasted three weeks, with the first two weeks used as adaptation period, and the third week for measurements of the total tract digestibility and urine production.

The animals were fed four different rations consisting of silage (restricted to 15 kg DM d^{-1}) and 4.5 kg DM of protein-rich concentrates d^{-1}. Silages and concentrates were the same as in Experiment 1, but the rations were different. The silages offered to the animals consisted of only IM (100IM), 20 or 60% of IM replaced by SPP (20SPP and 60SPP, respectively) or 60% of IM replaced by SPR (60SPR). The percentage of silage in the diet varied between 73 and 75% (depending on silage intake).

The silage was sampled during weighing. In the weeks of data collection feed refusals were weighed and sampled daily. Feed intake was measured during 48 hours (Sunday to Tuesday) and faeces and urine were collected quantitatively during 48 hours (Monday to Wednesday). To prevent mixture of urine with manure, cows were fitted with a bladder catheter (Barht). Urine was acidified with sulphuric acid to a pH between 2 and 3. Urine and faeces were proportionally sampled daily and samples were stored at –18 °C until analysis. Before analysis the samples of both days were combined.

Laboratory analyses

In Experiment 1, the silages and faeces were air-dried at 70 °C and in Experiment 2, the silages and faeces were freeze-dried. The protein-rich concentrates were freeze-dried in both experiments. Subsequently, the silages, concentrates and faeces were analysed for dry matter (DM), ash, neutral detergent fibre (NDF) and Kjeldahl N as described by Van Vuuren *et al*. (1993) and expressed in g kg^{-1} DM. *In vitro* organic matter digestibility (d_{OM}) of the silages and concentrates was determined according to Tilley & Terry (1963) and expressed in percentage. In Experiment 1, crude fat (CFAT) and crude fibre (CF) in silage, concentrates and faeces were determined according to Van Vuuren *et al*. (1993) and expressed in g kg^{-1} and gross energy (GE, in MJ kg^{-1} DM) was determined using a bomb calorie meter (NEN-ISO 1928). In Experiment 2, indigestible acid detergent fibre (IADF) in silage, concentrates and faeces was determined according to Penning & Johnson (1983) and expressed in g kg^{-1}. Urine was analysed for Kjeldahl N. In the feed refusals of both trials, DM and ash were determined as described by Van Vuuren *et al*. (1993).

Calculations

Organic matter (OM) in feed and faeces was calculated as $1000 - \text{ash}$ and crude protein concentration (CP) was calculated as 6.25 * N. Nitrogen free extract (NFE) was calculated as $1000 - \text{ash} - \text{CP} - \text{CF} - \text{CFAT}$.

In Experiment 2, the N balance was calculated as total N intake, minus N in milk, urine and faeces. The N that remains is called unrecovered N. In Experiment 1, the unrecovered N also includes the N in urine, as the balance was calculated as N intake minus N in milk and faeces.

Results were statistically analysed with the ANOVA procedure for a Latin square design using Genstat 5 (Genstat 5 Committee, 1993), with cow * period as a block structure and the diets as treatments. Treatment means were compared with the Student's *t*-test and significance was declared at $P < 0.05$.

Results

Laboratory analyses of forages

Crude protein concentration was highest in IM and lowest in SPR. Concentration of NDF was highest in SPP and lowest in IM (Table 1). The d_{OM} was highest for IM and lowest for SPR. SPP and SPR were similar in composition except for CP (SPR much lower than SPP), NDF (SPR much lower than SPP) and NFE (SPR much higher than SPP).

Experiment 1

Voluntary intake of SPP was significantly lower than of SPR ($P < 0.05$; Table 2). If fed restricted, DM intake remained lowest on SPP ($P < 0.05$; Table 3). The OM intake was highest on SPR and CP intake was highest on IM ($P < 0.05$). The CP intake was lowest and the NFE intake was highest on SPR ($P < 0.05$).

Highest *in vivo* digestibility coefficients were observed for IM, whereas the lowest *in vivo* digestibility coefficients were observed for SPR, except for NFE, where the digestibility was higher for SPR than for SPP ($P < 0.05$; Table 4).

Furthermore, N efficiency (% of ingested N recovered in milk) was highest in SPR (Table 5). The percentage of N recovered in faeces was higher in SPR than in SPP ($P < 0.05$), which in its turn was higher than in IM ($P < 0.05$).

Table 1. Chemical composition and *in vitro* digestibility of the silages and concentrates, average of different experiments and treatments: IM is intensively managed grass, SPP is extensively managed species poor grass, SPR is extensively managed species rich grass, Concentrates 1 is protein-rich concentrates, Concentrates 2 is concentrates fed in milking parlour.

	IM	SPP	SPR	Concentrates 1	Concentrates 2
Ash (g kg^{-1} DM)	114	94	95	93	76
Organic matter (g kg^{-1} DM)	886	906	905	907	924
Crude protein (g kg^{-1} DM)	190	132	101	247	176
Neutral detergent fibre (g kg^{-1} DM)	527	624	563	311	300
Crude fibre (g kg^{-1} DM)	284	325	316	93	113
Crude fat (g kg^{-1} DM)*	37	22	26	49	49
Nitrogen free extract (g kg^{-1} DM)**	375	427	463	518	586
Gross energy (MJ kg^{-1} DM)*	18.5	18.4	18.1	18.6	18.6
d_{OM}*** (%)	75.8	57.4	54.5	82.6	84.6

* Crude fat and gross energy were not analysed in Experiment 2, values in the Table are averages of Experiment 1. Nitrogen free extract in Experiment 2 was estimated from average crude fat concentration in Experiment 1.

** Nitrogen free extract is estimated as 1000−ash−CP−CF−CFAT.

*** d_{OM} = *in vitro* digestibility.

Table 2. Voluntary intake per day of intensively managed grass (IM), species poor grass (SPP) and species rich grass (SPR) by dairy cows fed a fixed amount of concentrates (in Experiment 1).

	IM	SPP	SPR	s.e.d.
Silage intake *ad libitum* (kg DM d^{-1})	13.0^{ab}	12.0^{b}	13.2^{a}	0.57
Concentrates (kg DM d^{-1})	4.0	4.0	4.0	0
Concentrates milking parlour (kg DM d^{-1})	0.4	0.4	0.4	0
Nutrient intake				
Organic matter (kg d^{-1})	15.6^{ab}	14.9^{b}	16.1^{a}	0.51
Crude protein (kg d^{-1})	3.6^{a}	2.7^{b}	2.5^{c}	0.10
Neutral detergent fibre (kg d^{-1})	8.3	9.0	8.7	0.33

^{a,b,c} Means in the same row and within the same experiment with different superscripts differ significantly ($P < 0.05$). s.e.d. = standard error of difference.

Table 3. Average intake of nutrients in the digestibility study in Experiments 1 and 2 (including concentrates). Treatments as described in Material and methods.

	Experiment 1				Experiment 2				
	IM	SPP	SPR	s.e.d.	100IM	20SPP	60SPP	60SPR	s.e.d.
Silage (kg DM d^{-1})	12.0[a]	10.4[b]	11.6[a]	0.29	13.9[a]	13.6[a]	12.2[b]	12.8[ab]	0.44
Concentrates 1 (kg DM d^{-1})	4.0	4.0	4.0	0	4.5	4.5	4.5	4.5	0
Concentrates 2† (kg DM d^{-1})	0.29	0.29	0.29	0	-	-	-	-	-
Total intake (kg DM d^{-1})	16.3[a]	14.7[b]	16.0[a]	0.29	18.3[a]	18.1[a]	16.7[b]	17.3[ab]	0.40
Organic matter (kg d^{-1})	14.6[a]	13.4[b]	14.4[a]	0.26	16.3[a]	16.1[a]	15.0[b]	15.5[ab]	0.39
Crude protein (kg d^{-1})	3.4[a]	2.5[b]	2.3[c]	0.04	3.7[a]	3.6[b]	3.0[c]	2.9[c]	0.08
NDF (kg d^{-1})	7.8	7.9	7.9	0.22	8.6	8.7	8.3	8.4	0.30
Crude fibre (kg d^{-1})	4.1	4.0	4.1	0.10	4.0	4.0	3.8	4.1	0.16
Crude fat (kg d^{-1})	0.67[a]	0.46[c]	0.52[b]	0.01	-	-	-	-	-
NFE (kg d^{-1})	6.4[b]	6.5[b]	7.5[a]	0.13	7.8	7.9	7.6	7.9	0.21
IADF (kg d^{-1})	-	-	-	-	0.71[c]	0.82[c]	0.99[b]	1.35[a]	0.063
Gross energy (MJ d^{-1})	303[a]	274[b]	292[a]	5.5	-	-	-	-	-

[a,b,c] Means in the same row and within the same experiment with different superscripts differ significantly ($P < 0.05$). s.e.d. = standard error of difference.

NDF is neutral detergent fibre, NFE is nitrogen free extract, IADF is indigestible acid detergent fibre

† Concentrates fed in the milking parlour, during milking. Only fed in Experiment 1.

-: Not measured in the experiment.

Table 4. *In vivo* digestibility coefficients (%) in Experiments 1 and 2. Treatments as described in Material and methods.

	Experiment 1				Experiment 2				
Coefficients of	IM	SPP	SPR	s.e.d.	100IM	20SPP	60SPP	60SPR	s.e.d.
Dry matter	76.8[a]	64.0[b]	61.2[c]	1.01	75.8[a]	73.8[a]	68.9[b]	67.6[b]	0.84
Ash	59.3[a]	46.9[b]	38.7[c]	1.47	61.3[a]	59.6[a]	55.3[b]	51.5[c]	1.02
Organic matter	78.9[a]	65.7[b]	63.5[c]	0.97	77.6[a]	75.5[b]	70.4[c]	69.4[c]	0.82
Crude protein	71.1[a]	61.6[b]	55.5[c]	1.16	70.8[a]	70.2[a]	67.1[b]	64.5[c]	0.78
NDF	81.8[a]	62.6[b]	55.6[c]	1.19	84.8[a]	81.4[b]	73.8[c]	72.3[c]	1.07
Crude fibre	83.4[a]	63.7[b]	54.6[c]	1.14	-	-	-	-	-
NFE	80.4[a]	67.6[c]	70.3[b]	1.12	-	-	-	-	-
IADF	-	-	-	-	25.6[ab]	27.9[a]	22.2[bc]	19.9[c]	2.26
Gross energy	75.8[a]	63.0[b]	60.4[c]	1.09	-	-	-	-	-

[a,b,c] Means in the same row and within the same experiment with different superscripts differ significantly ($P < 0.05$). s.e.d. = standard error of difference.

- Crude fibre, crude fat and gross energy were not analysed in Experiment 2, as a result also NFE could not be calculated. IADF was not analysed in Experiment 1. NDF is neutral detergent fibre, NFE is nitrogen free extract, IADF is indigestible acid detergent fibre.

Table 5. The nitrogen intake and excretion in Experiments 1 and 2. % = percentage of intake.

	Experiment 1				Experiment 2				
	IM	SPP	SPR	s.e.d.	100IM	20SPP	60SPP	60SPR	s.e.d.
N intake	546[a]	396[b]	365[c]	6.3	604[a]	566[a]	481[b]	465[b]	13.9
% N in milk	20[b]	21[b]	24[a]	0.9	18[c]	19[bc]	20[b]	22[a]	0.4
% N in faeces	29[c]	38[b]	44[a]	1.2	29[c]	31[c]	33[b]	36[a]	0.6
% N in urine	-	-	-	-	41	42	43	36	3.4
% N unrecovered*	52[a]	40[b]	32[c]	1.5	12	8	5	7	3.1

[a,b] Means in the same row and within the same experiment with different superscripts differ significantly ($P < 0.05$). s.e.d. = standard error of difference.

* In Experiment 1 the unrecovered N is the ingested N that was not excreted in the milk and faeces, and in Experiment 2 the unrecovered N is the ingested N that was not excreted in milk, faeces and urine.

Experiment 2

Intake of DM and OM was highest on 100IM and 20SPP and lowest on 60SPP ($P < 0.05$; Table 3). Crude protein intake was highest on 100IM and lowest on 60SPR ($P < 0.05$). No significant differences were observed in NDF or CF intake ($P > 0.05$). Intake of IADF was highest on 60SPR and lowest on 100IM and 20SPP ($P < 0.05$). No differences were observed in NDF and CF intake.

Digestibility coefficients of the total diet of DM, OM, CP, NDF and IADF were all highest in 100IM or 20SPP or both and lowest on 60SPR ($P < 0.05$; Table 4). Digestibility coefficients for 60SPP were also significantly lower than those for 100IM ($P < 0.05$), except for IADF. Also, digestibility coefficients of OM and NDF for 20SPP were significantly different from those of 100IM ($P < 0.05$), but not the digestibility coefficients of DM, ash, CP and IADF.

Also in Experiment 2 the diet which contained SPR had the highest N efficiency and 100IM the lowest ($P < 0.05$), although not all differences were statistically significant. The highest proportion of N in urine was observed on 60SPP ($P < 0.05$; Table 5). Percentage of ingested N in faeces was highest in 60SPR (Table 5).

Discussion

Intake

The low VI of DM for SPP and 60SPP is in accordance with the observations in another experiment (Bruinenberg *et al.*, 2003a) and was probably caused by the high NDF concentration (Table 1) and the low NDF degradability (Bruinenberg *et al.*, 2003b) of SPP. A high NDF concentration in the diet is expected to increase resistance to physical breakdown and rumen fill, resulting in a lower voluntary intake (Armstrong *et al.*, 1986; De Visser *et al.*, 1998). This is confirmed by the relatively high DM intake of SPR, which had a relatively low NDF concentration compared to SPP. The high NDF intake on 100IM and 20SPP might seem contrary to a limitation of NDF for intake, but the rate of degradation of

NDF on IM was higher than that on SPP or SPR (Bruinenberg *et al.*, 2003c). No relationships were observed between other chemical characteristics or digestibility of the silages and VI.

Digestibility

Factors affecting the difference in digestibility between IM and SPP or SPR have been discussed before (Bruinenberg *et al*., 2002). In short, these factors include differences in stage of maturity and in forage species and differences in anatomical structure between forage species. In this discussion we will focus on the different indirect methods to estimate the *in vivo* digestibility in cows and the possibility to use those methods for SPP and SPR.

The *in vitro* digestibility of the diet was calculated from the *in vitro* digestibility of the silages and concentrates and the proportions of these different feed components in the diets. The *in vivo* OM digestibility of the total diet was in accordance with the calculated *in vitro* OM digestibility of the total diet (Figure 1). This was not expected, as normally the *in vivo* digestibility with lactating dairy cows is depressed when compared to the *in vitro* OM digestibility which is standardized towards wethers fed at maintenance level (Tilley & Terry, 1963; Steg *et al*., 1990). However, maybe the activity of the microbes in the rumen fluid used in the laboratory was low, resulting in a lower *in vitro* digestibility. However, some standards with a known *in vivo* digestibility are included in the analysis to correct for differences in activity of rumen fluid. Therefore, a possibility for the relatively low *in vitro* digestibility might be that the standards used to correct for the differences in activity of the rumen fluid were not appropriate. When the *in vitro* OM digestibility was corrected for digestibility depression on higher feeding levels, the difference between *in vitro* and *in vivo* digestibility even increased, the *in vivo* digestibility becoming higher than the *in vitro* digestibility. Because of a higher energy intake on the diets containing (mainly) intensively managed grass, than on the diets containing large proportions of semi-natural grass, the digestibility depression was calculated to be higher on the diets containing mainly intensively managed grass.

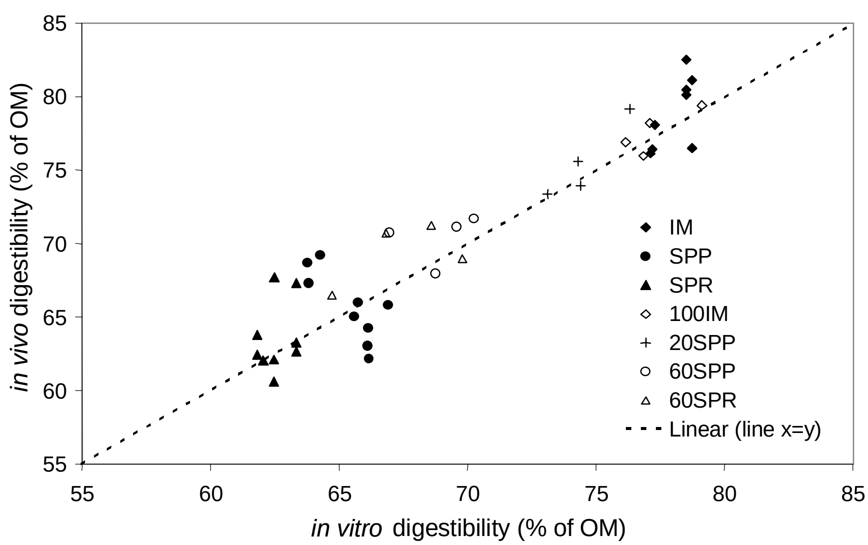

Figure 1. The relationship between the *in vitro* and the *in vivo* organic matter digestibility. For abbreviatons, see text.

The digestible OM (DOM) can also be estimated based on the chemical composition of silages (CVB, 2001), using the following equation:

$$\text{DOM (g kg}^{-1}\text{)} = 1027 - 0.77 * \text{CF} - 1.23 * \text{ASH} - 0.03 * \text{DM} - 0.3 * \text{D}$$

in which D = days after 1 April, other values in g kg^{-1}.

In practice this formula is not used anymore, because the estimation of DOM with near infrared reflectance spectroscopy (NIRS) is more efficient for routine analysis. However, NIRS is not appropriate for forages from semi-natural grasslands, and therefore, it is important to know if the DOM can be estimated based on the chemical composition. With the data in this study, it was also observed that the formula is not correct for any of the silages (Figure 2). It can therefore be concluded that for the silages in this study there was no relationship between the DOM estimated from the chemical composition and the DOM estimated from the *in vitro* digestibility. Differences were probably due to stage of maturity, botanical composition, cell wall concentration, or other differences among silages. CVB (2001a) already indicated that the formula should not be used for forages with a diverse botanical composition, but also for the intensively managed grass used in this experiment, the estimation was not correct.

For semi-natural grasslands, the D factor in the formula is an important cause for the incorrect estimation. The D corrects for the season effect, such as temperature. However, for semi-natural grasslands the late date of the first cut is probably more important than the advanced season, as a delayed first cut results in an advanced stage of maturity. The effect of D is, therefore, underestimated in the forages from semi-natural grasslands. Stage of maturity affects degradability and digestibility (Bosch *et al.*, 1992). Therefore, a correction of the formula for stage of maturity would probably improve the estimation of the DOM.

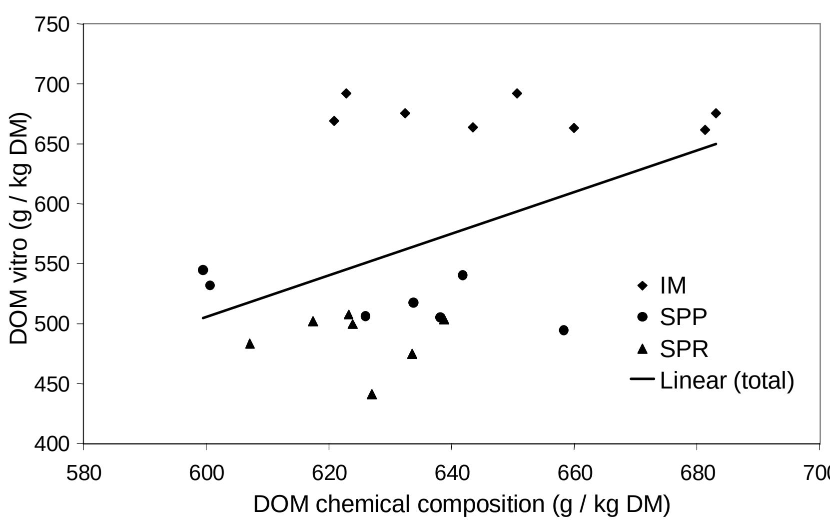

Figure 2. The relationship between the estimated DOM (based on chemical composition) and the measured DOM (based on *in vitro* OM digestibility).

Based on the *in vivo* OM digestibility of the diet and the *in vitro* digestibility of concentrates, the *in vivo* OM digestibility of the silage was calculated. Also for other nutrients such calculations were made, but assumptions had to be made for the digestibility of the nutrients of concentrates. Digestibilities of DM (d_{DM}), CP (d_{CP}) and NDF (d_{NDF}) of concentrates were assumed to be 80, 76 and 70%, respectively. Results of the calculations are shown in Table 6. When comparing the *in vitro* OM digestibility of the silages (Table 1) with the *in vivo* OM digestibility (Table 6), the *in vivo* digestibility was a few percentages higher. However, the differences are small and therefore it may be concluded that the *in vitro* digestibility of the silage indicates the actual value reasonably well. Because the *in vivo* digestibility of all diets was estimated correctly by the *in vitro* data, which is affirmed by Figure 1, it can also be concluded that there was no positive effect of replacing a proportion of the intensively managed grass by semi-natural forages on the OM digestibility. In addition, also from the comparisons between the different replacement percentages in the treatments it was concluded that the OM digestibility was additive. The *in vivo* OM digestibility on the treatments 20SPP, 60SPP and 60SPR was as expected from the *in vivo* OM digestibility based on the proportions by weight and OM digestibility of 100IM, SPP and SPR (Figure 3). The digestibilities of NDF and CP were not completely linearly correlated to percentage replacement (Figure 3; NDF). This was attributed to differences between animals, as the *in vivo* digestibility of SPP and SPR were measured in different animals than the digestibility of 60SPP and 60SPR.

Table 6. The digestibility (d, in %) of species poor (SPP) and species rich silage (SPR), calculated based on the digestibility of the treatments in Experiments 1 and 2. 'SPP a' based on 20SPP, 'SPP b' based on 60SPP.

	Experiment 1			Experiment 2			
	IM	SPP	SPR	IM	SPP a	SPP b	SPR
Dry matter	75.6	57.4	54.2	74.4	61.1	58.5	55.6
Organic matter	77.6	58.7	56.4	76.0	61.7	59.2	57.1
Crude protein	69.4	55.7	47.9	69.1	64.9	60.3	54.6
Neutral detergent fibre	86.0	59.5	50.3	89.6	67.6	65.7	61.9

Formula used, e.g.: d IM = (d diet IM – d concentrates * % concentrates) / % IM

The digestibility of concentrates is estimated based on *in vitro* OM digestibility: d_{OM} = 82.6%, d_{DM} = 80%, d_{CP} = 76% and d_{NDF} =70%. d_{NDF} of concentrates is based on the composition of the concentrates (Bruinenberg *et al*., 2003b) and the crude fibre digestibility of those components (CVB, 2001b).

Table 7. The digestibility of NDF (d_{NDF}, in %) of the silage, calculated based on the *in vitro* OM digestibility (with an assumed digestibility of cell contents of 95%), based on calculations in Table 6.

d_{NDF}	IM	SPP	SPR
in vitro	62.8	40.4	29.8
in vivo, Experiment 1	86.0	55.7	47.9
in vivo, Experiment 2	89.6	66.7	61.9

However, the difference between the *in vivo* and the *in vitro* digestibility was high for the digestibility of NDF (d_{NDF}). The *in vitro* d_{NDF} was calculated based on *in vitro* d_{OM}, the ratio NDF and non-NDF, with an estimated digestibility of the non-NDF fraction of 95% and it was compared to the *in vivo* d_{NDF} (Table 7). The *in vivo* digestibility of NDF was calculated with the assumption of a NDF digestibility of 70% for concentrates, which was based on the digestibility of CF of the main components of the concentrates (Bruinenberg *et al*., 2003b; CVB, 2001b). The *in vivo* d_{NDF} was quite similar in Experiment 1 and 2, but the calculated *in vitro* d_{NDF} was lower. This could have been due to the calculation methods. Maybe 95% digestibility of non-NDF, as estimated *in vitro*, is too high or the d_{NDF} in the concentrates is higher than estimated. A lower assumed digestibility of non-NDF (85% instead of 95%) increased the *in vitro* d_{NDF} with a few percentages, but the difference remains large. Furthermore, a higher assumed d_{NDF} of concentrates (80% instead of 70%) reduced the *in vivo* d_{NDF} of the three silages with a few percent, but even then the *in vitro* d_{NDF} remains lower. The calculations strongly suggest that the *in vivo* d_{NDF} is higher than the *in vitro* d_{NDF}, which could be due to an adaptation of rumen microbes to the diets.

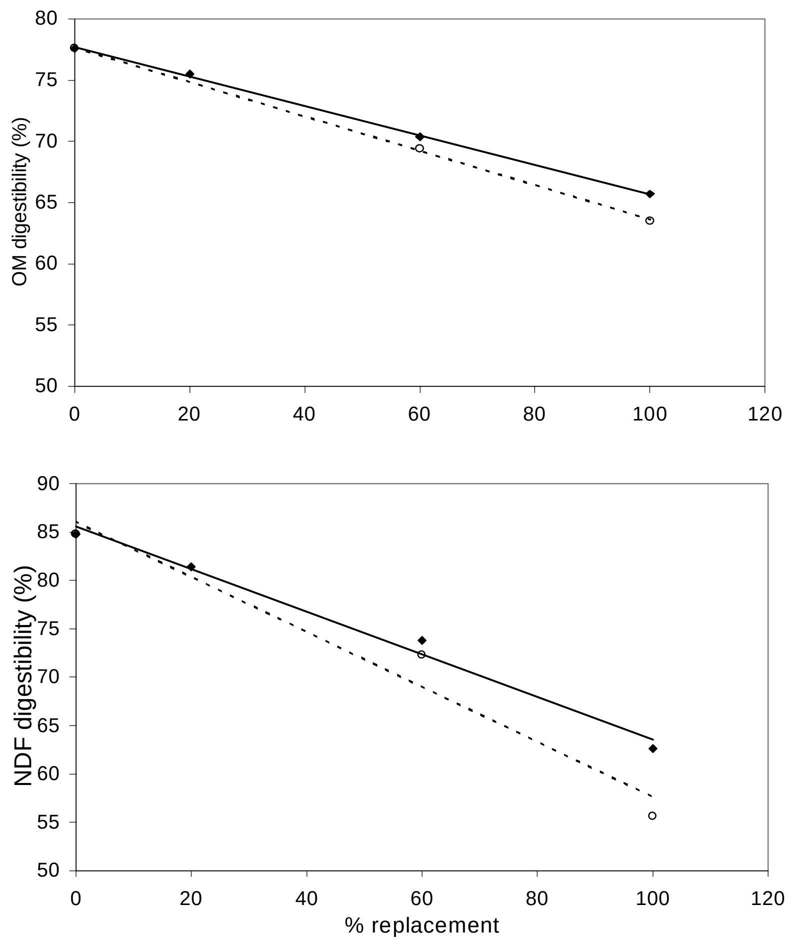

Figure 3. The decrease in OM and NDF digestibility with different replacement percentages, ♦ = replacement with species poor grass, ○ = replacement with species rich grass.

Metabolizable energy intake

In the Netherlands, three different formulae are in use to estimate the metabolizable energy value of grass forages (ME, in MJ kg^{-1}; Van Es, 1978; CVB, 2001).

$$ME^{1} = 15.9*DCP + 37.66*DFAT + 13.81*DCF + 14.64*DNFE - 0.63*SU \quad (1)$$
$$ME^{2a} = 14.2*DOM + 5.9*DCP \quad (2)$$
$$ME^{2b} = 15.1 * DOM \quad (3)$$

in which

DCP is digestible crude protein, DFAT is digestible crude fat, DCF is digestible crude fibre, DNFE is digestible nitrogen free extract and SU is sugars (only used if > 80 g kg^{-1}), DOM is digestible organic matter. All parameters in g kg^{-1}.

Also in other countries, the ME is often calculated based on the digestible nutrients in the feed (Van der Honing & Alderman, 1988; Beever *et al*., 2000).

In all cases ME^{2a} and ME^{2b} gave lower predictions than ME^{1}. However, when comparing formula ME^{1} and $ME^{2a \text{ or } b}$, total ME intake (MEI) maximally differed about 5 MJ day^{-1}, which is equivalent to approximately 1 kg of milk per day. Except for SPR, predictions of ME^{2b} differed more from ME^{1} than ME^{2a} did. Thus, although ME^{2a} gave lower values than ME^{1}, for the IM as well as SPP and SPR, the discrepancy between the two formulae remained small. Therefore, the formula can be used for forages from semi-natural grasslands.

Total DOM intake was higher on (60)SPR than on (60)SPP, although the digestibility of (60)SPP was higher than that of (60)SPR. This was due to the higher DM intake on (60)SPR. A higher DOM intake will also result in a higher MEI, and thus in a higher NE intake, resulting in a higher expected milk production. It would therefore be interesting to compare expected and actual milk output for the different treatments. However, differences in milk production between treatments could not be tested independently, because in our opinion a Latin square design, as used in these trials, is not suitable for measuring differences in milk production.

Nitrogen balance

Because of the short measuring periods, in this study the term unrecovered N is used instead of N retention. It is not likely that in two or three days N is retained in the body. Furthermore, variation in excreted N in urine or milk between days will have occurred, and finally, it was not clear if unrecovered N should be allocated to urine or milk.

No positive effects of mixing IM with SPP or SPR on efficiency of N utilization were observed. However, this could also be due to the statistical design. The high N intake in IM, 100IM and 20SPP resulted in a relatively low efficiency of N utilization for milk, whereas the low N intake on SPR resulted in a relatively high efficiency of N utilization for milk, even though milk production was reduced. The low N recovery in the milk on IM, 100IM and 20SPP coincided with high recoveries in the urine or high unrecovered N, which would result in high N losses to the environment. The high proportion of N in urine on 60SPP is not considered significant, as the unrecovered fraction is lower than on the other treatments in Experiment 2. As expected, recoveries in urine and unrecovered N were lowest in SPR. This was attributed to the low N intake and the low CP digestion on SPR.

Conclusions

In vitro digestibility gave a good indication of the *in vivo* digestibility. Also when intensively managed forage was combined with SPP or SPR, *in vivo* OM digestibility was additive. The *in vivo* NDF digestibility was higher than the calculated *in vitro* NDF digestibility. Our results confirm that the formula to predict DOM from the chemical composition (CVB, 2001a) is not valid for silages from semi-natural grasslands.

Although digestibility and CP concentration were higher in SPP than in SPR, in both trials DOM intake was higher on the diets with SPR thanks to the higher DM intake. Therefore, there may be more scope for the use of SPR than for SPP in diets of highly productive dairy cows.

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Chapter 8

Fermentation and degradation in the rumen of dairy cows fed on diets consisting of silage from an intensively managed sward and silages from semi-natural grasslands

Bruinenberg, M.H., Valk, H., Struik, P.C. & Van Vuuren, A.M. (2003) Fermentation and degradation in the rumen of dairy cows fed on diets consisting of silage from an intensively managed sward and silages from semi-natural grasslands (submitted to Journal of Agricultural Science, Cambridge)

Fermentation and degradation in the rumen of dairy cows fed on diets consisting of silage from an intensively managed sward and silages from semi-natural grasslands

Abstract

To assess the effect of grassland management on the ruminal digestion of silages, four lactating dairy cows, fitted with a rumen cannula, were fed different diets consisting of concentrates and different grass silages. The grass silages consisted of intensively managed grass (IM; a monoculture of *Lolium perenne*) in variable proportions replaced by silages harvested from two types of semi-natural grasslands. The silages from the semi-natural grasslands were either from a species poor grassland managed to stimulate nesting of birds (SPP) or from a grassland managed to increase plant species diversity (SPR). The roughage part of the diets was composed of 100% IM (100IM), or 20% (in dry matter) of IM replaced by SPP (20SPP) or 60% of IM replaced by SPP (60SPP), or SPR (60SPR). After an adaptation period of 16 days, diurnal patterns of the pH and concentrations of volatile fatty acids and ammonia were determined in rumen fluid. Total rumen content was evacuated and sampled three times during a normal feeding regime, and a fourth time after 13 hours of fasting. Contents were analysed for chemical composition and particle size. Intake was assessed during a five-days period and urine was collected during 48 hours and analysed for uric acids.

The pH in the rumen was highest on 60SPR and lowest on 100IM and 20SPP ($P < 0.05$), whereas VFA concentrations were lowest on 60SPP and 60SPR and highest on 100IM ($P < 0.05$). No differences in the ratio non-glucogenic : glucogenic fatty acids were observed among the diets. The NH_3 concentration was highest on 100IM and 20SPP and lowest on 60SPR ($P < 0.05$), reflecting differences in CP intake. The concentration of uric acids in the urine (mg per kg metabolic body weight) was highest on 100IM ($P < 0.05$). Rumen pool size of OM and DM did not differ among treatments, but pool size of NDF and IADF were highest on 60SPR ($P < 0.05$). Passage rate was high on 100IM and 60SPR, but no significant differences with the other treatments were established. Also, no significant differences were observed in rates of degradation. Clearance rate of large particles was highest on 60SPP and differed significantly from 60SPR ($P < 0.05$) only. No differences were observed in clearance rate of small particles. In conclusion, for most rumen fermentation characteristics measured in this study, no remarkably aberrant behaviour of the silages from semi-natural grassland was observed.

Keywords: Feed degradation, semi-natural grasslands, rumen fermentation and kinetics, cattle

Introduction

Semi-natural grasslands are different from intensively managed grasslands used to feed dairy cows in temperate regions. Firstly, many different, for intensively managed grasslands unusual, forage species may occur, grasses as well as dicots (Tallowin & Jefferson, 1998). Those forage species often have a higher cell wall or lignin concentration and a lower digestibility than the grass species occurring in intensively managed grasslands (mainly

Lolium perenne). Secondly, semi-natural grasslands are usually cut or grazed in a more advanced stage of maturity than the intensively managed grasslands (Korevaar, 1986; Bruinenberg *et al.*, 2002). Both aspects, the higher diversity of species and the more advanced stage at cutting, are associated with a reduced digestibility and thus a lower net energy value of forages (Armstrong *et al.*, 1986). Korevaar & Van der Wel (1997) also observed a reduced intake of silage of a mixture of mainly *Agrostis stolonifera*, *Lolium perenne*, *Poa trivialis* and *Alopecurus geniculatus* compared to silages consisting mainly of *L. perenne*. However, in that study the impact of differences in botanical composition on milk production was small (Korevaar & Van der Wel, 1997). In a performance trial with dairy cows a decrease in fat and protein corrected milk did not occur until 40% of silage from intensively managed grassland was replaced by silage from (species poor) semi-natural grasslands containing mostly grasses in an advanced stage of maturity (Bruinenberg *et al.*, 2003a). A decrease in intake only occurred if as much as 60% of the intensively managed silage was replaced (Bruinenberg *et al.*, 2003a). Such a decrease in intake was not observed if 60% of the silage was replaced by silage from species rich semi-natural grassland containing approximately 50% herbs. These contrasting results may be explained if more is known about rumen fermentation and kinetics on those diets. Rumen fermentation characteristics (pH, volatile fatty acids (VFA), NH_3) give an indication of the availability of energy and protein in the rumen, and differences in intake may be explained by differences in rumen degradation and passage rate. Also studying the amount of uric acids excreted in the urine is important, as this gives an indication of microbial protein synthesis in the rumen.

Information on rumen kinetics and fermentation of forages from semi-natural grasslands in dairy cows, in comparison to forages from intensively managed grasslands, is scarce. *In situ* or *in vitro*, herb rich forage of an advanced stage of maturity may have higher degradation rates than grass of the same age (Lopez *et al*., 1991; Bruinenberg *et al.*, 2003b). To obtain insight in rumen kinetics and rumen fermentation patterns in the rumen of dairy cows, two forages originating from different semi-natural grasslands were combined with intensively managed grass and concentrates, and fed to lactating dairy cows fitted with a rumen cannula. It was expected that the degradation and fermentation characteristics of the forages from semi-natural grasslands would differ from those of intensively managed grasslands, and that the combination of the forages from semi-natural grasslands with grass from intensively managed grasslands would result in a positive response on rumen fermentation patterns and rumen kinetics by reducing rate of degradation and passage through the rumen. Furthermore, as the surplus of nitrogen of diets with intensively managed grass will be reduced when some of the intensively managed grass is replaced by low protein silage, positive effects are expected on utilization of energy or protein in the rumen.

Material and methods

Cows and experimental design

In the experiment four lactating dairy cows, fitted with a rumen cannula (internal diameter 10 cm; Bar Diamond, Parma, ID) and with a milk production of 20-25 kg per day, were used.

Cows were housed in tied stalls on rubber mats and had free access to water. The experiment was carried out from May to August 2001 and was designed as a Latin square, with four periods, each of three weeks. The first two weeks of each period were used for adaptation of the cows to the diet and in the third week the measurements were carried out. The protocol of the experiment was approved of by the Ethical Committee for Animal Experiments of ID-Lelystad.

Treatments

Treatments were four different diets, consisting of one or combinations of two out of three different silages (Table 1). The silages were obtained by cutting intensively managed grassland (IM) and two semi-natural grasslands. One semi-natural grassland (species poor grassland; SPP) consisted of mainly mature grasses and the other semi-natural grassland (species rich grassland; SPR) consisted of 34% non-leguminous herbs, 11% legumes and 55% grasses. Grass from SPP was cut on 7 June 2000, grass from SPR on 21 June 2000 and grass from IM on 5 May 2000. All three types of grass were prewilted (wilting period < 72 hours) and subsequently ensiled in big bales (400-600 kg). For more details on the botanical composition and the management of the three grasslands, we refer to Bruinenberg *et al.* (2003a).

Table 1. The amount of different silages used in the four treatments diets and the chemical composition of the silages.

Diet	100IM	20SPP	60SPP	60SPR
Silage composition (g kg^{-1})				
Intensively managed silage	1000	800	400	400
Species poor silage	0	200	600	0
Species rich silage	0	0	0	600
Chemical composition (g kg^{-1} DM)				
Ash	115	111	103	103
Organic matter	885	889	897	897
Crude protein	191	181	159	139
Sugars	32	39	56	42
Neutral detergent fibre	524	540	568	547
Fermentable organic matter	561	531	471	464
Nutritive quality				
d_{OM} (proportion of OM)	0.757	0.721	0.646	0.628
Net energy (MJ kg^{-1} DM)	5.9	5.6	5.0	4.7

[1] Calculated based on *in situ* degradation characteristics (Bruinenberg *et al.*, 2003b).

d_{OM} = digestibility of the organic matter.

Table 2. Ingredients and composition of concentrates.

Ingredients	g per kg product	Chemical composition	($g\ kg^{-1}$ DM)
Rape seed, extracted	83	Ash	93
Wheat	74	Organic matter	907
Molasses, cane	50	Crude protein	247
Vinasses (beet)	50	Neutral detergent fibre	311
Palm oil fatty acids	2		
Premix minerals/ vitamins	8	Nutritive quality	
Chalk	6.2	d_{OM} (proportion OM)	0.826
Salt	1.9	Net energy ($MJ\ kg^{-1}$)	7.4
Magnesium oxide (80% MgO)	4		
Coconut expeller	133		
Maize glutenmeal	293.9		
Palmkernel expeller	150		
Soya beans extracted	18		
Soya beans extracted	125		
Mono calciumphosphate	1		

In addition to the silage, cows received 4.5 kg dry matter (DM) concentrates per day (composition in Table 2), offered during milking in two portions of 2.25 kg DM each. The silage was fed restricted in two portions a day, offered directly after the concentrates were eaten. In the morning the animals received 40% and in the evening they received 60% of the daily silage offer. Once every two weeks the four silage mixtures were prepared, weighed into individual plastic bags per cow per feeding time, and stored at –18 °C until one day before feeding. Silages were thawed for 24 h at environmental temperature.

Measurements, sampling and analysis

Intake was measured at four consecutive days in the third week of the experimental period. Samples of the silage mixtures were taken directly after mixing and stored at –18 °C. Production of urine, the pH of the rumen fluid, and VFA and NH_3 concentrations in the rumen, as well as the rumen contents were measured during the third week. Starting at 13:00 h on day 1, the urine was quantitatively collected for 48 h, using a bladder catheter. Urine was collected under sulphuric acid to maintain a pH < 3. After 48 h, samples of the acidified urine were stored at −18 °C until analysis for uric acid. Furthermore, starting on day 1, the rumen fluid was sampled at 16:00, 18:00, 20:00, 22:00, 24:00, 03:00, 06:00, 08:00, 10:00 and 13:00 h. The pH of the rumen fluid was measured directly and a subsample was taken, mixed with phosphoric acid in a ratio of rumen fluid:phosphoric acid of 5:1, and frozen at −18 °C until analysis of NH_3 and VFA. Rumen contents were evacuated manually at 4:00, 10:00 and 20:00 h on day 4, and at 9:00 h on day 5. Cows were deprived of food between 20:00 h, day 4, and 9:00 h, day 5. Rumen contents were weighed and two samples were taken, one for the analysis of the chemical composition and the other for the analysis of the particles size distribution in the rumen. Approximately two hours before the sampling of 20:00 h, 30 g

Cobalt EDTA, dissolved in water, was added in the rumen. Concentrations of Co-EDTA were determined in the rumen samples collected at 20:00 en 9:00 h, to estimate the passage rate of the rumen fluid.

After freeze-drying the samples of feed and rumen contents, DM, ash, NDF, sugars (only of feed) and Kjeldahl N were determined as described by Van Vuuren *et al*. (1993) and *in vitro* organic matter digestibility was determined according to Tilley & Terry (1963). Indigestible ADF (IADF) was determined as described by Penning & Johnson (1983). The VFA's and uric acids were analysed by high performance liquid chromatography, using a Merck polyspher OA-HY 51272 column. For VFA, the mobile phase was 0.0025 M sulphuric acid followed by RI-detection. For uric acids, the eluens was 0.005 M sulphuric acid and the detection method was UV at 283 nm. The NH_3 was determined with a modified Berthelot method (Robinson *et al.*, 1986). Organic matter (OM; g per kg) was calculated as 1000 – ash, and crude protein (CP) was calculated as 6.25*N. Fermentable organic matter (FOM) was calculated based on *in situ* degradation characteristics (Bruinenberg *et al*., 2003b).

The second (fresh) sample of the rumen contents was analysed for particle size distribution. From the sample two subsamples were taken, one for the analysis of the DM content, and the other for the analysis of the distribution of the particle size of the rumen contents. The particle size analysis was carried out by wet sieving of a fresh sample of the rumen contents, using four sieves with mesh sizes of 4 mm, 2.5 mm, 1 mm and 0.045 mm. The sieves were placed on top of each other, the one with the widest meshes on top. The subsample was placed in the top sieve, tap water was added, enough to cover the subsamples with water, the sieves with rumen contents were shaken for 15 minutes, and then water was removed. This procedure was repeated twice before the sieves were emptied and the different fractions were dried and weighed. The fraction of each particle size in the total rumen contents was then calculated based on their air-dried weight. Fractions were divided into fractions with large (> 2.5 mm) and small (< 2.5 mm) particle sizes.

Calculations and Statistics

Rumen turnover rates of different fractions were described by rate of intake, passage rate and degradation rate of OM and NDF. Rate of intake (% h^{-1}) of OM (k_{iOM}) and NDF (k_{iNDF}) were calculated as

$$k_i = (\text{kg ingested OM or NDF per day / kg OM or NDF in the rumen / 24 hours}) * 100\%.$$

in which kg OM or NDF in the rumen is average of the rumen evacuations at 4:00, 10:00 and 20:00 h on day 4 (during a normal feeding regime).
The passage rate (k_p, % h^{-1}) was calculated as

$$k_p = (\text{kg IADF intake per day / kg IADF in the rumen / 24 hours}) * 100\%.$$

in which kg IADF in the rumen is the average of the rumen evacuations at 4:00, 10:00 and 20:00 h on day 4.

The fractional degradability (% h^{-1}) of OM (k_{dOM}) or NDF (k_{dNDF}) was calculated as

$k_d = k_i – k_p$.

Furthermore, based on the disappearance of rumen contents after 13 hours of fasting, the rates of clearance (% h^{-1}) of rumen OM (k_{cOM}), NDF (k_{cNDF}), particles > 2.5 mm (k_{cL}) and particles < 2.5 mm (k_{cS}) were calculated as

k_c = [(ln(rumen contents at t=20:00) – ln(rumen contents at t=9:00)]/ 13 hours)] * 100%.

Results were statistically analysed with the ANOVA procedure for a Latin square design using Genstat 5 (Genstat 5 Committee, 1993), with cows*periods as the block structure and diets as the treatment. Treatment means were compared with the Student's *t*-test. Significance was determined at $P < 0.05$.

Results

Diet composition, quality and intake

The four silage mixtures differed in concentrations of CP, NDF, FOM and sugars and in the values for *in vitro* digestibility and net energy (Table 1). The digestibility of OM and the CP, FOM and net energy (NE) concentrations were highest in 100IM and lowest in 60SPR, whereas the NDF and sugar (SU) concentrations were lowest in 100IM and highest in 60SPP.

Concentrate intake was maximal, and therefore differences in total intake were caused by differences in silage intake (Table 3). Intakes of DM and OM were highest on 100IM and 20SPP and lowest on 60SPP ($P < 0.05$; Table 3). CP intake was highest on 100IM, and it was equally low on 60SPP and 60SPR. No significant differences were observed in intake of NDF. Sugar intake was highest on 60SPP ($P < 0.05$). Intake of IADF was highest on 60SPR and lowest on 100IM and 20SPP ($P < 0.05$).

Fermentation characteristics

The pH was highest for 60SPR, and lowest for 100IM and 20SPP ($P < 0.05$; Table 4). This was also reflected by the VFA concentrations in the rumen, which increased with a decrease in pH (R^2= 0.70, $P < 0.001$; n = 160). Type of silage had no effect on the molar proportions of acetate, propionate or butyrate, with one exception: the proportion of butyrate on 60SPP was significantly higher than on 100IM ($P < 0.05$). No significant differences were found for the ratio of non-glucogenic:glucogenic fatty acids (NGGR). The average concentration of NH_3 over the day reflected CP intake and was significantly highest in 100IM and 20SPP, and lowest in 60SPR ($P < 0.05$; Table 4). The NH_3 concentration in 60SPP was also significantly lower than in 100IM and 20SPP ($P < 0.05$). Over the day, the pH, VFA and NH_3 fluctuated, and for pH the daily pattern appeared to be different for 60SPP and 60SPR compared to 100IM and 20SPP (Figure 1). For NH_3 the common trend over the day was similar for all diets (Figure 2), although significant differences in levels were observed. Especially shortly after feeding the pH decreased (Figure 1) and the concentrations of NH_3 increased (Figure 2).

Table 3. Intake of dry matter, organic matter, crude protein, neutral detergent fibre, cellulose-indigestible acid detergent fibre, fermentable organic matter and particles larger or smaller than 2.5 mm on the four treatments. Abbreviations of the treatments as in Table 1. All values in kg per day.

	100IM	20SPP	60SPP	60SPR	s.e.d.
Diet					
Silage	14.0[a]	13.6[a]	12.2[b]	13.0[ab]	0.49
Concentrates	4.5	4.5	4.5	4.5	0
Total intake					
Dry matter	18.5[a]	18.1[a]	16.7[b]	17.5[ab]	0.49
Organic matter	16.4[a]	16.1[a]	15.0[b]	15.7[ab]	0.46
Crude protein	3.8[a]	3.6[b]	3.1[c]	2.9[c]	0.06
Sugars	0.80[b]	0.87[b]	1.02[a]	0.88[b]	0.04
Neutral detergent fibre	8.7	8.7	8.3	8.5	0.31
Indigestible acid detergent fibre	0.7[a]	0.8[a]	1.0[b]	1.4[c]	0.11
Fermentable organic matter	10.3	9.7	8.2	8.5	*
Particles > 2.5 mm	10.3	10.2	9.6	10.2	0.40
Particles < 2.5 mm	8.2[a]	7.9[b]	7.2[c]	7.3[c]	0.10

[a,b,c] Within a row, means not sharing common superscripts differ significantly ($P < 0.05$).

* Calculated based on averages (this table and Bruinenberg *et al*., 2003b) and assuming fermentable organic matter of concentrates was 520 g kg $^{-1}$, so no s.e.d. could be calculated. s.e.d. = standard error of difference.

Table 4. The pH and the concentration (mmol l^{-1}) of volatile fatty acids (VFA) and urea in the rumen for different silages. Numbers are the average over 10 times within 24 h. NGGR is (acetate + 2 * butyrate) / propionate. Abbreviations of the treatments as in Table 1.

Diet	100IM	20SPP	60SPP	60SPR	s.e.d.
pH	6.2[a]	6.2[a]	6.3[b]	6.5[c]	0.04
Total VFA	125[a]	119[a]	106[b]	104[b]	2.65
Acetate, mol%	71.0	70.9	70.9	71.1	0.38
Propionate, mol%	17.9	17.8	17.4	17.5	0.41
Butyrate, mol%	11.2[a]	11.3[ab]	11.8[b]	11.5[ab]	0.22
NGGR	5.3	5.3	5.5	5.4	0.16
NH_3	12.9[a]	12.3[a]	10.5[b]	8.3[c]	0.37

[a,b,c] Within a row, means not sharing common superscripts differ significantly ($P < 0.05$).

s.e.d. = standard error of difference.

Urine production was highest on 100IM and 20SPP and lowest on 60SPP and 60SPR (Table 5, $P < 0.05$). The total amount of uric acids produced in the urine per kg metabolic body weight were highest in 100IM ($P < 0.05$).

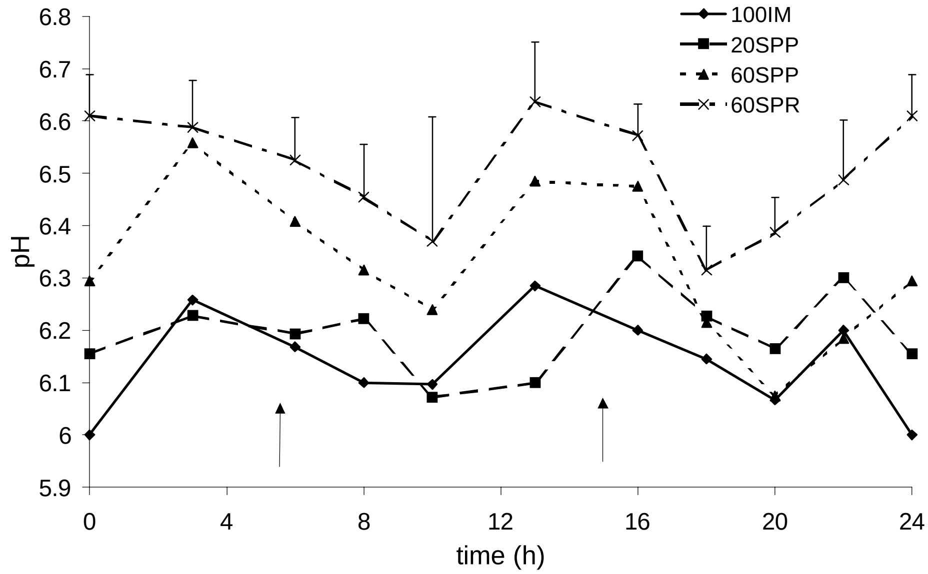

Figure 1. The pH patterns over the day of the four different diets. Arrows indicate the time of feeding. Vertical bars present standard error of difference.

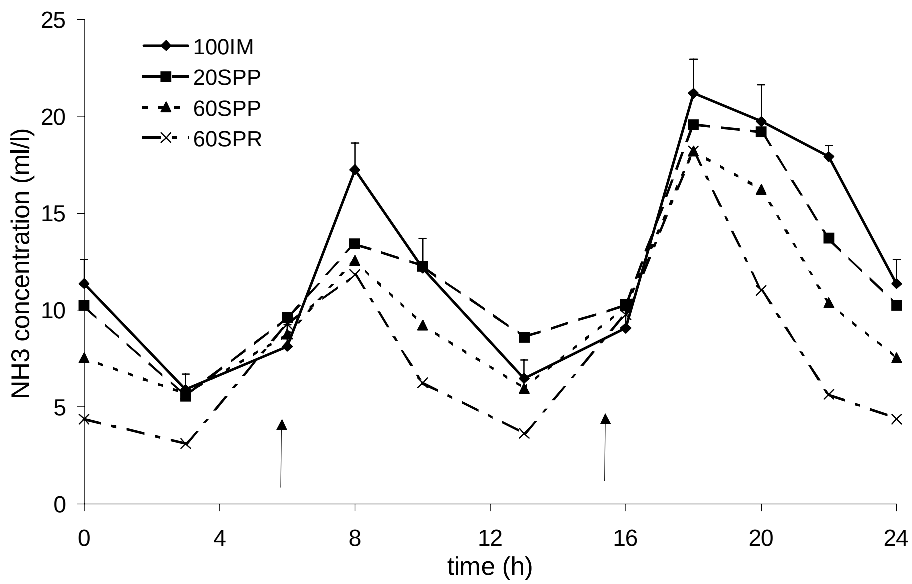

Figure 2. The NH_3 patterns on the four diets over the day. Arrows indicate the time of feeding. Vertical bars present standard error of difference.

Rumen kinetics

Rumen pool sizes of DM and OM did not differ significantly between diets (Table 6). The pool size of NDF was significantly larger on 100IM than on 60SPP or 60SPR. The pool size of IADF was lowest on 100IM and 20SPP and highest on 60SPR.

No significant differences in k_p among the different diets were observed, but the k_i of OM and NDF was significantly higher on 100IM (Table 7; $P < 0.05$). The k_d of NDF was significantly lower on 60SPR than on 100IM ($P < 0.05$). No differences in clearance rate after 13 hours of fasting among the different treatments were observed.

Table 5. The effect of treatment on urine production and uric acid. Abbreviations of the treatments as in Table 1, BW = body weight.

Diet	100IM	20SPP	60SPP	60SPR	s.e.d.
Urine production (kg d^{-1})	37.1[a]	35.2[a]	26.7[b]	23.8[b]	2.27
Uric acid (mg kg^{-1} $BW^{3/4}$)	22.7[a]	15.8[b]	13.9[b]	14.7[b]	2.77

[a,b] Within a row, means not sharing common superscripts differ ($P < 0.05$). s.e.d. = standard error of difference.

Table 6. Average pool size of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and cellulose-indigestible acid detergent fibre (IADF). Abbreviations of the treatments as in Table 1.

	100IM	20SPP	60SPP	60SPR	s.e.d.
Rumen pool size (kg)					
DM	13.9	14.2	14.0	13.8	0.50
OM	12.3	12.7	12.5	12.3	0.47
NDF	6.1[a]	6.7[ab]	7.0[b]	7.1[b]	0.31
IADF	1.2[a]	1.5[a]	1.8[ab]	2.4[b]	0.30

[a,b,c] Within a row, means not sharing common superscripts differ ($P<0.05$). s.e.d. = standard error of difference.

Table 7. Passage rate of indigestible acid detergent fibre (IADF) and turnover of organic matter and neutral detergent fibre (NDF), calculated from the rumen evacuation data. k_p is passage rate, k_i is rate of intake, k_d is rate of degradation (calculated from k_i and k_p) and k_c is clearance rate after 13 hours of fasting (rates in % h^{-1}). Abbreviations of the treatments as in Table 1.

	100IM	20SPP	60SPP	60SPR	s.e.d.
IADF					
k_p [1]	2.7	2.3	2.5	2.8	0.50
Organic matter					
k_{iOM}	5.7[a]	5.4[ab]	5.1[b]	5.5[ab]	0.22
k_{dOM}	3.0	3.0	2.7	2.7	0.33
k_{cOM}	5.7	5.2	5.2	5.0	0.52
NDF					
k_{iNDF}	6.1[a]	5.5[b]	5.1[b]	5.0[b]	0.26
k_{dNDF}	3.4[a]	3.2[ab]	2.6[ab]	2.3[b]	0.40
k_{cNDF}	6.3	5.7	5.0	4.7	0.66
Fluid (Co-EDTA)					
k_c	12.8	12.4	11.2	12.5	0.65

[a,b] Means with a different superscript are significantly different ($P < 0.05$). s.e.d. = standard error of difference.

[1]: Calculated as $k_p = k_i$ IADF = (intake IADF / rumen IADF content I / 24h)* 100%

$k_d = k_i - k_p$, k_p used in this table based on intake.

k_c = 100* [ln (Co-EDTA in rumen fluid at t=20) − ln (Co-EDTA in rumen fluid at t=9)] / 13

To calculate the clearance rate of particles, a model as presented in Figure 3 was used. Before as well as after fasting, the highest proportion of large particles was observed on 60SPR and the lowest proportion for 20SPP (before fasting) and 60SPP (after fasting) ($P < 0.05$; Table 8). Rumen clearance rate of the particles > 2.5 mm was highest on 60SPP and lowest on 60SPR ($P < 0.05$), but clearance rate of particles < 2.5 mm was relatively low on 60SPP and relatively high on 60SPR (differences statistically not significant).

Discussion

To obtain insight in rumen kinetics and rumen fermentation patterns of forages from semi-natural grasslands, four diets with differing proportions of forages semi-natural grasslands in the diet were fed to lactating dairy cows fitted with a rumen cannula. Compared to diets consisting solely of intensively managed grass and concentrates, the inclusion of forages from semi-natural grasslands in the diet was expected to result in differences in rumen fermentation and kinetics because of a reduction of degradation rate, passage rate and nitrogen surplus in the rumen. This discussion consists of four sections. Firstly the intake of the different diets is discussed, as intake determines rumen fermentation processes and flows of digesta. Secondly the rumen fermentation patterns are discussed, subsequently the kinetics of nutrients and particles in the rumen are examined, and finally conclusions are drawn about the behaviour of forages from semi-natural grasslands in the rumen of dairy cows.

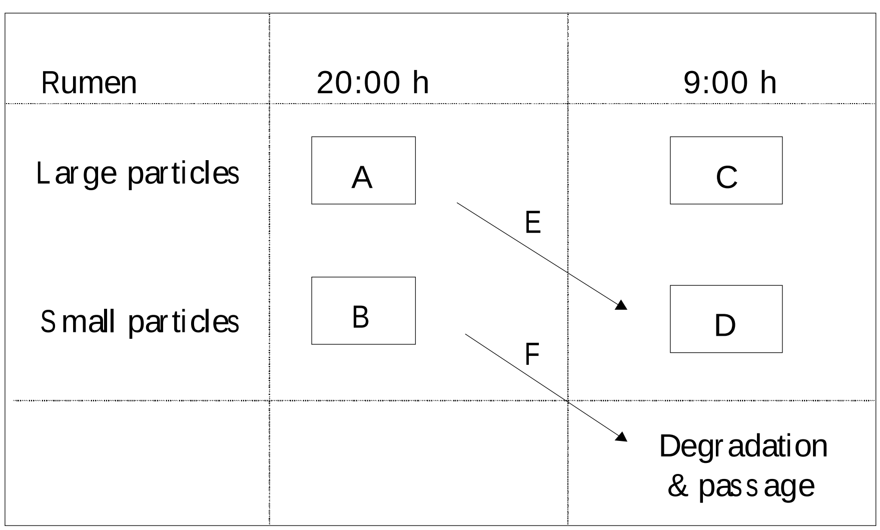

Figure 3. Pools and flows of particles in the rumen between 20:00 and 9:00 h (fasting period). A and B indicate the pool of large and small particles before fasting, C and D indicate this pool after fasting, and E and F indicate the flow from both pools during the fasting period. The values of A, B, C, D and F are shown in Table 8.

Intake

On 60SPP, intake was lower than on 100IM, which confirmed earlier results (Bruinenberg *et al*., 2003a). This reduction was attributed to the low degradation rate of SPP as has been observed *in vitro*, using the gas production technique, as well as *in situ*, using the nylon bag technique (Bruinenberg *et al*., 2003b). Compared to 60SPP, the intake of 60SPR was relatively high, which was attributed to the presence of herbs, having higher *in vitro* and *in situ* degradation rates than grasses (Lopez *et al*., 1991; Bruinenberg *et al*., 2003b). Rumen pool sizes and *in vivo* degradation and their relationships with intake will be discussed later in this chapter.

Fermentation characteristics

No relationships were found between rumen pH and any of the intakes of chemical components. On 60SPR, rumen pH was higher than was expected from OM or sugar intake, or even from VFA concentrations in the rumen. The reason for this high pH was not found. The relatively higher pH was probably caused by some nutritional factor that was not analysed, such as concentration of tannins, which can be present in herbs (Mertens, 1998; Rezvani Moghaddam & Wilman, 1998; Scehovic, 2000), and thus in 60SPR. The presence of tannins may increase saliva production (Van Soest, 1994), which results in the adding of more buffer into the rumen, and thus in a higher pH.

As expected, the pH and the VFA concentration were inversely correlated (R^2 = 0.70) and the highest VFA concentrations were observed on 100IM and 20SPP, reflecting the

Table 8. Distribution of particle size in the ingested feed and in the rumen and rumen clearance rate of the different sizes particles. Distribution in the rumen before (t=20) and after fasting (t=9). Abbreviations of the treatments as in Table 1.

Diet	100IM	20SPP	60SPP	60SPR	s.e.d.
Rumen pool size (before fasting)					
> 2.5 (A)	5.2[ab]	4.3[b]	4.6[ab]	5.8[a]	0.53
< 2.5 (B)	10.6	11.0	11.4	10.4	0.60
Rumen pool size (after fasting)					
> 2.5 (C)	2.0[ab]	1.9[ab]	1.4[b]	2.9[a]	0.43
< 2.5 (D)	5.6	5.9	7.0	5.8	0.75
Rumen clearance rate of different sized particles					
> 2.5 mm (E)	7.3[ab]	5.6[ab]	9.3[a]	5.3[b]	1.6
< 2.5 mm (F)	5.0	4.7	4.0	5.0	0.9

[a,b] Figures with a different superscript are significantly different (P < 0.05). s.e.d. = standard error of difference.
(A…F) are pools and flows as shown in Figure 3.

relatively high *in vitro* digestibility. The VFA concentration on 100IM was comparable to values in the literature (e.g. Bosch *et al.*, 1992a; De Visser *et al.*, 1993). As expected, the relationship between FOM and VFA was approximately linear. The relationship between CP and VFA concentration was even better, which was attributed to the relationship between protein concentration of the silages and their OM degradability.

Cell walls in the diet are known to favour acetate over propionate and butyrate (Miller, 1979; Bannink *et al.*, 2000), but no differences were observed between diets. Also the proportion of propionate was rather consistent over the diets in this study. The proportion of butyrate was significantly higher on 60SPP, but the difference was small.

On all diets a decrease in pH was observed after feeding, with a slight increase in pH several hours later (Figure 1). However, after feeding the pH started to increase earlier on 60SPR than on the other diets. This was caused by the lower production of VFA's on this diet, because of lower supply of available fermentable material, and thus a more rapid exhaustion of degradable energy in the rumen. As mentioned earlier, perhaps also an increase in saliva production played a role here. The pH pattern on 60SPP and 60SPR had a different form than on 100IM and 20SPP. This could not be attributed to differences in intake pattern, because intake patterns were not observed to be different among diets.

The NH_3 concentration in the rumen increased with higher CP intake and with higher ratio CP intake / OM intake. After feeding, the NH_3 concentration in the rumen instantly increased, and within two hours after feeding the NH_3 concentration decreased. This indicates that degradation of protein from the diet occurs directly after feeding. The decline in NH_3 concentration occurred faster after feeding on SPR60 than on the other treatments. This is attributed to the low CP concentration and to a low rumen availability of CP. Part of the protein may be intertwined with lignin or other cell wall material (Iiyama *et al.*, 1993), and SPP and SPR contained larger proportions of lignin and cell walls than IM (Table 1; Bruinenberg *et al.*, 2003b). Just before feeding, the NH_3 concentration on 60SPR was relatively low compared to the other diets, but concentrations (> 2 mmol l^{-1}) were probably still sufficient for efficient microbial degradation and protein synthesis in the rumen. An inhibited microbial protein synthesis would be shown in a reduced uric acid excretion in the urine, as uric acid is an indicator of the production of microbial protein in the rumen (Johnson *et al*., 1998; Chen *et al*., 1992). Indeed, a reduction in uric acid excretion in the urine was not observed (Table 5).

The quantity of uric acids in the urine is usually influenced by FOM intake. This was also observed in this study, although the quantity of uric acids on 20SPP was low, compared to uric acids excreted on 100IM, 60SPP and 60SPR. The low quantity of uric acids on 20SPP could not satisfactorily be explained by the available feed and intake characteristics. Maybe attachment of microbes to large forage particles delayed the passage rate of the microbes, thus increasing recycling of energy and N in the rumen and thereby causing a larger quantity of energy and nitrogen to be used for maintenance of the microbial population rather than for growth (Clark *et al*., 1992). Indeed, the k_p was observed to be lower on 20SPP than on the other treatments, although differences were not significant (Table 7).

Rumen kinetics

Pool sizes. The NDF pool size was in accordance with NDF pool sizes observed by Bosch *et al.* (1992b). The NDF pool in the rumen is probably a factor limiting intake (De Visser *et al.*, 1998). A lower CP intake resulted in a larger NDF pool. This is due to a decrease in CP concentration with advancing stage of maturity (Beever *et al.*, 2000), resulting in a positive relationship between CP concentration (and thus between CP intake) and degradability of NDF. Intake of NDF had no effect on the NDF pool, as the insignificant decrease in NDF intake for the four diets (Table 3) was accompanied with significantly increased NDF pools (Table 6).

Nutrient flows. In this study IADF is used to determine the k_p. The use of IADF as marker is also described by Tamminga *et al.* (1989). The k_p's of IADF on the different treatments were calculated to be between 2.3 and 2.8% h^{-1} (Table 7). Passage rates of OM in rumen and duodenum fistulated cows fed fresh grass have been observed to be between 3.2 and 5.1% h^{-1} (Van Vuuren *et al.*, 1993), and passage rates for OM were higher than the passage rates for NDF (Van Vuuren *et al.*, 1993). Therefore, it is likely that k_p differs among nutrients and thus passage rates of OM and NDF may have been higher or lower than k_p of IADF measured in this study. Based on the *in situ* degradation of the silages (Bruinenberg *et al.*, 2003b), passage rates of OM and NDF were calculated to be lower than the k_p of IADF (k_p of OM: 1.0-1.6% h^{-1}, k_p of NDF 1.5-1.8% h^{-1}).

Because intake and chemical composition of the four diets differed, differences in k_p were expected. Indeed, differences for k_p among the four diets were observed, but difference remained statistically insignificant. Intake of DM (DMI) is often related to rumen clearance rate, which is the sum of k_d and k_p. The animal can achieve higher DMI by either an increase of k_d or k_p or both. In this experiment, for three of the four treatments, DMI was related to k_d of OM and NDF, and not to k_p. For 60SPR, DMI was higher in relationship to k_{dNDF}. In this treatment, the relatively high k_p resulted in maintaining a certain level of DMI.

Two methods were used to estimate disappearance from the rumen, i.e. the k_i and the k_c. On most diets, k_{iOM} was approximately equal to k_{cOM}, and k_{iNDF} was approximately equal to k_{cNDF}. Small differences between k_i and k_c were attributed to differences in the normal and in the fasting situation. Sometimes, in the fasting situation k_{cOM} and k_{cNDF} were smaller than k_{iOM} and k_{iNDF} (60SPR). For a better explanation of this phenomenon, more measurements during fasting should have been taken. However, this could have led to other disturbances in rumen kinetics.

The k_{iNDF} increased with higher CP intakes, due to the interrelationship of CP concentration of the silage with degradability of the NDF, and thus with rumen NDF pool, as discussed earlier. The k_{iOM} also had relationships with CP intake, but on 60SPR the k_{iOM} was higher than expected from CP intake.

Particle size. The relatively low intake of large particles on 60SPP was due to the low DM intake of the diet, which resulted in a relatively high proportion of concentrates in the diet.

No relationship was observed between k_c of large particles (k_{cL}) and NDF pool size in the rumen before fasting, and differences in k_{cL} between the different diets could therefore not be attributed to nutrients in the rumen. The high k_{cL} on 60SPP was not expected, as the diet consisted mostly of mature grasses, causing a more difficult reduction of particles. However, on tropical, less digestible grasses, more time is spent on chewing, resulting in greater breakage (Mtengeti *et al*., 1996). The vascular structure of the forage in 60SPP probably also stimulated chewing and rumination, resulting in the high k_{cL}. More time spent on mechanical reduction through rumination would also explain the relatively low intake of the diet. On 20SPP, the k_{cL} was lower than on both 100IM and 60SPP, although it was expected that 20SPP would show values between 100IM and 60SPP. The reason for this was not clear – maybe the quantity of SPP ingested was not large enough to stimulate rumination, but large enough to reduce comminution. The k_{cL} of 60SPR was low compared to 100IM and 60SPP. This was not expected, because usually fibre in dicots is more easily degraded into small particles than fibre in grasses. This may be due to junctions between cells, instead of the parallel girder system of vascular bundles found in grasses (Wilson, 1985). However, probably 60SPR does not stimulate rumination, and k_{cL} was reduced because of the high IADF pool in the rumen on 60SPR (Table 6). Plant parts containing IADF, e.g. the lignified xylem tissue (Wilson & Hatfield, 1997), are difficult to degrade, resulting in a long retention time in the rumen. The low k_{cL} on 60SPR (Table 8) is contradictory to the relatively low rumen pool size (Table 6) and high k_p (Table 7) on 60SPR. The contrasting results could be due to differences in rumen behaviour with fasting, compared to the normal situation.

The high k_{cL} on 60SPP resulted in the low k_c of small particles in this ration (k_{cS}), as reduced large particles shift towards the small particle pool, whereas the low k_{cL} on 60SPR resulted in a relatively high k_{cS}. High values of k_{cS} indicate a fast degradation or passage, but the quantity of small particles should be corrected for the flow from the large particle pool. If the pool size of small particles is corrected for the flow from the large particles, clearance rate of small particles increases, but the same trends are observed for the different treatments (k_{cS}: 100IM: 7.8, 20SPP: 7.4, 60SPP: 6.4, 60SPR: 7.8), still due to differences in comminution of the large pool.

Conclusions

Based on rumen fermentation and kinetics, it can be concluded that forages from semi-natural grasslands behave in general in the same way as intensively managed forages. The concentration of VFA and NH_3 in the rumen and the ratio between the different VFA's were not remarkably different from expectations based on FOM and CP intake. However, the k_p and k_i of herb rich forage were higher than expected, which could explain why a reduction of intake on 60SPR did not occur, despite the low digestibility and high IADF concentration. Furthermore, the rate of clearance of large particles on 60SPP was higher than expected, probably indicating more rumination activity.

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Chapter 9

General discussion

General discussion

Introduction

In temperate regions, fresh or ensiled grass is an important component in the diet of dairy cows. Often this grass is produced on grasslands with high nitrogen fertilization, which results in a high grassland productivity (Hopkins, 2000; Valk, 2002). The grass is harvested in a young and immature stage, and it thus has a high quality, i.e. high digestibility and high protein concentration (Beever *et al*., 2000). However, some research has suggested that dairy cows fed high quality grass do not reach the milk output which is predicted based on their energy intake (Beever *et al*., 1989; Beever & Reynolds, 1994; Valk *et al*., 2000).

Furthermore, recently the interest in forages from semi-natural grasslands has grown (Chapter 4), thanks to governmental regulations, which stimulate farmers to adapt their management to improve the biodiversity of the grasslands in terms of numbers and diversity of birds and plants. Also some non-governmental organizations active in the field of nature conservation try to stimulate biodiversity on grasslands that they have purchased.

Semi-natural grasslands managed for nature conservation have to comply with certain management rules defined by legislation (Korevaar, 1986). Therefore, they often do not receive fertilizer, and are harvested later in the year. These measures result in a more advanced stage of maturity, and thus a lower quality at cutting, causing lower digestibility and lower protein concentration (Tallowin & Jefferson, 1999). Moreover, the botanical composition is often more diverse (Korevaar & Van der Wel, 1997; Jefferson & Robertson, 2000), and often forage species are found which usually do not occur in intensively managed production grasslands. The combination of the late harvesting and the diverse botanical composition in semi-natural grasslands complicates the estimation of the nutritive value of the forage (Chapter 4). Farmers are therefore reluctant to use the forages for their dairy cows. Probably, if more is known about the nutritive value of forages from semi-natural grasslands, farmers will be less reluctant to integrate this type of forage in the diets dairy cows, and this will stimulate the use and development of those types of grasslands.

Thus, there were two objectives in this study, which will be discussed in this general discussion. The general discussion is split into two parts. Firstly, the proper prediction of nutritive value of forages from intensively managed grasslands and the use of these forages are discussed. Specific objectives were to investigate the energy value of intensively managed grass and to analyse why milk production is lower than predicted by the current feeding value formulae when cows are mainly fed with highly digestible grass. Secondly, the emphasis is put on the prediction of nutritive value of extensively managed grasslands. Specific objectives were to investigate the nutritive value of forages from semi-natural grasslands, and to obtain insight in possibilities to integrate those forages in the diet of highly productive dairy cows.

The discussion will include evaluation systems, such as the metabolizable energy (ME) system and the protein evaluation system (true protein digested in the small intestine; DVE) for these types of grass (supply and requirements), but also nutritional characteristics, such as fermentation and degradation of the forages in the rumen and their relation to voluntary intake. Finally, some guidelines for the use of forages in diets of highly productive dairy cows are given.

The feed evaluation of intensively managed grass

Based on twelve different performance trials, it was found that in grass-fed dairy cows the energy *input* was usually higher than the energy *output* (Chapter 2). The treatments in the trials were divided into two different sets of treatments, one with proportions of grass varying between 40 and 65%, and one with the proportion of grass varying between 80 and 90%. It was observed that in those two sets of treatments different factors caused the discrepancy between energy input and output (Chapter 2). Results were sometimes even opposite between those sets: e.g. an increase in crude fibre concentration in the grass increased the overestimation of the milk production in the 40-65% grass set of treatments, whereas it decreased the overestimation in the 80-90% grass set of treatments. Therefore, to explain the observed discrepancy between input and output, those sets of treatments should be discussed separately. In this general discussion, only the diets with more than 80% grass are discussed, because those diets can be discussed more straightforward than diets also including other feed components than grass and concentrates.

In Chapter 3, the metabolizable energy intake (MEI) was calculated from the gross energy in feed, faeces, urine and produced methane. The MEI was thus not calculated based on chemical components of the diet, and therefore, emphasis was put on the utilization of energy in the cow's body. Diets consisted for 91-100% of fresh grass. Also in this study, discrepancies between the net energy (NE) input and the NE output were observed. From Chapters 2 and 3 it was concluded that the energy requirements for maintenance may be underestimated on grass-based diets (Chapter 3), and that the composition of the diet and the composition of the grass affect the energy metabolism (Chapter 2).

Diets with 80-100% grass

Increased maintenance requirements on diets with 80-100% grass compared to maintenance requirements on more balanced diets may be caused by several factors, e.g. increased costs for nitrogen (N) excretion (Tyrrell *et al*., 1970), or more energy required for chewing and rumination of grass (Susenbeth *et al*., 1998). Furthermore, extensive proteolysis or deamination in the rumen may cause a reduced quantity of protein absorbed in the small intestine (Beever *et al*., 1989). Another factor that might play a role is composition of the grass and the lack of diversity in a diet containing mostly intensively managed grass (*Lolium perenne* with a high digestibility). This lack of diversity could cause a suboptimal supply of nutrients in the rumen, due to an imbalance of N and C, resulting in a reduced efficiency of utilization in the rumen (Clark *et al*., 1992). Increased maintenance requirements on grass-based diets are therefore connected to the diet fed to the animals.

The high protein concentration in grass results in high nitrogen (N) surpluses in the body and thus in high excretions in the urine. Martin & Blaxter (1965) calculated that the synthesis and excretion of urea in sheep requires 13.1 kJ NE g^{-1} excreted N. Metabolized protein and

NH_3 transferred through the rumen wall will finally be excreted in the urine or in the milk. With regard to the excess of rumen N, the degraded protein balance (OEB; Tamminga *et al.*, 1994) could be a base for the calculation of the energy costs for N excretion. On average OEB in the grass was 40 g kg^{-1} DM (Table 1), i.e. a surplus of N of 6.4 g. Costs of excretion are then 84 kJ NE per kg of grass, which is approximately 1.3% of the average NE value (6540 kJ kg^{-1} DM) of grass.

Three problems occur with this calculation:

1. A positive OEB may be corrected by feeding supplements with a negative OEB, or a low OEB. It is therefore not justified to correct the NE value of grass with its OEB value, but the total diet should be taken into account.
2. The requirements for synthesis of urea from NH_3 are probably higher (18 instead of 13.1 kJ NE g^{-1} N; Tyrrell *et al.*, 1970) than calculated by Martin & Blaxter (1965), which was attributed to recycling of urea in the gastro-intestinal tract.
3. In fact, all N digested in the gastrointestinal tract will ultimately be excreted in milk or urine. Therefore, the OEB is not a correct measure for the actual costs of N excretion.

The amount of protein excreted in faeces (the difference between CP and digestible CP (DCP)) is not of importance for insight in energy requirements for N excretion, although it should be kept in mind that also some endogenous protein is found in the faeces. The DCP can be allocated to milk, maintenance or pregnancy and surpluses. Protein in the body is not utilized with 100% efficiency, and discarded protein will finally be excreted in the urine, together with the surpluses. The main part of the DCP is thus excreted in the urine. The average DCP concentration of grass was 151 g kg^{-1} (Table 1, based on Chapter 2). One kg of milk contains 34 g of protein (Table 1; average as calculated in Chapter 2), i.e. 5.3 g of nitrogen. Theoretically, based on the NE content, 1 kg of grass (NE value 6540 kJ; Table 1) can be converted into 2.14 kg of milk, and thus 73 g of milk protein, or 11.4 g of nitrogen will be excreted in the milk. The N excretion in the urine is then 12.8 g (151/6.25 – 11.4 g). The excretion of 1 g N requires 18 kJ NE (Tyrrell *et al.*, 1970), and thus the energy requirements for the excretion of surplus N would be 230 kJ per kg of grass used for milk, 3.5% of the NE value. In Chapter 3, it was suggested to add those requirements for nitrogen to the maintenance requirements of dairy cows fed on grass. An alternative method is to link the amount of nitrogen that has to be excreted in the urine to the NE value of grass, with the assumption of an efficiency of 60% to convert ME into NE.

Table 1. The average composition and the range of grass and the protein excretion in milk in the 80-90% grass groups as used in Chapter 2.

	CP	DCP	DVE	OEB	NE	Protein g kg^{-1} milk
Mean	195	151	93	40	6540	34
Minimum	134	93	78	−9	5820	31
Maximum	281	241	106	110	7090	37

CP is crude protein, DCP is digestible CP, DVE is true protein digested in small intestine, OEB is degraded protein balance, NE is net energy. CP, DCP, DVE and OEB in g kg^{-1} DM, NE in kJ kg^{-1} DM.

The ME formulae of grass would then be corrected for DCP concentration, e.g.

$$ME = 14.2*DOM + 5.9*DCP * \{1- [30*(\text{digestible N}-11)]/(14.2*DOM+5.9*DCP)\}$$

where 30 is the requirement for the excretion of 1 g N in kJ ME, digestible N is calculated as DCP/6.25, 11 is the N excreted in the milk, based on 2.1 kg of milk with a protein concentration of 3.35%, produced from 1 kg of grass. The effect of the correction formula is shown in Figure 1. In this figure, the DOM concentration was fixed on 712g kg^{-1} DM, the average in Chapter 2.

Another disadvantage of the high N concentration of grasses is the extensive proteolysis in the rumen (Beever *et al*., 1989). With high quality forages containing substantial proportions of N, gross oversupply of ammonia in relation to microbial requirements may occur (Beever & Reynolds, 1994), resulting in impaired rumen function due to imbalanced supply of nitrogen and carbohydrates. The amount of microbial protein in the small intestine may then be lower than expected. Furthermore, heifers fed on 75% concentrates have been observed to have greater tissue N retention than heifers fed 75% alfalfa silage (Reynolds *et al*., 1991). This could possibly be linked to the extensive proteolysis of protein-rich forages in the rumen.

Another factor affecting the requirements of dairy cows on grass-based diets, is the energy connected with intake and digestion. For high quality grass, Susenbeth *et al*. (1998) observed requirements for chewing and rumination to be 10% of the ME value of the grass. Furthermore, silage-based diets are normally associated with larger gastro-intestinal tracts (Agnew *et al*., 1998), which is probably linked to the large bulk volume of digesta transferred throughout the gastrointestinal tract on grass-based diets. The maintenance of the larger gastrointestinal tract, and the large bulk volume require energy. It is difficult to validate these requirements, but it may be expected that forage-based diets result in higher maintenance requirements for digestion than diets containing more concentrates or other feeds. Reynolds *et al*. (1991) observed higher metabolic activity of visceral tissues on diets with 75% alfalfa silage compared to diets with 75% concentrates. This was attributed to differences in rumination and digesta movement.

Although rumination and digestion require energy, the ability to digest forages is an essential characteristic of the ruminant, and ruminants need certain amounts of fibre in their diet to maintain an optimal rumen environment (Mertens, 1997). Too little fibre in the diet leads to decreased chewing activity, which leads to less salivary buffer excretion and thus lower pH and altered rumen fermentation (Mertens, 1997). To examine whether dairy cows consume enough fibre in their diets, the structure index (SI) has been developed (CVB, 2001a). For fresh grass the SI is calculated as

$$SI = 1.70+0.01*(CF-210)$$

In this formula, CF is crude fibre in g kg^{-1} DM.

The average SI of the total diet should at least be1 (CVB, 2001b). For all treatments used in the dataset the SI of the diet was sufficient, being 1.6 on average. As most grasses were highly

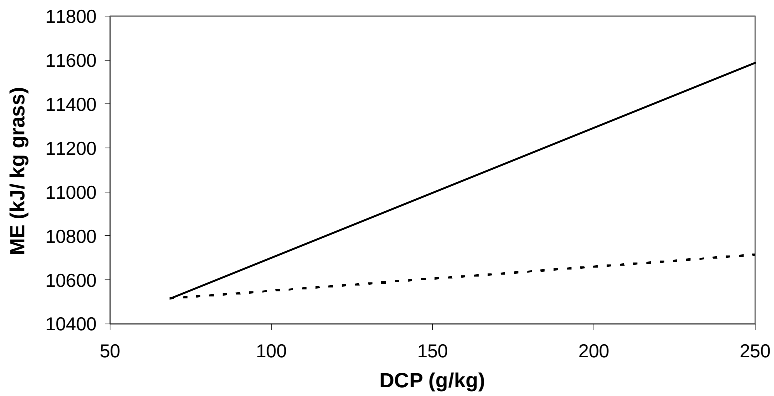

Figure 1. The effect of the correction formula on ME concentration (DOM concentration fixed on 712 g kg^{-1}, the average in Chapter 2). Solid line is regular equation, dotted line is equation corrected for crude protein.

digestible, it could be questioned whether the fibre in the diet was effective. Milk fat concentration and production can indicate if enough effective fibre is present in the diet (Mertens, 1997). As no milk fat depression was observed on the diets used (on average 45 g milk fat kg^{-1} milk), presumably effective fibre was sufficient.

Thus, theoretically, the rations with high quality grass contained enough fibre. However, in Chapter 2, lower discrepancies were observed for grasses with higher fibre concentrations. Therefore, there could be a small effect. Furthermore, high DOM concentrations coincided with an overestimation of milk production, and DOM is usually negatively correlated to CF. It is expected that high DOM and low CF in the forage lead to high passage and degradation rates in the rumen, which reduce the efficiency of fermentation. If high quality grass is fed, maybe supplementation with roughage of a lower quality would stimulate rumination and contractions of the rumen, and thus would increase utilization of nutrients in the rumen.

The cultivation of grass with a lower digestibility will result in higher fibre concentrations, thus would stimulate rumination, and lower CP concentration, thus reducing nutrients imbalance in the rumen and energy costs for nitrogen excretion. However, high maintenance requirements for grass-based diets would remain, as more fibre in the diet would still result in higher requirements for digestion (Susenbeth *et al*. 1998). In the meantime, a correction for high CP contents in the ME formulae would probably improve the estimation of the ME value of grasses.

Consequences for diets with high amount of grass

In Chapter 7, it was observed that the results of the simplified formula to estimate the ME value of intensively managed grass was similar to results of estimations of the ME value with the formulae, based on DCP, digestible CF, digestible crude fat and digestible nitrogen-free extract, that is also used for other feeds. The simplified formula even resulted in somewhat lower values, but this is not disturbing, as the milk production is already overestimated (Valk *et al*., 2000). If a correction factor for a surplus of CP is added to the formula, the difference between the common ME formula and the simplified formula increases, but the difference between the expected and the actual milk output will decrease. Therefore, it would be advisable to insert a correction factor in the formula to estimate the ME value of high quality grass, which can be used independently of the proportion of grass in the diet.

In Chapter 3, it was suggested to increase the maintenance requirements of dairy cows by 10% mainly to correct for the energy required for nitrogen excretion. As we suggested here to correct the estimation of the ME value for the energy costs for the excretion of the estimated nitrogen surplus, this maintenance correction does not seem to be necessary. However, energy required for intake, rumination and digestion, and the reduction of efficiency of energy utilization because of the imbalance of nutrients on the rumen and the use of amino acids for energy also have to be taken into account. Therefore, an increase of maintenance requirements of 10% on diets with 80-100% grass would still be a sensible method to better estimate the milk production of dairy cows on grass based diets. When both corrections were applied on our dataset, the discrepancy decreased, although it did not completely disappear (Figure 2). This was attributed to weight gain of animals in the experiments of the dataset.

Too high N concentrations in forage should be avoided. When such forages are produced anyway, it would be best to feed such forages in combination with forages or feeds with a low protein concentration, such as maize silage. Also the inclusion of straw or forages from semi-natural grasslands would have a positive effect by increasing the ingested fibre and decreasing the ingested N.

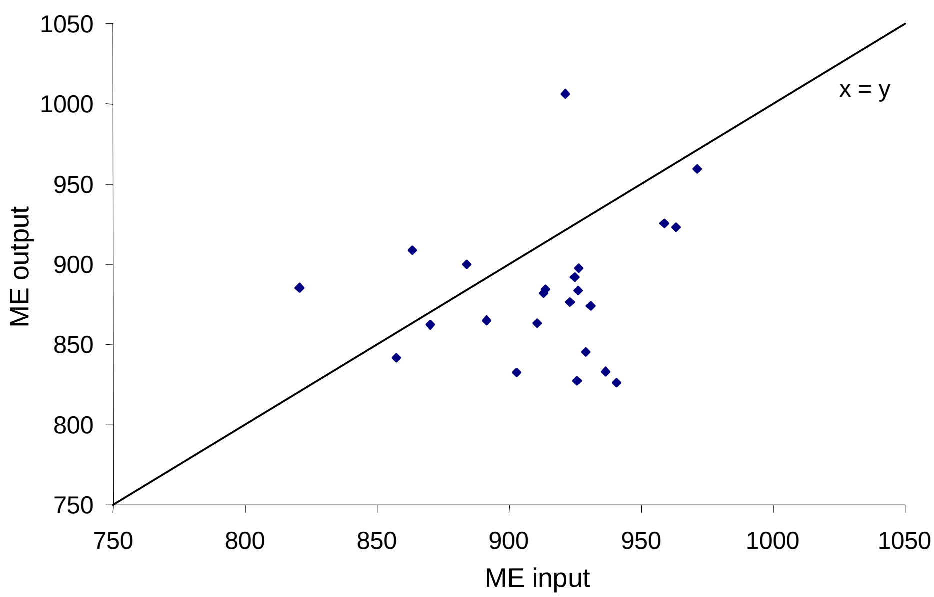

Figure 2. The relationship between the corrected metabolizable energy input and the corrected metabolizable energy output on grass-based diets.

The nutritional value of forages from semi-natural grasslands

Forages from semi-natural grasslands are usually characterized by a variety of stages of maturity and by a diversity of forages species. As was discussed in Chapter 4, an advanced stage of maturity and a diversity of forage species often results in low digestibility and degradability of the forages, and furthermore, intake may be reduced. However, dicotyledonous forage species may have a higher digestibility than grasses harvested on the same date (Peeters & Janssens, 1998), and for some dicotyledonous species intake may be higher than expected due to the rapid degradation (Derrick *et al*., 1993). In this part of the general discussion observations about the voluntary intake are discussed, in combination with degradation (*in situ* and *in vitro*) and rumen kinetics (*in vivo*). Then the Dutch protein evaluation system is applied to indicate the availability of protein in the rumen, followed by the *in vivo* fermentation characteristics and the consequences for the availability of energy when forages from semi-natural grasslands are included in the diet. Subsequently, the digestibility (*in vivo* and *in vitro*) and the ME value of the different forages are discussed and related to the potential milk production and energy requirements of the animals on diets, including forages from semi-natural grasslands. Finally, conclusions are drawn about the integration and use of forages from semi-natural grasslands in the diets of dairy cows. Throughout the discussion, it should be kept in mind that only two forages from semi-natural grasslands were investigated, which were harvested on a specific date from a specific location. This may raise the question whether the forages are representative for forages from semi-natural grasslands.

Voluntary intake and rumen kinetics

In Chapter 6, a low *in vitro* digestibility of the species rich silage (SPR) was observed, but the intake was higher than of the species poor silage (SPP), although not statistically significantly so. Also in the other experiments (Chapters 7 and 8) intake of the SPR was higher than that of SPP. The average intakes of the different diets are shown in Table 2.

Thus, diets with a large proportion of SPP were ingested in lower quantities than diets containing large proportions of intensively managed grass silage (IM) and SPR (Table 2). This was probably due to reduced passage or degradation rates (Bowman *et al*., 1991; Carro *et al*., 1991; Nandra *et al*., 1993). In Chapter 5, the *in situ* degradation and the *in vitro* gas production rates are described, and in Chapter 8, some information is given on the *in vivo* degradation and passage rate. Although the values of rate of *in situ* OM degradation (k_{dOM}) and maximal rate of gas production of the cell wall fraction (R_m) of the silages are not absolutely comparable, they both give an indication of rate of degradation. For k_{dOM} as well as for R_m, the highest values were found for IM and the lowest for SPP, with intermediate values for SPR (Table 3). The k_d and R_m of SPR were thus higher than that of SPP. The high degradation rate of SPR was attributed to the presence of dicotyledonous species (legumes + herbs was 47% of botanical composition on SPR; Chapter 6), such as *Ranunculus acris*, *Crepis biennis* or *Anthriscus sylvestris*, which had high gas production rates (Chapter 5). The high k_d of dicotyledonous species (Chapter 5; Lopez *et al*., 1991) is attributed to their anatomical structure, i.e. a reticulate venation, resulting in relatively little vascular tissue per

Table 2. The intake of dry matter (DMI), organic matter (OMI), crude protein (CPI), NDF (NDFI) and DOM (DOMI) and the average *in vitro* OM digestibility (d_{OM}) of the different diets. All values in kg d^{-1}, except for the grass silage and d_{OM}: those are in proportions.

Data in	Diet	grass silage	DMI	OMI	CPI	NDFI	DOMI	d_{OM}
Chapter 6*	100IM	0.55	21.3	19.4	3.8	9.3	14.6	0.75
	20SPP	0.56	21.4	19.5	3.7	9.5	14.2	0.73
	40SPP	0.56	21.1	19.2	3.9	9.1	13.8	0.72
	60SPP	0.56	19.9	18.2	3.5	8.7	12.9	0.71
	60SPR	0.55	21.3	19.4	3.6	9.0	13.6	0.70
Chapter 7	IM	0.75	17.3	15.4	3.5	8.2	12.0	0.78
	SPP	0.74	16.3	14.8	2.6	8.8	9.5	0.64
	SPR	0.75	17.5	15.8	2.4	8.8	9.6	0.61
Chapter 8	100IM	0.76	18.3	16.3	3.7	8.6	12.7	0.78
	20SPP	0.75	18.1	16.1	3.6	8.7	12.1	0.75
	60SPP	0.73	16.7	15.0	3.0	8.3	10.4	0.69
	60SPR	0.74	17.3	15.5	2.9	8.4	10.5	0.68

100IM in Chapter 6 is intensively managed grass silage, concentrates and maize silage, 20 / 40 / 60SPP in Chapter 6 is as 100IM, but 20 / 40 / 60% of intensively managed silage replaced by species poor silage, and 60SPR in Chapter 6 is as 100IM, but 60% of intensively managed grass replaced by species rich silage. IM is intensively managed grass silage and concentrates, SPP is species poor silage and concentrates, SPR is species rich silage and concentrates. 100IM, 20SPP, 60SPP and 60SPR in Chapter 8 are as in the treatments from Chapter 6, but without maize silage.

* The NDF concentration and digestibility of the concentrates fed in the milking parlour are estimated to be 300 g kg^{-1} and 75%. In Chapters 6 and 8, intake was restricted, although not all animals ingested all the offered silage. In Chapter 7 intake was *ad libitum*.

unit volume, and many junctions between short veins, causing an easy fragmentation of fibre into small particles (Wilson, 1985).

The advanced stage of maturity of SPP resulted in low digestible grass with long vascular bundles (Wilson, 1985), and thus in the relatively low k_d and R_m. This type of grass requires more time spent on chewing and rumination (Chapter 8; Mtengeti *et al*., 1996), as cell wall content of the diet is highly correlated with rumination time (Bosch *et al*., 1992). Cell wall concentration of SPP was high (Chapters 5, 6, 7 and 8). Since there is a limitation in the maximum time spent on chewing (Van Vuuren, 1993), more time required for chewing and rumination will decrease intake (Bosch *et al*., 1992), explaining the lower intake on diets with large proportions of SPP.

In vivo, no differences in rate of degradation were observed (Table 4), but effects of the silages were diluted, as they were fed in a mixture with intensively managed silage and concentrates. However, the passage rates for 20SPP and 60SPP were lower than for the other diets. The low k_i and k_p on 60SPP explain the reduced intake on diets with SPP. Intake was not reduced for SPR. For sheep fed *Spergula arvensis*, Derrick *et al*. (1993) observed a high intake, which was attributed to the physical construction of the dicotyledonous species. The high intake was combined with a high fibre excretion in faeces, suggesting a fast breakage and a quick passage out of the rumen (Derrick *et al*., 1993), before the nutrients were degraded. In Chapter 7, a high fibre excretion was also observed on SPR, suggesting some similarities of the diet with *Spergula arvensis*. The high IADF and NDF in the rumen (Chapter 8) seem contradictory to this hypothesis. However, not all cell walls will be turned over quickly, as highly lignified stems will be difficult to reduce to small particles and thus remain in the rumen for a longer period. The degradation and rumen clearance rates of NDF were relatively low on 60SPR (Chapter 8).

The *in vivo* degradation rate is a combination of the different diet components. Degradation rate of concentrates is expected to be high, and, therefore, *in vivo* degradation rate should be higher than if only based on the combination of silages (Table 4). However, the calculated *in situ* k_d of the mixed silages was higher than the *in vivo* k_d of the mixed diets (including concentrates). This is probably due to imperfections of the *in situ* technique, as incubations of feeds do not represent the real situation within the rumen (Beever *et al*., 2000). Furthermore, the method to calculate the *in vivo* k_d results in the accumulation of errors in the k_d. For example, when k_p was estimated based on the excretion of IADF in the faeces instead of k_i of IADF, the k_p was calculated to be 2.0, 1.7, 1.9 and 2.1 for 100IM, 20SPP, 60SPP and 60SPR, respectively. This resulted in a higher k_d compared to calculations based on k_i of IADF. The differences in k_p were due to a lower excretion of IADF in the faeces, compared to intake of IADF. This could be due to some degradation of IADF in the rumen, to irregular excretion of IADF in the faeces, or to the method of sampling.

Table 3. Average rates of degradation of OM or gas production rates of the cell wall fraction, as calculated from *in situ* and *in vitro* experiments. k_{dOM} is the rate of degradation of organic matter in the rumen (% h^{-1}), and R_m is the maximal relative rate of degradation of the cell wall fraction (ml g^{-1} OM h^{-1}).

	IM	SPP	SPR
k_{dOM}	4.7	2.7	3.8
R_m	18.4	12.7	15.3

Table 4. Degradation as calculated for the *in vivo* experiments. k_d silage is the rate of degradation of the silage (% h^{-1}), based on the calculations of the *in situ* trial, k_i is the rate of intake (% h^{-1}), k_p is the passage rate (% h^{-1}), and k_d is the rate of degradation (% h^{-1}), which is calculated as $k_i - k_p$. Treatments as in Table 2.

	100IM	20SPP	60SPP	60SPR
k_d silage	4.7	4.3	3.5	4.2
k_i	5.7	5.4	5.1	5.5
k_p	2.7	2.3	2.5	2.8
k_d	3.0	3.0	2.7	2.7

Energy and protein in the rumen

The rates of degradation can be used to estimate availability of energy and protein in the rumen. Important characteristics in this respect are the rumen fermentable OM (FOM), the true protein digested in the small intestine (DVE) and the rumen degradable protein balance (OEB). The FOM *in situ* (FOM, in g/kg) was calculated as

$$FOM = [W + D * (k_{dom}/(k_{dom} + k_p))] * OM/1000$$

in which W is the soluble fraction, D is the degradable fraction, k_{dOM} is the rate of OM degradation, k_p is the passage rate, which is assumed to be 4.5% h^{-1}, and OM is the organic matter fraction.

The calculation used above is different from the formula to estimate FOM based on chemical composition (Tamminga *et al.*, 1994). The FOM was highest in IM compared to SPR and SPP, and FOM was higher in SPR than in SPP ($P < 0.05$; Table 5).

To calculate the DVE, several other calculations have to be made, i.e. the proportion of rumen undegraded protein (%RUP), the intestinally digestible RUP (IDP) and the amount of RUP absorbed in the small intestine (DVBE). Based on the *in situ* measurements in Chapter 5, %RUP (in % of total protein), IDP (in % of total protein) and DVBE (in g kg^{-1}) are calculated as:

$$\%RUP = U_{CP} + (D_{CP} * k_p) / (k_p + k_{d,CP})$$
$$IDP = \%RUP - U_{CP}$$
$$DVBE = CP * (1.11 * \%RUP/100) * (IDP/100)$$

in which U_{CP} is the percentage of the undegradable CP fraction, D_{CP} is the proportion of the degradable CP fraction, and k_{dCP} is the degradability of CP in % h^{-1} (values as given in Chapter 5).

The method to estimate %RUP described above is different from calculation rules (Tamminga *et al.*, 1994; CVB, 2001a). These calculation rules are also applied to estimate differences between the measured (based on *in situ* degradation; DVE^1) and calculated DVE (DVE^2).

To calculate DVE, also the digestible microbial protein synthesised in the rumen (DVME) and DVE loss to substitute for endogenous protein losses in digestion (DVMFE) are required. The calculations of DVME and DVMFE were based on FOM and the calculation rules given by Tamminga *et al*. (1994). The digestible protein (DVE, in g kg^{-1}) was calculated as

$$DVE = DVBE + DVME - DVMFE$$

The DVE based on the *in situ* degradation (DVE^1) was highest in IM (Table 5), although it was lower than expected based on calculation rules for DVBE (Tamminga *et al.*, 1994; DVE^2, Table 5). With both calculations it is clear that the DVE of SPP and SPR are low, and these should be compensated by concentrates or protein rich grass if fed to dairy cows.

Table 5. Predicted feeding values of intensively managed grass (IM), species poor grass (SPP) and species rich grass (SPR), measured in two periods, (1) and (2). It was assumed that forages are fed in a diet with the OEB of the total diet > 0, justifying the application of the calculation formulae for DVE.

	IM1	IM2	SPP1	SPP2	SPR1	SPR2	s.e.d.
FOM	510^{a}	480^{a}	307^{c}	330^{c}	378^{b}	390^{b}	16.3
RBP	29.0^{c}	32.5^{c}	57.6^{ab}	58.1^{a}	53.1^{b}	54.5^{ab}	2.21
IDP	19.4^{ab}	20.9^{ab}	19.8^{ab}	25.3^{a}	17.7^{b}	22.0^{ab}	2.78
DVE^{1}	66.5	64.5	22.0	35.7	21.0	25.5	-
DVE^{2}	75.3	72.8	34.9	42.8	27.5	26.0	-
OEB^{1}	38.8	33.2	−11.2	−14.9	−19.0	−18.6	-
OEB^{2}	33.5	32.1	9.8	7.1	−6.3	−3.0	-

FOM is fermentable organic matter (g kg^{-1}), RBP is rumen bypass protein (%), IDP is intestinally digestible protein (% of protein fraction), DVE is digestible protein (g kg^{-1})

[a,b,c] Different superscripts in row depict significant differences ($P < 0.05$). s.e.d. = standard error of difference.

DVE^{1}: DVBE calculated according to *in situ* measurements

DVE^{2}: DVBE calculated according to CVB (2001a) and Tamminga *et al*. (1994)

OEB1: MREN calculated according to *in situ* measurements

OEB2: MREN calculated according to CVB (2001a) and Tamminga *et al*. (1994)

To calculate the OEB, the amount of microbial protein possibly synthesised in the rumen based on available nitrogen (MREN) and based on available energy (MREE) are required (Tamminga *et al*., 1994). The MREN, MREE and OEB are calculated as

$$MREN = CP * (1-1.11 * \%RUP/100)$$
$$MREE = FOM * 0.15$$
$$OEB = MREN - MREE$$

in which %RUP is the fraction of undegraded feed CP in total feed CP, as calculated from *in situ* measurements (OEB^{1}) or calculation rules (OEB^{2}). For SPP and SPR, calculations of OEB based on the *in situ* measurements were lower than those based on the calculation rules (Table 5).

From Table 5, it can be concluded that the *in situ* (and therefore probably also the actual) DVE^{1} and OEB^{1} of forages from semi-natural grasslands were mostly lower than the DVE^{2} and OEB^{2} according to calculation rules (Tamminga *et al*., 1994). This is probably due to an overestimation of the degradability of forages from semi-natural grasslands, which on its turn is attributed to an underestimation of the effect of fibre and lignin, as the forages are mostly harvested in an advanced stage of maturity. This should be taken into account if such forages are used in diets of dairy cows.

The FOM, DVE and OEB were highest on IM, and FOM was lowest on SPP, whereas DVE and OEB were lowest on SPR. In the performance trial the highest milk productions were observed on 100IM and 20SPP, which is in accordance with the FOM concentration of the feed. The lowest FOM concentrations were observed on SPP, but performance on 60SPP

Table 6. The average requirements of true protein digested in small intestine (DVE) and the supply of DVE and degraded protein balance (OEB) in the different treatments in the performance trial as presented in Chapter 6. Treatments as in Table 2. Units in g kg^{-1}, unless stated otherwise.

	100IM	20SPP	40SPP	60SPP	60SPR
Animal characteristics					
Protein yield	930	937	886	897	853
Weight (kg)	611	602	631	604	622
Requirements					
DVE milk	1467	1479	1390	1409	1333
DVE maintenance	115	115	117	115	117
DVE total	1582	1594	1507	1524	1449
Intake					
DVE intake 1	1824	1736	1876	1707	1801
DVE intake 2	1884	1806	1939	1769	1771
OEB intake 1	481	357	354	228	212
OEB intake 2	462	444	431	438	423

DVE intake 1 and OEB intake 1: based on the data of the *in situ* experiments (Chapter 5).

DVE intake 2 and OEB intake 2: as calculated in Chapter 6, according to the calculation rules.

was equal to the performance on 60SPR. This was because of the higher ME value of SPP compared to SPR. It should be kept in mind that also maize silage was fed in the performance trial, and that the diets were mixtures of different ingredients. The DVE values based on the *in situ* measurements are lower than those values based on the calculation rules (Tamminga *et al.*, 1994), but the DVE supply was still enough to fulfil requirements (Table 6). In all treatments, the OEB was positive, although for the diets containing forages from semi-natural grasslands, the OEB supply was lower if calculations were based on *in situ* measurements compared to the calculation rules described by Tamminga *et al*. (1994).

Rumen fermentation

Total volatile fatty acids (VFA) were highest on 100IM or 20SPP, and lowest on 60SPP and 60SPR (Table 7), which reflected differences in FOM intake. Calculations (based on Van Soest, 1994) also indicated that the production of VFA per kg OM was highest on IM and lowest on 60SPP and 60SPR. The VFA production was 4.5, 4.3, 3.9 and 3.9 moles per kg OM for 100IM, 20SPP, 60SPP and 60SPR, respectively. Most energy was thus available on diets with high proportions of IM. Furthermore, in the performance trial (Chapter 6), milk production was also observed to be highest on 100IM and 20SPP, compared to the other treatments, and this was attributed to the higher energy intake. The nitrogen supply was relatively low in 60SPR (Tables 2, 6). Ruminal NH_3 levels dropped below 2.15 mmol litre^{-1} just before feeding (Chapter 8), but also the VFA availability was low at this point. Still, the ratio NH_3: VFA was lower on 60SPR (0.07) than on the other treatments (0.10 or 0.11), but since no reduction in uric acid excretion in the urine was observed in this diet (Chapter 8), a shortage of nitrogen did probably not occur.

Table 7. Total rumen contents measured during rumen evacuations (Chapter 8). Treatments as in Table 2. s.e.d. = standard error of difference.

	100IM	20SPP	60SPP	60SPR	s.e.d.
Rumen content (kg)	115	115	114	106	3.9
Dry matter (kg)	14.0	14.2	13.9	13.7	0.52
Liquid (kg)	101.2	101.2	100.4	92.7	3.5
Volatile fatty acids					
Acetate (mol)	8.9^{a}	8.7^{a}	7.5^{b}	7.0^{b}	0.32
Propionate (mol)	2.2^{a}	2.2^{a}	1.9^{b}	1.7^{b}	0.07
Butyrate (mol)	1.3^{a}	1.3^{ab}	1.2^{bc}	1.1^{c}	0.04
Total volatile fatty acids (mol)	12.4^{a}	12.2^{a}	10.6^{b}	9.7^{b}	0.40
NH_3 (mol)	1.3^{a}	1.3^{a}	1.1^{a}	0.7^{b}	0.13

Differences in VFA patterns give insight in milk production, since propionate is the main precursor for glucose in the cow's body (Beever & Reynolds, 1994), and thus for milk production. Differences in the ratio NG:G between treatments were only significant for 60SPR, compared to 100IM, and there was a trend ($P < 0.10$) for a higher ratio NG:G on 60SPP. This was expected, as on diets rich in fibre, acetate and butyrate predominate (Beever & Reynolds, 1994), resulting in a higher ratio NG:G.

Energy value and energy requirements for dairy cows

In Chapter 7, the *in vivo* digestibility was approximately similar to the *in vitro* digestibility. Although this observation can be questioned, as discussed in Chapter 7, it suggests that for the forages from semi-natural grasslands the *in vitro* digestibility is an adequate predictor of the *in vivo* digestibility. Furthermore, in Chapter 7 it was observed that the ME values did not differ much between the official and the simplified formula. In that respect, the simplified ME formula can thus also be used for the forages from semi-natural grasslands, meaning that the ME value was correctly estimated in the performance trial.

However, in Chapter 6, differences were observed between expected and actual milk output, indicating discrepancies somewhere in the energy evaluation system (Table 8). For diets in which forages from semi-natural grasslands were included, the overestimation of the

Table 8. The net energy (NE) intake (NEI), the total NE output (in milk and maintenance; NEO) and the energy surplus (surplus: NEI / NEO) for the five treatments as described in Table 2 (data of Chapter 6).

	100IM	20SPP	40SPP	60SPP	60SPR
NEI	140	136	131	120	128
NEO	131	126	120	115	119
Surplus	1.07	1.08	1.09	1.04	1.08

performance of the animals was not expected, because the mixture with low quality grass was expected to increase rumen retention time, resulting in an improved digestion and absorption of the intensively managed grass silage. Therefore, an adequate prediction of the energy value was expected.

In the first part of this discussion, the discrepancy observed for diets based on high quality grass, was attributed to the high CP concentration of the forages, but in SPP and SPR the CP concentrations were low, and for those treatments discrepancies were also observed. Two explanations are given for this observation.

1) The CP intake was in all treatments substantial (Table 2), as in the diets with more than 40% of the grass silage consisting of semi-natural silage, the animals received protein-rich concentrates. Thus, in all treatments, protein supply was above requirements (Table 6), and some extra energy for nitrogen excretion would have been required. In Chapter 7, the animals on SPP and SPR had lower CP intakes (Table 2) and they produced more milk than was expected based on the energy value. However, the experiment was designed as a Latin square, and therefore, those data are not robust.

2) The amount of low quality forage was higher, and although this was expected to improve the rumen environment and degradation kinetics, low quality forages require more energy for chewing and rumination. Requirements for chewing and rumination can be up to 30% of the ME value of the forage (Susenbeth *et al*, 1998), leaving a relatively low amount of ME value for the maintenance and milk production. Therefore, the actual ME supply to the mammary gland may be lower than on the diets with higher percentages of intensively managed grass. The reason for the highest efficiency on 60SPP probably was the low intake, which probably forced cows to be more efficient with their energy.

Maybe also some other factors could have caused the discrepancy, such as an unfavourable ration between different nutrients, different time of degradation of the proteins and carbohydrates in the rumen, resulting in a suboptimal ration between the different VFA's and NH_3. Furthermore, the amino acid supply to the intestine might have been suboptimal for milk production.

In Chapter 2, high fibre concentrations in the grass were negatively correlated to the discrepancy between energy intake and energy output with the 80-90% grass diets. It would therefore be expected that a small proportion of semi-natural grass in a diet based on high quality grass, would reduce the discrepancy, and this would probably be because of the stimulation of rumination by fibre. This was also suggested by Ferris *et al*. (2000), who included straw in the diet to stimulate chewing activity, stabilize rumen environment and improve the efficiency of digestion and absorption of nutrients. However, increasing levels of straw inclusion resulted in a linear decline in milk yield (Ferris *et al*., 2000), due to the lower energy intake.

Furthermore, a decrease of DOM also resulted in a smaller error in Chapter 2, but this is usually inversely related to the fibre concentration. Furthermore, this could be positively related to CP concentration of the grass, as high CP concentrations are often related to immature grass (Beever *et al*., 2000), and thus to a high digestibility.

In Chapter 7, the excretion of N in the urine was measured or calculated. The digestible N (DN) of the total diet was calculated based on data from Chapter 7. With the assumption of a digestibility of N from concentrates of 76% (which is 8% lower than the digestibility of OM), the DN consumption from the silage component was calculated, and energy costs were allocated to each feed based on the percentage of DN intake of the total diet. Energy requirements for N excretion per kg grass were thus calculated for the 7 different treatments (Table 9). Since in Experiment 1 the urine had not been collected, the N excretion in the urine was calculated based on the average percentage of N excretion in urine from unrecovered N + N in urine from Experiment 2.

As expected, costs for nitrogen excretion in the urine per kg silage are linearly correlated to N concentration per kg silage (Figure 3). In the first part of the discussion, for high quality grass, it was calculated that 230 kJ per kg grass was used for N excretion. In the high quality diets in Table 9 (IM and 100IM), costs for nitrogen excretion on high quality grass was approximately similar. The somewhat lower value may be explained by the fact that we did not add unrecovered N to the excretion of N in the urine.

Table 9. Daily nitrogen (N) intake and N excretion and energy requirements for N excretion. CP is crude protein, DCP is digestible CP, DN is digestible nitrogen. Treatments as described in Table 2.

	IM	SPP	SPR	100IM	20SPP	60SPP	60SPR
Concentrates intake (kg)	4.3	4.3	4.3	4.5	4.5	4.5	4.5
Silage intake (kg)	11.6	10.4	11.6	13.9	13.6	12.2	12.8
CP intake (kg)	3.3	2.5	2.3	3.7	3.6	3.0	2.9
N intake (g)	528	400	368	592	576	480	464
N intake via concentrates (g)	167	167	167	178	178	178	178
N intake via silage (g)	361	233	201	414	398	302	286
DCP intake (kg)	2.3	1.5	1.3	2.6	2.5	2.0	1.9
DN intake (g)	368	240	208	416	400	320	304
DN intake via concentrates (g)	127	127	127	135	135	135	135
DN intake via silage (g)	241	113	81	281	265	185	169
N digestibility of silage	0.67	0.48	0.40	0.68	0.67	0.61	0.59
DN from silage (%)	0.65	0.47	0.39	0.68	0.66	0.58	0.56
N excretion in urine (g)	211	127	95	234	222	195	152
Energy costs (kJ NE)	3798	2286	1710	4212	3996	3510	2736
DN in urine (%)	0.57	0.53	0.46	0.56	0.56	0.61	0.75
Energy costs attributed to silage	2469	1074	667	2864	2637	2036	1532
Energy costs (kJ NE) / kg silage intake	213	103	58	206	194	167	120

Intake was restricted.

Digestibility of CP from concentrates was estimated to be 8% lower than digestibility of OM for concentrates, thus on average 76%. Digestibility of CP is assumed to be similar for both types of concentrates.

Digestible N of silage calculated from total DN intake and estimated DN of concentrates.

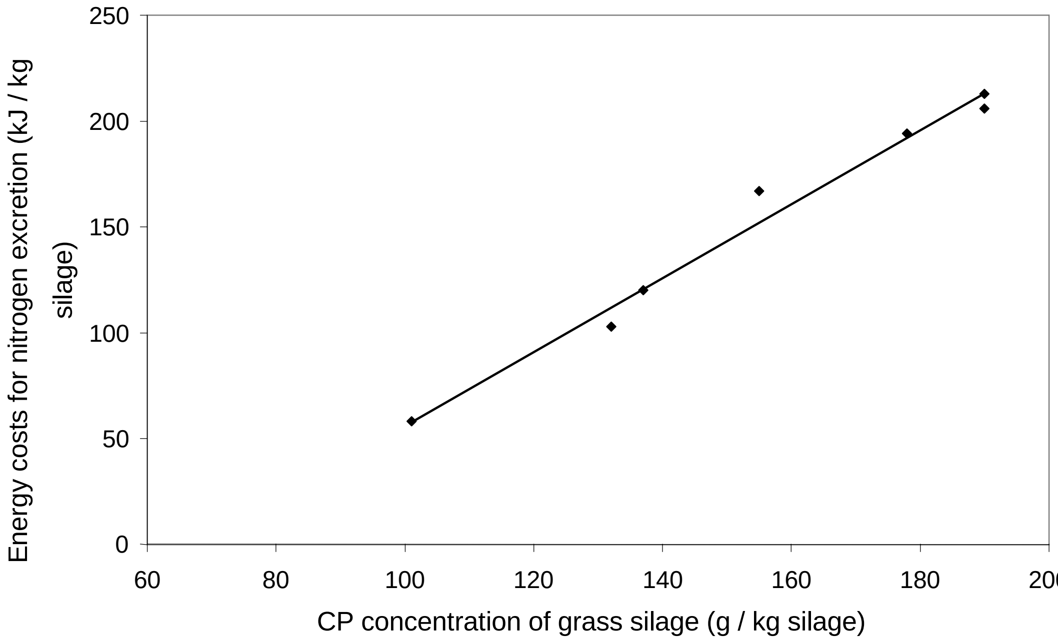

Figure 3. The relationship between the crude protein (CP) concentration and the energy costs for nitrogen excretion in the urine.

For the low quality grass diets (SPP and SPR), requirements for N excretion were lower than for IM and 100IM. As regression formulae to estimate the DCP concentration of grasses are not applicable for grass with a diverse botanical composition (CVB, 2001a), it would be difficult to use a correction factor for N excretion for those types of grass. In this thesis only two types of forages from semi-natural grasslands are discussed, and the forages were each cut on one specific date. Therefore, it is not possible to develop a regression formula to estimate DCP, which has a broad applicability, i.e. can be used for several types of forages from semi-natural grassland. More research is needed for such a formula. In the specific case investigated here, it would be possible to incorporate a correction factor for the excretion of nitrogen, but the impact is low: a reduction of the ME value of 1.4% for SPP and 0.8% for SPR (based on an NE value of 7.6 and 7.4 MJ kg^{-1} DM grass, respectively).

Requirements for chewing and rumination for untreated straw was up to 30% of the ME provided by the feed (Susenbeth *et al.*, 1998). The quality of the forages from semi-natural grasslands was somewhere intermediate between high quality grass and straw, and therefore a requirement of 20% of the ME value of the feed for chewing and rumination would be reasonable. A correction method for chewing and rumination based on NDF would be advisable, but as no respiration trials were carried out for the purpose of this thesis, it was not possible to estimate a value based on the measured data.

Furthermore, since the *in vivo* digestibility of low quality forages is lower, the excretion of faeces is high. A higher bulk volume of digesta in the gastro-intestinal tract is thus expected on the diets with semi-natural grass. Therefore, more energy may be required to mix the digesta in the rumen and to move the digesta throughout the gastro-intestinal tract (Agnew *et al.*, 1998). This would be another reason why a correction factor of 20% of the ME value of

Table 10. Extra requirements for the chewing of low quality grass. DMI is dry matter intake in kg d^{-1}, CE is chewing energy (MJ kg^{-1}), EE is extra energy for chewing in total diet (MJ d^{-1}), EE / MW is extra energy for chewing (kJ) per kg metabolic body weight, MR is maintenance requirements (kJ kg^{-1} MW d^{-1}), MRC is correction for maintenance requirements (kJ kg^{-1} MW d^{-1}) and MRC/MR is the proportional increase of maintenance requirements.

	ME	DMI	CE	EE	EE / MW*	MR	MRC	MRC/MR
Species poor silage	7.8	10.4	1.6	16.7	138	499	637	1.28
Species rich silage	7.4	11.6	1.5	17.4	144	499	643	1.29

*A cow of 600 kg is assumed; 121 kg metabolic weight.

grass would be tenable. In Table 10, the calculated energy costs for chewing of SPP and SPR (20% of ME value) are reallocated to maintenance requirements.

The estimate of additional requirements of almost 30% per kg metabolic weight is probably too high, as in the original maintenance requirements some energy for chewing, rumination and digestion is already included (Van Es, 1978). It was also indicated that grazing requires extra energy, mainly for walking on the pastures. No firm data were found to confirm the requirements of additional energy in the trials discussed in this thesis, and therefore, no firm conclusions can be drawn.

Thus, though high NDF concentration and high protein concentration in a grass are expected to be inversely correlated, both factors may result in higher maintenance requirements. A certain amount of fibre is required in the diet of ruminants, and because it is generally believed that intensively managed grass may have too little fibre to stimulate rumination, a small proportion of semi-natural grass in the diet will be positive for the rumen environment. In Chapter 8, it was mentioned that stimulation of rumination did not seem to occur with 20% replacement, because of the low particle size reduction in this diet. Therefore, a higher percentage replacement should be considered. In Chapter 6, milk production was reduced starting from replacement percentages of 40. Therefore, the optimal replacement percentage seems to be between 20 and 40%. Replacement of approximately 30% of intensively managed grass by forages from semi-natural grassland is therefore expected to be optimal. Rumination and thus salivation could then be increased, resulting in a higher pH in the rumen, and an optimal degradation of the ingesta, possibly without a reduction in milk production. Furthermore, the nitrogen surplus is lower, and use of nutrients in the rumen might therefore be more efficient.

Guidelines for the use of semi-natural grass.

In this thesis, dairy cows in the second part of their lactation (after 100 days) were used, and they were supplemented with protein-rich concentrates. Under practical conditions, this was probably not necessary (Table 6). In Chapter 6, the intake did not decrease when small proportions of intensively managed grass were replaced by semi-natural grass, but also maize silage was fed. Maize silage often results in a higher milk production, due to higher intakes (Valk *et al*., 1990). Therefore, the maize silage in the ration makes the drawing of firm conclusions concerning an optimal ratio between intensively managed grass: semi-natural forage in the diet complicated. However, the inclusion of maize silage in the treatments makes the experiment applicable to the practical situation, as it is quite common to feed a mixed ration including maize silage.

Milk production decreased earlier than DM intake, due to reduction of energy content of the diet. It was also observed that forage containing herbs was ingested in higher proportions than semi-natural forages containing mostly grasses, although milk production was reduced, reflecting energy intake. It is difficult to translate the observations in this thesis to other types of semi-natural grass, although it is expected that the effects of vegetations containing many mature grasses will be comparable to the observations on SPP and that vegetations containing many herbs will be comparable to the observations on SPR. A confirmation of this assumption requires more research.

Integration of forages from semi-natural grasslands is also possible for either youngstock, dry cows, or high-producing cows, although the diet should then be adapted to the specific circumstances. Requirements for youngstock and dry cows are lower than for lactating cows, and therefore, the demand for energy intake is lower, and thus easier to meet with low quality forages. For high-producing cows, it would be more difficult to integrate this kind of forage in their diet, as the energy content is clearly lower. However, if the total composition of the diet is of high quality, a small part of low quality forage may work well, as it could stimulate rumen function. Furthermore, if semi-natural grass is fed in larger quantities, corrections for protein should be made through the supplements.

Conclusions

For intensively managed grass, the estimation of the nutritional value is not correct. This can be attributed to the requirements of dairy cows fed grass, or on characteristics of the grass. However, it probably is a combination of both. As the protein value of grass is often high, this could be corrected in the ME formulae, by adding a correction factor for the surplus of digestible crude protein in the grass, and this would account for grass, independent of the diet it is part of. Furthermore, forage-based diets contain high concentrations of fibre and consequently require rumination and extra energy for digestion. If an imbalance of nutrients in the rumen occurs, resulting in a less efficient utilization of the available energy, increased maintenance requirements on grass-based diets may be additive to corrected ME values of grass. On diets with high percentages (> 80%) of grass, it is therefore suggested to increase maintenance requirements with an extra 10%, thus from 293 kJ towards 322 kJ NE per kg metabolic weight. This would be additive to increased maintenance requirements for grazing animals, as already suggested by CVB (2001b). Increased maintenance requirements, as suggested in this thesis, would give a more reliable estimation of the milk production of dairy cows on grass-based diets.

For semi-natural grass, intake was lower on the forage consisting mainly of mature grasses than on forage consisting of intensively managed grass or herb-rich grass. The calculation rules of the DVE / OEB system were not correct for forages from semi-natural grasslands, as the values calculated based on *in situ* measurements were lower than the values calculated with the calculation rules. This should be accounted for when using such forages. Furthermore, the ME value of the forages was correctly estimated if the simplified formula was compared to the original formula, but performance was lower than expected (Chapter 6). This was partly attributed to the N surplus, and partly to extra energy required for rumination and digestion. Therefore, a correction in maintenance requirements, as suggested for intensively managed grass, and the allocation of energy costs for nitrogen excretion may be advisable. This work has shown that the inclusion of forages from semi-natural grass in the diet of dairy cows is possible, especially if the semi-natural forages are fed in small amounts. Taking the described observations into account, replacement of intensively managed grass by forages from semi-natural grasslands until a maximum of 30% seems to offer best possibilities for optimization of the diet.

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Summary

Introduction

In temperate regions, the diet of high producing dairy cows often contains concentrates, maize silage and grass. Usually, the grass is harvested early, and has a high digestibility and high protein concentrations, and thus a high metabolizable energy (ME) concentration. Cows are therefore expected to reach high milk production on diets containing this type of grass. Recently, the interest in the use of forages from semi-natural grasslands also increased. On semi-natural grasslands, often the cutting date is delayed, and fertilization is restricted, resulting in high cell wall concentrations, low protein concentrations and a low digestibility, and thus a low ME concentration. This would result in a relatively low milk production if this type of grass is included in the diet of dairy cows.

For intensively managed grass as well as for semi-natural forages, difficulties occur concerning the estimation of its nutritive value. For the intensively managed grass with a high quality, the actual milk production is lower than expected based on the estimated energy value and DM intake of the grass. The semi-natural forages with a low quality are hardly investigated in order to estimate the feeding value of those forages in diets of dairy cows, and nutritional characteristics such as *in vivo* digestibility, degradability and voluntary intake in dairy cows are mostly unknown.

In this thesis, firstly the evaluation of intensively managed grass is discussed and subsequently, the nutritive value of forages from semi-natural grassland is discussed. Since those two types of grass are different, the thesis is split in two parts. In the first part, only intensively managed grass is discussed, whereas in the second part the forages from semi-natural grasslands are discussed, in combination with intensively managed grass.

Part I: the feed evaluation of intensively managed grass

To investigate why the actual milk production is lower than the expected milk production based on energy intake on grass-based diets, in Chapter 2, twelve feeding trials with intensively managed grass were collected. The treatments in the trials were split into two groups. In the first group, diets consisted for 80-90% of fresh grass, and the remaining 10-20% was concentrates. In the second group the diets consisted for 40-65% of grass, the remaining proportion of the diets consisted of concentrates and other supplements, such as maize silage. Of both groups the energy intake in grass and supplements was calculated, and the energy output in milk and maintenance was calculated. Discrepancies between energy input and output were correlated to variables. It was observed that correlations between discrepancy and variables were different for both groups. For the 40-65% grass group, crude protein decreased the discrepancy, whereas crude fibre increased the discrepancy. However, in the 80-90% grass group, crude fibre decreased the discrepancy. It was concluded that grass with a high quality does not always show in the 80-90% grass diets, which could be due to a surplus of nitrogen in those diets or to more energy required by the gastro-intestinal tract.

In Chapter 3, respiration trials with lactating dairy cows fed fresh or frozen grass were collected, and reanalysed. The trials were carried out in the 1970s in Lelystad and Wageningen, the Netherlands, and in the 1990s in Hillsborough, Northern Ireland. The diets of the animals consisted of 90-100% fresh or frozen grass. Also in these trials discrepancies were observed between energy input and energy output. Two possibilities were considered to have caused this discrepancy. The first was a reduced efficiency of energy utilization for milk (k_l), and the second was increased maintenance requirements (ME_m) of dairy cows on grass based diets. Carrying out a regression analysis with free intercept, it was calculated that the ME_m should be increased together with the k_l. However, an increased efficiency did not seem possible, as there was also no evidence in the literature, and therefore the k_l was fixed at 0.6, the value used in the Dutch VEM-system. Carrying out the regression analysis with a fixed k_l, the ME_m increased approximately 10%, compared to the value used in the VEM-system. Calculations indicated that this is approximately equal to the energy costs for nitrogen excretion in the urine, and therefore, it was advised to increase ME requirements for maintenance by 10% for dairy cows on grass based diets.

Part II: the nutritional value of forages from semi-natural grasslands

To start the second part of the thesis, first a review is given about the factors influencing digestibility and intake (Chapter 4). In this chapter, the variation of species and of stages of maturity together with the late harvesting are indicated as problems to estimate the nutritive value. Forage species found on semi-natural grasslands often have lower digestibilities than *Lolium perenne*, even if harvested in the same stage of maturity. Furthermore, digestibility is difficult to estimate from chemical composition, because the species often differ in relation between chemical composition and digestibility, compared to species used at intensively managed grasslands. A delayed harvest on semi-natural grasslands results in higher ratios stem:leaf of the grasses, and the digestibility of the plant organs declines. Dicotyledonous species appear to maintain their digestibility longer than grass species, and these species may also have higher intakes than expected based on their digestibility, which may be due to a fast rate of degradation. Therefore, intake and digestibility of forages from semi-natural grasslands may be higher than expected based on the harvesting date. In order to indicate possibilities of forages from semi-natural grasslands in diets of ruminants, calculations were made concerning nutritive requirements and energy intake of different grasses. A fixed DM intake was assumed. It was calculated that performance would be reduced. However, based on literature, there are possibilities to include forages from semi-natural grasslands in the diets of ruminants, although decreased performance should be accounted for. However, effects on animal performance might be smaller than expected, due to possible higher intakes and a higher *in vivo* digestibility than estimated based on chemical analysis. To test the effects of the inclusion of forages from semi-natural grasslands in the diet of dairy cows, several experiments were carried out. Those experiments are described in the Chapters 5-8.

Chapter 5 reports two methods to estimate degradation of three forages in the rumen, i.e. the *in situ* technique, using nylon bags and an *in vitro* technique, using a gas production apparatus. Silages were an intensively managed grass silage (intensively managed; IM), a silage from a grassland managed to stimulate nesting of birds (species poor; SPP) and a silage form a grassland managed to maintain botanical diversity (species rich; SPR). Apart from the three silages, also some dried samples of the fresh material were used and some individual grass and dicotyledonous species. It was observed that the intensively managed grass had the highest degradation and gas production rate and SPP the lowest, whereas SPR was intermediate. With the individual species (only with the gas production technique) it was observed that the dicotyledonous species had a high gas production rate, especially if compared with mature grass species.

In Chapter 6, results of a performance trial with lactating dairy cows on five different treatments are reported. Cows were fed diets containing concentrates, maize silage and grass silage, and treatments were different compositions of the grass silage. The grass silages were the silages as described above, IM, SPP and SPR, and the different compositions of the grass silages were: 100% IM (100IM), 80% IM and 20% SPP (20SPP), 60% IM and 40% SPP (40SPP), 40% IM and 60% SPP (60SPP) and 40% IM and 60% SPR (60SPR). Milk production did not differ significantly among the treatments, but differences in protein and fat concentrations were observed. As a result, fat and protein corrected milk also decreased at replacement percentages from 40% onwards. A reduction in intake was only observed with 60% replacement of IM by SPP. Intake of 60SPR was similar to intake of 100IM. Therefore, the potential intake of SPR appeared to be high, although milk production was reduced. It was concluded that small proportions of intensively managed grass can be replaced by forages from semi-natural grasslands without a decrease in milk production, but at higher percentages, milk production may be decreased due to the lower energy intake, as semi-natural forages have lower energy density. Furthermore, at high replacement percentages, also dry matter intake may be reduced.

The digestibility and the voluntary intake of different diets containing the grass silages as described above (IM, SPP, SPR) is discussed in Chapter 7. Digestibility was assessed in two different trials, and voluntary intake was assessed in one trial. In trial one, the different grass silages were fed solely, only supplemented with concentrates during milking. Treatments were IM, SPP and SPR. In this trial also the voluntary intake of either of the silages was assessed. In trial two, IM was fed solely or fed in combination with either SPP or SPR, with the treatments 100IM (100% IM), 20SPP (80% IM, 20% SPP), 60SPP (40% IM, 60% SPP) and 60SPR (40% IM, 60% SPR). The grass silage was supplemented with concentrates. *In vivo* digestibility was highest in IM or 100IM and lowest in SPR or 60SPR. The *in vivo* digestibility was approximately similar to the *in vitro* digestibility, in both trials, indicating no differences in efficiency of utilization with different replacement percentages. Digestibility could not be estimated based on chemical composition. Voluntary intake of IM and SPR in Experiment 1 was approximately similar, although the (partly restricted) intake of 60SPR in Experiment 2 was (not significantly) lower than of 100IM.

In Chapter 8, the rumen fermentation and kinetics were studied in four cannulated, lactating dairy cows fed intensively managed grass and forage from semi-natural grasslands.

It was observed that the pH in the rumen was mainly depending on FOM intake, as well as the volatile fatty acids production. The NH_3 concentration in the rumen followed CP intake or the ratio CP intake / OM intake. The intake rate and the passage rate of the diet with SPR appeared to be relatively high, as well as on the diet consisting solely from IM. The intake rate was low on the diet containing a large proportion of SPP. However, on that diet, the clearance rate of large particles (> 2.5 mm) was high, which could indicate a high rumen activity. For most rumen fermentation and kinetics characteristics measured in the experiment, the forages from semi-natural grasslands behaved in the same way as the grass from intensively managed grasslands.

In Chapter 9, the results and data of the experiments on the Chapter 2-8 are combined. For intensively managed grass, with high concentrations of protein, it was suggested to correct the ME-formula for the nitrogen surpluses in the forage. This surplus is related to the protein excreted in milk. Also chewing, rumination and digestion require energy. Therefore, apart from the correction of the ME value for nitrogen surpluses, it is also suggested to increase maintenance requirements by 10%. A low fibre concentration of grass was observed to increase the overestimation of the milk production. Therefore, the inclusion of straw or forages from semi-natural grasslands may have a positive effect on digestion, as this would increase the fibre concentration of the diet. Another positive effect of such an inclusion is that it would decrease the nitrogen surplus. Thus, although the energy density of forages from semi-natural grasslands is lower than of intensively managed grass, the inclusion of the forages may be favourable for rumen function and to reduce nitrogen surpluses.

Also with forages from semi-natural grasslands, the output in milk was lower than expected based on the calculated energy value, although the regular calculation rules were also found to be applicable for these forages. This could be due to chewing and rumination of the material, but also to the nitrogen surpluses, which also occur in those diets, due to high nitrogen concentrations of the concentrates fed. Energy costs for nitrogen excretion in the urine were calculated from the nitrogen balance and the intake of nitrogen in grass silage. Energy costs were especially high in IM, but also in SPP and even in SPR energy would have been required for N excretion. Therefore, maybe the same correction factor could be used for forages from semi-natural grasslands as for intensively managed grass.

This work described problems concerning the use of intensively managed as well as semi-natural grasslands in the diet of dairy cows. It advised an increase of maintenance requirements of 10% for dairy cows on grass based diets, together with a correction for protein concentrations in the ME formulae. Furthermore, it indicates that for semi-natural grasslands the DVE/OEB system is not correctly estimated, which should be taken into account when using such forages in diets for dairy cows. However, there is scope for using forages from semi-natural grasslands in the diet of dairy cows. Replacement of intensively managed grasslands by forages from semi-natural grasslands until a maximum of 30% seems to offers best possibilities for optimization of the diet.

Samenvatting

Inleiding

Het rantsoen van hoogproductieve melkkoeien bestaat vaak uit krachtvoer, maïssilage en gras of grassilage. Het gevoerde gras is meestal afkomstig van een grasland dat zwaar bemest is en vroeg in het jaar gemaaid wordt, omdat op deze manier een hoge kwaliteit verkregen wordt, dus een hoge verteerbaarheid en een hoog eiwitgehalte. In theorie zouden koeien op dit gras een hoge melkproductie kunnen halen. In de laatste jaren is ook de belangstelling voor beheergraslanden toegenomen. Beheergraslanden worden vaak later in het seizoen gemaaid dan intensief beheerde graslanden, zodat vogels en planten zich kunnen voortplanten. Op dit soort graslanden komen ook andere plantensoorten voor, die vaak een lagere verteerbaarheid hebben dan Engels raaigras, zelfs in hetzelfde groeistadium. Het late maaien en de afwijkende plantensoorten resulteren in een lagere verteerbaarheid en een lager eiwitgehalte, en dus een lagere kwaliteit van het gras. Melkveehouders gebruiken dit soort gras liever niet voor hun koeien, omdat door de lage kwaliteit van het gras een lagere melkproductie verwacht wordt.

Zowel het intensief geproduceerde gras als het gras afkomstig van beheergraslanden leveren problemen op met betrekking tot de schatting van de voederwaarde. Voor het intensief geproduceerde gras is het probleem dat de melkproductie van koeien lager is dan verwacht wordt op basis van de energieopname in gras en krachtvoer. Beheergras is nog nauwelijks onderzocht, en van voederwaardekarakteristieken zoals de *in vivo* verteerbaarheid, de afbraaksnelheid en de vrijwillige opname door melkkoeien is dan ook weinig bekend.

In dit proefschrift werd allereerst de voederwaardering van intensief geproduceerd vers gras in het rantsoen van melkkoeien onderzocht, en vervolgens werd de voederwaarde van beheergras besproken. Vanwege het verschil in opzet van het onderzoek, en de verschillen tussen de grassoorten is het proefschrift in twee delen verdeeld. In het eerste deel wordt aan de hand van voeder- en respiratieproeven het gebruik van intensief geproduceerd gras voor melkkoeien bediscussieerd. In het tweede deel wordt het gebruik van gras afkomstig van beheergraslanden in rantsoenen van melkkoeien bediscussieerd aan de hand van proeven met melkkoeien, waarin o.a. de vertering, pensfermentatie en opname bestudeerd zijn.

Deel I: de voederwaardering van intensief geproduceerd gras

Om te onderzoeken waarom de werkelijke melkproductie lager is dan de verwachte productie gebaseerd op drogestofopname en energiewaarde van het voer, zijn in Hoofdstuk 2 12 voederproeven verzameld, waarin de individuele voeropname en de melkproductie gemeten waren. In deze proeven zaten verschillende behandelingen die onderverdeeld zijn in rantsoenen met 40-65% gras en rantsoenen met 80-90% gras. Bij de 40-65% gras groep bestond de rest van het rantsoen uit krachtvoer en andere supplementen, zoals maïssilage of bietenpulp, en bij de 80-90% gras groep bestond de rest van het rantsoen uit krachtvoer. Van beide groepen werden de input in voer en de output in melk en onderhoud bepaald.

Vervolgens werden de discrepanties tussen input en output berekend en gecorreleerd met verschillende variabelen, zoals samenstelling van het gras, samenstelling van het rantsoen of de melkproductie. Correlaties tussen de discrepantie en de variabelen waren verschillend voor de beide groepen. In de groep met 40-65% gras had ruw eiwit een negatief effect op de discrepantie, terwijl ruwe celstof een positief effect op de discrepantie had. In de groep met 80-90% gras had ruwe celstof juist een negatief effect op de discrepantie. Het bleek dat de voederwaarde van gras met een hoge kwaliteit tegenvalt in de rantsoenen met 80-90% gras, wat veroorzaakt zou kunnen zijn door de overmaat van stikstof in deze rantsoenen of door een verhoging van de energie die nodig is voor de vertering van het voer in het maagdarmkanaal.

In Hoofdstuk 3 werden respiratieproeven met lacterende melkkoeien verzameld en opnieuw geanalyseerd. De proeven werden uitgevoerd in de jaren ’70 in Wageningen en Lelystad en in de jaren ’90 in Hillsborough, Noord-Ierland. De rantsoenen van de dieren bestonden uit 90 tot 100% vers of bevroren gras. Ook in deze proeven werden discrepanties gevonden tussen de energie-input en de energie-output. Er zijn twee mogelijke redenen om de discrepantie te verklaren. De eerste is een verminderde efficiëntie van energie gebruik voor melk (k_l), en de tweede is een verhoogde onderhoudsbehoefte van melkkoeien op gras gebaseerde rantsoenen. Een regressieanalyse met een vrij intercept gaf aan dat zowel de onderhoudsbehoefte als de k_l omhoog zouden moeten gaan. Omdat er in de literatuur geen aanwijzingen waren om de k_l te verhogen, leek een verhoogde efficiëntie niet mogelijk. De k_l werd daarom gefixeerd op 0.6, wat de waarde is die in het VEM-systeem gebruikt wordt. Het uitvoeren van de regressieanalyse met een gefixeerde k_l resulteerde vervolgens in een stijging van de onderhoudsbehoefte van 10%, vergeleken met de waarde die gebruikt wordt in het VEM-systeem. Dit is ongeveer gelijk aan de berekende energiekosten voor uitscheiding van stikstof in de urine. Er werd daarom geadviseerd om de ME behoefte met 10% te verhogen.

Deel II: de voederwaarde van ruwvoer van beheergraslanden

Als een soort inleiding op het tweede deel van het proefschrift, werd eerst een overzicht gegeven van de factoren die de verteerbaarheid en opname van planten door herkauwers beïnvloeden (Hoofdstuk 4). In dit hoofdstuk werd de variatie in plantensoorten en in ontwikkelingsstadia aangegeven als probleem om de voederwaarde te schatten. Plantensoorten op beheergraslanden hebben vaak een lagere verteerbaarheid dan Engels raaigras, zelfs als ze in hetzelfde ontwikkelingsstadium gemaaid worden. Verder is de verteerbaarheid moeilijk te schatten op basis van de chemische samenstelling, omdat de soorten verschillen in relatie tussen de chemische samenstelling en de verteerbaarheid, vergeleken met soorten op intensief geproduceerd grasland. Het later maaien op beheergraslanden resulteert in een hogere stengel:blad verhouding, en daarnaast gaat de verteerbaarheid van de plantorganen omlaag. Dicotylen lijken hun hoge verteerbaarheid langer vast te houden dan grassoorten, en ze lijken ook beter opgenomen te worden dan verwacht zou worden op basis van hun verteerbaarheid, wat veroorzaakt zou kunnen zijn door een hoge afbraaksnelheid. De opname en verteerbaarheid van ruwvoer van beheergraslanden zou daarom hoger kunnen zijn dan verwacht wordt op basis van de maaidatum. Om de mogelijkheden van het gebruik van ruwvoer afkomstig van beheergraslanden in het rantsoen van herkauwers aan te geven, werden de energiebehoefte van herkauwers en de energieopname van verschillende grassen, bij een vastgestelde drogestofopname, berekend. Bij het gebruik van gras van beheergraslanden zou de melkproductie of groei afnemen vergeleken met een rantsoen van alleen intensief gras. In de literatuur zijn echter wel voorbeelden te vinden waarbij beheergras gebruikt wordt voor rantsoenen van herkauwers. Er lijken dus wel mogelijkheden te zijn om beheergras te gebruiken. Daarbij zou echter wel rekeningen gehouden moeten worden met verminderde producties. Effecten op de productie zouden echter kleiner kunnen zijn dan verwacht, door een relatief hoge opname, en door een mogelijke onderschatting van de *in vivo* verteerbaarheid. Om na te gaan wat de precieze effecten zijn van de integratie van beheergras in rantsoenen van melkkoeien, zijn er verschillende experimenten uitgevoerd, waarbij o.a. gekeken is naar de verteerbaarheid en pensfermentatie. Deze experimenten zijn beschreven in de Hoofdstukken 5 tot 8.

In Hoofdstuk 5 worden twee methoden om de afbraak van silages in de pens te meten beschreven, namelijk het gebruik van nylon zakjes gevuld met de silages, die gedurende 3 tot 264 uur in de pens geïncubeerd werden (*in situ*), en de gasproductietechniek (*in vitro*), waarbij de silages gedurende 72 uur in penssap geïncubeerd werden. De silages waren een intensief geproduceerde grassilage (intensively managed; IM), een silage afkomstig van een weidevogelgrasland (species poor; SPP) en een silage afkomstig van een kruidenrijk grasland (species rich; SPR). Naast de drie silages, zijn er in de gasproductietechniek ook nog gedroogde monsters van het verse materiaal gebruikt, en een aantal individuele gras- en dicotyle soorten. Het intensief geproduceerde gras had de hoogste afbraak- en gasproductiesnelheid, en het weidevogelgras de laagste. Het kruidenrijke gras had een hogere afbraak- en gasproductiesnelheid dan SPP, maar een lagere dan IM. Van de individuele soorten bleken met name de dicotyle soorten een hoge afbraaksnelheid te hebben, vooral in vergelijking met de grassen in een vergevorderd ontwikkelingsstadium.

In Hoofdstuk 6 worden de resultaten van een productieproef met lacterende melkkoeien op vijf verschillende behandelingen weergegeven. De koeien kregen rantsoenen bestaande uit krachtvoer, maïssilage, en gras, en de behandelingen waren verschillende samenstellingen van het grassilage. De grassilages waren dezelfde als hierboven beschreven: IM, SPP en SPR, en de verschillende samenstellingen van het grassilage waren: 100% IM (100IM), 80% IM en 20% SPP (20SPP), 60% IM en 40% SPP (40SPP), 40% IM en 60% SPP (60SPP) en 40% IM en 60% SPR (60SPR). De melkproductie was niet verschillend voor de verschillende rantsoenen, maar verschillen in vet- en eiwitconcentraties werden wel waargenomen. Hierdoor werden bij vervangingspercentages vanaf 40% ook de verschillen in meetmelk (gecorrigeerd voor vet- en eiwitconcentraties) waargenomen. Een verlaging van de (deels beperkte) opname trad alleen op bij 60SPP. De opname van 60SPR was gelijk aan de opname van 100IM, wat een potentieel hoge opname van SPR suggereert. De hoeveelheid meetmelk van de koeien op 60SPR was lager dan van 100IM, wat veroorzaakt werd door het lagere energiegehalte van SPR. Kleine proporties van intensief gras kunnen dus vervangen worden door gras van beheergebieden zonder een afname in melkproductie, maar bij hogere percentages zal de melkproductie omlaag gaan door de lagere energieopname. Bij hogere vervangingspercentages zou ook de drogestofopname omlaag kunnen gaan.

De verteerbaarheid en de vrijwillige opname van de verschillende soorten gras zoals hierboven beschreven (IM, SPP en SPR) worden bediscussieerd in Hoofdstuk 7. De verteerbaarheid werd geschat in twee experimenten, en de vrijwillige opname in één experiment. Beide experimenten waren opgezet als een Latijns vierkant. In het eerste experiment werden de silages afzonderlijk gevoerd, alleen aangevuld met krachtvoer tijdens het melken. De behandelingen waren IM, SPP en SPR. In deze proef werd ook de vrijwillige opname van de silages bepaald. In het tweede experiment was IM de basis van het rantsoen, en werd dit als enig ruwvoer aangeboden, of gemengd met SPP of SPR. Behandelingen waren 100IM (100% IM), 20SPP (80% IM en 20% SPP), 60SPP (40% IM en 60% SPP) en 60SPR (40% IM en 60% SPR). Het grassilage werd aangevuld met krachtvoer. De *in vivo* verteerbaarheid was het hoogst in IM of 100IM en het laagst in SPR of 100SPR. De *in vivo* verteerbaarheid was ongeveer gelijk aan de *in vitro* verteerbaarheid, wat betekent dat de efficiëntie van het energiegebruik in de pens niet afwijkend is als er beheergras gevoerd wordt. Echter, voor de celwanden was de vertering *in vivo* hoger dan *in vitro*. Verteerbaarheid kon niet geschat worden aan de hand van de chemische samenstelling. In Experiment 1 was de vrijwillige opname van SPR ongeveer gelijk aan de vrijwillige opname van IM. In Experiment 2 was de (deels beperkte) opname van 60SPR echter (niet significant) lager dan die van 100IM. De rantsoenen SPP als 60SPP werden beide relatief slecht opgenomen.

In Hoofdstuk 8 werd de pensfermentatie en -kinetiek bekeken in vier gecannuleerde, lacterende melkkoeien die op de vier rantsoenen stonden zoals hierboven beschreven (Hoofdstuk 7, Experiment 2). De pH en de productie van vluchtige vetzuren in de pens bleken daarbij voornamelijk afhankelijk te zijn van de fermenteerbare organische stofopname. De NH_3 concentratie in de pens was gerelateerd aan de opname van ruw eiwit en de verhouding opname van ruw eiwit : opname van organische stof. De opnamesnelheid en de passagesnelheid bleken relatief hoog te zijn op 60SPR en 100IM, terwijl de opnamesnelheid laag was op 60SPP. Op 60SPP was de verdwijningssnelheid van de grote deeltjes (> 2.5 mm) echter hoog, wat zou kunnen duiden op een hoge pensactiviteit. Voor de meeste pensfermentatie- en penskinetiekkarakteristieken in dit experiment, leken de silages van de beheergraslanden zich op dezelfde manier te gedragen in de pens als gras van intensief beheerde graslanden.

Tenslotte zijn alle resultaten en data van de Hoofdstukken 2-8 gecombineerd in Hoofdstuk 9. Voor het intensieve gras, met hoge eiwitconcentraties, wordt gesuggereerd de formule om de metaboliseerbare energie te schatten, te corrigeren met de overmaat aan eiwit van het gras. Deze overmaat is gerelateerd aan de hoeveelheid eiwit uitgescheiden in de melk. Ook kauwen, herkauwen en vertering kosten energie voor de herkauwer. Daarom wordt gesuggereerd om, naast een correctie van de formule voor metaboliseerbare energie voor de stikstof overmaat, ook de onderhoudsbehoefte met 10% te verhogen. Een lage ruwe-celstofconcentratie van het gras bleek positief gecorreleerd te zijn met de overschatting van de melkproductie. De integratie van stro of gras van beheergraslanden zou dus een positief effect kunnen hebben op de vertering, omdat dit de ruwe-celstofconcentratie van het rantsoen verhoogt. Een ander positief effect van dit soort voeders is dat deze de overmaat van stikstof zouden verlagen. Hoewel het energiegehalte van beheergras lager is dan dat van intensief geproduceerd gras zou het beheergras dus bevorderend kunnen werken op het functioneren van de pens, en zou het de stikstofovermaat kunnen verminderen.

Ook bij de proeven met beheergras bleek de melkproductie lager te zijn dan verwacht werd aan de hand van de energieopname, hoewel de gangbare berekeningsmethoden wel geschikt bleken te zijn voor dit soort voeders. De reden voor de incorrecte inschatting was mogelijk het kauwen en herkauwen van dit soort materiaal, maar ook in dit geval was er sprake van een overmaat aan stikstof, omdat de dieren een eiwitrijk krachtvoer kregen om te compenseren voor de lage ruw-eiwitconcentratie van het beheergras. De energiekosten voor de stikstofuitscheiding in de urine werden berekend uit de stikstofbalans en de opname van stikstof in grassilage. De energiekosten waren vooral hoog in 100IM, maar ook in SPP, en zelfs in SPR moet energie nodig zijn geweest voor de stikstofexcretie. Daarom zou voor beheergras dezelfde correctiemethode gebruikt kunnen worden als voor intensief geproduceerd gras.

In dit proefschrift worden de problemen met betrekking tot het gebruik van zowel intensief als van beheergras in het rantsoen van melkkoeien besproken. Een verhoging van de onderhoudsbehoefte van 10% is gesuggereerd, samen met een correctiefactor voor de overmaat aan eiwit in de formule om de metaboliseerbare energie te schatten. Verder is aangegeven dat het DVE / OEB systeem de eiwitwaarde van beheergras niet goed schat, waar rekening mee gehouden zou moeten worden als dit soort gras gebruikt wordt in het rantsoen van melkkoeien. Er is echter wel toekomst voor het gebruik van dit soort gras in het rantsoen van melkkoeien. Een vervanging van maximaal 30% van het intensief geproduceerde gras door beheergras, lijkt de beste mogelijkheden te bieden.

Curriculum vitae

Martine Henriëtte Bruinenberg werd op 26 september 1975 te Alphen aan den Rijn geboren. Na de lagere school ging zij naar het voormalige Christelijk Lyceum te Alphen aan den Rijn, waar zij in 1993 haar VWO diploma haalde. In hetzelfde jaar begon zij aan de studie Zoötechniek aan de Landbouwuniversiteit Wageningen (nu Wageningen Universiteit) te Wageningen. Na een stage aan de Universitas Gadjah Mada in Yogyakarta, Indonesië, en het uitvoeren van twee afstudeervakken, Dierlijke Productiesystemen en Veevoeding, studeerde zij in september 1998 af met als specialisatie Dierlijke Productie Systemen. In diezelfde maand begon zij als assistent in opleiding (AIO) bij de leerstoelgroep Gewas- en Onkruidecologie van Wageningen Universiteit, gedetacheerd bij ID Lelystad / ID TNO Diervoeding te Lelystad. Hier verrichtte zij het onderzoek zoals dat in dit proefschrift beschreven staat. Vanaf december 2002 werkt zij als onderzoeker grasland en voedergewassen bij het Praktijkonderzoek Veehouderij te Lelystad.