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RESEARCH ARTICLE

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Fatty acid intake and rumen fatty acid composition is affected by pre-grazing herbage mass and daily herbage allowance in Holstein dairy cows

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Abstract

The objective of this study was to investigate the effect of level of pre-grazing herbage mass (HM) and daily herbage allowance (DHA) on the fatty acid (FA) intake and composition of ruminal content of grazing dairy cows. Four rumen fistulated Holstein-Friesian dairy cows were allocated to either a high or low HM (1700 *vs* 2600 kg DM ha^{-1}) and within herbage mass treatment further allocated to a high or low DHA (20 *vs* 16 kg of DM cow^{-1} day^{-1}) in a 4 × 4 Latin square design. Total FA intake and linolenic acid (LNA) intake was higher for cows on high DHA ($p < 0.05$). Ruminal oleic acid, linoleic and LNA were not affected by treatments. Ruminal stearic acid (C18:0) and vaccenic acid (VA) concentrations were higher at low HM (43.6 and 14.8 g/100 g of FA respectively; $p < 0.01$) compared to high HM (42.0 and 12.5 g/100 g of FA respectively for C18:0 and VA). Cows grazing high DHA had higher ruminal concentration of VA (15.3 g/100 g of FA; $p < 0.01$) than low DHA (12.1 g/100 g of FA). Regarding milk FA composition, only some of the milk FA varied across treatments, being the VA and LNA concentrations higher at low HM ($p < 0.05$). These data suggest that low HM and high DHA, at least within the range studied here, promotes the accumulation of ruminal VA which could be available for subsequent conversion within the mammary gland to the human health promoting c9,t11 isomer of conjugated linoleic acid.

Additional key words: conjugated linoleic acid; linolenic acid; rumen fatty acids metabolism.

Introduction

It is now accepted that dietary intake of some polyunsaturated fatty acids (PUFA) can have positive effects on human health. For example, n-3 fatty acids (FA) have been demonstrated to be anticarcinogenic and antiatherogenic, while the c9,t11 isomer of conjugated linoleic acid (CLA) has also been showed to be anticarcinogenic, antidiabetic and antiadipogenic (Parodi, 1999; Hamazaki *et al.*, 2003; Lock & Bauman, 2004). Milk fat from dairy cows is one of the most important sources of c9,t11-CLA in the human diet (Lock & Bauman, 2004; Schroeder *et al.*, 2004) and these beneficial PUFA are in greater abundance with the milk of grazing dairy cows (Kelly *et al.*, 1998; Kraft *et al.*, 2003). Pasture is an important source of PUFA, particularly α-linolenic acid (LNA; C18:3 n-3; Palladino *et al.*, 2009a), which is one of the main precursors of c9,t11-CLA in milk.

The PUFA concentration in milk is dictated by supply of precursors from the diet and by the extent of ruminal biohydrogenation (Dewhurst *et al.*, 2006). Some CLA isomers are synthesized by ruminal bacteria as an intermediary in the biohydrogenation of LNA and linoleic acid (LA; C18:2 n-6). The majority of milk c9,t11-CLA is synthesised *de novo* in the mammary

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Abbreviations used: ADF (acid detergent insoluble fibre); BCS (body condition score); BW (body weight); CLA (conjugated linoleic acid); CP (crude protein); DHA (daily herbage allowance); DMI (dry matter intake); FA (fatty acid); FAME (methyl esters of fatty acids); HM (pre-grazing herbage mass); LA (linoleic acid); LCFA (long chain FA); LNA (linolenic acid); MCFA (medium chain FA); NDF (neutral detergent insoluble fibre); OM (organic matter); PUFA (polyunsaturated fatty acids); SFA (saturated FA); VA (vaccenic acid); VFA (volatile fatty acids); WSC (water soluble carbohydrate).

gland from the desaturation of another product of ruminal biohydrogenation, vaccenic acid (VA; t11-C18:1), catalysed by Δ9-desaturase (Griinari & Bauman, 1999; Lock & Garnsworthy, 2003). The extent to which PUFA are hydrogenated in the rumen is affected by the degree of unsaturation (Scollan *et al.*, 2001), forage/concentrate ratio (Loor *et al.*, 2004), ruminal pH (Schroeder *et al.*, 2004) and rumen retention time or passage rate (Dewhurst *et al.*, 2003). While, many of these variables could, potentially, be affected by changes in pasture management (*e.g.,* pasture allowance may affect DMI and fatty acid intake and/or passage rate), to the authors' knowledge, there are no published data on this topic. It is expected that changes in herbage mass and daily herbage allowance may affect grass quality and dry matter intake, and thus, potentially, biohydrogenation of PUFA. Therefore, the objective of this study was to evaluate the effect of daily herbage allowance (kg of DM cow^{-1} d^{-1}) and pregrazing herbage mass (kg of DM ha^{-1}) on rumen and milk FA composition of grazing dairy cows.

Material and methods

Experimental site and pasture

The study was carried out at Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland (52°09´N; 8°16´W) during 2007 as part of a larger experiment (Palladino *et al.*, 2009b). The objective was to evaluate the effect of contrasting levels of pregrazing herbage mass (kg of DM ha^{-1}) and daily herbage allowance (kg of DM cow^{-1} d^{-1}) on the milk FA composition of grazing dairy cows. A predominantly perennial ryegrass (*Lolium perenne* L.) pasture was used during the experimental period.

Experimental design, animals and management

Four rumen fistulated Holstein-Friesian dairy cows (67 ± 9.5 days in milk; lactation number, 4.3 ± 0.50; body weight (BW), 591 ± 41.1; and body condition score (BCS), 2.38 ± 0.144) were allocated to one of two levels of herbage mass (HM; 1700 and 2600 kg DM ha^{-1}; measured above 40 mm) and within HM to one of two levels of daily herbage allowance (DHA; 16 and 20 kg of DM cow^{-1} d^{-1}; measured above 40 mm). Thus there were the four treatment groups *viz.* HH (high HM, high DHA); HL (high HM, low DHA); LH (low HM, high DHA); and LL (low HM, low DHA). The study was arranged in a 4 (treatments) × 4 (periods; 14 days for adaptation and 7 days for collecting samples and measurements) Latin square design, and run from June to August. Cows grazed a new paddock on a daily basis after the morning milking. Each fistulated cow grazed in its corresponding paddock as part of a group of cows used for a larger experiment (Palladino *et al.*, 2009b). Concentrate (0.4 kg cow^{-1} d^{-1}) was offered in the milking parlour in two equal feeds at both morning and afternoon milkings (concentrate composition on a fresh weight basis was (g kg^{-1} of concentrate) ground citrus pulp, 305; barley, 237; maize gluten, 249; soybean meal, 140; vitamins-mineral, 43; and fat, 26).

Measurements

Herbage mass and chemical composition. The HM (kg DM ha^{-1} above 40 mm) was estimated by cutting 4 strips (1.2 × 10 m) for each HM treatment area twice weekly with an Agria machine (Etesia UK Ltd., Warwick, UK). Ten compressed sward surface height measurements were recorded before and after harvesting using an electronic plate meter (Urban & Caudal, 1990) with a plastic plate (30 × 30 cm and 4.5 kg m^{-1}, Agrosistèmes, Choiselle, France). The herbage harvested was weighed, and samples were collected (approximately 0.1 kg) for DM determination (oven dried at 105°C over 24 h) and for chemical composition (including FA analysis; stored at –20°C). Sward density (kg DM ha^{-1} cm^{-1}) was calculated as HM (kg DM ha^{-1}) divided by the difference between pre and post sward height (cm).

Pregrazing compressed sward height was measured daily throughout the experiment by recording approximately 20 heights across the two diagonals of each grazing area. The DHA for each treatment was calculated by multiplying mean pregrazing sward heights [above 40 mm, *i.e.* pregrazing compressed height (mm) – 40 mm] by the sward density and adjusting the area per cow daily.

Grass samples were stored at –20°C before being freeze-dried and milled through a 1-mm sieve prior to chemical analysis. The herbage samples and the concentrate were analyzed for concentration of ADF, NDF (Van Soest, 1963), ash, CP (Leco FP-428, Leco Australia Pty Ltd., Baulkham Hills, New South Wales, Australia)

Table 1. Herbage mass (HM), chemical and fatty acid composition (mean values ± SD) of selected herbage samples and concentrate offered

Item	HM		DHA		Concentrate
	High	Low	High	Low	
Herbage mass (kg of DM ha^{-1})	2580 ± 517.1	1726 ± 303.6	2270 ± 585.3	2036 ± 634.3	—
OM digestibility (g kg^{-1} of DM)	820 ± 14.7	844 ± 13.6	832 ± 21.5	832 ± 16.9	—
Chemical composition (g kg^{-1} of DM)					
Crude protein	189 ± 21.0	245 ± 21.6	218 ± 40.2	216 ± 33.7	182 ± 17.2
Neutral detergent insoluble fibre	435 ± 24.1	399 ± 30.1	422 ± 39.6	412 ± 24.3	248 ± 40.8
Acid detergent insoluble fibre	283 ± 23.9	276 ± 21.1	275 ± 26.2	284 ± 26.2	—
Ash	70.1 ± 6.03	77.1 ± 8.53	74.0 ± 9.13	73.2 ± 7.28	100 ± 6.6
Fatty acid composition (g kg^{-1} of DM)					
C14	1.8 ± 1.51	1.0 ± 0.68	1.5 ± 1.47	1.2 ± 0.79	1.5 ± 0.18
C16	10.7 ± 1.53	13.5 ± 3.07	12.5 ± 1.62	11.8 ± 3.82	30.1 ± 0.07
c9-C16:1	1.8 ± 0.93	1.8 ± 0.67	2.0 ± 0.68	1.5 ± 0.85	2.7 ± 0.10
C18	0.8 ± 1.35	0.6 ± 0.27	0.9 ± 1.18	0.5 ± 0.41	1.7 ± 0.60
c9-C18:1	0.6 ± 0.54	1.0 ± 0.28	0.9 ± 0.37	0.8 ± 0.52	16.4 ± 0.04
C18:2	6.2 ± 1.07	6.8 ± 1.29	6.5 ± 1.29	6.6 ± 1.17	19.2 ± 0.10
C18:3	28.2 ± 5.43	30.4 ± 10.83	29.7 ± 8.08	29.0 ± 9.65	3.4 ± 0.57
Total	50.1 ± 7.76	55.0 ± 15.51	54.0 ± 9.85	51.3 ± 15.44	75.9 ± 0.01

DHA: daily herbage allowance. OM: organic matter. DM: dry matter.

and organic matter (OM) digestibility (Morgan *et al.*, 1989). The chemical composition is shown in Table 1.

Rumen fermentation parameters. Rumen samples were taken twice weekly (non-consecutive days) during each sampling period at four time points within each sample day (0700, 1100, 1500 and 2000 h). Samples were collected from different rumen locations, mixed, and approximately 50 g of rumen content finally sampled. Two sub-samples were collected, one for concentration of volatile fatty acids (VFA) (stored at –20°C and acidified with concentrated H_2SO_4) and the other for FA (stored at –20°C) analysis. The VFA profile was measured following the procedure of Ranfft (1973). Ruminal pH was also recorded using a glass electrode and a pH meter (pHM2 standard pH meter-radiometer; Radiometer, Copenhagen, Denmark) on the digesta sample at initial collection.

Milk production and composition. Milking took place at 07:00 h and 16:00 h daily. Individual milk yields (kg) were recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, protein and lactose concentrations were determined for each animal using a MilkoScan 203 (DK-3400, Foss Electric, Hillrød, Denmark).

Fatty acid analysis. The FA composition of the herbage, concentrate, and rumen samples (freeze-dried samples) was analyzed by gas chromatography using a one-step methylation procedure (Sukhija & Palmquist, 1988). The methyl esters of FA (FAME) were injected by auto-sampler on a Varian GC 3800 equipped with a flame ionization detector. The FAME (injected using a 10:1 split ratio) were separated on a fused silica capillary column (100 m × 0.25 mm i.d × 0.39 μm film thickness; Varian Fame Select CP 7420, Varian Inc., Palo Alto, CA, USA). The C19:0 FAME (nonadecanoic acid methyl ester, Sigma-Aldrich Ireland Limited, Co Wicklow, Ireland) was used as internal standard and a FAME mixture C14:0-C:22:0 (Supelco, Sigma-Aldrich Ireland Limited, Co Wicklow, Ireland) as external standard. The injector temperature was held at 250°C, and the detector temperature at 300°C. The initial oven temperature was 140°C (held for 10 min) and then increased to 240°C at a rate of 4°C min^{-1} (held for 17 min). Nitrogen (N_2) was used as the carrier gas and column flow was held at 2 ml min^{-1}. Results for herbage and concentrate samples are shown in Table 1.

To determine milk FA composition, samples from p.m. milking were centrifuged at 4°C (978 × g) for 20 min, the cream was extracted, flushed with N_2 during 10-15 s and stored at –20°C overnight. The following morning samples were heated to 40°C and centrifuged at 30°C (978 × g) for 10 min. The extracted fat was stored at –20°C until subsequent FA analysis. The FA composition of the milk fat after methylation was analyzed by gas chromatography using an adaptation of the method of Christie (1982). Approximately 1 mg of milk fat was dissolved in dried n-hexane. Methyl aceta-

te (20 μL) and 1 M sodium methoxide in anhydrous methanol (20 μL) were added following neutralization with a saturated solution of oxalic acid in methanol (30 μL). After centrifugation, an aliquot of the upper layer was taken for gas chromatography. Milk and rumen FAMEs were injected by auto-sampler on a Varian GC 3800 equipped with a flame ionization detector. FAME (injected using a 10:1 split ratio) were separated on a fused silica capillary column (100 m × 0.25 mm i.d × 0.39 μm film thickness; Varian Fame Select CP 7420, Varian Inc., Palo Alto, CA, USA). The external standards used for FAME identification were Larodan Mixture ME 100 (Larodan, Malmö, Sweeden) and 9c11t + 10t12c CLA methyl ester (Larodan, Malmö, Sweeden). The injector temperature was held at 250°C, and the detector temperature 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^{-1} (held for 5 min) and then increased to 240°C (4°C min^{-1}; held for 15 min). Nitrogen (N_2) was used as a carrier gas. The pressure of the column was held at 40 psi (held for 8 min) and then increased to 65 psi at a rate of 2 psi min^{-1} (held for 24 min).

Dry matter intake. DM intake was measured using the n-alkane technique described by Mayes *et al.* (1986), and modified by Dillon & Stakelum (1989). Cows were dosed twice daily for 12 d before both morning and evening milkings with a paper pellet (Carl Roth, GmbH, Karlesruhe, Germany) containing 500 mg of dotriacontane (C_{32}-alkane). Fecal grab samples were collected twice daily, from day 7 to day 12, prior to both morning and evening milkings. During the same period, herbage samples were manually collected with a Gardena hand shears (Acu 60, Gardena International GmbH, Ulm, Germany) at approximately the same height to which cows grazed, after each morning and evening milking for each treatment, to provide a representative sample of the herbage grazed during the same period. A concentrate sample was collected daily and both herbage and concentrate were frozen (–20°C) after collection. Fecal grab samples were thawed and bulked (10 g of each collected sample) by cow. Faecal samples were dried at 40°C for 48 h, milled through a 1-mm screen. The n-alkane concentration of the dosed pellets, feces, herbage, and concentrate were determined as described by Dillon (1993).

Statistical analysis

Statistical analyses were carried out using the Statistical Analysis Software package (SAS version 9.1, SAS Institute, 2002). Data were analyzed using the MIXED procedure and statistically significant differences were detected using the PDIFF command adjusted by the Tukey test. Cow was the experimental unit. For variables related to FA intake, and milk FA, cow was used as a random effect and the model include terms for HM, DHA and period. For ruminal FA composition, the model also included terms for time of sampling and a procedure for repeated measures was used. Several covariance structures were tested using the Bayesian Information Criterion (BIC) and the best fit used. All appropriate interactions were tested for inclusion in the final statistical model. Statistically significant differences were declared at $p < 0.05$ and trends were declared at $p < 0.10$.

Results

Animal performance

No differences were found between treatments for estimated DMI or animal performance. With regards to estimated FA intake, only DHA affected intake of FA, being higher for cows on high DHA (total FA and LNA intake; $p < 0.05$). Also LA intake tended to be higher at high DHA ($p < 0.10$; Table 2). Moreover, cows grazing the low HM tended to have a greater FA intake (Total, LNA and LA; $p < 0.10$).

Rumen fermentation parameters and FA composition

Table 3 shows the effect of HM and DHA on ruminal fermentation parameters and FA composition of ruminal content**.** There was a HM × DHA interaction ($p < 0.01$) for pH with LL having the lowest pH (5.77) and HL the highest pH value (5.93). No effect of either HM or DHA was found on rumen pH, total VFA or individual VFA (Table 3). However, rumen pH tended to be lower in the low HM treatment ($p < 0.10$).

There was a HM × DHA interaction for some of the FA measured in the ruminal content. The highest ruminal concentration of C18:0 was in cows grazing the LL treatment (44.3 g/100 g of FA) whilst cows in HH and LH were similar (43.0 and 42.8 g 100/g of FA; $p < 0.01$). The HL treatment had the lowest C18:0 concentration (40.9 g/100 g FA). In turn, the highest

Table 2. Effect of herbage mass (HM) and daily herbage allowance (DHA) on animal performance

Item	HM		SED	DHA		SED	Significance		
	High	Low		High	Low		HM	DHA	HM × DHA
DM intake (kg d^{-1})	18.6	19.2	2.37	19.9	17.9	2.37	NS	NS	NS
Milk production (kg d^{-1})	22.8	22.6	0.46	22.7	22.7	0.65	NS	NS	NS
Milk fat (%)	3.95	3.74	0.214	3.90	3.80	0.323	NS	NS	NS
Fat yield (kg d^{-1})	0.86	0.86	0.067	0.89	0.83	0.067	NS	NS	NS
Milk protein (%)	3.28	3.34	0.063	3.35	3.27	0.077	NS	NS	NS
Protein yield (kg d^{-1})	0.74	0.75	0.012	0.76	0.73	0.012	NS	*	NS
Efficiency (kg of milk kg^{-1} of DM)	1.15	1.09	0.150	1.04	1.20	0.150	NS	NS	NS
Total fatty acids intake (kg d^{-1})	0.98	1.15	0.078	1.19	0.94	0.078	†	*	NS
Linolenic acid intake (kg d^{-1})	0.54	0.64	0.012	0.66	0.53	0.012	†	*	NS
Linoleic acid intake (kg d^{-1})	0.11	0.14	0.002	0.14	0.11	0.002	†	†	NS

SED: standard error of the differences. NS = not significant ($p > 0.10$); †, *: significant at $p < 0.10$, $p < 0.05$, respectively.

Table 3. Effect of herbage mass (HM) and daily herbage allowance (DHA) on rumen fermentation variables and concentration of fatty acids in ruminal contents

Item	HM		SED	DHA		SED	Significance		
	High	Low		High	Low		HM	DHA	HM × DHA
Rumen fermentation variables									
Rumen pH	5.86	5.80	0.037	5.82	5.85	0.039	†	NS	**
Total VFA, mmol L^{-1}	76.7	82.3	5.44	80.9	78.0	5.44	NS	NS	NS
Acetate, mmol L^{-1}	48.1	51.2	3.38	50.4	48.9	3.38	NS	NS	NS
Propionate, mmol L^{-1}	15.0	16.0	1.13	15.9	15.2	1.13	NS	NS	NS
Butyrate, mmol L^{-1}	10.8	11.9	0.84	11.5	11.1	0.82	NS	NS	NS
Acetate:Propionate ratio	3.30	3.25	0.069	3.25	3.31	0.074	NS	NS	NS
Fatty acids (g/100 g of fatty acids)									
C14:0	2.6	2.2	0.15	2.4	2.4	0.15	**	NS	*
iso-C15:0	2.3	1.9	0.11	2.0	2.2	0.11	**	*	*
anteiso-C15:0	5.5	4.7	0.21	5.0	5.2	0.21	**	NS	NS
C15:0	4.0	3.5	0.16	3.7	3.9	0.16	**	NS	NS
C16:0	20.9	20.2	0.22	20.1	21.1	0.09	NS	†	NS
c9-C16:1	0.6	0.4	0.06	0.5	0.5	0.06	**	NS	†
C17:0	0.6	0.6	0.06	0.6	0.6	0.06	NS	NS	NS
C18:0	42.0	43.6	0.60	42.9	42.6	0.60	**	NS	**
c9-C18:1	2.5	3.0	0.46	2.7	2.7	0.52	NS	NS	NS
t11-C18:1 (VA)	12.5	14.8	0.47	15.3	12.1	0.49	**	**	*
c9,c12-C18:2 n-6 (LA)	1.8	1.8	0.22	1.8	1.8	0.22	NS	NS	†
c9,t11-C18:2 (CLA)[a]	—	—	—	—	—	—	—	—	—
C18:3 n-3 (LNA)	0.9	0.7	0.21	0.8	0.8	0.22	NS	NS	NS

SED: standard error of the differences. NS = not significant ($p > 0.10$); †, *, **: significant at $p < 0.10$, $p < 0.05$ and $p < 0.01$, respectively. [a]CLA was not detected in most of the samples and was present on average at a concentration of <0.2% for all treatment groups.

ruminal concentration of VA was found in cows grazing the LH treatment (17.0 g/100 g of FA; $p < 0.05$) and the lowest in the HL treatment (11.5 g/100 g of FA).The HM and DHA affected most of the FA recorded. However, oleic acid (c9-C18:1), LA and LNA did not differ between treatments and c9,t11-CLA was not detected in ruminal samples. The low HM treatment resulted in higher concentration of C18:0 and VA ($p < 0.01$). Additionally, cows grazing high DHA had higher ruminal concentration of VA ($p < 0.01$).

Table 4. Effect of herbage mass (HM) and daily herbage allowance (DHA) on milk concentration of fatty acids

Item, g kg^{-1} of total fatty acids	HM		SED	DHA		SED	Significance		
	High	Low		High	Low		HM	DHA	HM × DHA
C4:0	94	79.6	8.93	90.5	83.2	10.2	NS	NS	NS
C6:0	43.9	46.7	0.91	47.3	43.3	0.81	NS	NS	NS
C8:0	23.1	24.9	2.46	24.2	23.8	2.83	NS	NS	NS
C10:0	41.9	43.4	6.09	43.5	41.7	6.63	NS	NS	NS
C12:0	41.6	32.3	5.87	37.8	36.1	5.87	NS	NS	NS
C14:0	100.1	97.7	0.5	101.5	96.3	0.44	NS	*	*
c9-C14:1	11.2	9.4	1.19	10.2	10.4	1.64	NS	NS	NS
C15:0	9.3	9.4	0.2	9.2	9.6	0.97	NS	NS	NS
C16:0	231.9	217.9	8.60	223.6	226.2	13.9	**	NS	NS
c9-C16:1	16.9	15.4	0.91	16.6	15.8	1.23	NS	NS	NS
C17:0	5.1	4.3	1.01	4.6	4.7	1.01	NS	NS	NS
C18:0	62.9	77.4	9.34	69	71.3	9.34	NS	NS	NS
c9-C18:1	180.6	197	22.77	190	187.6	29.05	NS	NS	NS
t11-C18:1 (VA)	22.0	29.6	0.86	24.9	26.7	1.2	*	NS	NS
c9,c12-C18:2 n-6 (LA)	8	9	1.56	8.4	8.6	1.56	NS	NS	NS
c9,t11-C18:2 (CLA)	10.8	14.8	2.5	12.9	12.6	1.89	NS	NS	NS
C18:3 n-3 (LNA)	3.6	5.6	0.69	4.3	4.9	0.69	*	NS	NS
Total SFA[a]	676.3	615.4	24.42	653.8	638	30.77	*	NS	NS
Total UFA[b]	269.8	297.7	33.46	284.6	282.9	33.46	NS	NS	NS
UFA:SFA	0.42	0.49	0.064	0.45	0.46	0.064	NS	NS	NS
n-6	11.8	14.1	0.63	13.3	12.6	4.1	†	NS	NS
n-3	3.8	6.0	0.68	4.9	5	0.68	*	NS	NS
n-3:n-6	0.42	0.47	0.66	0.44	0.45	0.894	NS	NS	NS
SCFA[c]	152.4	160	6.65	160	160	7.11	NS	NS	NS
MCFA[d]	476.1	419.1	22.26	452.3	443	22.26	*	NS	NS
LCFA[e]	301.3	349.8	44.37	323.2	327.9	44.37	NS	NS	NS
Δ9 desaturase index[f]	0.09	0.09	0.011	0.09	0.09	0.011	NS	NS	NS

SED: standard error of the differences. NS = not significant ($p > 0.10$); †, *, **: significant at $p < 0.10$, $p < 0.05$ and $p < 0.01$, respectively. [a] SFA: saturated fatty acids. [b] UFA: unsaturated fatty acids. [c] SCFA: short chain fatty acids; sum of C4:0 to C8:0. [d] MCFA: medium chain fatty acids; sum of C10:0 to C16:1. [e] LCFA: long chain fatty acids; sum of C17:0 to C22:6. [f] C14:1 / (C14:0 + C14:1).

Milk fatty acid composition

The effect of treatment on milk FA composition is presented in Table 4. There was a HM × DHA ($p < 0.05$) interaction for milk concentration of C14:0, being the highest on HH (data not shown). Concentration of C16:0 was higher at high HM ($p < 0.01$) and consequently, saturated FA (SFA) and medium chain FA (MCFA) were also higher ($p < 0.05$). Contrary to this, VA and LNA were higher at low HM ($p < 0.05$). As a consequence of the higher concentration of LNA, total n-3 PUFA were higher at low HM ($p < 0.05$) whilst the n-6 PUFA tended to be lower ($p < 0.10$) on this treatment. The DHA only affected C14:0, which was higher at high DHA ($p < 0.05$).

Discussion

Few studies have shown an effect of different DHA and HM on milk FA composition. Given that intake of precursors is the main factor influencing PUFA content of milk, as long chain FA (LCFA) can not be synthesized *de novo* in mammary gland (Jensen, 2002), it is likely that variation in DHA and HM could affect the FA intake of cows at pasture and consequently PUFA content in milk. In the present study, only DHA affected FA intake (total and LNA), being higher for cows grazing pastures at high DHA. This effect was unexpected because there were no differences in estimated DMI between treatments although DMI at high DHA was numerically higher (+2 kg DMI). Moreover, even

though pasture FA composition was not statistically analysed, total FA in grass for the different treatments remained similar. Therefore, the higher intake of total FA and LNA was probably a consequence of a higher DMI at high DHA.

No differences were found for any of the ruminal fermentation related variables across treatments. Only pH at low HM tended to be lower than high HM ($p < 0.10$). Parga *et al.* (2000) found lower pH values for cows offered a leafy sward (pH 6.02; higher DM digestibility than control sward) compared to a control sward (pH 6.18; lower DM digestibility and leaf proportion than leafy sward) independent of the DHA (12 *vs* 18 kg DM cow^{-1} d^{-1}) allocated. These authors also found lower content of acetate and higher content of propionate in cows fed the leafy sward. In our study, VFA did not differ amongst treatments. Despite the large divergence in HM (2580 *vs* 1726 kg DM ha^{-1} for high and low HM respectively) generated among the pastures used in our study, OM digestibility was similar between treatments. These results may partially explain the absence of differences in ruminal fermentation parameters.

Even though biohydrogenation rate was not measured in this study, rumen FA profile can provide an approximation of the level of dietary FA biohydrogenation (Loor *et al.*, 2005). The C18:0 and VA were all higher at low HM while VA was also significantly higher at high DHA in comparison to high HM and low DHA respectively. This effect may be a consequence of the higher LNA intake (the precursor of both through biohydrogenation) registered at low HM and high DHA.

Rumen pH can affect *in vitro* biohydrogenation and, under low ruminal pH conditions, progression of the hydrogenation steps from VA to C18:0 can be inhibited (Troegeler-Meynadier *et al.*, 2003; Qiu *et al.*, 2004; Ribeiro *et al.*, 2007). In our study, the ruminal pH tended to be lower at low HM, in coincidence with the highest VA concentration. Nevertheless, the magnitude of pH differences between treatments recorded would seem not to be of biological importance and, in all cases, ruminal pH was lower than 5.9 which would lead to increased concentrations of VA in the rumen. In fact, Qiu *et al.* (2004) found that, at low pH (5.8), accumulation of VA occurs and C18:0 decreases in concentration. Loor *et al.* (2004) concluded that a low dietary forage:concentrate ratio reduces the biohydrogenation of unsaturated FA and increases the flow of *trans*-C18:1 into the small intestine. Troegeler-Meynadier *et al.* (2006) stated that low pH inhibits the isomerisation of LA to c9,t11-CLA and also the reduction of VA to C18:0. Consequently, it is expectable that the accumulation of VA increases under the grazing conditions studied (high quality herbage which promotes low ruminal pH in comparison to low quality herbage). With regards to the treatments under study, differences in ruminal VA content may be more related to PUFA intake rather than differences in ruminal pH.

As mentioned before, low pH (<6.0) but also high amounts of LNA in the diet can inhibit the isomerisation of LA to c9,t11-CLA in rumen (Troegeler-Meynadier *et al.*, 2006). Therefore, it was expectable that c9,t11-CLA has not been detected in either rumen samples. Coincident with our results, Ribeiro *et al.* (2007) did not observe c9,t11-CLA in continuous culture using alfalfa as substrate, probably because of the relatively low LA coming from herbage based diets. It is important to note that LA is the precursor of c9,t11-CLA in rumen and, at the present study, the predominant dietary FA available to the animals was LNA (Griinari & Bauman, 1999). In agreement with previous studies (Kay *et al.*, 2004; Mosley *et al.*, 2006), most of the milk c9,t11-CLA was likely synthesized *de novo* in the mammary gland by the Δ9 desaturase enzyme. In fact, the absence of difference in milk c9,t11-CLA may be related to the similar Δ9 desaturase activity estimated across treatments (Palladino *et al.*, 2009b) although other factors as substrate availability may affect Δ9 desaturase activity. In our study, the differences in rumen VA content were probably too low to affect milk FA composition.

Despite the differences in rumen FA recorded, only some of the milk FA varied across treatments. For example, the concentration of C16:0 in milk was higher at high HM whilst VA and LNA were higher at low HM. Given that approximately 50% of C16:0 in milk is synthesized *de novo* in mammary gland, it is difficult to explain the effect of HM. The greatest concentration of C16:0 in milk resulted in a higher concentration of MCFA for the high HM. Additionally, VA and LNA concentration in milk were higher in cows grazing at low HM. With respects to DHA, in our experiment the herbage allowance decreased from 20 to 16 kg cow^{-1} with the quality of the herbage remaining similar and without any change in milk VA, c9,t11-CLA nor LNA content. The absence of statistically significant differences was probably due to the similarity in herbage quality between treatments. In contrast, Elgersma *et al.* (2004) found a rapid decline in c9,t11-CLA and VA in

milk from grazing cows when a 50% decrease in DHA was invoked. In their experiment, DHA was reduced from 44 to 22 kg cow^{-1} inducing a great change in terms of herbage quantity.

Boufaied *et al.* (2003) argued that biohydrogenation may be reduced by increased fibre content in the pasture or by the conservation process (hay), probably as a consequence of a reduction in DM digestibility which can affect the accessibility of ruminal bacteria to the FA. These arguments support our results since NDF and ADF content in the different treatments used in this trial were quite similar and only small differences were found in FA rumen content. The greater LNA and n-3 PUFA content of milk from cows grazing low HM can be explained by the high intake of LNA achieved by this treatment. This study suggests that DMI, particularly FA intake, and not altered ruminal biohydrogenation, seems to be the most important factor affecting milk FA concentration under the grazing conditions employed here (high quality pastures). However, DMI and FA intake did not explain the absence of differences with regards to DHA treatments. It is possible that changes in ruminal biohydrogenation that were not detected due to the rumen sampling procedure may explain partially the similarity between treatments.

Under the conditions employed here, changes in HM and DHA did not largely affect the composition of FA in the milk of grazing dairy cows. Even though FA intake and rumen FA profile varied between treatments, it seems that differences in the magnitude recorded here, are not of sufficient biological importance to invoke differences in the concentration of either c9,t11-CLA or n-3 PUFA in milk. The high intake of LNA recorded and the low pH achieved in all the treatments led to an accumulation of VA in the rumen. As LNA was the major FA in the diet and, this is not an important precursor of c9,t11-CLA in the rumen (Lee & Jenkins, 2011), most of the c9,t11-CLA in milk was most likely synthesized *de novo* in the mammary gland. It seems that changes in FA intake, rather than changes in ruminal metabolism (*i.e.* pH, or rumen FA profile), may have a greater effect on milk FA composition, especially VA and LNA (but not c9,t11-CLA) when high quality pastures are grazed. It is likely that increasing the concentration of health promoting FA in milk through changes in either HM or DHA of high quality pastures alone may be difficult. Further research is required to design grazing strategies that enhance the level of the beneficial fatty acids in milk.

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