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

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Abstract

The discrepancy between the estimated feeding value of fresh grass and the output per kg grass in terms of milk and maintenance was studied by evaluating 12 experiments with grass-fed dairy cows. The percentage grass in the diets varied between 40 and 90. Intake and milk production were recorded daily.

Per treatment a number of parameters relating to the composition of the grass, characteristics of the animals, and composition of the total diet were determined. The correlation between each of these parameters and the measured discrepancy was calculated. The digestible organic matter in the grass (DOM), intake of grass, intestinal digestible protein in the total diet, percentage milk protein and body weight gain correlated well with the discrepancy. It was concluded that energy input from grass and energy output in milk production were significantly different ($P < 0.05$). For the diets with 80–90% grass a high DOM increased discrepancy.

It was furthermore concluded that the maintenance requirements of lactating dairy cows fed grass-based diets are probably higher than the currently used values This was ascribed to energy requirements in the gastro-intestinal tract and to nitrogen excretion.

Additional keywords: ruminant nutrition, feed evaluation, grass-based diets

Introduction

In temperate regions, fresh grass is one of the main components in the diet of dairy cows. Due to high rates of nitrogen (N) fertilizer, this grass – mainly *Lolium perenne* – is usually highly digestible and contains large proportions of protein and small proportions of cell wall material, suggesting a high quality of the grass. Grass of such a composition is expected to enable a high production performance of lactating cows. However, when feeding fresh grass of a high quality to dairy cows Valk *et al.* (2000) observed that the cows produced less milk than was predicted from their net energy intake. This phenomenon has also been described for ensiled grass: Thomas

& Gill (1988) concluded that cattle offered diets containing high proportions of grass silage had a low efficiency of energy utilization. So for cows on diets based on fresh or ensiled grass, discrepancies are found 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 know why grass-fed dairy cows do not attain their expected output.

The feeding value of grass is expressed in terms of net energy for lactation (NE_L; Van Es, 1978), which is estimated using 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) may be caused by an underestimation of the energy requirements for maintenance and production when cows receive forage-based diets, or by an overestimation of the energy content of the grass. Since the introduction of the feed unit system for dairy cows (VEM) 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 by Bruinenberg *et al.* (2002) suggested a 10% increase in maintenance requirements of dairy cows fed on grass compared with current calculation methods.

To find possible explanations for the observed discrepancy and formulate possible corrections to improve the feed evaluation system the results of a number of performance experiments with dairy cows fed fresh grass were evaluated. Three hypotheses were considered:

1. The feed evaluation formula for grass is incorrect, and a new formula has to be developed to replace the present one;
2. The present formula calculates the potential energy value, but in some cases this energy is not fully utilized due to factors like an unbalanced nutrient supply. In that case a correction factor should be used;
3. The energy requirements of dairy cows on grass-based diets are higher than previously assumed, as was already suggested by Bruinenberg *et al.* (2002). So the equation for the estimation of energy requirements should be changed.

The study is based on 12 feeding trials with lactating dairy cows fed on grass-based diets. The differences between potential milk production, based on dry matter intake and calculated energy value of the ingested feed, and actual milk output were calculated. To explain the discrepancy between potential and actual energy output, this discrepancy was related to variables such as nutrient content of the grass, diet composition and production level.

Material and methods

Experimental details

Data from 12 experiments with stall-fed, multiparous lactating dairy cows were used. In these experiments feed intake, diet composition, milk production and live weight of the animals were recorded. In all experiments the cows were fed fresh grass

– mainly *Lolium perenne* – with a percentage grass in the dry matter (DM) varying between 40 and 90. Feeding was *ad libitum*. Nine experiments had been carried out at ID-Lelystad (Experiments 1–9) and three at the Research Institute for Animal Husbandry in Lelystad (Experiments 10–12). Details of the experiments are summarized in Table 1. For the abbreviations and units used in this paper see Table 2.

The results of Experiments 5, 10, 11 and 12 have not been published. Therefore, some additional information is given about the treatments.

Experiment 5 included five feeding treatments: (i) grass + concentrates, (ii) grass + dried sugar beet pulp, (iii) grass + ensiled pressed sugar beet pulp, (iv) grass + corn-cob-mix without husks, and (v) grass + corn-cob-mix with husks (H. Valk, unpublished data). In Experiments 10 and 11 the cows were split over three treatment groups. The treatments included grasses from different plots, fertilized with 150 or 300 kg N ha^{-1} per year. Part of the grass fertilized with 150 kg N ha^{-1} per year 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} per year (1500–2000 kg ha^{-1}; R.L.G. Zom, unpublished data). In Experiment 12 two feeding treatments were compared: grass fertilized with 150 kg N ha^{-1} per year and

Table 1. Details of the 12 experiments.

Expt. No.	Year	No. of treat-ments	% grass	FPCM[1] kg day^{-1}	Duration weeks	No. of animals per treatment[2]	Treat ments[3]	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	H. 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] These numbers may be lower than the numbers in the references. This is the result of heifers being excluded from the calculations.
[3] A: 80–90% grass; B: grass supplemented with maize silage and concentrates; C: grass supplemented with beet pulp concentrates; D: grass supplemented with maize concentrates; E: grass supplemented with beet pulp and maize concentrates.

Table 2. Abbreviations and units used.

Abbreviation	Variable	Unit
BW	Body weight	kg
ASH	Crude ash	g per kg DM
CF	Crude fibre	g per kg DM
CFAT	Crude fat	g per kg DM
CP	Crude protein	g per kg DM
DCP	Digestible crude protein	g per kg 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 per kg DM
DVE	Digestible protein in the intestine, amount of DVE in the grass	g per kg DM
DVE diet	Average DVE in the total diet	g per kg DM
FCM	Fat corrected milk	kg
FL	Feeding level	–
FPCM	Fat and protein corrected milk	kg
GE	Gross energy	kJ
% grass	Percentage 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 fat in milk	%
ML	Percentage lactose in milk	%
MP	Percentage 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 per kg DM
OEB	Undegradable protein balance in the rumen. OEB in the grass	g per kg DM
OEB diet	Average OEB in the total diet	g per kg DM
OM	Organic matter	g per kg 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 per kg DM
VEM	Feed unit for dairy cows	–

grass fertilized with 300 kg N ha^{-1} per year, both cut at the same DM yield (1500–2000 kg ha^{-1}; R.L.G. Zom, unpublished data).

Variables measured

The following grass-related variables were measured: crude ash (ASH), nitrogen (N), crude fibre (CF), and sugars (SU). As crude fat (CFAT) in forages is seldom measured, we assumed a fat concentration of 40 g per kg DM in all forages for the calculation of gross energy (GE). 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. The digestibility of OM (%OMD) was measured using the method of Tilley & Terry (1963). The same parameters were determined in the concentrates.

Digestible organic matter (DOM) was calculated from OM and %OMD. Digestible crude protein (DCP) was calculated using standard calculation methods (Anon., 2000a). The 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 the grass ($NE_{L,\,grass}$) was calculated from the gross energy (GE), the metabolizable energy (ME) and the metabolizability of the feed (100 * ME/GE = q), using standard calculation methods (Van Der Honing & Alderman, 1988; Van Es, 1978; Anon., 2000a). The NE_L was calculated using the following equation:

$$NE_L = \{0.6 * [1 + 0.004 * (q - 57)] * 0.9752 * ME\} \quad (1)$$

where

NE_L = the net energy value (kJ),

q = 100 * metabolizable energy / gross energy,

ME = metabolizable energy (kJ), which for grass = 14.2 * DOM + 5.9 * DCP.

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

In Experiments 1–9 body weight of the animals (BW) was recorded twice a day, after milking. In Experiments 10–12 body weight was recorded once a day (at the same time) on three subsequent days during three different 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) was calculated for each cow by subtracting its weight at the start from its weight at the end of the experiments. Furthermore, for each cow the milk production (kg per day) was measured, together with the percentages fat (MF), protein (MP), and lactose (ML) in the milk. From these parameters the fat and protein corrected milk production (FPCM) was calculated according to Anon. (2000b). Other animal-related variables that were calculated 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). The minimum, maximum and mean values of the grass-, diet- and animal-related variables have been summarized in Table 3.

The discrepancy between the net energy input and the net energy output

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

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

Table 3. Minimum, mean and maximum values of the calculated variables for the two grass diet groups.

Variable[1]	Unit	80–90% grass diets			40–65% grass diets		
		Min.	Mean	Max.	Min.	Mean	Max.
Grass related							
ASH	g per kg DM	87	104	131	99	131	116
CP	g per kg DM	134	195	281	175	221	281
CF	g per kg DM	194	219	245	194	219	232
NFE	g per kg DM	354	442	509	354	415	492
SU	g per kg DM	63	125	177	63	94	146
%OMD	%	74	79	84	77	79	81
DOM	g per kg DM	651	712	746	669	700	729
DCP	g per kg DM	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 per kg DM	78	93	106	93	96	105
OEB	g per kg DM	–9	40	110	13	58	110
Diet related							
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 per kg DM	82	94	105	57	88	99
OEB diet	g per kg DM	–7	35	87	–11	23	42
Animal related							
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

[1] For abbreviations see Table 2.

where

$NE_{L,\,required}$ = the daily energy requirement (kJ),
BW = body weight (kg),
CM = 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 by means of the formulae used in the NE_L system, which is based on the chemical composition of supplements (Van Es, 1978). The energy output 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

$Output_{grass}$ = the energy output (kJ) per kg DM of grass,
$NE_{L,\,required}$ = the daily energy requirements (kJ),
DMI_{supp} = the DM intake of supplements (kg),
$NE_{L,\,supp}$ = the estimated energy content of the supplements (kJ),
DMI_{grass} = 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.

$$Discrepancy = NE_{L,grass} - Output_{grass} \quad (4)$$
$$\%\ Discrepancy = 100 * (Discrepancy\ /\ NE_{L,\,grass}) \quad (5)$$

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

Statistical analysis

Firstly, the treatment averages 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 in two different groups:
1. The diets with 80–90% grass (n = 23),
2. The diets with 40–65% grass (n = 19).

For each diet group the percentage variance of the discrepancy that can be explained by each of the different variables was calculated using linear regression analysis. Significance was determined with the F-test.

Results

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

In the 12 experiments a series of treatments was tested, covering a wide range in diet composition, feeding level, and milk production. As a result the data set contained a large variation in % Discrepancy and in 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, $NE_{L,\,grass}$ has been plotted against $Output_{grass}$.

Most diets overestimated the energy input, i.e. the energy output in terms of milk production was lower than expected on the basis of estimated energy intake. Only for some of the diets containing maize silage the energy input was underestimated compared with the output. The NE_L input was significantly ($P < 0.01$) higher than the NE_L output.

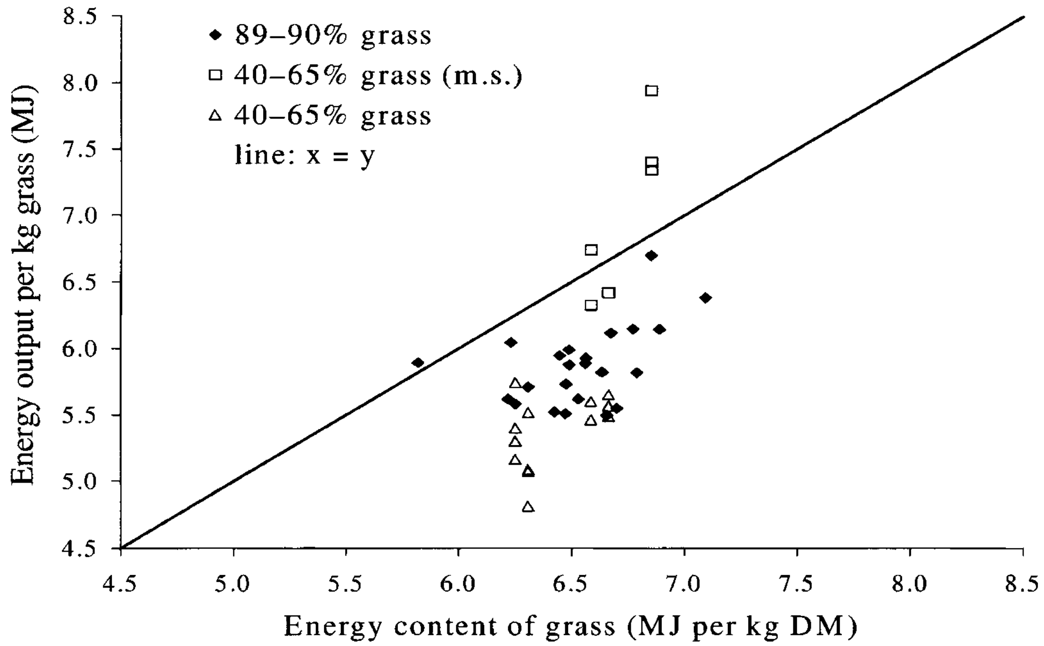

Figure 1. Relation between energy input and energy output. The line x = y indicates the ideal situation in which energy input equals energy output. The further away a data point is from this line, the larger the discrepancy. 40–65% grass (m.s.) = grass diets that included maize silage.

Correlations with discrepancy

The correlations (R^2) between the discrepancy and the factors for the two diet groups are shown in Table 4.

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

Diets with 40–65% grass
Compared with the 80–90% grass diet group, the correlations of the % Discrepancy with grass-related variables appeared to have an opposite effect: CF had a positive effect ($P < 0.001$) and %OMD a negative effect ($P < 0.05$). The % Discrepancy was also negatively correlated with q ($P < 0.001$), ME ($P < 0.01$) and DVE ($P < 0.001$). Of the diet-related variables, $DMI_{grass,}$ % grass, $NE_{L, supp}$ and DVE diet were positively correlated ($P < 0.001$) with the % Discrepancy whereas DMI_{supp} was negatively correlated ($P < 0.001$). Of the animal-related variables, milk protein, BW change and

Table 4. The percentages (R^2) of the discrepancy variance accounted for by the variables in the two grass diet groups.

Variable[1]	80–90% grass diets (n = 23)		40–65% grass diets (n = 19)	
	R^2	Level of significance[2]	R^2	Level of significance[2]
ASH	36.3 (–)[3]	**	–	
CP	1.4	NS	21.1 (–)	*
CF	15.8 (–)	*	51.5 (+)	***
NFE	20.8 (+)	*	–	
SU	22.4 (+)	*	–	
%OMD	13.6 (+)	*	26.9 (–)	*
DOM	31.8 (+)	**	3.4	NS
ME	12.0	NS	37.6 (–)	**
GE	–		–	
q	12.5	NS	59.8 (–)	***
DVE	–		46.9 (–)	***
OEB	4.4	NS	16.0	NS
DMI_{grass}	47.1 (+)	***	76.3 (+)	***
DMI_{supp}	12.3	NS	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	NS	5.0	NS
Milk	–		5.6	NS
MF	–		8.8	NS
MP	14.4 (+)	*	64.4 (+)	***
FPCM	–		15.3	NS
BW	8.3	NS	9.1	NS
BW change	31.9 (+)	**	55.9 (+)	***
GPCM	35.0 (+)	**	77.6 (+)	***
GPMW	34.4 (+)	**	70.9 (+)	***

[1] For abbreviations see Table 2.
[2] * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant.
[3] (–) = discrepancy decreases; (+) = discrepancy increases.

DVE diet were positively ($P < 0.001$) correlated with the % Discrepancy. In this analysis, correlations were strong, 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 these outliers, correlations were less strong or disappeared completely.

Discussion

Compared with $Output_{grass}$, $NE_{L, grass}$ was overestimated. This means that for cows on grass-based diets farmers overestimate the expected milk yield. It therefore is desirable to have insight into the causes of this discrepancy, as it could indicate how to produce grass with a composition that enables a more efficient milk production, or how to adjust the diet. If it is shown that certain factors cannot be manipulated this could mean that a lower milk production has to be accepted. To test the earlier mentioned hypotheses, the observed discrepancies are discussed in relation to grass-related, diet-related and animal-related variables.

Correlation of discrepancy with grass-related variables; hypothesis 1

Hypothesis 1 reads: The feed evaluation formula for grass is incorrect.

If this hypothesis were true, a positive correlation would be expected between discrepancy and grass variables. However, such correlations were not always evident. Some statistically significant correlations were observed but they were often contradictory. For the 80–90% grass diets DOM had the highest positive correlation (i.e. a higher chance for overestimating milk production) and ASH the highest negative correlation. DOM and ASH were also mutually correlated [$R^2 = 0.372$ ($P < 0.01$) in the 80–90% grass diet group], which is caused by the relation of ASH with OM. A high OM content will probably result in a high DOM value. As a high DOM content is correlated with a relatively low CF content [$R^2 = 0.201$ ($P < 0.05$) in the 80–90% grass diet 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 accounts for much of total DOM, and although correlations between discrepancy and digestible CP were not observed, a N surplus probably did occur. A relative deficiency of available energy reduces microbial growth 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 concentrates alone, because of optimization of availability of fermented substrate and increased rate of passage of digesta from the rumen (Clark *et al.*, 1992). So 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.

For the 40–65% grass diets a high grass quality (high ME, q, CP and DVE, and low CF, most of which are mutually correlated) decreased the discrepancy, which is the opposite from what was observed for the 80–90% grass diets. 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 not calculated correctly, which was observed only in the 80–90% grass diets and not in the 40–65% ones. As the correlations between the discrepancy and the grass-related variables did not hold when the outliers in the 40–65% grass group were excluded, the negative effect of the high quality grass on discrepancy was probably due to specific effects of feeding maize silage. The reasons for these effects will be discussed below.

Correlation of discrepancy with diet-related variables; hypothesis 2

Hypothesis 2 reads: The present formula calculates the potential energy value, but in some cases this energy is not fully utilized due to factors like an unbalanced nutrient supply.

If this hypothesis would be valid, positive correlations between discrepancy and diet-related variables would be expected. Such correlations were indeed found.

In the 80–90% grass diet group the discrepancy was positively correlated with DMI_{total}. This was expected because with a higher intake more energy would be required in the gastro-intestinal tract to support the contractions of rumen and intestine. Higher levels of nutrition are related to a higher blood flow and a higher oxygen consumption in the viscera (Burrin *et al.*, 1989). However, with sheep, the oxygen consumption per g of liver tissue did not differ between animals fed restricted on a maintenance level and animals fed *ad libitum*, which suggested that the level of feed intake did not affect tissue metabolic activity (Burrin *et al.*, 1989). This could explain why an effect of DMI_{total} was not observed in the 40–65% grass diet group. In the 80–90% grass diet group DMI_{total} was positively correlated with DMI_{grass} (R^2 = 0.703; $P < 0.001$). A high DMI_{grass} and a high % grass resulted in a higher discrepancy in both groups. Some literature data suggest higher energy requirements when cows are fed forage-based diets. A forage-rich diet will probably result in a larger digestive tract, which could lead to increased maintenance costs because of the accompanying higher oxygen consumption (Agnew *et al.*, 1998). Also experiments with beef cattle fed 75% alfalfa or 75% concentrates showed that weight gain is more efficient with a concentrate diet than with an alfalfa diet, which was ascribed to intense metabolic activity in gut and liver with forage-based diets (Reynolds *et al.*, 1991).

For both grass diet groups the discrepancy was negatively correlated with DMI_{supp} although it was most prominent for the 40–65% grass diet group. A possible explanation for the lower correlation found for the 80–90% grass diet group is probably the lower variation in percentage grass (and thus supplements) in the diets of this group. This percentage varied between 80.9 and 91.3, with an average of 87.9, and a median of 89.5. So 50% of the diets had a percentage grass higher than 89.5. From the statistical analysis of the DMI_{supp} values in the 80–90% grass diet group it appeared that the residuals were not randomly distributed. In the 40–65% grass diet group the variation was larger and the residuals were more randomly distributed. However, the lower discrepancy with high DMI_{supp} was probably due to the positive effect of maize silage. Although the cows on maize silage diets were in the same stage of lactation as the cows on the other diets, in four diets with maize silage the energy content of the grass was underestimated compared with the output. In diets with maize silage, high-quality grass reduced the discrepancy, whereas in the 80–90% grass diet 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 (1) a positive effect of slowly degradable starch on milk yield (Nocek & Tamminga, 1991), (2) the equalization of the degradation of energy and protein in the rumen (Tamminga, 1992) and thus to a more efficient production of microbial protein (Clark *et al.*, 1992), or (3) improved utilization of protein and energy (Moran & Stockdale, 1992).

Another possible explanation is that the estimated feeding value of the supplements was not correct, which is confirmed by the high positive correlation between the discrepancy and $NE_{L,\ supp}$. This correlation remains statistically significant even when the three outliers (with their negative discrepancy) were excluded from calculations ($R^2 = 0.675$; $P < 0.001$; not shown). The absence of correlations in the 80–90% grass diet group can be explained by the low variation in supplements: 7.0 to 7.2 MJ per kg DM.

In both diet groups the discrepancy was positively correlated with DVE diet, but the correlation was not statistically significant for the 80–90% grass diet group. If DVE diet is high, a N surplus will probably occur. Surplus N has to be converted in the liver to urea and has to be excreted in the kidneys. Both processes require energy. According to Valk (1994), 221 g more N in the 80–90% grass diets (compared with 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 via the saliva, increasing the internal pool of N. So this turnover of N with a high rate of ureogenesis in the liver could require more energy than expected from theoretical calculations. On the other hand, the fact that in the Krebs cycle ketoacids resulting from de-aminated amino acids are used as energy sources (Satter *et al.*, 1998) partly compensates for the energy costs of N excretion.

Kirkpatrick *et al.* (1997) found a (statistically non-significant) lower N-retention for beef cattle fed diets based on grass silage only, compared with 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 proportion of grass silage are associated with high urinary energy losses due to increased N excretion from inefficient utilization of the 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.

The positive correlations found between discrepancy and diet-related variables, and data from the literature (e.g. Agnew *et al.*, 1998; Reynolds *et al.*, 1991) confirm the hypothesis, so we can conclude that with high amounts of grass the chances 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-related factors; hypothesis 3

Hypothesis 3 reads: The energy requirements of dairy cows on grass-based diets are higher than previously assumed.

If this hypothesis were valid, positive correlations would be expected between the discrepancy and the animal-related variables.

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

Also pregnancy of the cows can have some effect. An energy bonus for pregnancy is usually given starting at six months of pregnancy (Van Es, 1978; Anon., 2000b). This state of pregnancy was not reached in any of the 12 experiments, so pregnancy did not account for extra energy in this study.

Furthermore, there was a high positive correlation between discrepancy and milk protein. Animals with an energy shortage usually show this by a lower protein content in the milk (Blaxter, 1962), so it was clear that discrepancy was not accompanied by an energy shortage. An energy surplus would be more likely. However, this energy surplus was not used for the production of milk.

Possibilities for adaptation of the energy evaluation of grass

The correlations between the discrepancy and the grass-related variables CP, CF, DOM, OEB and 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 statistically significant. Because discrepancy was positively correlated with DOM in the 80–90% grass diet group, it can be concluded that with a high DOM, and thus a low CF in the grass, the chances of discrepancy increase. The statistically significant correlation of discrepancy with DOM disappears in the 40–65% grass diet group, and the correlation with %OMD even becomes negative. These effects may be attributed to proportions of grass in these diets that are too low to observe a significant effect of chemical composition, or they may be attributed to an effect of the composition of the total diet on the discrepancy. It would therefore also be likely to support Hypothesis 2, i.e. that in some situations the grass is not fully utilized. This could be caused by the high amounts of grass in the diet or by the balance of the diet. Hypothesis 2 can be combined with part of Hypothesis 3: higher maintenance requirements for dairy cows on grass-based diets. In a previous study an increase of 10% was suggested (Bruinenberg *et al.*, 2002). Higher requirements could be caused by (1) increased energy requirements in the gastro-intestinal tract for movement of the digesta, and (2) the imbalance of this type of diet resulting in a reduction of ruminal microbial efficiency and increased energy costs for N excretion. Furthermore, some extra requirements for BW gain during the second half of the lactation may be expected.

Conclusions

There is a difference between the estimated energy input from grass-based diets and the energy output in milk and maintenance. Especially the proportion of grass in the diet affected this discrepancy, probably either due to an imbalanced nutrient supply or to the higher maintenance requirements on a grass-based diet. Furthermore, in diets with more than 80% grass a high DOM in the grass increased discrepancy.

The estimated feeding value of grasses in diets with more than 35% supplements does not appear incorrect. 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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