Fatty acid intake and milk fatty acid composition of Holstein dairy cows under different grazing strategies: Herbage mass and daily herbage allowance

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ABSTRACT

The objective of this study was to investigate the effect of level of 1) pregrazing herbage mass (HM) and 2) level of daily herbage allowance (DHA) on the performance and fatty acid (FA) composition of milk from grazing dairy cows. Sixty-eight Holstein-Friesian dairy cows were allocated to either a high or low pregrazing HM (1,700 vs. 2,400 kg of DM/ha; >40 mm), and within HM treatment, cows were further allocated to either a high or low DHA (16 vs. 20 kg of DM/d per cow; >40 mm) in a 2 × 2 factorial design. Pregrazing HM did not affect dry matter intake (17.5 ± 0.75 kg/d), milk production (22.1 ± 0.99 kg/d), milk composition (milk fat, $3.88 \pm 0.114\%$; milk protein, $3.28 \pm 0.051\%$), body weight (525 ± 16 kg), or body condition score (2.65 ± 0.064). Increasing DHA increased dry matter intake ($+1.5$ kg/d) but did not affect any other variable measured. Cows grazing the low HM or high DHA had a higher daily intake of total FA ($+0.12$ and $+0.09$ kg/d, respectively, for the low HM and high DHA), α -linolenic acid (LNA; $+0.08$ and $+0.05$ kg/d, respectively, for the low HM and high DHA), and linoleic acid ($+0.01$ for both the low HM and high DHA) compared with either the high HM or low DHA. Milk conjugated linoleic acid (*cis*-9, *trans*-11 isomer) was not affected by treatment (13.0 ± 0.77 g/kg of total FA); however, large variation was recorded between individual animals (range from 5.9 to 20.6 g/kg of total FA). Milk concentrations of LNA were higher for animals offered the low HM (5.3 g/kg of total FA), but across treatments, milk concentrations of LNA were low (4.9 ± 0.33 g/kg of total FA). The present study indicates that changes in HM and DHA do not have a great effect on the milk FA composition of grazing dairy cows. Further enhancement of the beneficial FA content in milk purely from changes in grazing strategy may be difficult when pasture quality is already high.

Key words: conjugated linoleic acid, linolenic acid, herbage mass, daily herbage allowance

INTRODUCTION

There is increasing interest in enhancing the polyunsaturated fatty acid (PUFA) composition of the human diet, particularly the n-3 fatty acid (FA) and conjugated linoleic acid (CLA) content. Indeed, both these groups of PUFA are thought to confer positive effects on human health (Parodi, 1999; Hamazaki et al., 2003; Lock and Bauman, 2004). For humans, milk fat from dairy cows is one of the principal dietary sources of CLA (Khanal and Olson, 2004; Schroeder et al., 2004), a collective term for geometric and positional isomers of the essential dietary PUFA linoleic acid (LA), of which the *cis*-9, *trans*-11 isomer is the most predominant in ruminant tissues. Furthermore, concentrations of the essential n-3 PUFA α -linolenic acid (LNA; C18:3n-3) and its longer chain derivatives are present in ruminant tissue and milk (Lock and Bauman, 2004).

Many studies have shown that increased pasture intake leads to elevations in the CLA and n-3 FA concentrations of milk (Kelly et al., 1998; Dhiman et al., 1999; Kraft et al., 2003), potentially because of higher concentrations of LNA in particular in fresh herbage compared with either conserved forages or cereal-based concentrate feeds (Schroeder et al., 2004; Dewhurst et al., 2006). Conjugated linoleic acid is synthesized by ruminal microorganisms during the ruminal biohydrogenation of LNA and LA. The majority of tissue and milk CLA is formed through *de novo* mammary tissue synthesis from the desaturation of another product of this ruminal biohydrogenation process, vaccenic acid (VA; *trans*-11 C18:1), to *cis*-9, *trans*-11 CLA. This latter reaction is catalyzed by the actions of the enzyme stearoyl coenzyme A, otherwise known as Δ^9 -desaturase (Lock and Garnsworthy, 2003), and is responsible for up to 90% of the *cis*-9, *trans*-11 CLA found in milk fat (Pipero et al., 2002; Lock and Garnsworthy, 2003). Furthermore, there is evidence of a synergistic relationship between dietary LA and n-3 PUFA intake

Received May 20, 2009.

Accepted July 4, 2009.

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on the synthesis of *cis*-9, *trans*-11 CLA in dairy cows (AbuGhazaleh et al., 2007).

Because the PUFA content in fresh forage is highly variable (Palladino et al., 2009), the milk PUFA content in grazing cows can be influenced by many factors, including the species and cultivars grazed as well as the quantity and quality of the herbage available. For example, seasonal effects on milk PUFA concentration have been identified, in which CLA was at a higher concentration in milk during spring and early summer compared with autumn, coincident with seasonal variation in the herbage PUFA content (Chilliard et al., 2001; Lock and Garnsworthy, 2003; Dewhurst et al., 2006). Furthermore, reducing the daily herbage allowance (**DHA**) from 20 to 16 kg of DM per cow reduced the concentration of CLA in milk (Stanton et al., 1997; Dewhurst et al., 2003). Additionally, Elgersma et al. (2004) recorded lower concentrations of milk CLA and VA when herbage allowance was reduced by 50%. Despite this, other authors have failed to observe any effect of modifying the pasture allowance on milk PUFA content (Dewhurst et al., 2006).

The equivocation between studies has resulted in a lack of clear information for producers on grazing management practices to optimize the concentration of health-promoting PUFA in milk across the season. Additionally, to the authors' knowledge, there is little or no published information on the effect of variation in pregrazing herbage mass (**HM**) on milk FA composition. Indeed, an understanding of how HM and DHA interact to affect sward structure, and thus milk yield and composition, is essential to successfully manipulate the concentration of beneficial FA in milk from grazing cows.

The objective of this study was therefore to examine the effect of contrasting levels of pregrazing HM (kg of DM/ha) and DHA (kg of DM/d per cow) on the milk FA composition of grazing dairy cows during 4 different stages of lactation (**SL**).

MATERIALS AND METHODS

The experiment was carried out at Moorepark Dairy Production Research Center, Fermoy, Co. Cork, Ireland (52°09' N; 8°16' W), during 2007. The soil type is a free-draining acid brown earth of sandy loam to loam texture. A predominantly perennial ryegrass (*Lolium perenne* L.) pasture was used during the experimental period. The swards were on average 5 yr old.

Animals and Experimental Design

The objective of this study was to investigate the effect of 2 levels of pregrazing HM (1,700 vs. 2,400

kg of DM/ha; >40 mm), and within this, 2 levels of DHA (16 vs. 20 kg of DM/d per cow; >40 mm) on the milk FA composition of grazing dairy cows. Sixty-eight Holstein-Friesian dairy cows (20 primiparous and 48 multiparous) were selected from the Moorepark spring-calving dairy herd and balanced by calving date (February 7; SD 15.6), lactation number (2.8; SD 1.84), and the following data, which were collected during wk 2 and 3 of lactation: milk yield (28.1; SD 4.26), BW (513; SD 64.2), and BCS (2.8; SD 0.41). Animals were then randomly allocated within block to 1 of 4 treatments in a 2 × 2 factorial design replicated over 4 time points (April, May, July, and August) representing different SL, although these time points also represented differences in the stage of grass growth. Cows commenced the study at 74 DIM (SD 14.7). The 4 treatments were **HH** (high HM, high DHA), **HL** (high HM, low DHA), **LH** (low HM, high DHA), and **LL** (low HM, low DHA). Fresh herbage was allocated to each treatment group on a daily basis after the morning milking. A small quantity of concentrate (0.4 kg/cow per day) was offered in the milking parlor in 2 equal feedings at both the morning and evening milking (concentrate composition, on a fresh weight basis, was ground citrus pulp, 0.305 kg/kg of concentrate; barley, 0.237 kg/kg of concentrate; corn (*Zea mays*) gluten, 0.249 kg/kg of concentrate; soybean meal, 0.14 kg/kg of concentrate; vitamin-mineral mix, 0.043 kg/kg of concentrate; and fat, 0.026 kg/kg of concentrate. Chemical analyses are shown in Table 1).

Sampling, Measurements, and Analyses

HM Determination. Herbage mass (grass cut at >40 mm above ground level) was calculated by cutting 4 strips (1.2 × 10 m) for each herbage allowance area twice weekly with an Agria machine (Etesia UK Ltd., Warwick, UK) to determine sward density and HM. Ten grass height measurements were also recorded before and after harvesting by using an electronic plate meter (Urban and Caudal, 1990) with a plastic plate (30 × 30 cm and 4.5 kg/m; Agrosistèmes, Choiseille, France). The herbage harvested was weighed, and subsamples were collected (approximately 0.1 kg) for DM determination at 95°C over 16 h. Additional subsamples were collected for chemical composition (including FA analysis) and stored at −20°C before being freeze-dried. To calculate the sward density, the following equation was used:

$$\text{Sward density} = \{ \text{HM (kg of DM/ha)} \div [\text{precutting height} - \text{postcutting height (kg of DM/cm per ha)}] \}.$$

Pre- and Postgrazing Sward Heights. Pregrazing sward height was measured daily throughout the ex-

Table 1. Herbage mass and chemical composition of selected herbage samples and concentrate offered $\pm$ SD¹

Item	Treatment ²				Concentrate ³
	HH	HL	LH	LL	
Herbage mass, kg of DM/ha	2,518 $\pm$ 456.8	2,271 $\pm$ 436.1	1,836 $\pm$ 190.3	1,554 $\pm$ 167.7	—
OM digestibility, g/kg of DM	845 $\pm$ 17.0	844 $\pm$ 24.9	863 $\pm$ 14.6	862 $\pm$ 7.9	—
CP, g/kg of DM	199 $\pm$ 22.4	183 $\pm$ 12.6	263 $\pm$ 14.0	257 $\pm$ 24.2	186 $\pm$ 16.7
NDF, g/kg of DM	412 $\pm$ 20.0	396 $\pm$ 24.4	369 $\pm$ 23.6	392 $\pm$ 23.2	248 $\pm$ 40.8
ADF, g/kg of DM	246 $\pm$ 22.8	251 $\pm$ 36.6	257 $\pm$ 25.1	261 $\pm$ 12.6	—
Ash, g/kg of DM	70.2 $\pm$ 5.50	65.8 $\pm$ 2.07	73.4 $\pm$ 9.96	79.1 $\pm$ 3.05	100 $\pm$ 6.6

¹Means calculated from 8 samples per treatment.²Treatments: HH = high herbage mass (HM), high daily herbage allowance (DHA); HL = high HM, low DHA; LH = low HM, high DHA; LL = low HM, low DHA.³Concentrate = concentrate offered.

periment by recording approximately 20 heights across the 2 diagonals of each grazing area. The DHA for each treatment was calculated by multiplying pregrazing sward heights (>40 mm) by sward density. A similar number of sward heights were recorded after grazing for each treatment.

Milk Production and Composition. Milking commenced at 0700 and 1600 h daily. Individual milk yields (kg) were recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, protein, and lactose concentrations were calculated weekly for each animal with a MilkoScan 203 instrument (DK-3400, Foss Electric, Hillrød, Denmark). Body weight was recorded weekly with a portable electronic weighing scale. Body condition score was assessed weekly and was scored by 1 experienced technician on a scale of 1 to 5 (Lowman et al., 1976).

Measurement of DMI and Feed Sample Analysis. Dry matter intake of all individual cows was estimated by using the *n*-alkane technique described by Mayes et al. (1986) and modified by Dillon and Stakelum (1989). Cows were dosed twice daily, before both the morning and evening milking, for 12 d with a paper pellet (Carl Roth GmbH, Karlsruhe, Germany) containing 500 mg of dotriacontane (C₃₂-alkane). Fecal grab samples were collected twice daily, from d 7 to 12, before both the morning and the evening milking and were subsequently frozen at -20°C . During the same period, herbage samples were manually collected with a Gardena hand shears (Acu 60, Gardena International GmbH, Ulm, Germany) at the approximate height to which cows grazed after each morning and evening milking, to allow a representative sample of the herbage actually grazed. A sample of concentrate was collected daily, and both herbage and concentrate were frozen at -20°C after collection. Before analysis, fecal grab samples were thawed and both morning and evening samples were bulked (10 g of each) by cow. Fecal samples were then dried at 40°C for 48 h and milled

through a 1-mm screen. The *n*-alkane concentrations of the dosed pellets, feces, herbage, and concentrate were determined as described by Dillon (1993).

Chemical Analysis of Herbage. Samples were stored at -20°C before being freeze-dried and milled through a 1-mm sieve before chemical analysis. The herbage samples and the concentrate were analyzed for DM content, ADF, NDF (Ankom Technology, Macedon, NY), ash, CP (Leco FP-428, Leco Australia Pty Ltd., Baulkham Hills, New South Wales, Australia), and OM digestibility (Morgan et al., 1989). Chemical analyses of both herbage and concentrate are shown in Table 1.

FA Analysis. The FA composition of the herbage and concentrate samples was analyzed by gas chromatography, using a one-step methylation procedure (Sukhija and Palmquist, 1988). Samples were injected by autosampler on a Varian GC 3800 instrument (Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector. Methyl esters of FA (injected using a 10:1 split ratio) were separated on a fused-silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.39- μm film thickness; Varian Fame Select CP 7420, Varian Inc.). The injector temperature was held at 250°C , and the detector temperature was held at 300°C . The initial oven temperature was 140°C (held for 10 min) and was then increased to 240°C at a rate of $4^{\circ}\text{C}/\text{min}$ (held for 17 min). Nitrogen was used as the carrier gas and the column flow was held at 2 mL/min. Results are shown in Table 2.

To determine milk FA composition, samples from the evening milking were centrifuged at 4°C ($978 \times g$) for 20 min, and the cream was extracted, flushed with N₂ for 10 to 15 s, and stored at -20°C overnight. The following morning, samples were warmed to 40°C and centrifuged for 10 min ($978 \times g$; 30°C). The extracted fat was then stored at -20°C until FA analysis. The FA composition of the milk fat was analyzed by gas chromatography by using an adaptation of the method

Table 2. Fatty acid composition of selected herbage samples and concentrate offered (g/kg of DM $\pm$ SD)

Fatty acid, g/kg of DM	Treatment ¹				Concentrate ²
	HH	HL	LH	LL	
C14	2.2 $\pm$ 1.98	1.0 $\pm$ 0.31	0.9 $\pm$ 0.49	1.4 $\pm$ 0.89	1.5 $\pm$ 0.18
C16	12.5 $\pm$ 3.08	11.1 $\pm$ 3.80	14.4 $\pm$ 1.28	15.0 $\pm$ 2.71	30.1 $\pm$ 0.07
C16:1	2.2 $\pm$ 0.37	1.5 $\pm$ 0.79	1.9 $\pm$ 0.23	2.5 $\pm$ 0.90	2.7 $\pm$ 0.10
C18	1.2 $\pm$ 1.74	0.2 $\pm$ 0.13	0.4 $\pm$ 0.30	0.7 $\pm$ 0.39	1.7 $\pm$ 0.60
C18:1	1.0 $\pm$ 0.45	0.6 $\pm$ 0.54	0.8 $\pm$ 0.08	1.0 $\pm$ 0.22	16.4 $\pm$ 0.04
C18:2 (linoleic acid)	6.5 $\pm$ 1.58	6.2 $\pm$ 0.53	7.2 $\pm$ 1.00	6.9 $\pm$ 1.32	19.2 $\pm$ 0.10
C18:3n-3 (α -linolenic acid)	30.6 $\pm$ 3.22	26.7 $\pm$ 4.02	33.8 $\pm$ 7.45	35.5 $\pm$ 10.38	3.4 $\pm$ 0.57
Total	56.2 $\pm$ 2.96	47.3 $\pm$ 8.57	59.4 $\pm$ 9.92	63.1 $\pm$ 15.84	75.9 $\pm$ 0.01

¹Treatments: HH = high herbage mass (HM), high daily herbage allowance (DHA); HL = high HM, low DHA; LH = low HM, high DHA; LL = low HM, low DHA.

²Concentrate = concentrate offered.

of Christie (1982). Approximately 1 mg of milk fat was dissolved in dried *n*-hexane. Methyl acetate (20 μ L) and 1 *M* sodium methoxide in anhydrous methanol (20 μ L) were added after neutralization with a saturated solution of oxalic acid in methanol (30 μ L). After centrifugation, an aliquot of the upper layer was taken for analysis by gas chromatography. Samples were injected by autosampler on a Varian GC 3800 instrument equipped with a flame-ionization detector. The FA methyl esters (injected using a 10:1 split ratio) were separated on a fused-silica capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.39- μ m film thickness; Varian Fame Select CP 7420, Varian Inc.). The injector temperature was held at 250°C, and the detector temperature was held at 260°C. The initial oven temperature was 140°C (held for 5 min), increased to 180°C at a rate of 4°C/min (held for 5 min), and then increased to 240°C (4°C/min; held for 15 min). Nitrogen was used as a carrier gas. The pressure of the column was held at 275 kPa (held for 8 min) and then increased to 448 kPa at a rate of 14 kPa/min (held for 24 min).

Weather

Total rainfall for the experimental period (from April to August) was similar to the previous 10-yr average, although the distribution differed. The rainfall for April and May was lower than the 10-yr average (17 vs. 74 mm, and 56 vs. 73 mm for April and May, respectively), whereas total rainfall for June (106 mm) and July (105 mm) was greater than the 10-yr average (64 and 50 mm for June and July, respectively). Total rainfall for August was similar to the 10-yr average (94 and 98 mm, respectively). The mean temperature during April was 11.3°C (2.7°C warmer than the 10-yr average), and May (12.1°C) and June (14.5°C) were 0.8°C warmer than the 10-yr average. The mean temperatures for July (14.9°C) and August (15.3°C) were colder than

the averages for the previous 10 yr (−0.7 and −0.4°C for July and August, respectively).

Statistical Analysis

Statistical analyses were carried out using the SAS package, version 9.1 (SAS Institute, 2002) according to the factorial design used. Data were analyzed using the MIXED procedure, and the existence of a statistically significant difference between least squares means was tested by the PDIF command incorporating the Tukey test for pairwise comparison of treatment means. For all variables, cow was included as a random effect, and the model used was as follows:

$$Y_{ijk} = \mu + H_i + D_j + L_k + (H_i \times D_j) + (H_i \times L_k) + (D_j \times L_k) + (H_i \times D_j \times L_k) + e_{ijk}$$

where Y_{ijk} represents the response; μ is the mean; H_i is the HM ($i = 1$ to 2); D_j is the DHA ($j = 1$ to 2); L_k is the SL or month ($k = 1$ to 4); $H_i \times D_j$ is the interaction between HM and DHA; $H_i \times L_k$ is the interaction between HM and SL; $D_j \times L_k$ is the interaction between DHA and SL; $H_i \times D_j \times L_k$ is the interaction between HM, DHA, and SL; and e_{ijk} is the residual error term. Significant differences were declared at $P < 0.05$ and trends were declared at $P < 0.10$. Additionally, regression analyses were performed to examine the relationship between various FA in milk and between FA intake and milk FA concentration by the REG procedure of SAS.

RESULTS

Animal Performance

DMI, BCS, and BW Change. There were interactions between DHA and SL ($P < 0.05$); HM and SL

Table 3. Effect of herbage mass and daily herbage allowance on animal performance

Item	Treatment ¹				SED ²	Significance		
	HH	HL	LH	LL		HM	DHA	HM × DHA
DMI, kg/d	18.3	17.2	18.2	16.2	1.07	NS	*	NS
BCS	2.75	2.61	2.55	2.66	0.091	NS	NS	†
BW, kg	529	542	521	510	22.6	NS	NS	NS
Milk production, kg/d	22.5	21.8	22.3	21.5	1.40	NS	NS	NS
Milk fat, %	3.83	3.90	3.92	3.84	0.162	NS	NS	NS
Fat yield, kg/d	0.85	0.83	0.87	0.82	0.058	NS	NS	NS
Milk protein, %	3.33	3.29	3.31	3.20	0.073	NS	NS	NS
Protein yield, kg/d	0.75	0.71	0.73	0.69	0.048	NS	NS	NS
Efficiency, kg of milk/kg of DMI	1.21	1.25	1.22	1.30	0.040	NS	*	NS

¹Treatments: HH = high herbage mass (HM), high daily herbage allowance (DHA); HL = high HM, low DHA; LH = low HM, high DHA; LL = low HM, low DHA.

²Standard error of the difference.

† $P < 0.10$; * $P < 0.05$.

($P < 0.01$); and HM, DHA, and SL ($P < 0.01$). The highest DMI was recorded in the second SL (May) for cows at a high DHA (19.8 kg of DM/d; $P < 0.05$). The DHA affected DMI, being higher for cows grazing at a high DHA (+1.5 kg of DM/d; Table 3). Moreover, DMI varied with SL, with the highest intake (18.7 kg of DM/d) recorded during the second SL (Table 4).

Despite the differences in DMI, no differences were recorded for BCS for either DHA or HM, but BCS was affected by SL ($P < 0.01$; Table 4), decreasing as SL progressed (from 2.75 to 2.60). In contrast, mean BW increased with SL, increasing from 517 kg in the first SL (April) to 542 kg in the last SL (August; $P < 0.01$).

Milk Production. Data regarding the effect of treatment on milk production-related variables are presented in Table 3. Despite the differences recorded for DMI, no difference ($P > 0.05$) was detected between treatments in milk production. However, the low-DHA treatment displayed the highest efficiency (kg of milk/kg of DMI) because of the lower DMI recorded for that treatment.

No differences were found for milk fat (%) and protein (%). As expected, SL affected all milk-related variables (Table 4). Milk production, fat yield, protein yield, and efficiency decreased as the SL progressed. In contrast, milk fat and milk protein (%) increased in the last 2 SL recorded (July and August).

FA Intake. There was an interaction between HM and SL, with the highest FA intake (1.3, 0.77, and 0.15 kg/d of total FA, LNA, and LA, respectively) for cows at a low HM in the last SL ($P < 0.01$). There was an interaction between DHA and SL for total FA intake ($P < 0.01$), with the highest intake in the last SL for cows at both a low and a high DHA (1.2 kg/d). The LNA intake was also higher at a low and a high DHA during the last SL (0.65 kg/d; $P < 0.01$). Additionally, LA intake was higher in the second SL for both the high and low DHA (0.10 and 0.13 kg/d for the high and low DHA, respectively; $P < 0.01$). Both HM and DHA affected daily intake of FA (Table 5). Cows on the low-HM treatment had higher total FA ($P < 0.05$), LNA

Table 4. Effect of stage of lactation on animal performance

Item	Stage of lactation ¹				SED ²	Significance
	I	II	III	IV		
DMI, kg/d	16.9 ^b	18.7 ^a	17.1 ^b	17.1 ^b	0.21	**
BCS	2.75 ^a	2.65 ^b	2.58 ^c	2.60 ^{bc}	0.025	**
BW, kg	517 ^c	518 ^c	524 ^b	542 ^a	2.1	**
Milk production, kg/d	28.4 ^a	23.6 ^b	19.5 ^c	16.5 ^d	0.29	**
Milk fat, %	3.72 ^b	3.76 ^b	3.98 ^a	4.04 ^a	0.083	**
Fat yield, kg/d	1.06 ^a	0.88 ^b	0.77 ^c	0.66 ^d	0.216	**
Milk protein, %	3.24 ^b	3.12 ^c	3.38 ^a	3.38 ^a	0.025	**
Protein yield, kg/d	0.92 ^a	0.74 ^b	0.66 ^c	0.56 ^d	0.010	**
Efficiency, kg of milk/kg of DMI	1.67 ^a	1.25 ^b	1.12 ^c	0.95 ^d	0.021	**

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Stage of lactation: I = April; II = May; III = July; IV = August.

²Standard error of the difference.

** $P < 0.01$.

Table 5. Effect of herbage mass and daily herbage allowance on intake of fatty acids and transfer to milk

Item	Treatment ¹					Significance		
	HH	HL	LH	LL	SED ²	HM	DHA	HM × DHA
Total fatty acid intake, kg/d	0.99	0.92	1.13	1.02	0.060	*	*	NS
Linolenic acid intake, kg/d	0.52	0.50	0.63	0.56	0.034	**	*	NS
Linoleic acid intake, kg/d	0.12	0.11	0.13	0.12	0.007	*	*	NS
Linolenic acid transferred to milk, ³ %	0.70 ^{ab}	0.67 ^b	0.64 ^b	0.82 ^a	0.060	NS	†	*

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Treatments: HH = high herbage mass (HM), high daily herbage allowance (DHA); HL = high HM, low DHA; LH = low HM, high DHA; LL = low HM, low DHA.

²Standard error of the difference.

³Total amount of linolenic acid in milk/linolenic acid intake.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

($P < 0.01$), and LA ($P < 0.05$) intakes than those on the high-HM treatment. With regard to DHA, animals on the high DHA had the highest intake of total FA, LNA, and LA ($P < 0.05$). Total FA intake was highest in the last SL (1.15 kg/d; Table 6) and was lowest in the third SL (0.86 kg/d; $P < 0.01$). The same pattern was observed for LNA (0.47 and 0.62 kg/d for the third and fourth SL, respectively; $P < 0.01$). Because no differences in DMI were found for the last 2 SL, this variation can be explained by the higher content of total FA and LNA of the pasture in the fourth SL (38.8 and 69.1 g of FA/kg of DM vs. 27.4 and 48.8 g of FA/kg of DM for total FA and LNA, respectively).

Milk FA Composition

The concentration of FA in milk is presented in Table 7. There were interactions between HM and DHA for C10:0, C12:0, C14:0, and *cis*-9 C18:1 ($P < 0.05$). Cows in the HL treatment presented the lowest milk content of *cis*-9 C18:1 (160.4 g/kg of total FA). The highest concentration of *cis*-9 C18:1 was recorded for cows grazing in the LL treatment (185.8 g/kg of total FA). The high HM increased the proportion of C14:0 ($P < 0.05$) and C15:0 in milk ($P < 0.01$). This led to

an increase in medium-chain FA (MCFA), which were higher at a high HM ($P < 0.05$), and there was also a trend toward a higher content of saturated FA (SFA; $P < 0.10$). The milk content of LNA was higher in cows in the low-HM treatment ($P < 0.01$). No effect of DHA was recorded for any FA. Only MCFA tended to be higher in milk from cows grazing in the high-DHA treatment ($P < 0.10$). Despite an absence of treatment differences for CLA content, there was evidence of substantial variation among cows across treatments, with a minimum CLA concentration of 5.9 g/kg of total FA and a maximum of 20.6 g/kg of total FA being recorded (concentrations are the average of the 4 SL per cow).

Cows in the HL treatment presented the highest content of SFA (680.4 g/kg of total FA) and the lowest content of unsaturated FA (UFA; 246.9 g/kg of total FA), whereas the LL treatment showed a contrasting response (638.2 and 281.4 g/kg of total FA for SFA and UFA, respectively; $P < 0.05$). Consequently, the UFA:SFA ratio was higher for cows in the LL treatment (0.45 vs. 0.38 g/kg of total FA for the HL treatment; $P < 0.05$). Because most of the SFA were MCFA, cows in the HL treatment had a higher content of MCFA (460.4 g/kg of total FA) and a lower content of long-chain FA (LCFA; 280.1 g/kg of total FA) compared with those

Table 6. Effect of stage of lactation on fatty acid intake and transfer to milk

Item	Stage of lactation ¹				SED ²	Significance
	I	II	III	IV		
Total fatty acid intake, kg/d	1.00 ^c	1.06 ^b	0.86 ^d	1.15 ^a	0.012	**
Linolenic acid intake, kg/d	0.58 ^b	0.54 ^c	0.47 ^d	0.62 ^a	0.007	**
Linoleic acid intake, kg/d	0.12 ^b	0.14 ^a	0.11 ^c	0.12 ^c	0.002	**
Linolenic acid transferred to milk, ³ %	0.82 ^a	0.65 ^b	0.85 ^a	0.52 ^c	0.050	**

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Stage of lactation: I = April; II = May; III = July; IV = August.

²Standard error of the difference.

³Total amount of linolenic acid in milk/linolenic acid intake.

** $P < 0.01$.

in the LL treatment (422.0 and 319.1 g/kg of total FA for MCFA and LCFA, respectively; $P < 0.05$).

The SL affected all milk FA variables measured (Table 8). Vaccenic acid was lowest (19.4 g/kg of total FA; $P < 0.01$) during the second SL and was highest during the first and fourth SL (25.6 and 23.8 g/kg of total FA, respectively; $P < 0.01$). In addition, concentrations of CLA were lowest in the first and second SL (9.3 and 10.1 g of CLA/kg of total FA, respectively) and were highest (18.6 g/kg of total FA) during the final SL. The LNA content was lowest in the second SL (4.3 g of LNA/kg of total FA) and in the third (5.5 g/kg of total FA; $P < 0.01$). In accordance with these results, the greatest milk concentrations of both n-3 and n-6 PUFA were recorded during the last SL ($P < 0.01$). The LCFA

were lowest during the second SL (222.1 g/kg of total FA) and were highest during the fourth SL (358.6 g/kg of total FA; $P < 0.01$). In agreement with the pattern of the CLA data, the Δ^9 -desaturase index increased from the first to the last SL (from 0.07 to 0.11; $P < 0.01$). In addition, there was a significant relationship between concentrations of VA and CLA in milk [intercept = 4.5 ± 0.87 ; slope (VA) = 0.36 ± 0.035 ; $R^2 = 0.30$; $r = 0.55$; $P < 0.01$].

Relationship Between FA Intake and Milk Concentrations of FA

No relationship was observed between milk concentrations of VA and dietary FA intake (total and LNA

Table 7. Effect of herbage mass and daily herbage allowance on milk fatty acid composition

Item, g/kg of total fatty acids	Treatment ¹				SED ²	Significance		
	HH	HL	LH	LL		HM	DHA	HM × DHA
C4:0	91.6	104.0	97.4	98.7	6.36	NS	NS	NS
C6:0	47.9	53.7	50.8	49.0	3.24	NS	NS	†
C8:0	26.1	28.9	27.9	25.6	1.77	NS	NS	†
C10:0	49.5 ^{ab}	53.8 ^a	51.5 ^{ab}	45.0 ^b	3.69	NS	NS	*
C12:0	42.1 ^{ab}	44.6 ^a	44.1 ^{ab}	36.9 ^b	2.93	NS	NS	*
C14:0	102.3 ^{ab}	105.7 ^a	102.9 ^a	93.2 ^b	3.62	*	NS	*
C14:1	10.4	10.2	10.0	8.9	0.76	NS	NS	NS
C15:0	10.3	10.5	9.6	9.0	0.43	**	NS	NS
C16:0	216.8	212.1	214.1	207.5	6.56	NS	NS	NS
C16:1	13.9	13.9	14.1	13.3	1.30	NS	NS	NS
C17:0	4.0	4.1	3.6	3.8	0.31	NS	NS	NS
C18:0	63.7	60.2	59.5	66.9	3.91	NS	NS	†
<i>Cis</i> -9 C18:1	173.2 ^{ab}	160.4 ^b	168.0 ^{ab}	185.8 ^a	9.65	NS	NS	*
<i>Trans</i> -11 C18:1 (vaccenic acid)	22.6	22.3	22.4	24.1	1.86	NS	NS	NS
<i>Cis</i> -9, <i>cis</i> -12 C18:2n-6 (linoleic acid)	7.4	6.7	7.0	7.1	0.60	NS	NS	NS
<i>Cis</i> -9, <i>trans</i> -11 C18:2 (conjugated linoleic acid)	12.8	12.5	12.9	13.7	1.08	NS	NS	NS
C18:3n-3 (α -linolenic acid)	4.7	4.3	5.0	5.6	0.46	**	NS	NS
Saturated fatty acids (SFA) ³	658.4 ^{ab}	680.4 ^a	665.1 ^{ab}	638.2 ^b	13.99	†	NS	*
Unsaturated fatty acids (UFA) ⁴	261.2 ^{ab}	246.9 ^b	256.6 ^{ab}	281.4 ^a	10.95	†	NS	*
UFA:SFA	0.41 ^{ab}	0.38 ^b	0.40 ^{ab}	0.45 ^a	0.025	†	NS	*
n-6 ⁵	11.9	11.5	12.0	11.0	1.08	NS	NS	NS
n-3 ⁶	5.0	4.6	5.8	6.2	0.57	**	NS	NS
n-3:n-6	0.54	0.51	0.61	0.67	0.073	*	NS	NS
SCFA ⁷	165.6	186.7	176.1	173.4	10.66	NS	NS	NS
MCFA ⁸	455.5 ^a	460.4 ^a	455.8 ^a	422.0 ^b	11.04	*	†	*
LCFA ⁹	298.4 ^{ab}	280.1 ^b	289.3 ^{ab}	319.1 ^a	14.38	NS	NS	*
Δ^9 -Desaturase index ¹⁰	0.09	0.09	0.09	0.09	0.005	NS	NS	NS

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Treatments: HH = high herbage mass (HM), high daily herbage allowance (DHA); HL = high HM, low DHA; LH = low HM, high DHA; LL = low HM, low DHA.

²Standard error of the difference.

³Saturated fatty acids: sum of C10:1, C14:1, C16:1, C17:1, C18:1, vaccenic acid, linoleic acid, conjugated linoleic acid, and α -linolenic acid.

⁴Unsaturated fatty acids: sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, and C24:0.

⁵n-6: sum of linoleic acid, *trans*-9, *trans*-12 C18:2n-6, γ -C18:3n-6, C20:3n-6, and C20:4n-6.

⁶n-3: sum of α -linolenic acid, C20:5n-3, and C22:6n-3.

⁷Short-chain fatty acids: sum from C4:0 to C8:0.

⁸Medium-chain fatty acids: sum from C10:0 to C16:1.

⁹Long-chain fatty acids: sum from C17:0 to C22:6.

¹⁰ Δ^9 -Desaturase index: C14:1/(C14:0 + C14:1).

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

Table 8. Effect of stage of lactation on milk fatty acid composition

Item, g/kg of total fatty acids	Stage of lactation ¹				SED ²	Significance
	I	II	III	IV		
C4:0	119.3 ^a	107.6 ^a	84.7 ^b	80.1 ^b	6.27	**
C6:0	59.8 ^a	60.8 ^a	42.8 ^b	38.0 ^b	2.99	**
C8:0	31.5 ^a	34.6 ^a	22.8 ^b	19.6 ^b	1.57	**
C10:0	60.3 ^b	65.9 ^a	40.6 ^c	33.0 ^d	2.79	**
C12:0	40.0 ^b	54.6 ^a	34.8 ^c	29.2 ^d	1.96	**
C14:0	104.3 ^b	117.6 ^a	92.7 ^c	89.4 ^c	2.76	**
C14:1	8.4 ^b	10.7 ^a	9.8 ^a	10.6 ^a	0.430	**
C15:0	10.4 ^a	9.5 ^{ab}	9.2 ^b	10.2 ^a	0.34	**
C16:0	187.8 ^b	220.4 ^a	213.5 ^a	228.8 ^a	5.94	**
C16:1	14.6 ^{ab}	10.8 ^b	12.9 ^b	16.9 ^a	1.17	**
C17:0	4.0 ^b	2.9 ^c	3.8 ^b	4.9 ^a	0.30	**
C18:0	60.0 ^b	47.7 ^c	68.3 ^a	74.4 ^a	2.97	**
<i>Cis</i> -9 C18:1	171.9 ^b	127.8 ^c	177.0 ^b	210.8 ^a	8.21	**
<i>Trans</i> -11 C18:1 (vaccenic acid)	25.6 ^a	19.4 ^b	22.4 ^{ab}	23.8 ^a	1.61	**
<i>Cis</i> -9, <i>cis</i> -12 C18:2n-6 (linoleic acid)	6.6 ^b	5.1 ^c	7.7 ^{ab}	8.9 ^a	0.58	**
<i>Cis</i> -9, <i>trans</i> -11 C18:2 (conjugated linoleic acid)	9.3 ^c	10.1 ^c	14.0 ^b	18.6 ^a	0.92	**
C18:3n-3 (α -linolenic acid)	4.8 ^{ab}	4.3 ^b	5.5 ^a	5.1 ^{ab}	0.41	**
Saturated fatty acids (SFA) ³	690.4 ^b	724.8 ^a	616.4 ^c	610.5 ^c	12.33	**
Unsaturated fatty acids (UFA) ⁴	258.3 ^b	201.5 ^c	269.1 ^b	317.3 ^a	10.08	**
UFA:SFA	0.39 ^c	0.29 ^d	0.45 ^b	0.52 ^a	0.021	**
n-6 ⁵	12.0 ^a	7.0 ^b	13.4 ^a	14.0 ^a	1.05	**
n-3 ⁶	5.4 ^a	4.5 ^b	5.9 ^a	5.8 ^a	0.42	**
n-3:n-6	0.56 ^b	0.77 ^a	0.52 ^b	0.49 ^b	0.066	**
SCFA ⁷	210.6 ^a	203.0 ^a	150.4 ^b	137.6 ^b	10.18	**
MCFA ⁸	445.1 ^b	501.1 ^a	421.9 ^c	425.6 ^{bc}	8.58	**
LCFA ⁹	292.8 ^b	222.1 ^c	313.4 ^b	358.6 ^a	12.88	**
Δ^9 -Desaturase index ¹⁰	0.07 ^d	0.08 ^c	0.09 ^b	0.11 ^a	0.002	**

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Stage of lactation: I = April; II = May; III = July; IV = August.

²Standard error of the difference.

³Saturated fatty acids: sum of C10:1, C14:1, C16:1, C17:1, C18:1, vaccenic acid, linoleic acid, conjugated linoleic acid, and α -linolenic acid.

⁴Unsaturated fatty acids: sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, and C24:0.

⁵n-6: sum of linoleic acid, *trans*-9, *trans*-12 C18:2n-6, γ -C18:3n-6, C20:3n-6, and C20:4n-6.

⁶n-3: sum of α -linolenic acid, C20:5n-3, and C22:6n-3.

⁷Short-chain fatty acids: sum from C4:0 to C8:0.

⁸Medium-chain fatty acids: sum from C10:0 to C16:1.

⁹Long-chain fatty acids: sum from C17:0 to C22:6.

¹⁰ Δ^9 -Desaturase index: C14:1/(C14:0 + C14:1).

** $P < 0.01$.

intake; $P > 0.05$). Similarly, no relationship was found between dietary intake and milk concentrations of LNA (total and LNA intake; $P > 0.05$). There was a positive, although weak, relationship between milk concentrations of CLA and total dietary FA intake [intercept = 8.3 ± 1.70 ; slope (total FA intake) = 4.3 ± 1.61 ; $R^2 = 0.025$; $r = 0.17$; $P < 0.01$] and between milk CLA and dietary LNA intake [intercept = 8.7 ± 1.58 ; slope (LNA intake) = 7.4 ± 2.73 ; $R^2 = 0.025$; $r = 0.17$; $P < 0.01$].

DISCUSSION

Because it is now well established that the CLA and n-3 PUFA content in milk is increased under grazing-based production systems when compared with TMR-based systems, understanding how to further increase

these beneficial FA in milk from grazing dairy cows is an important objective of grassland research. Therefore, the objective of this study was to investigate the effect of different grazing strategies, based on both the quality and quantity of herbage offered, on the milk FA composition of grazing dairy cows.

DMI and Animal Performance

Several authors have indicated a positive relationship between DHA, grass DMI, and milk production (Le Du et al., 1979; Stakelum, 1986a; Mayne et al., 1987; Ribeiro et al., 2005; McEvoy et al., 2008). In the present study, although grass DMI increased when cows were offered a high DHA, this was not reflected in increased milk production (Table 3), which agrees with the find-

ings of other studies (Stakelum, 1986b,c; Kuusela and Khalili, 2002). Many factors can affect grass DMI and milk production, including season, SL, species grazed (legumes or grasses), and level of production (Vazquez and Smith, 2000), which could explain the disparity in findings among previous studies. The lack of difference in the other production-related variables studied (BW, BCS, milk composition) is contrary to the findings of some previous studies (Combellas and Hodgson, 1979; Le Du et al., 1979; McEvoy et al. 2008).

FA Intake and Milk FA Composition

Milk from cows grazing pastures is richer in PUFA, especially CLA and n-3 PUFA, compared with TMR systems (Chilliard et al., 2001; Kraft et al., 2003; Dewhurst et al., 2006). Even when fat supplements are fed with the objective of increasing the intake of CLA precursors in TMR diets, milk concentration of CLA is below that recorded under grazing systems (Dhiman et al., 1999). The most effective strategy to increase the CLA and n-3 PUFA content of milk appreciably is therefore strategic supplementation of grazing cow diets (Murphy et al., 1995; Lawless et al., 1998; Flowers et al., 2008). This is an expensive approach, and supplementation in general, particularly from midlactation onward, is not in harmony with low-cost pasture-based production systems. To this end, there is a requirement to examine regimens to improve the nutraceutical composition of milk through improved pasture management. Because LCFA cannot be synthesized *de novo* in the mammary gland (Ney, 1991; Jensen, 2002), the intake of precursors is the main approach to increasing the concentrations of these PUFA in milk. Accordingly, it is not unreasonable to assume that variation in the 2 main pasture-related factors we examined, pregrazing HM and DHA, could affect the FA intake of grazing cows. Indeed, we did find differences in FA intake between treatments and across the season (Tables 5 and 6). Cows grazing a low HM had a higher intake of total FA, LNA, and LA, which was related to the high herbage content of both LNA and total FA in the LH and LL treatments (Table 2). This is not surprising, given that longer regrowth periods are necessary to generate pastures with greater pasture cover, and several authors have demonstrated that FA content in grasses declines as the duration of the regrowth period increases (Dewhurst et al., 2001; Elgersma et al., 2003b). In addition, cows grazing a high DHA had a higher intake of FA, but this seemed to be more closely related to the higher DMI than to differences in pasture quality. With regard to differences in FA intake across the season, the variation seemed to be more a function of differences in FA composition of the pasture than differences in DMI.

For example, DMI in the third and fourth SL were similar (17.1 kg), but total FA intake differed because of changes in total FA content in the pasture (48.8 vs. 69.1 g of FA/kg of DM in the third and fourth SL, respectively). Similar changes in FA content across the season were reported previously for perennial ryegrass (Dewhurst et al., 2001).

Although we recorded differences in FA intake between treatments, this was not reflected in differences in milk for most of the FA (Table 7). In particular, there was no effect of diet on milk concentrations of either CLA or VA during any of the periods measured. Consistent with this result, a poor relationship was found between FA intake (total and LNA) and milk CLA. In contrast with our results, however, Elgersma et al. (2003a) reported that milk concentrations of both VA and CLA were affected in cows grazing perennial ryegrass cultivars genetically selected for either high or low LNA content. Furthermore, Elgersma et al. (2004) found a rapid decline in milk CLA when cows grazed a low DHA, but in their study, the high DHA was 44 kg of DM/cow per day, whereas the low DHA was 22 kg/cow per day. Dewhurst et al. (2006), after an extensive review of the published literature, could not establish any effect of varying DHA on milk concentrations of either CLA or LNA. Despite the lack of difference in CLA between treatments in the current study, we observed significant variation among cows (coefficient of variation = 0.49). In agreement, several authors found high variation in CLA between individual animals, and these differences between animals were consistent across time (Lawless et al., 1999; Kelsey et al., 2003; Lock and Garnsworthy, 2003). In addition, it is important to note that higher variation in Δ^9 -desaturase activity, as measured by the desaturation index, was observed in this study under the grazing conditions used than with comparable TMR-based systems (Kelly et al., 1998). This grazing effect on enzyme activity could potentially mask differences in experiments carried out on grazing systems.

Milk CLA content increased as the SL progressed from 9.3 (first SL) to 18.6 g/kg of total FA (last SL). A similar pattern was found for the Δ^9 -desaturase index. A previous study by Lock and Garnsworthy (2003) demonstrated a seasonal variation in CLA content of milk attributable to changes in Δ^9 -desaturase activity. In that study, the authors estimated Δ^9 -desaturase activity as the ratio of C14:1/(C14:0 + C14:1), which has been identified as the most appropriate index, given that milk C14:0 is entirely synthesized *de novo* in the mammary gland and, consequently, all C14:1 is exclusively a product of Δ^9 -desaturase-mediated desaturation. Additionally, Kelsey et al. (2003) concluded that there was a relationship between SL and the activity

of the Δ^9 -desaturase enzyme (a slight increase as the season progressed). Nevertheless, in the study by Lock and Garnsworthy (2003), the variation in Δ^9 -desaturase activity recorded seemed to be more closely related to changes in the diet across the season (cows receiving fresh grass instead of conserved forages), rather than to physiological changes in the Δ^9 -desaturase activity attributable to the SL. Previous work has shown a negative effect of some n-3 FA on the expression of genes associated with the Δ^9 -desaturase enzyme system (Bu et al., 2007; McGettrick et al., 2007; Waters et al., 2008). In the present study, we found a poor relationship between FA intake (total and LNA intake) and CLA in milk, which suggests that the FA intake from pasture did not greatly affect the activity of the Δ^9 -desaturase enzyme. Although VA is the precursor of de novo synthesis of CLA in the mammary gland, it exhibited a seasonal pattern of concentration in milk that was not consistent with the linear increase in CLA. Moreover, we found a relatively weak relationship between CLA and VA ($R^2 = 0.30$). Recent studies examining gene expression in the mammary gland showed a poor relationship between desaturase indexes and expression of genes involved as stearyl-coenzyme A desaturase (Bionaz and Loores, 2008).

The milk LNA was higher at a low HM, in accordance with the higher n-3 PUFA content ($P < 0.01$) and n-3:n-6 ratio. In agreement, the highest LNA intake was recorded for cows at a low HM. However, no relationship was found between FA intake and LNA concentrations in milk. In fact, contradictory results were also found in terms of the effect of SL in that LNA content in milk was highest at the third SL, whereas dietary intake of LNA for the same SL was the lowest. These results were also reflected in the data recorded for LNA transferred to milk (Table 6). Therefore, the reason the low HM led to a higher milk LNA content remains unclear. More factors seem to be affecting LNA content in milk than merely absolute dietary FA intake (i.e., factors affecting biohydrogenation). Additionally, it is important to note that the overall LNA content of milk was relatively low in our study compared with other similar studies (Murphy et al., 1995; Lawless et al., 1998; Flowers et al., 2008). This may have been related to the well-documented high degree of ruminal biohydrogenation for this FA. Typical values for dietary LNA biohydrogenation range between 0.85 and 1.0 (Scollan et al., 2001; Dewhurst et al., 2003). Furthermore, Dewhurst et al. (2006) concluded that LNA recovery in milk was higher for hay-based diets than for silage- or fresh grass-based diets. Fresh grass (such as the perennial ryegrass pasture used in this trial) is highly fermented in the rumen because of the low content and high degradability of its fiber fraction. It is

probable that in cows grazing fresh grass, FA are more exposed to biohydrogenation compared with grass hay, and this may be the explanation for the low recovery of LNA in milk in the present study.

Both milk n-6 and n-3 presented similar variation across the experiment, being lowest at the second SL. However, the n-3:n-6 ratio was higher in the second SL. This variation in the n-3:n-6 ratio could be explained by the degree of variation in n-3 PUFA across the season, which was lower when compared with n-6 PUFA.

The SCFA remained unchanged between treatments but varied across the season, being higher during early lactation, whereas the concentration of LCFA was highest during the last SL measured. These results were unexpected because it is known that LCFA in milk are generally greater during the first months of lactation (Jensen, 2002; Chilliard et al., 2003). On the other hand, cows grazing either the high-HM pasture or a high DHA had a higher content of MCFA. Additionally, significant interactions were found between HM and DHA for MCFA and LCFA. Cows in the LL group had the lowest MCFA and the highest LCFA content. It is likely that cows grazing in the LL treatment consumed less dietary fiber, which provides the precursors for SCFA and some MCFA (Jensen, 2002; Chilliard et al., 2003). Finally, UFA content was higher later in the year, in accordance with the highest UFA:SFA ratio. These results are due to a significant increase in the magnitude of the Δ^9 -desaturase enzyme index during the last SL, which was manifested in higher milk concentrations of *cis*-9 C18:1, CLA, and VA (unsaturated LCFA).

CONCLUSIONS

The results of the present study indicate that changes in HM and DHA (at least of the magnitude studied) did not greatly affect the composition of milk FA in grazing dairy cows, despite variation between treatments in dietary FA intake. Milk concentrations of CLA were unaffected by treatment, and only SL seemed to affect the CLA content as the grazing season progressed, which was mirrored by an associated increase in the Δ^9 -desaturase enzyme activity index. We also recorded large variation in milk CLA between individual animals, which may have contributed to the absence of significant differences between treatments. Milk LNA was higher at a low HM, but these differences seem to have no practical importance because the content of LNA in milk was low across the whole experiment, independent of treatment. Enhancing the concentration of health-promoting FA in milk through manipulation of grazing management alone (under the high-quality pasture conditions used here) will be difficult; thus,

future research should focus more on identifying the biological basis for the significant interanimal variation in milk FA concentration recorded here and in other studies.

ACKNOWLEDGMENTS

This work was funded by the Irish Department of Agriculture, Food and Fisheries Research Stimulus Fund (Dublin, Ireland).

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