Added-value products: healthier milk from forage, scope for breeders?

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Abstract. Plant lipids are a major source of beneficial fatty acids (FA) in milk. Forage quality differences can affect rumen metabolism and there could be opportunities to change the composition of ruminant products through species or cultivar choice. Twelve dairy cows were used in two stall-feeding trials with fresh grass to evaluate the effect of diploid perennial ryegrass (*Lolium perenne*) cultivars on milk FA composition during the growing season.

Six cultivars were cut daily during three 14-day periods at the same target yield. The experiments consisted of two 3x3 Latin square trials, in each of which 3 cultivars were fed to 2 groups of three cows. The six cultivars differed significantly in concentrations of water-soluble carbohydrates, neutral-detergent fibre and crude protein. However, there were no significant differences among the six cultivars in FA concentration or proportions of FA. No variation in DM intake, milk production and composition or milk FA composition was found. The latter may be due to the lack of variation in grass FA concentration and composition in the cultivars studied.

Introduction

Dietary components that improve human health are gaining increased attention. Animal fats contain mainly saturated fatty acids (SFA) and increasing the proportion of (poly)unsaturated fatty acids (PUFA) would improve its value. Besides, ruminant products contain conjugated linoleic acid (CLA), especially C18:2 c-9 t-11, with perceived health benefits for consumers. Many studies with supplements to animal diets have been conducted to study effects on milk and meat, but there are very few direct comparisons allowing a precise evaluation of the effects of the basal forage diet on milk fatty acid (FA) composition (Chilliard *et al.* 2000). Little is known about the fat content and composition of forages and factors influencing them. Elgersma *et al.* (2003a) evaluated the effects of grass cultivar and season on the FA pattern of the fat fraction of perennial ryegrass (*Lolium perenne* L.). In two cultivars, Barlet and Magella, the ash, neutral detergent fibre (NDF), carbon (C) and nitrogen (N) concentrations did not differ but cv. Barlet had higher levels of linolenic acid (C18:3) and lower levels of linoleic acid (C18:2) than cv. Magella. In six other cultivars, there were differences for leaf blade and stem proportions and ash, NDF and N concentrations, but not for FA pattern (Elgersma *et al.* 2003a). Grass quality differences can affect rumen metabolism and there could be opportunities to change the composition of ruminant products through grass cultivar choice. To test this hypothesis, these six cultivars were fed to dairy cows in a stall-feeding trial with fresh grass to evaluate the effect of grass cultivar on milk FA composition during the growing season.

Materials and methods

Experimental design

The trial was carried out at Wageningen University, The Netherlands as part of a larger experiment. Fresh grass of eight cultivars of perennial ryegrass was stall-fed to 12 highly productive Holstein Friesian cows (Tas *et al.* 2005). The experimental period

covered 14 weeks, divided into seven periods (P1-7) of two weeks. In periods 1, 3, 5 and 7, two cultivars (Barlet and Magella) were fed (Elgersma *et al.* 2003b). In periods 2, 4 and 6, six cultivars were fed. Three groups of 2 replicate cows were fed three cultivars during the 3 periods in a 3 x 3 Latin Square design (LS 1), and six other cows were fed three other cultivars (LS 2).

Grass sampling procedures

Six diploid perennial ryegrass (*Lolium perenne* L.) cultivars were sown. The heading date of cvs A, B and C is in the period 25 – 28 May, whereas cvs D, E and F have their heading date during 7-9 June. The experimental design was randomized blocks with 21 replicates, each consisting of six adjacent plots (Elgersma *et al.* 2003a; Smit *et al.* 2005). The plots were cut daily between 13.30 and 14.30 p.m. during periods 2, 4 and 6, starting on 23 June, 21 July and 18 August. Samples of four days during the second week of each period, days 10-13, were pooled and the NDF concentration was determined (Van Soest *et al.* 1991) as well as ash, ADL, CP, and WSC, C and N (Dumas) and the C/N ratio was calculated. At day 11 of each period, a grass sample was collected to determine the fatty acid composition. The procedure for fat extraction was described by Elgersma *et al.* (2003).

Cows and intake measurements

Twelve Holstein Friesian dairy cows were used. Six cows were fitted with a rumen fistula (Taweel *et al.* 2005). Replicate cows were selected on date of calving and assigned randomly to the cultivars. The cows were fed fresh grass *ad libitum*, supplemented with concentrates at a fixed level (4 kg DM/d) at milking (Tas *et al.* 2005). Feed residues were removed before each milking. Half of the grass that was cut early afternoon was stored in containers in the barn, and half was stored overnight at 4 °C. From 15 h on, freshly cut grass was fed in portions till 22 h, and from 06 h onward cut grass was fed from the cooling unit. The DM intake (DMI) was recorded daily for each cow during the experimental period. The first five days were used for adaptation and the average intake per period was calculated from days 6-13. Although in general longer-term trials would have allowed the rumen biota more time to adjust to the change in diet, there is repeated evidence that changes in milk FA composition in response to diet occur within days (e.g., Elgersma *et al.* 2004). Feed residues were removed daily at 0600 and 1600 and collected for each cow. Samples of refusals and diet samples were dried at 70 °C for 24 h to determine DMI.

Milk sampling procedures

At each milking, yield was determined and milk was sampled. Two samples were stored at 4 °C with a preservative until analysed for fat, protein, lactose and urea content by mid-infrared. Each sample was a composite of consecutive evening and morning milking (1:1 ratio). On day 11, a milk sample of 200 ml was stored at -20 °C for FA analysis (Elgersma *et al.* 2003b).

Determination of fatty acids

The fatty acid methyl esters (FAME) were analysed on a Carlo Erba 5200 gas chromatograph, equipped with a flame ionisation detector (FID), detector temperature 280°C. The carrier gas was hydrogen, inlet pressure of 200 kPa. Samples were injected by split injection (split ratio 50:1). A 100m fused-silica column coated with 100% cyanopropyl polysiloxane (Varian-Chrompack, CP-Sil 88, i.d. 0.25mm; df 0.2 µm) was used isothermally at 175 °C. Identification of the FA was based upon retention times using standards of methyl esters. A mixture of the standards of the individual FAME was used to determine calibration factors. The areas of the peaks in the chromatogram

were calculated and normalized using calibration factors. Proportions (Pi) of individual FA were calculated.

Statistical analyses

Data were processed using the statistical package Genstat 5 release 5.4. A Latin Square analysis of variance was used and LS1 and LS2 were analysed separately. The cow was the experimental unit. Effects of cow and period on DMI, MP, milk FA and rumen VFA concentrations were analysed.

Results and discussion

The six cultivars were rather variable in their morphological and chemical characteristics (Table 1). Cvs E and B had a lower WSC concentration ($P < 0.001$) than the other cultivars, while cvs A and F had the highest ($P < 0.001$) WSC and the lowest ($P < 0.01$) NDF concentrations. Cv. F had the lowest, and cv. E the highest CP concentration ($P < 0.001$). Despite the variation in quality parameters among the cultivars, their DMI did not differ, and milk production and composition were similar (Table 2). The fact that DMI, MP and MC were similar enabled an unbiased comparison of the effects of grass cultivar quality and composition on milk FA composition in this study. Both experiments contained a high sugar variety (HSV): intermediate-heading cv. A in LS1 and late-heading cv. F in LS2 (Taweel *et al.* 2005). In contrast to some evidence reported in literature (e.g., Miller *et al.* 2001), in this study the variation in WSC between the cultivars did not affect intake. There was even a tendency for a lower DMI of the HSV, as discussed by Tas *et al.* (2005).

Despite differences for ash, NDF, CP, WSC and N concentrations (this study) and leaf blade and stem proportions, the grass cultivars did not differ in FA composition (Elgersma *et al.* 2003a).

The milk had a favourable FA composition from a health viewpoint, with low SFA levels. The concentrations of FA in milk did not differ among the six cultivars (Table 3), which may be due to the lack of sufficient variation in grass lipid composition between the cultivars and throughout the season (Elgersma *et al.* 2003a). However, in the intermittent periods, milk fat from cows fed cv. Barlet, with a consistently higher C18:3 concentration than cv. Magella, did contain less SFA and more PUFA and CLA (Elgersma *et al.* 2003b). This may suggest that variation in lipid composition of the diet would be needed to affect milk lipid composition, but that plant morphological or chemical characteristics such as ash, NDF, CP, and WSC concentration do not affect milk FA if the herbage FA concentration is not different (this study). Further research is needed to test this hypothesis, as there is also evidence that in herbage, the N concentration is positively related with the total FA and C18:3 concentrations (Elgersma *et al.* 2005).

Table 1. Herbage yield (> 5 cm), leaf blades and quality parameters of six diploid perennial ryegrass cultivars, mean values from pooled samples taken during days 10-13 during each of three periods (P2, P4, P6) from June-August, standard error of difference, and significance among cultivar means (NS = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

WSC = water-soluble carbohydrates, CP = crude protein, NDF = neutral-detergent fibre, ADL = acid-detergent fibre

Cultivar	HY (kg DM/ha)	Leaf blades (% of DM)	WSC	CP	NDF (g/kg DM)	ADL	ASH	C/N (g/g)
A	2173	82	192	160	399	16.7	110	17.2
B	2159	80	152	159	429	18.2	121	17.4
C	2149	78	170	157	423	16.5	113	17.6
D	2099	81	172	156	412	17.2	118	17.7
E	1993	86	158	166	414	16.9	119	16.5
F	2185	88	195	150	400	15.3	116	18.4
Mean	2126	83	173	158	413	16.8	116	17.5
s.e.d	27	1	5	2	6	1.09	5.4	0.7
Sign.	***	***	***	***	**	NS	*	**

Table 2. Herbage dry matter intake (DMI) (kg DM/d) of six perennial ryegrass cultivars, milk production (MP) (kg/d), milk composition, and fat- and protein-corrected milk yield (FPCM = $(0.337 + 0.116 \times \text{fat}\% + 0.06 \times \text{protein}\%) \times \text{MP}$), averaged over two replicate cows and three 14-day periods in two Latin Square (LS) experiments. Standard error of means and significance among cultivar means are indicated (NS = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Cultivar	DMI	MP	Fat	Protein	FPCM
LS1					
A	16.2	26.9	40.5	34.3	27.0 ^{ab}
C	17.4	28.1	39.2	33.7	27.8 ^a
E	17.4	26.3	38.3	34.1	26.0 ^b
s.e.m.	0.4	1.2	1.1	0.4	1.2
Sign	NS	NS	NS	NS	*
LS2					
B	16.6	28.2	37.9	32.9	27.5
D	16.8	27.9	38.5	33.8	27.3
F	16.1	26.6	39.7	33.4	26.6
s.e.m.	0.4	0.9	0.9	0.5	0.8
Sign	NS	NS	NS	NS	NS

Table 3. Milk fatty acid composition (mg/g fat) of milk produced from cows stall-fed fresh herbage of six perennial ryegrass cultivars during three 14-day periods in two Latin Square (LS) experiments, averaged over two replicate cows. SFA = saturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids, CLA = conjugated linoleic acid.

Cultivar	SFA	MUFA	PUFA	CLA
LS1				
A	650	290	39	12.6
C	650	289	41	15.3
E	637	297	45	17.4
LS2				
B	666	275	40	13.6
D	659	279	42	16.2
F	663	277	40	14.5

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