ORIGINAL ARTICLE

Milk casein and fatty acid fractions in early lactation are affected by nutritional regulation of body condition score at the beginning of the transition period in primiparous and multiparous cows under grazing conditions

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Summary

The objective was to evaluate the effect of body condition score (BCS) at 30 days before calving (−30 days) induced by a differential nutritional management, parity and week of lactation (WOL) on milk yield and composition, and milk casein and fatty acid composition. Primiparous and multiparous Holstein cows with high BCS (PH, $n = 13$; MH, $n = 9$) and low BCS (PL, $n = 9$; ML = 8) under grazing conditions were sampled at WOL 2 and 8 (before and after peak of lactation). Milk yield was greater in multiparous than in primiparous cows and tended to decrease from WOL 2 to 8 only in ML cows. Milk protein, fat and casein yields were greater in multiparous than in primiparous cows and decreased from WOL 2 to 8. Milk casein concentration in milk protein was greater in MH cows than in ML, PH and PL cows at WOL 2. Milk κ-casein was greater, and β-casein was less in multiparous than in primiparous cows. As lactation progressed, proportion of casein fractions were not altered. Only κ-casein fraction was affected by BCS at −30 days as PL showed a higher concentration than PH. The *de novo* (4:0–15:1) and mixed-origin fatty acids (16:0–16:1) in milk fat increased, whereas preformed fatty acids (≥17:0) decreased from WOL 2 to 8. Saturated (SAT) fatty acids tended to be greater and monounsaturated fatty acids (MUFA) were less in multiparous than in primiparous cows. High-BCS cows had greater concentrations of polyunsaturated (PUFA), conjugated linoleic acid (CLA) as well as n-6 and n-3 fatty acids in milk fat than low-BCS cows. The results indicate that casein and fatty acid fractions in milk were affected by parity and may be modified by a differential nutritional management during the pre-calving period (BCS at −30 days) in cows under grazing conditions.

Introduction

Nutritional, physical and flavour properties of milk are influenced by its casein and fatty acid contents (Palmquist et al., 1993; Ostersen et al., 1997). Furthermore, in order to respond to consumer demands and human health recommendations that require certain milk properties and components (e.g. conjugated

linoleic acid, CLA), a better understanding of milk biosynthesis is needed (Lock and Shingfield, 2004; Bauman et al., 2006).

Milk protein consists of 80% of casein that comprises four proteins: αs1-, αs2-, κ- and β-caseins (Farrell et al., 2004). In early lactation, energy and nitrogen (N) intake is often inadequate to support the high rates of milk protein output (Bequette et al., 1998). In these circumstances, depletion of skeletal muscle protein is important to supplement dietary and microbial protein to maintain an adequate supply of amino acids (AA) to the mammary gland and carbon for gluconeogenesis in the liver (Black et al., 1990). Few studies have analysed the effect of the body condition score (BCS) on milk protein. Ostersen et al. (1997) did not detect any effect of BCS at calving on αs1-, αs2-, κ- and β-casein concentrations in primiparous cows when cows were classified according to BCS at calving.

Milk fatty acids originate from three major sources: synthesized *de novo* in the mammary gland, absorbed from the gastrointestinal tract (directly from the diet or formed in the rumen by biohydrogenation or bacterial synthesis) and released from body fat (Stoop et al., 2009). At the onset of lactation, cows are in negative energy balance, and therefore, adipose fatty acids are mobilized and incorporated into milk fat (Palmquist et al., 1993; Kelsey et al., 2003; Kay et al., 2005). The changes in milk fatty acid composition over lactation reflect shifts in the uptake and/or synthesis activity related to cow energy status changes (Van Knegsel et al., 2005; Stoop et al., 2009) and could differ between parities (Kelsey et al., 2003). Moreover, milk from cows with high BCS classified at calving or 1 month before calving contained increased amounts of long-chain and unsaturated fatty acids and reduced amounts of short- and medium-chain fatty acids whether the cows were managed on confinement (Pedron et al., 1993; Agenas et al., 2003) or in grazing systems (Stockdale et al., 2005).

The prevailing endocrine profile of the periparturient cow includes major reductions in plasma concentrations of insulin and insulin-like growth factor-I, which, together with insulin resistance in peripheral tissues, must permissively facilitate, if not actively promote, net mobilization of AA and fatty acids from these tissues, directing nutrient partitioning towards the mammary gland (Bell et al., 2000). We have previously reported the effects of classified BCS on the metabolic endocrinology of the transition dairy cow (Meikle et al., 2004; Cavestany et al., 2005) and hypothesized that other factors not

related to metabolic reserves (BCS at calving) could be partially involved in the obtained outcome, which could be the result of a differential animal capacity to face the negative energy balance during this period (i.e. individual intake behaviour or mobilization reserves capacity).

Therefore, our hypothesis was that greater body energy reserves reflected in greater BCS at the beginning of the transition period (−30 days) induced by nutritional treatment during the dry period (−100 to −30 days) would provide the mammary gland with more energy and synthesis precursors (i.e. long-chain fatty acids, AA) that would result in a modified milk casein and fatty acid fraction profiles (i.e. increase in beneficial fatty acids in milk and a better casein profile from a manufacturer's perspective). This response could be different according to parity, as primiparous cows would need extra energy and AA for growth simultaneously with the lactation demands (Rémond et al., 1991) and would have a lower dry matter intake (DMI) capacity (Ingvartsen, 1994; Maekawa et al., 2002). In addition, the effect of nutritional regulated BCS at −30 days and parity could be differential before (week of lactation 2, WOL 2) than after (WOL 8) peak of lactation, which could be partly due to an increased DMI during mid-lactation in cows with greater BCS and/or to a reduction in the partitioning of nutrients towards replenishment of tissue stores in these cows. Thus, the objective was to evaluate the effect of body condition score (BCS) at 30 days before calving (−30 days) induced by a differential nutritional management, parity and WOL on milk yield and composition, and milk casein and fatty acid composition.

Materials and methods

Animal experimentation was approved by the Animal Experimentation Committee of the Universidad de la República Oriental del Uruguay. Detailed descriptions of the effect of BCS at −30 days on milk production, metabolic and endocrine profiles, and days to first ovulation in primiparous and multiparous Holstein cows under grazing conditions have been reported previously (Adrien et al., 2012).

Experimental design

Holstein cows with two to five parturitions (multiparous cows, M; $n = 32$) and cows without previous parturitions (primiparous cows, P; $n = 30$) (average 305 days corrected milk yield: 6000 and 4800 kg, respectively, for herd of Uruguay) with fall

parturitions (March, April and May) were selected (according to age, body weight, lactation number, previous milk yield and expected calving date) from the herd (200 dairy cows) of the experimental dairy farm of School of Agronomy (Paysandú, Uruguay).

Cows were blocked at −100 days according to their expected calving date and body weight and randomly assigned to different nutritional treatments to induce the desired BCS (0.5 units of difference between low and high BCS respectively) at −30 days. Therefore, to achieve the desired BCS at −30 days, cows grazed paddocks of a long-term established pasture with a forage allocation of 7, 14 or 20 kgDM/day to lose, maintain or gain BCS respectively. The pasture had a maximal herbage mass of 1200 kgDM/ha with an estimated DM composition of 12.7% crude protein (CP), 56.7% neutral detergent fibre (NDF), 24.3% acid detergent fibre (ADF) and 5.23 MJ/kgDM of net energy of lactation (NEL). Body condition score was evaluated every 15 days, and the amount of forage offered was altered to achieve the desired BCS at −30 days. Cow BCS was measured according to a scale of 1 to 5 (1 = thin, 5 = fat; Edmonson et al., 1989), and all observations were made by the same observer.

Only cows that responded to nutritional treatments and achieved the desired BCS were considered in the study, and this was defined as follows:

primiparous and multiparous cows with high BCS (PH, $n = 13$, MH, $n = 9$) had to gain an additional 0.5 units of BCS, while primiparous cows with a low BCS (PL, $n = 9$) had to lose 0.5 units of BCS and multiparous cows with low BCS had to maintain BCS (ML = 8) at least in two subsequent observations from −100 to −30 days. Primiparous cows had greater BCS than multiparous cows at the initiation (−100 days) of the experiment (3.80 ± 0.74 vs. 2.91 ± 0.68). There were no differences between BCS within parity groups before the nutritional treatments were applied, but they did differ from −85 to −30 days. At −30 days and calving, BCS was different between high and low cows within parity (Table 1). From −30 to +60 days, BCS was assessed every 15 days during the pre-partum and weekly during the post-partum, and body weight was measured at 30-day intervals.

From −30 days to calving, primiparous and multiparous cows were managed separately and were fed a diet formulated according with their NRC requirements (NRC, 2001). Cows were offered once a day a diet that included whole-plant maize silage (4.2 or 5.1 kgDM for primiparous or multiparous cows respectively) and a commercial concentrate (3.7 and 4.6 kgDM for primiparous or multiparous cows respectively) and had *ad libitum* access to *Setaria italica* hay. This diet resulted in an estimated

Table 1 Body condition score (BCS), milk yield and composition in primiparous and multiparous Holstein cows with low (PL, ML) and high (PH, MH) BCS at −30 days of lactation

	Treatments*					P^+				
	PL	PH	ML	MH	SEM	p	BCS (P)	WOL	P* WOL	BCS (P)* WOL
Body condition score						<0.01	<0.01	<0.01	<0.01	<0.01
At −30 days	2.98[b]	3.43[a]	2.89[b]	3.44[a]	0.07					
At calving	2.95[b,c]	3.18[a]	2.68[b]	3.12[a,c]	0.07					
at 15 days	2.84[b]	3.06[a]	2.74[b]	2.89[a,b]	0.09					
At 56 days	2.69[a]	2.86[a]	2.60[a]	2.63[a]	0.13					
Milk yield										
kg/day	23.7	24.0	29.4	28.9	0.5	<0.01	0.91	0.28	0.64	<0.01
Protein										
kg	0.74	0.76	0.95	0.94	0.03	<0.01	0.78	<0.01	0.30	0.42
%	3.1	3.1	3.2	3.1	0.1	0.89	0.26	<0.01	0.44	0.65
Fat										
kg	0.81[a]	0.95[a,b]	1.06[b]	1.09[b]	0.05	<0.01	0.21	<0.01	0.05	0.03
%	3.5[x]	3.9[y]	3.6[x,y]	3.5[x]	0.1	0.29	0.04	<0.01	0.41	0.09
Milk SCC										
Linear score	1.73	1.57	1.85	2.08	1.25	0.02	0.35	0.86	0.63	0.76
(num. × 1000)	(53.7)	(37.2)	(70.8)	(120.2)						

[+]P, parity; BCS, body condition score at −30 days; WOL, week of lactation.

[a,b]BCS at −30 days × parity means within a row with different superscripts differ (p < 0.05).

[x,y]BCS at −30 days × parity means within a row with different superscripts differ (0.05 < p ≤ 0.10).

*Data represent least squares means ± SEM for the BCS at −30 days within parity effect.

chemical composition of 9.5% CP, 54.2% NDF, 30.8% ADF and 5.56 MJ/kgDM of NEL, for both groups. From calving to 60 days, primiparous and multiparous cows grazed separately a second-year pasture, mixture of *Festuca arundinacea*, *Trifolium repens* and *Lotus corniculatus*, with a forage allowance of 30 kgDM/cow in weekly plots allocated twice per day. Pasture composition for the whole period averaged 25% DM, 14.2% CP, 49.0% NDF and 24.5% ADF. Cows were also supplemented individually with 3.1 kgDM of whole-plant corn silage (31.1% DM, 6.9% CP, 65.0% NDF and 33.0% ADF) and 3.7 kgDM of a commercial concentrate (89% DM, 18.1% CP, 19.2% NDF and 12% ADF) after the morning milking. In addition, cows received 1.3 kgDM of the same commercial concentrate in the parlour during each milking.

Data collection and sample analyses

Cows were milked twice a day (5:00 AM and 3:00 PM), and yields at each milking were recorded. Samples (10 ml) for milk composition were obtained from four consecutive milkings per week until 8 weeks of lactation (WOL) from each cow and were stored in snap tubes containing preservative (Lactopol®, Rodolfo Benzo, Uruguay). Samples were taken to the laboratory and immediately placed in a 37 °C water bath for 10 min, homogenized and a representative aliquot was subsequently analysed for fat, protein and lactose by mid-infrared spectrophotometry (Milko-Scan, Foss Electric, Hillerød, Denmark) and for somatic cell count (SCC) using a Fossomatic (Foss Electric). During WOL 2 and 8, milk samples were obtained for casein and fatty acid composition. Samples for casein determination were skimmed by centrifugation (1878 *g* at 5 °C for 20 min). A 100 μl volume of skim milk was mixed with 900 μl of sample buffer and stored at −20 °C. The sample buffer (pH = 7.5) consisted of 17.5 Mm 1,3-bis [tris (hydroxymethyl)-methylamino] propane containing 7 M urea and 0.5% of 2-mercaptoethanol. For individual fatty acid composition, milk samples were stored at −20 °C without preservative until analysed.

Casein fraction determination

Milk total protein and whey content were analysed by Kjeldahl method (AOAC, 1980). Whey protein was obtained from skim milk by isoelectric precipitation at pH 4.6 by the addition of 1 M HCL.

Caseins (α s-1, α s-2, β and κ) were separated in a single run by a modification of the method used by Visser et al. (1991). Caseins were isolated by RP-HPLC with an Apex WP ODS column (7 μm, 4.6 × 250 mm, Jones Chromatography, Mid-Glamorgan, UK) maintained at 46 °C (Damián et al., 2008). The eluent consisted of a gradient of acetonitrile–water–trifluoroacetic acid developed by mixing volume-based solutions of 100-900-1 (solution A) and 900-100-0.7 (solution B) of these compounds respectively. Proteins were eluted with a series of linear gradients of solution B in solution A: 28.7–42.5% solution B for 15 min, 42.5–48.8% solution B for 15 min and 48.8–28.7% solution B for 15 min. The column was re-equilibrated between samples with 28.7% solution B in solution A for 15 min. Flow rate was 1 ml/min, and the eluted peaks were detected by UV absorbance at 214 nm. All samples were run in duplicate and were routinely identified by comparison of retention times with authentic standards purchased from Sigma (αs-casein C6780; β-casein C6905; κ-casein C0406; St. Louis, MO, USA). The intra- and interassay coefficients of variation (CV) for each peak measured were in average 7% and 5% respectively. The area of each casein fraction peak was quantified and reported as a proportion of the sum of the area of all four casein fractions (Trujillo et al., 2000).

Fatty acid fraction determination

Milk fat was extracted according to Folch et al. (1957), and fatty acid methyl esters were prepared by the transmethylation procedure described by IUPAC 2.301 (Mossoba et al., 1996). Fatty acid methyl esters were quantified using a gas chromatograph (Agilent Technologies 6890, Palto Alto, CA, USA) connected to a mass spectrometer (Agilent Technologies 5973) equipped with a flame ionization detector and a CP 2560 fused silica SP 2560 capillary column (100 m × 0.25 mm i.d. with 0.2-μm film thickness; Supelco, Bellefonte, PA, USA). Gas chromatograhy, column, oven, gas variables and fatty acid identification were done as previously described (Moore et al., 2004, 2005). The samples were run in duplicate, and FAME standard (Supelco 47885-U, Bellefonte; 37 FAME from C4:0 to C24:0) was analysed at regular intervals for quality control purposes and to determine recovery and correction factors for individual fatty acids. The intra- and interassay CV for each analyte measured were in average 3–6% respectively. The fatty acid composition of milk fat is expressed as grams of each individual fatty acid per kilograms of total fatty acids. The CLA isomers are reported as the sum of all

linoleic isomers with conjugated cis- and trans-double bonds.

Statistical analysis

Milk yield and composition, and milk casein and fatty acid profiles were analysed as a randomized block design with repeated measures (Mixed procedure; SAS Institute, Cary, NC, USA), with WOL as the repeated effect and first-order autoregressive as the covariance structure. The model included the effects of parity, BCS at −30 days nested within parity, WOL and their interactions as fixed effects and block and cow as random effects. The Kenward–Rogers procedure was used to adjust the denominator degree of freedom. Tukey–Kramer tests were conducted for mean separation. Means were reported as least squares means with their respective pooled standard errors and were considered to differ when $p \leq 0.05$, and trends were identified when $0.05 < p \leq 0.10$. Pearson's correlations were performed to examine means' associations between variables (Corr procedure, SAS Institute). For the statistical analysis of SCC data, the absolute values were transformed into somatic cell linear scores applied a $\log_{10}$ transformation to normalize the data.

Results

Milk yield and composition

Milk yield was greater in multiparous than in primiparous cows (29.2 vs. 23.9 ± 0.5 kg/day; $p < 0.01$) and was not affected by BCS at −30 days within parity or WOL (Table 1). However, there was an interaction of BCS at −30 days with WOL ($p < 0.01$) as

milk yield tended ($p = 0.09$) to decrease from WOL 2 to 8 only in ML cows.

Milk protein and milk fat yields were greater ($p < 0.01$) in multiparous than in primiparous cows (0.94 vs. 0.75 ± 0.02 kg/day and 1.08 vs. 0.88 ± 0.04 kg/day for milk protein and fat, respectively, Table 1) and decreased ($p < 0.01$) from WOL 2 to 8 (0.89 vs. 0.81 ± 0.02 kg/day and 1.05 vs. 0.90 ± 0.03 kg/day for milk protein and fat respectively). Milk protein and fat yields were not affected by BCS at −30 days within parity. However, milk fat yield was affected by the interactions between parity and WOL ($p = 0.05$) and between BCS at −30 days within parity and WOL ($p = 0.05$) as milk fat yield tended to decrease ($p = 0.09$) from WOL 2 to 8 only in ML cows (data not shown).

Milk fat percentage was affected by BCS at −30 days within parity ($p = 0.04$) and tended to be less ($p < 0.10$) in PL than in PH cows, while no differences were observed between treatments in multiparous cows (Table 1). All groups decreased ($p < 0.05$) fat concentrations from WOL 2 to 8, except for MH (data not shown). Milk SCC was greater ($p = 0.02$) in multiparous than in primiparous but was not affected by BCS at −30 days or WOL (Table 1).

Nitrogen determination by Kjeldahl

Casein represented 78.5 ± 1.2% of milk total protein. Milk from the multiparous cows contained more casein (797.4 vs. 772.4 ± 6.1 g N/kg total N) and less whey (202.6 vs. 227.6 ± 6.1 g N/kg total N) than milk from the primiparous cows ($p = 0.02$, Table 2). Casein content decreased from WOL 2 to 8

Table 2 Milk protein fractions and casein components in primiparous and multiparous Holstein cows with low (PL, ML) or high (PH, MH) body condition score (BCS) at −30 days of lactation

Variable	Treatments*					P^+				
	PL	PH	ML	MH	SEM	p	BCS (P)	WOL	P * WOL	BCS (P)* WOL
Nitrogen fractions g N/kg total N										
Casein	777.2	767.6	780.2	814.7	10.9	0.02	0.33	0.03	0.57	0.10
Whey protein	222.8	232.4	219.8	185.4	6.1	0.02	0.35	0.03	0.57	0.10
Caseins fractions (g/kg detected)										
αs-1-casein	368.8	393.5	395.7	398.0	14.9	0.24	0.71	0.61	0.27	0.62
αs-2-casein	109.7	70.0	84.4	77.0	9.5	0.30	0.20	0.70	0.62	0.90
β-casein	428.7	462.6	427.6	432.3	7.0	0.04	0.13	0.77	0.07	0.94
κ-casein	97.4[a]	69.5[b]	94.5[a]	89.9[a,b]	3.9	0.02	0.03	0.67	0.96	0.15

[+]P, parity; BCS, body condition score at −30 days; WOL, week of lactation.
[a,b]BCS at −30 days × parity means within a row with different superscripts differ ($p < 0.05$).
*Data represent least squares means ± SEM for the BCS at −30 days within parity effect.

(794.6 vs. 775.2 ± 6.1 g N/kg total N, p = 0.03), whereas whey content increased (205.4 vs. 224.8 ± 6.1 g N/kg total N, p = 0.03; Table 2) but there was a trend (p = 0.10) of an interaction between BCS at −30 days within parity and WOL because these changes were only significant for PH cows. Milk from the MH cows contained more casein and less whey than milk from cows in the other treatment groups at WOL 2 (p < 0.05; Fig. 1a,b).

Casein fractions

The proportion of *β*-casein was less (429.9 vs. 445.6 ± 4.0 g/kg; p = 0.04) and *κ*-casein greater (92.2 vs. 83.5 ± 2.5 g/kg; p = 0.02) in milk from multiparous than in milk from primiparous cows (Table 2). As lactation progressed, proportions of the individual casein fractions were not altered (Table 2), but there was a trend (p = 0.07) of an interaction of parity with WOL on *β*-casein, as it was greater in primiparous than in multiparous cows due primarily to a greater concentration in PH than in

ML and MH cows at WOL 8 (p < 0.05; Fig. 1c). The *κ*-casein was affected (p = 0.03) by BCS at −30 days within parity as its proportion in milk was less for PH than for PL and ML cows (p < 0.05; Table 2).

Fatty acid fractions

Palmitic acid and all individual *de novo* fatty acids except butyric acid increased with WOL (Table 3). The proportion of *de novo* and mixed-origin fatty acids (95% palmitic acid) increased from WOL 2 to 8 (168.7 vs. 226.4 ± 10.3 mg/g and 291.3 vs. 324.2 ± 6.9 mg/g, respectively, p < 0.01; Table 4; Fig. 2). In contrast, preformed fatty acids (51% oleic and 27% stearic acid) decreased (543.4 vs. 454.2 ± 16.3 mg/g; p < 0.01) during the same interval (Fig. 2). The decrease in preformed fatty acids at WOL 8 was mainly due to a decrease in stearic acid.

Mixed-origin fatty acids tended (p = 0.10) to be affected by BCS at −30 days within parity as the concentration of these fatty acids was greater in milk fat from ML than in milk fat from MH cows
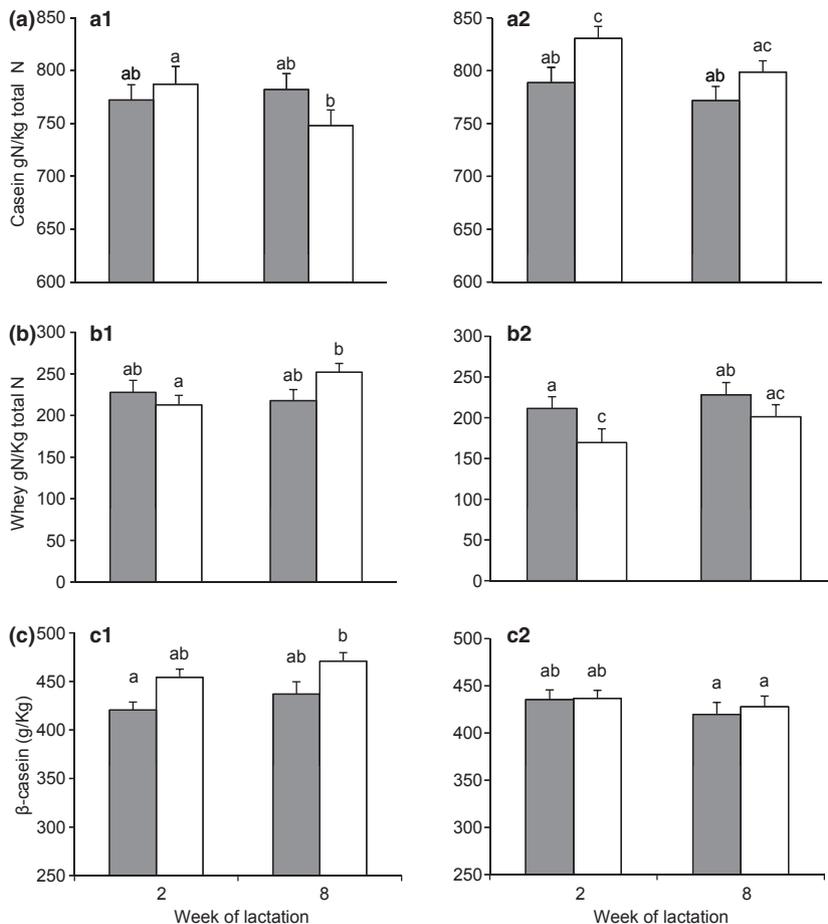
Fig. 1 Casein (a), whey (b) and *β*-casein concentrations (c) in milk at weeks of lactation 2 and 8 in primiparous (a1, b1 and c1) and multiparous (a2, b2 and c2) cows with low (3.0, grey) or high (3.5, white) body condition score at −30 days of lactation. Data are least square means ± pooled standard error. Letters above bars indicate Tukey's test differences for the interaction between body condition score at −30 days within parity and week of lactation; a, b, c: p < 0.05.

Table 3 Milk fatty acid composition in primiparous and multiparous Holstein cows with low (PL, ML) or high (PH, MH) body condition score (BCS) at −30 days of lactation

Fatty acid (mg/g)	Treatments*					P^+				
	PL	PH	ML	MH	SEM	p	BCS (P)	WOL	P * WOL	BCS(P)*WOL
4:0	6.91	5.20	6.05	5.42	0.77	0.64	0.62	0.12	0.15	0.85
6:0	7.89	7.27	8.85	6.69	0.80	0.79	0.32	<0.01	0.58	0.21
8:0	7.01	6.52	7.47	8.91	1.13	0.21	0.76	<0.01	0.56	0.94
10:0	17.72	18.67	22.27	17.68	2.56	0.44	0.59	<0.01	0.28	0.87
12:0	24.67	22.77	30.30	24.04	4.0	0.32	0.35	<0.01	0.25	0.87
13:0	1.28	1.30	1.54	1.38	0.18	0.33	0.88	<0.01	0.46	0.71
14:0	98.30	94.41	120.30	108.43	8.54	0.12	0.46	<0.01	0.18	0.92
14:1	4.91	5.81	8.07	6.41	0.95	0.06	0.51	<0.01	0.06	0.93
15:0	16.74^{a,b}	17.72^{a,b}	19.08^a	16.23^b	0.72	0.61	0.05	0.06	0.16	0.55
16:0	285.66	291.47	312.36	282.22	10.86	0.29	0.22	<0.01	0.39	0.48
16:1	12.80^{a,b}	16.10^{a,b}	16.55^a	8.43^b	1.22	0.13	<0.01	0.52	0.50	0.07
17:0	18.18	23.03	17.84	24.60	2.59	0.93	0.24	<0.01	0.56	0.92
18:0	147.19	115.77	142.11	134.32	13.94	0.62	0.26	<0.01	0.25	0.72
18:1*cis*	272.65	273.68	198.15	254.60	15.87	0.02	0.17	0.12	0.13	0.45
18:1 *trans*	39.09	46.84	39.66	46.48	8.77	0.99	0.74	0.48	0.05	0.09
18:2 (n-6)	17.38	20.02	18.04	22.62	1.50	0.26	0.16	0.30	0.39	0.25
18:2(CLA)	8.52^{a,b}	10.24^b	6.17^a	10.30^b	0.70	0.15	0.02	0.81	0.26	0.06
18:3 (n-3)	2.93^a	4.56^b	2.47^a	5.02^b	0.24	0.99	<0.01	0.13	0.75	0.03
18:3 (n-6)	0.78	0.59	0.28	0.79	0.35	0.66	0.66	0.11	0.16	0.86
20:5 (EPA)	0.10	0.01	0.02	0.06	0.04	0.86	0.37	0.79	0.58	0.93
22:6 (DHA)	0.04	0.09	0.02	0.01	0.04	0.93	0.48	0.20	0.14	0.59
Others†	10.18	18.77	24.91	13.40	8.54	0.50	0.52	0.71	0.66	0.83

$^+$P, parity; BCS, body condition score at −30 days; WOL, week of lactation; CLA, conjugated linoleic acid; EPA, eicospentaenoic acid; DHA, docosa-hexaenoic acid.

^{a,b,c}BCS at −30 days × parity means within a row with different superscripts differ (p < 0.05).

*Data represent least squares means $\pm$ SEM for the BCS at −30 days within parity effect.

†Others = 11 + 12:1 + 13 + 15:1 + 17:1 + 18:2 + 19 + 19:1 + 20 + 20:1 + 20:2 + 20:3n6 + 20:3n3 + 20:4n6 + 20:4n3 + 21 + 22 + 22:1cis + C22:4 + 22:5 +24.

(Table 4), and this was associated with the increased concentration of C16:1 in milk fat from ML in relation to MH cows (p < 0.01, Table 3).

Saturated fatty acids (SAT) tended to be greater (p = 0.06) and monounsaturated (MUFA) fatty acids were less (p = 0.04) in milk fat from multiparous than in milk fat from primiparous cows (663.5 vs. 621.5 ± 13.4 mg/g and 295.9 vs. 340.1 ± 12.4 mg/g respectively). This resulted in a saturated/unsaturated ratio (SAT/UNSAT) that was greater (p = 0.03) in milk fat from multiparous than in milk fat from primiparous cows (2.4 vs. 1.7 ± 0.2 mg/g; Table 4), which increased (p = 0.03) as lactation progressed (1.5 vs. 2.0 ± 0.3 respectively). However, there was an interaction between parity and WOL on the proportion of SAT and MUFA as SAT increased (p = 0.04) and MUFA decreased (p = 0.03) from WOL 2 to 8 (Fig. 3) only in primiparous cows. Parity and WOL did not affect milk polyunsaturated fatty acid (PUFA) concentrations (Table 4) in milk fat. Milk PUFA concentrations were greater in high-BCS cows, but this effect was evident at WOL 2 for multiparous cows and at WOL 8 for primiparous cows (Fig. 3c). Changes in PUFA concentrations were associated with increases (p < 0.01) in α-Linolenic acid (n-3) PUFA, especially in linolenic acid in both PH and MH cows (Tables 3 and 4). Although there were no differences in linoleic acid between treatment groups, n-6 PUFA tended (p = 0.10) to be affected by BCS at −30 days within parity as milk fat from MH cows tended to have greater concentrations of n-6 PUFA than milk fat from ML cows (Table 4).

Trans-fatty acids in milk fat tended (p = 0.07) to increase from WOL 2 to 8 only in MH cows (Fig. 4a2). This contributed to the trend of the proportion of *trans*-fatty acids in milk fat to be greater (p = 0.08) at WOL 8 in MH than in ML and PL cows (Fig. 4a). Milk CLA concentration was greater (p =

Table 4 Milk fatty acid components in primiparous and multiparous Holstein cows with low (PL, ML) or high (PH, MH) body condition score (BCS) at −30 days of lactation

Variable	Treatments*					P^+				
	PL	PH	ML	MH	SEM	p	BCS (P)	WOL	P* WOL	BCS (P)* WOL
Fatty acid origin, mg/g of fatty acid										
De novo (4:0–15:1)	187.9	182.4	225.8	194.1	17.1	0.14	0.58	<0.01	0.21	0.98
Mixed origin (16:0 + 16:1)	298.5[x,y]	307.5[x,y]	334.0[y]	290.9[x]	10.5	0.35	0.10	<0.01	0.42	0.30
Preformed (>17:0)	518.8	514.8	444.4	517.4	25.4	0.16	0.31	<0.01	0.26	0.75
Fatty acid saturation, mg/g fatty acid										
Saturated	635.3	607.7	697.8	629.3	20.6	0.06	0.16	0.13	0.04	0.55
Monounsaturated	329.3	351.0	267.8	323.9	19.0	0.04	0.22	0.18	0.03	0.68
Polyunsaturated	35.2[a]	41.8[b]	32.3[a]	45.7[b]	2.6	0.84	0.02	0.68	0.84	0.11
Saturated/Unsaturated	1.8	1.6	2.9	1.9	0.3	0.03	0.11	0.32	0.03	0.46
n-3	3.2[a]	4.6[b]	2.8[a]	5.3[b]	0.3	0.70	<0.01	0.13	0.93	0.04
n-6	19.1[y]	22.0[x,y]	19.0[y]	25.4[x]	1.7	0.31	0.10	0.54	0.71	0.19
n-6/n-3	5.5	4.9	6.0	5.5	0.7	0.42	0.71	0.16	0.53	0.89
trans	44.2	52.6	45.7	52.1	9.0	0.95	0.74	0.51	0.07	0.08
14:1/14:0	0.05	0.06	0.07	0.06	0.01	0.20	0.45	0.26	0.07	0.98
16:1/16:0	0.05[x]	0.06[x]	0.05[x]	0.03[y]	0.01	0.20	0.07	0.13	0.34	0.24
18:1/18:0	1.70	2.88	1.93	2.10	0.44	0.49	0.21	0.35	0.36	0.58

[+]P, parity; BCS, body condition score at −30 days, WOL, week of lactation.
[a,b,c]BCS at −30 days × parity means within a row with different superscripts differ (p < 0.05).
[x,y]BCS at −30 days × parity means within a row with different superscripts differ (0.05 < p ≤ 0.10).
*Data represent least squares means ± SEM for the BCS at −30 days within parity effect.

0.02) in high-BCS cows (Table 4), but there was a trend (p = 0.06) of an interaction between BCS at −30 days within parity and WOL as CLA concentration decreased from WOL 2 to 8 only in milk fat from PL cows. Thus, the proportion of CLA in milk tended to be greater in PH than in PL cows at WOL 8 (Fig. 4b1). In addition, milk fat from ML cows had a reduced concentration of CLA in relation to MH cows at WOL 2 (Fig. 4b2).

There were no consistent patterns in product-to-substrate ratios and the apparent Δ9-desaturase activity (Table 4). The milk 14:1/14:0 fatty acid ratio tended (p = 0.07) to increase from WOL 2 to 8 only in primiparous cows (0.05 vs. 0.07 ± 0.01 mg/g, Table 4), and the 16:1/16 ratio tended (p = 0.07) to be less in MH than in ML, PH and PL cows (Table 4).

Discussion

This study demonstrated that nutritionally regulated BCS at 30 days pre-partum had impacted on milk casein and fatty acid fractions. Milk κ-casein differed between BCS groups, and the magnitude of this effect was greater in primiparous than in multiparous cows. In addition, increased individual FA concentration of C18:3 and CLA contributed to increased amounts of

PUFA, n-3 and CLA in milk fat when cows had higher BCS at 30 days pre-partum. Moreover, at 2 WOL, multiparous cows with higher BCS had increased amount of PUFA and CLA. These results suggest that different energy intake or differential partitioning of nutrients among tissues during the early post-partum period could depend – at least partially – on animal development (primiparous vs multiparous cows), which in turn interacts with the nutritional management during the pre-partum period.

Milk casein content was greater at WOL 2 in our study, in agreement with a previous report (Ng-Kwai-Hang et al., 1982) that found greater milk casein content during the first 10 days in milk. In contrast, Ostersen et al. (1997) found that casein reached its maximum at mid-lactation. While we did not detect changes in casein fractions between WOL 2 and 8, Ostersen et al. (1997) and Kroeker et al. (1985) reported decreased/increased proportions of αs-casein/β-casein, respectively, along lactation. Contradictory studies were found on κ-casein: decreased proportions (Ostersen et al., 1997) or no changes (Kroeker et al., 1985) were reported. Differences in milk casein concentrations during lactation could be attributed to changes in the number of secretory cells, which peaks in early lactation (14 days) (Capuco et al., 2001), activity of mammary cells (Na-dependent transport),
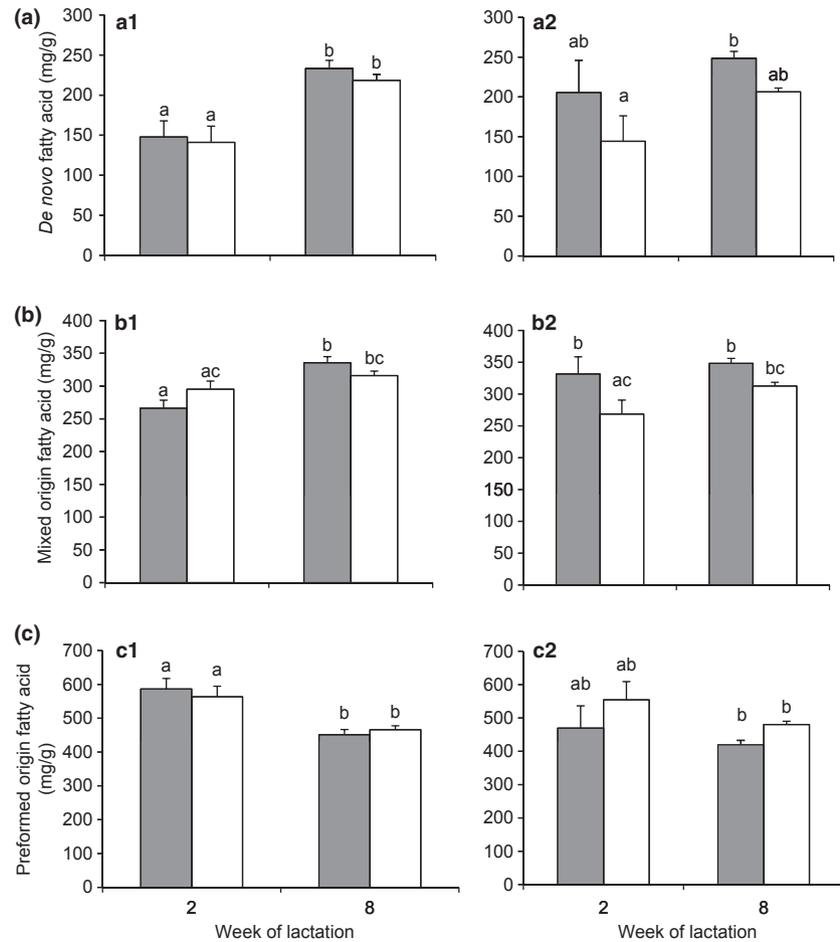
Fig. 2 Milk fatty acid origin at weeks of lactation 2 and 8 in primiparous (a1, b1 and c1) and multiparous (a2, b2 and c2) cows with low (3.0, grey) or high (3.5, white) body condition score at −30 days of lactation. Origins were calculated as (a) *de novo* (4:0–15:1), (b) mixed-origin (16:0 + 16:1), and (c) preformed (≥17:0) fatty acids. Data are least square means ± pooled standard error. Letters above bars indicate Tukey's test differences for the interaction between body condition score at −30 days within parity and week of lactation; a, b, c: $p < 0.05$.

extra-/intracellular AA concentration and casein gene expression that is regulated by hormonal pathways (Maas et al., 1997).

Milk casein concentration (in total milk protein) was greater in multiparous than in primiparous cows; this could be due to greater cell content and cellular differentiation in the mammary gland of multiparous cows (Miller et al., 2006). In addition, multiparous cows generally have a greater DMI than primiparous cows (Ingvartsen, 1994; Maekawa et al., 2002), which could result in greater circulating peptides and AA, sources for casein synthesis in early-lactation cows (Bequette et al., 1998; Chibisa et al., 2008). Moreover, the reduced plasma insulin concentrations found in multiparous cows (Adrien et al., 2012) could have promoted an enhanced proteolysis in this category (Bell et al., 2000). In agreement with our results, several researchers (Kroeker et al., 1985; Ng-Kwai-Hang et al., 1987; Jõudu et al., 2008) have reported that milk from multiparous cows contained less β-casein and more κ-casein than

milk from primiparous cows. Crudden et al. (2005) reported that β-casein is the most susceptible casein isoform to degradation by plasmin activity and that plasmin activity increases with increased SCC. Indeed, multiparous cows presented greater SCC, as was reported by others (Laevens et al., 1997; Sedere-vicius et al., 2006). No other casein fractions differed between parities in our study, which disagrees with reports that milk αs-casein content increased in multiparous cows (Kroeker et al., 1985; Ng-Kwai-Hang et al., 1987). Contradictory results regarding the effect of physiological factors (WOL and parity) on casein fractions may be due to feeding practices (TMR vs. grazing) and/or determination analyses (electrophoresis vs. HPLC).

Expression of casein (αs1-, αs2-, κ- and β-caseins) genes is affected by a complex hormonal regulation of both transcriptional and post-transcriptional events (Martin and Groslaude, 1993). Insulin stimulates casein gene expression (Menzies et al., 2009), and the impact on individual caseins can vary (Bobe
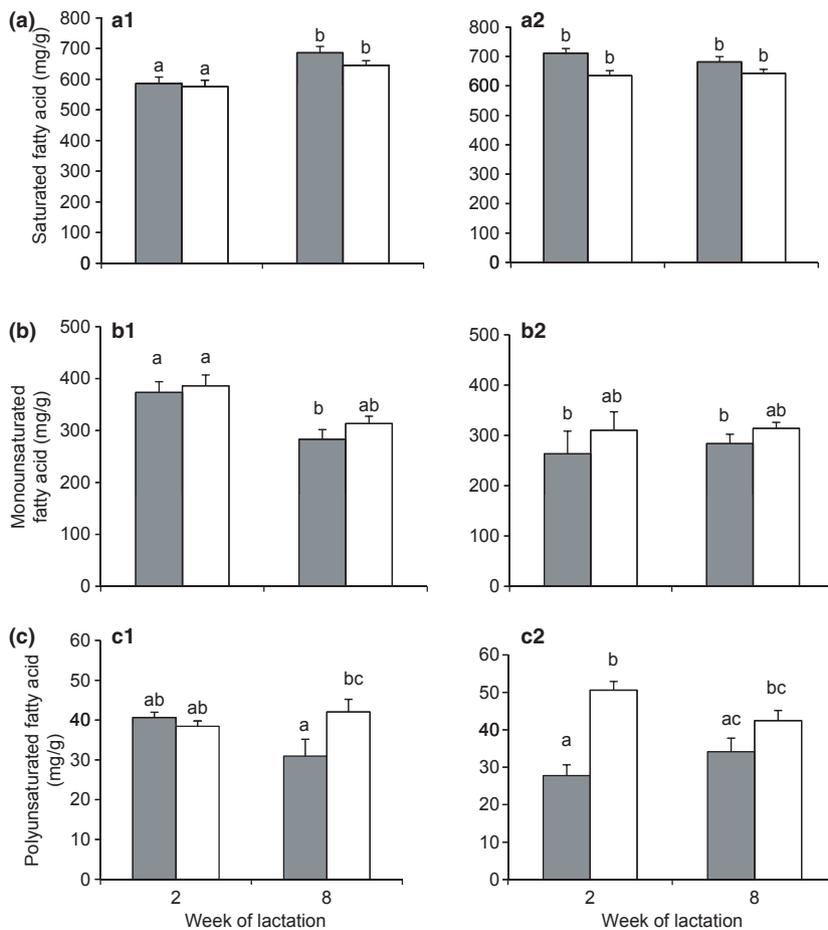
Fig. 3 Saturated (a), monounsaturated (b) and polyunsaturated (c) milk fatty acids at weeks of lactation 2 and 8 in primiparous (a1, b1 and c1) and multiparous (a2, b2 and c2) with low (3.0, grey) or high (3.5, white) body condition score at −30 days of lactation. Data are least square means ± pooled standard error. Letters above bars indicate Tukey's test differences for the interaction between body condition score at −30 days within parity and week of lactation; a, b, c: $p < 0.05$.

et al., 2007). Insulin concentrations were greater at the end of the nutritional treatment in PL than in PH cows (Adrien et al., 2012), and this may have contributed to the greater κ-casein content in milk from these cows.

Fatty acid origin (*de novo*, mixed origin, preformed) was clearly affected by WOL because greater concentrations of preformed fatty acids were found at WOL 2, which is consistent with previous reports (Stanton et al., 1997; Kelsey et al., 2003; Kay et al., 2005). When long-chain fatty acids are available either from the diet or from body fat mobilization, there is a decrease in the percentage of *de novo* and mixed-origin fatty acids in milk fat (Chilliard et al., 2000). Long-chain fatty acids (with C16 or more carbon atoms) are potent inhibitors of mammary fatty acid synthesis and act through a direct inhibition of acetyl CoA activity (Barber et al., 1997). Indeed, the greater concentrations of preformed fatty acids occurred when NEFA concentrations were greatest at WOL 2 in these cows (Adrien et al., 2012).

Parity did not affect fatty acid origin (*de novo*, mixed origin, preformed), but SAT content tended to be greater in milk from multiparous cows and the ratio of SAT/UNSAT was greater in milk from multiparous than in milk from primiparous cows. Greater concentrations of SAT were found in multiparous cows at WOL 2, which could be explained by a greater proportion of *de novo* SAT (data not shown). Indeed, Miller et al. (2006) reported greater mammary DNA concentration and abundance of fatty acid synthase in multiparous than in primiparous cows during early lactation, but not at mid- or late lactation. Greater concentrations of MUFA in primiparous cows were mainly due to the greater content of oleic acid (C18:1) in these cows, which could be associated with a greater body fat mobilization (Adrien et al., 2012) as is a predominant preformed fatty acid in adipocytes and is the primary fatty acid released from adipocytes during lipolysis (Rukkwamsuk et al., 2000). Except for Kelsey et al.'s (2003) study, which reported milk fatty acids in Hol-
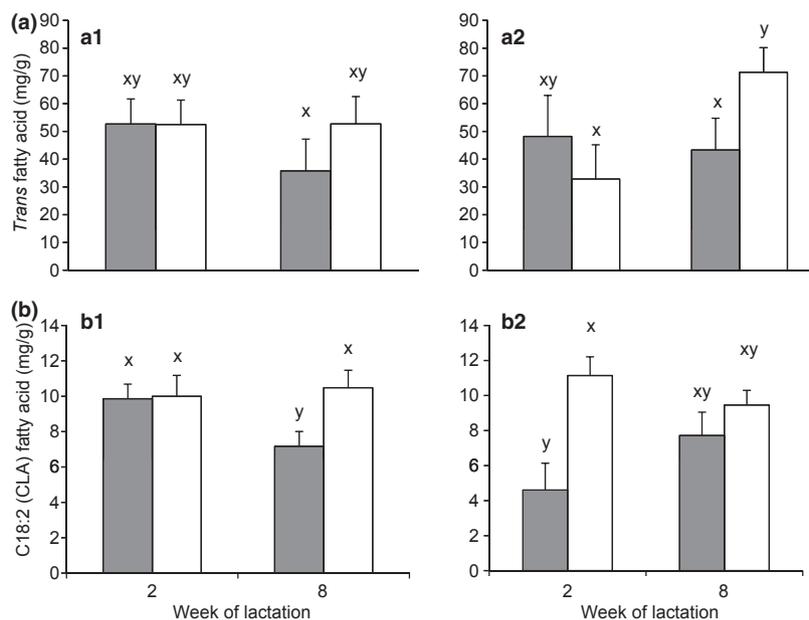
Fig. 4 Milk *trans* (a) fatty acids and C18:2 conjugated linoleic acid (CLA; b) at weeks of lactation 2 and 8 in primiparous (a1, b1 and c1) and multiparous (a2, b2 and c2) with low (3.0, grey) or high (3.5, white) body condition score at −30 days of lactation. Data are least square means ± pooled standard error. Letters above bars indicate Tukey's test differences for the interaction body condition score at −30 days within parity and week of lactation; *x*, *y*: 0.5 < p ≤ 0.10.

stein and Brown Swiss cows fed TMR, we have not found any other study that had systematically examined the effect of parity on milk fatty acid composition. Except for oleic acid, in contrast to Kelsey et al. (2003), we did not find differences in milk individual fatty acids according to parity in Holstein cows under grazing conditions.

No effect of BCS at 30 days pre-partum on proportions of *de novo* and preformed fatty acids was detected. However, ML cows tended to present greater mixed-origin fatty acids (on average at 2 and 8 WOL) than MH cows, mostly due to a greater proportion of palmitoleic acid (C16:1). Although other studies reported less SAT and greater UNSAT in milk fat of cows with high BCS at calving (Pedron et al., 1993) or 1 month before calving (Stockdale et al., 2005), we did not detect an effect of BCS at 30 days pre-partum in SAT or MUFA in milk fat. However, concentrations of PUFA in milk fat were greater in high-BCS cows. According to Chilliard et al. (2000), PUFA are not synthesized by ruminant tissue; therefore, their concentration in milk depends firstly on the PUFA content of the diet and secondly on the amount of PUFA that escape ruminal biohydrogenation. Grazing diets contain a high proportion of PUFA (50–75%) of total fatty acids as α-linolenic acid (Dewhurst et al., 2006). The presence of forage in the diet of ruminants has been associated with an increased rumen PUFA outflow, which is likely due to a faster rate of passage that limits forage PUFA hydrogenation (Dewhurst et al., 2006). We suggest

that increased DMI, reflected in changes in BW and metabolic profiles reported by Adrien et al. (2012), could explain in part the increased PUFA in milk fat from our high-BCS cows.

The milk CLA concentration was greater in high-BCS cows. Vaccenic acid (trans-11 C18:1) is the predominant source of endogenously synthesized *cis*-9, *trans*-11 CLA in the mammary gland through the action of mammary Δ9-desaturase. A small amount of the milk CLA originates from biohydrogenation/ metabolism of unsaturated fatty acids by rumen bacteria. The increased linolenic acid (C18:3) content in milk from high-BCS cows in our study could probably resulted from increased DMI of high-quality pastures (40–80 g/100 g fatty acids; Dewhurst et al., 2001) that increased ruminal production of the *trans*-11 C18:1 (vaccenic acid). Large amounts of *trans*-11 C18:1 absorbed post-ruminally in grazing cows and its subsequent desaturation to CLA by the enzyme Δ9-desaturase in mammary gland could explain the greater CLA content in milk reported in cows with greater BCS (Griinari and Bauman, 1999). Vaccenic acid (88% of *trans*-fatty acids) tended to increase from WOL 2 to 8 only in MH cows. The MH cows presented better energy status at WOL 8 (Adrien et al., 2012) probably due to a faster recovery of DMI in early lactation. Besides, n-6 and n-3 were greater in MH cows, which could be due to a greater intake of their precursors (C18:2 and C18:3) in fresh grass (Moghadasian, 2008). Our results are in agreement with Lock and Bauman's

(2004) study, which reported that fatty acids EPA and DHA are absent or at a minimal level on grazing dairy cow diets and, consequently, they are typically present in very low amounts in ruminant products (<0.1% of total fatty acids).

This study showed that casein and fatty acid fractions in milk were affected by cow's development and may be modified by a differential nutritional management during the pre-calving period (BCS at −30 days) in cows under grazing conditions. Altered casein and fatty acid profiles could have practical benefit to manufactures and consumers. Higher β-casein content, which is the most abundant milk protein and is involved in binding calcium phosphate, would increase milk calcium levels, while greater κ-casein content, which is linked to smaller micelles, would determine better heat stability and improved cheese-making properties (Jimenez Flores and Richardson, 1988; Dalgleish et al., 1989). In addition, increased PUFA, particularly CLA and n-3 that have bioactive properties (e.g. antiobesity, anticarcinogenic and promotion of the immune system; Bauman et al., 2006), may increase milk quality; moreover, these fatty acids had been related to rheological (melting) properties, which influence numerous aspects of character and quality of manufactured dairy products (Palmquist et al., 1993). Nutritional regulation of BCS at the beginning of the transition period could be a useful strategy for milk manipulation at early–mid-lactation in multiparous and primiparous cows in grazing conditions.

Acknowledgements

References

Adrien, M. L.; Mattiauda, D. A.; Artegoitia, V.; Carriquiry, M.; Bentancur, O.; Meikle, A., 2012: Nutritional regulation of body condition score at the initiation of the transition period in dairy cows under grazing conditions: milk production, resumption of postpartum ovarian cyclicity and metabolic parameters. *Animal* **6**, 292–299.

Agenas, S.; Burstedt, E.; Holtenius, K., 2003: Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. *Journal of Dairy Science* **86**, 870–882.

AOAC., 1980: *Official Methods of Analysis*, 13th edn. Association of Official Analytical Chemists, Washington, DC.

Barber, M. C.; Clegg, R. A.; Travers, M. T.; Vernon, R. G., 1997: Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta* **1347**, 101–126.

Bauman, D. E.; Mather, I. H.; Wall, R. J.; Lock, A. L., 2006: Major advances associated with the biosynthesis of milk. *Journal of Dairy Science* **89**, 1235–1243.

Bell, A. W.; Burhans, W. S.; Overton, T. R., 2000: Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. *Proceedings of the Nutrition Society* **59**, 119–126.

Bequette, B. J.; Backwell, F. R. C.; Crompton, L. A., 1998: Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. *Journal of Dairy Science* **81**, 2540–2559.

Black, A. L.; Anand, R. S.; Bruss, M. L.; Brown, C. A.; Nakagari, J. A., 1990: Partitioning of amino acids in lactating cows: oxidation to carbon dioxide. *Journal of Nutrition* **120**, 700–709.

Bobe, G.; Lindberg, G. L.; Freeman, A. E.; Beitz, D. C., 2007: Short Communication: composition of milk protein and milk fatty acids is stable for cows differing in genetic merit for milk production. *Journal of Dairy Science* **90**, 3955–3960.

Capuco, A. V.; Wood, D. L.; Baldwin, R.; Mcleod, K.; Paape, M. J., 2001: Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. *Journal of Dairy Science* **84**, 2177–2187.

Cavestany, D.; Blanc, J. E.; Kulcsar, M.; Uriarte, G.; Chilibroste, P.; Meikle, A.; Febel, H.; Ferraris, A.; Krall, E., 2005: Studies of the transition cow under a pasture-based milk production system: metabolic profiles. *Journal of Veterinary Medicine Series A. A Physiology, Pathology, Clinical Medicine* **52**, 1–7.

Chibisa, G. E.; Gozho, G. N.; Van Kessel, A. G.; Olkowski, A. A.; Mutsvangwa, T., 2008: Effects of peripartum propylene glycol supplementation on nitrogen metabolism, body composition, and gene expression for the major protein degradation pathways in skeletal muscle in dairy cows. *Journal of Dairy Science* **91**, 3512–3527.

Chilliard, Y.; Ferlay, A.; Mansbridge, R. M.; Doreau, M., 2000: Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Annales de Zootechnie* **49**, 181–205.

Crudden, A.; Fox, P. F.; Kelly, A. L., 2005: Factors affecting the hydrolytic action of plasmin in milk. *International Dairy Journal* **15**, 305–313.

Dalgleish, D. G.; Horne, D. S.; Law, A. J. R., 1989: Size-related differences in bovine casein micelles. *Biochimica et Biophysica Acta* **991**, 383–387.

Damián, J. P.; Sacchi, I.; Reginensi, S.; De Lima, D.; Bermúdez, J., 2008: Cheese yield, casein fractions and major components of milk of Saanen and Anglo-Nubian dairy goats. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **60**, 1564–1569.

Dewhurst, R. J.; Scollan, N. D.; Youell, S. J.; Tweed, J. K. S.; Humphreys, M., 2001: Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. *Grass and Forage Science* **56**, 68–74.

Dewhurst, R. J.; Shingfield, K. J.; Lee, M. R. F.; Scollan, N. D., 2006: Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* **131**, 168–206.

Edmonson, A. J.; Lean, L. J.; Weaver, L. D.; Farver, T.; Webster, G., 1989: A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* **72**, 68–78.

Farrell, H. M. Jr; Jimenez-Flores, R.; Bleck, G. T.; Brown, E. M.; Butler, J. E.; Creamer, L. K., 2004: Nomenclature of the proteins of cows' milk—sixth revision. *Journal of Dairy Science* **87**, 1641–1674.

Folch, J.; Lees, M.; Sloane, G.; Stanley, H., 1957: A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Griinari, J. M.; Bauman, D. E., 1999: Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: M. Yurawecz, M. Mossoba, J. Kramer, M. Pariza, G. Nelson (eds), *Advances in Conjugated Linoleic Acid Research*, Vol. 1. AOCS Press, Champaign, IL, pp. 180.

Ingvartsen, K. L., 1994: Models of voluntary food intake in cattle. *Livestock Production Science* **39**, 19–38.

Jimenez Flores, R.; Richardson, T., 1988: Genetic engineering of the caseins to modify the behavior of milk during processing: a review. *Journal of Dairy Science* **71**, 2640–2654.

Jõudu, I.; Henno, M.; Kaart, T.; Püssa, T.; Kärt, O., 2008: The effect of milk proteins contents on the rennet coagulation properties of milk from individual dairy cows. *International of Dairy Journal* **18**, 967–970.

Kay, J. K.; Weber, W. J.; Moore, C. E.; Bauman, D. E.; Hansen, L. B.; Chester-Jones, H.; Crooker, B. A.; Baumgard, L. H., 2005: Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. *Journal of Dairy Science* **88**, 3886–3893.

Kelsey, J. A.; Corl, B. A.; Collier, R. J.; Bauman, D. E., 2003: The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. *Journal of Dairy Science* **86**, 2588–2597.

Kroeker, E. M.; Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E., 1985: Effects of environmental factors and milk protein polymorphism on composition of casein fraction in bovine milk. *Journal of Dairy Science* **68**, 1752–1757.

Laevens, H.; Deluyker, H.; H.Schukken, Y.; Meulemeester de, L.; Vandermeersch, R.; Muelenaere de, E.; Kruif de, A., 1997: Influence of parity and stage of lactation on somatic cell count in bacteriologically negative dairy cows. *Journal of Dairy Science* **80**, 3219–3226.

Lock, A. L.; Bauman, D. E., 2004: Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* **39**, 1197–1206.

Lock, A. L.; Shingfield, K. J., 2004: Optimising milk composition. In: E. Kebreab, J. Mills, D. E. Beever (eds), *Dairying—Using Science to Meet Consumers' Needs,* Vol 29. Br. Society of Animal Science, Nottingham University Press, Loughborough, UK, pp. 107–188.

Maas, J. A.; France, J.; McBride, B. W., 1997: Model of milk protein synthesis. A mechanistic model of milk protein synthesis in the lactating bovine mammary gland. *Journal of Theoretical Biology* **187**, 363–378.

Maekawa, M.; Beauchemin, K. A.; Christensen, D. A., 2002: Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *Journal of Dairy Science* **85**, 1165–1175.

Martin, P.; Groslaude, F., 1993: Improvement of milk protein quality by gene technology. *Livestock Production Science* **35**, 95.

Meikle, A.; Kulcsar, M.; Chilliard, Y.; Febel, H.; Delavaud, C.; Cavestany, D., 2004: Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. *Reproduction* **127**, 727–737.

Menzies, K. K.; Lefevre, C.; Macmillan, K. L.; Nicholas, K. R., 2009: Insulin regulates milk protein synthesis at multiple levels in the bovine mammary gland. *Functional & Integrative Genomics* **9**, 197–217.

Miller, N.; Delbecchi, L.; Petitclerc, D.; Wagner, G. F.; Talbot, B. G.; Lacasse, P., 2006: Effect of stage of lactation and parity on mammary gland cell renewal. *Journal of Dairy Science* **89**, 4669–4677.

Moghadasian, M. H., 2008: Advances in dietary enrichment with n-3 fatty acids. *Critical Reviews in Food Science and Nutrition* **48**, 402–410.

Moore, C. E.; Hafliger, H. C.; Mendivil, O. B.; Sanders, S. R.; Bauman, D. E.; Baumgard, L. H., 2004: Increasing amounts of conjugated linoleic acid (CLA) progressively reduce milk fat synthesis immediately postpartum. *Journal of Dairy Science* **87**, 1886–1895.

Moore, C. E.; Kay, J. K.; VanBaale, M. J.; Collier, R. J.; Baumgard, L. H., 2005: Effect of conjugated linoleic

acid on heat stressed Brown Swiss and Holstein cattle. *Journal of Dairy Science* **88**, 1732–1740.

Mossoba, M. M.; Yurawecz, M. P.; Roach, J. A. G.; McDonald, R. E.; Flickinger, B. D.; Perkins, E. G., 1996: Analysis of cyclic fatty acid monomer 2-alkenyl-4,4-dimethyloxazoline derivatives by gas chromatography–matrix isolation–flourier transform infrared spectroscopy. *Journal of Agriculture and Food Chemistry* **44**, 3193–3196.

National Research Council, 2001: *Nutrient Requirements of Dairy Cattle, 7th rev. ed.* Natl Acad Sci., Washington, DC.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G., 1982: Environmental influences on protein content and composition of bovine milk. *Journal of Dairy Science* **65**, 1993–1998.

Ng-Kwai-Hang, K. F.; Hayes, J. F.; Moxley, J. E.; Monardes, H. G., 1987: Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. *Journal of Dairy Science* **70**, 563–570.

Ostersen, S.; Foldager, J.; Hermansen, J. E., 1997: Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *Journal of Dairy Research* **64**, 207–219.

Palmquist, D. L.; Beaulieu, A. D.; Barbano, D. M., 1993: ADSA foundation symposium: milk fat synthesis and modification. Feed and animal factors influencing milk fat composition. *Journal of Dairy Science* **76**, 1753–1771.

Pedron, O.; Cheli, F.; Senatore, E.; Baroli, D.; Rizzi, R., 1993: Effect of body condition score at calving on performance, some blood parameters, and milk fatty acid composition in dairy cows. *Journal of Dairy Science* **76**, 2528–2535.

Rémond, B.; Cisse, M.; Ollier, A.; Chilliard, Y., 1991: Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. *Journal of Dairy Science* **74**, 1370–1381.

Rukkwamsuk, T.; Geelen, M. J. H.; Druip, T. A. M.; Wensing, T., 2000: Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. *Journal of Dairy Science* **83**, 52–59.

Sederevicius, A.; Balsyte, J.; Lukauskas, K.; Kazlauskaite, J.; Biziulevicius, G. A., 2006: An enzymatic cow immunity-targeted approach to reducing milk somatic cell count: 3. A comparative field trial. *Food and Agricultural Immunology* **17**, 1–7.

Stanton, C.; Lawless, F.; Kjellmer, G.; Harrington, D.; Devery, R.; Connolly, J. F.; Murphy, J., 1997: Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content. *Journal of Food Science* **62**, 1083–1086.

Stockdale, C. R.; Doyle, P. T.; Wijesundera, C.; Williams, R. P. W., 2005: Effects of body condition at calving and peri-parturient nutrition on the composition of milk fat produced by grazing dairy cows. *Australian Journal of Dairy Technology* **60**, 244–251.

Stoop, W. M.; Bovenhuis, H.; Heck, J. M. L.; van Arendonk, J. A. M., 2009: Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *Journal of Dairy Science* **92**, 1469–1478.

Trujillo, A. J.; Casals, I.; Guamis, B., 2000: Analysis of major caprine milk proteins by reverse- phase high-performance liquid chromatography and electrospray ionization-mass spectrometry. *Journal of Dairy Science* **83**, 11–19.

Van Knegsel, A. T. M.; van den Brand, H.; Dijkstra, J.; Tamminga, S.; Kemp, B., 2005: Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle—Review. *Reproduction Nutrition Development* **45**, 665–688.

Visser, S.; ChSlangen, J.; Rollema, H. S., 1991: Phenotyping of bovine milk proteins by reversed-phase high-performance liquid chromatography. *Journal of Chromatography* **548**, 361–370.