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Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation

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Summary

Milk fatty acid (FA) profiles were determined in Holstein cows (n = 27) fed total mixed rations (TMR) ad libitum (G0) or diet composed by TMR (50% dry matter [DM] offered) plus grazing of pasture with 6 hr of access time to paddock in one session (G1) or 9 hr in two sessions (G2) at 45 days in milk (DIM). Moreover, milk FA was determined at 65 DIM when G0 cows turned out to G1 diet without adaptation period (Post-G0), G1 remained as controls. Milk FA was quantified using gas chromatography and mass spectrometry. Preformed FA at 45 DIM was greater (+27%) for G2 than G0 cows (p < .05). Stearic acid (C18:0) was 30% greater for G2 cows (p < .05). De novo FA was lowest for G2 cows (p < .05). Conjugated linoleic acid (CLA) did not differ (p < .12), while vaccenic acid (C18:1*trans*) was twofold greater for grazing treatments (p < .01). Linolenic acid [C18:3(n-3)] was greatest for G2 and lowest for G0 cows (p < .01). Omega 6 FA was greater for G0 than grazing cows, mainly due to linoleic acid [18:2*cis*(n-6); p < .05]. These results determined that n-6/n-3 ratio was almost three-fold greater for G0 than grazing cows (p < .001). When diet of G0 cows changed to include pasture (Post-G0), preformed FA increased (p < .05), explained mainly by the increase (p < .05) of stearic (C18:0) and C18:1*trans*, while de novo FA tended to decrease (p < .1). Moreover, the amount of CLA and C18:3(n-3) tended to increase (p < .1) in Post-G0 cows. Offering 50% of dietary DM from pasture modified milk FA profile in early lactation potentially beneficial for human health. When TMR-fed cows were turned out to 50% pasture, milk FA profile reflected dietary change without need of an adaptation period.

KEYWORDS
dairy cattle, fat composition, grazing pasture

1 | INTRODUCTION

Milk fat is one of the main sources of essential fatty acids (FA) for human consumption. There is an increasing consumers trend to select foods with elevated content of polyunsaturated fatty acids (PUFA), particularly omega-3 (n-3) and conjugated linoleic acid (CLA), due to their postulated beneficial properties for health (i.e., antioxidants, carcinogenesis and atherogenesis inhibition, and antidiabetic effects; Pariza, Park, & Cook, 2001; Bauman, Mather, Wall, & Lock, 2006). In addition, FA profile has an important impact on the technological aptitude and sensory characteristics of milk and its derivatives (Croissant, Washburn, Dean, & Drake, 2007; Palmquist, Beaulieu, & Barbano, 1993).

Animal nutrition has been reported as the most influential factor capable of modifying milk fat composition (Artegoitía et al., 2013; Dewhurst, Shingfield, Lee, & Scollan, 2006). In confined systems where nutrition is based on the use of total mixed ration (TMR), numerous studies have evaluated the effect of different supplements such as

marine or vegetable oils, oilseeds or inert lipids protected from ruminal degradation on milk FA profiles (AbuGhazaleh & Holmes, 2007; AbuGhazaleh, Schingoethe, Hippen, & Kalscheur, 2003; Flowers, Ibrahim, & AbuGhazaleh, 2008; Gao, Sun, & Li, 2009; Loor, Ferlay, Ollier, Doreau, & Chilliard, 2005; Zunong, Tuerhong, Okamoto, Hongo, & Hanada, 2009) on milk production and milk FA profile. These authors reported a decrease in de novo and mixed FA and an increase in preformed FA, CLA and n-3 FA. In that sense, the research was focused on the effects of several supplementation strategies with less attention placed on changes in milk FA profile due to variations in pasture intake (Dewhurst et al., 2006). It has been reported that the inclusion of fresh pastures in the diet has the potential to increase monounsaturated fatty acid (MUFA) and PUFA content in milk fat (Dewhurst et al., 2006). Indeed, it has been shown that grazing cows had increased amounts of n-3 and CLA compared with TMR-fed cows (Schroeder et al., 2003).

Grazing systems have an advantageous position in the global milk production systems as the inclusion of pasture in the diet reduces the cost of milk production (Chilibroste, 2011) and enhance the image of milk products from a consumer point of view (Croissant et al., 2007). However, dry matter intake (DMI) in grazing systems is usually limited in comparison with confined TMR systems, being insufficient to maintain the high milk production that could be achieved with the genetic potential (Chilibroste, Mattiauda, Bentancur, Soca, & Meikle, 2012; Kolver & Muller, 1998). Thus, supplementation with concentrates or TMR has been included in grazing production systems (Wales et al., 2013). Imbalances between herbage production and herd requirements occur during the year (Chilibroste, Soca, Mattiauda, Bentancur, & Robinson, 2007; Wales et al., 2013), generating alternate periods when cows are kept in confinement and fed only TMR with periods were grazing is combined with supplementation. The supplementation of grazing cows with TMR increases milk yield, fat and protein content even compared with concentrate supplementation (Bargo, Muller, Delahoy, & Cassidy, 2002; Wales et al., 2013).

Research on milk FA profile in mixed dairy production systems (TMR plus pasture) is limited and even less where different grazing strategies are considered. Loor, Soriano, Lin, Herbein, and Polan (2003) evaluated the use of TMR combined with morning or afternoon grazing and found that pasture inclusion, particularly during daylight, enhances linolenic acid n-3 [C18:3(n-3)] content. Morales-Almaraz et al. (2010) reported increases in linolenic acid (C18:3) and CLA with 12 hr vs. 6 hr of grazing, but a loss in fat yield. Moreover, there is scarce information about the adaptation of TMR cows switched to a mixed system (grazing plus TMR). Khanal, Dhiman, and Boman (2008) reported a decrease in short and medium chain FA with an increase in CLA and C18:3 when cows were switched from TMR to pasture, although CLA increase took 23 days to reach the maximum after turning cows out to pasture.

The hypothesis of this work was that milk FA fractions (mainly C18:3 and CLA) would be modified by early lactation feeding strategies. At the same time, turning out to a mixed system (TMR + grazing) after TMR feeding in early lactation would provide pasture FA that would be reflected on milk FA profile. Thus, the first objective of this work was to compare milk FA profile of early lactation multiparous cows fed either TMR ad libitum or a diet composed by TMR (50% dry matter [DM] offered) and pasture grazing with either 6 or 9 hr of access time to paddock in one or two grazing sessions respectively. In addition, a second objective was to evaluate if milk FA profile is modified when cows fed TMR in early lactation are changed to a mixed diet composed by TMR and grazing.

2 | MATERIALS AND METHODS

2.1 | Animals and pre-calving management

The experimental protocol was evaluated and approved by the Honorary Committee for Animal Experimentation in Uruguay (CHEA—UdelaR). Twenty-seven multiparous Holstein cows (4.5 ± 1.81 lactations) from the dairy herd of the Experimental Station Dr. M. A. Cassinoni (EEMAC) of the School of Agronomy were used. Cows were blocked by lactation number, expected calving date, body condition score (BCS) and body weight (BW) 30 days pre-calving. From −8 to −4 weeks relative to calving, the herd was managed to achieve a BCS at calving between 3 and 3.5 points (1–5 scale, Edmonson, Lean, Weaver, Farver, & Webster, 1989). From week −4 to calving, a ration with corn silage and concentrates was offered to prevent BCS losses and calving illness. During this period, BCS and BW were determined weekly. Cow BCS before calving (−4 ± 3 day of calving) was 3.2 ± 0.2 and BW was 753.0 ± 51.0 kg.

2.2 | Treatments and experimental design

The experimental design was a randomised block design. Cows were randomly assigned during the first 60 days in milk (DIM) to one of the following three treatments as described by Fajardo et al. (2015): TMR ad libitum (G0) or a diet composed by TMR (50% DM of ad libitum intake) and grazing of pasture with either 6 hr of access time to paddock in one grazing session (08:00–14:00; G1) or 9 hr of access time to paddock in two sessions (08:00–14:00 and 17:00–20:00; G2). Treatments G0 (n = 9), G1 (n = 10) and G2 (n = 8) accessed daily to the same offer of total DM differing on the dietary source (TMR vs. TMR + pasture). From 61 to 90 DIM, a second period was established in which G0 cows were turned out to G1 treatment routine with no adaptation period (Post-G0; n = 8) and G1 cows (n = 8) remained as a control group. Both groups, Post-G0 and G1 had 6 hr of access to paddocks in one grazing session (08:00–14:00) and were supplemented with 50% TMR. Different treatments for cows were managed independently.

2.3 | Pasture

After calving, cows grazed a second-year perennial pasture of *Festuca arundinacea*, *Trifolium repens* and *Lotus corniculatus* in a paddock 1.7 km away from the milking parlour. A new grazing area was assigned weekly with a mean herbage allowance of 15 kg DM/day/cow over 4 cm above ground level. Herbage mass (kg DM/ha) was

estimated using the comparative yield method adapted from Haydock and Shaw (1975) with a five-point scale and three replicates in representative areas of the pasture. All measurements were according to Fajardo et al. (2015).

Cows of G0 were fed with a TMR with a forage/concentrate ratio of 45/55. The diet was formulated according to National Research Council (2001) for a target milk production of 40 kg/day and 30.7 kg DM/day was offered daily, with a refusal goal of 15%. For grazing treatments, the proportion of TMR (the same used in G0) and pasture in the diet (DM basis) were 73% TMR and 27% pasture for G1 cows and 66% TMR and 34% pasture for G2 cows (Fajardo et al., 2015).

The TMR was offered twice a day, 40% in the morning and 60% in the afternoon to G0 treatment. For G1 and G2 treatments, TMR was offered once a day during the afternoon. Cows of G0 treatment were kept always in confinement (open sky stalls with a ground floor and grouped feed bunks) with free access to TMR and water. The animals of G1 treatment had access to the paddock from 08:00 to 14:00 hr and stayed after the afternoon milking in confinement with access to TMR and water until 04:00 hr. The animals of G2 treatment had access to paddock from 08:00 to 14:00 hr and from 17:00 to 20:00 hr, after that in confinement with access to TMR and water until 04:00 hr. Confinement for grazing cows (G1 and G2) was also in open sky stalls with grouped feed bunks. Chemical composition of TMR and pasture is presented in Table 1.

All cows were milked twice a day at 05:00 and 15:00 hr. Milk yield was registered individually at each milking with the use of Waikato® milk meters. Milk fat and protein contents were determined weekly during two consecutive milking by mid-infrared spectrophotometry

TABLE 1 Chemical composition and FA profile of TMR and pasture used in the experiment

Nutrient	TMR	Pasture of 45 DIM	Pasture of 65 DIM
DM, g/kg	492 ± 29.8	371 ± 21	279 ± 14
CP, g/kg DM	149 ± 23.7	146 ± 7.4	184 ± 13.9
NDF, g/kg DM	348 ± 41.2	531 ± 3.3	427 ± 3.0
ADF, g/kg DM	189 ± 31.0	272 ± 6.8	201 ± 2.5
Ash, g/kg DM	72 ± 8.8	10.2 ± 0.7	11.4 ± 0.5
NEL, Mcal/kg	1.63	1.5	1.7
FAs, g/100 g			
C10:0	0.02	0.26	0.46
C14:0	0.11	0.55	0.72
C16:0	19.74	23.15	27.58
C16:1	0.12	0.59	0.62
C18:0	4.63	6.32	6.7
C18:1*cis*	29.72	6.56	6.3
C18:2*cis*(n-6)	36.97	12.19	12.28
C18:2*trans*	0.12	nd	nd
C18:3(n-3)	2.35	20.01	20.61
C20:0	0.98	2.35	2.03
C20:1*cis*	0.45	nd	nd
C20:2*cis*(n-6)	0.49	nd	nd
C20:4(n-6)	0.32	nd	nd
C21:0	0.12	nd	nd
C22:0	1.1	4.96	3.87
C22:3	0.3	nd	nd
C23:0	0.31	0.97	0.8
C24:0	1.16	4.18	3.81
C25:0	0.17	0.98	1
C26:0	0.27	7.18	7.16
C28:0	0.11	4.65	3.79
n-3	2.34	20.92	20.81
n-6	37.84	12.42	12.69
n-6/n-3	16.06	0.60	0.61
Ether extract, g/100 g	7.28	2.05	1.57

nd, not detected; DIM, days in milk; DM, dry matter; FA, fatty acid; TMR, total mixed ration.

(*Near Infrared Reflectance Spectroscopy*-NIRS, Milko-Scan; Foss Electric, HillerØd, Denmark).

2.4 | Determination of fatty acid profile in milk and feed

Fatty acid profile was determined in milk samples (composed and representative of the two daily milking using Waikato® milk meters) and in TMR and pasture samples (Table 1) at 47 ± 12 DIM in G0, G1 and G2 groups and at 63 ± 11 DIM in Post-G0 and G1 groups. Milk fat was extracted according to Folch, Lees, Sloane, and Stanley (1957), and FA methyl esters were prepared by the trans-methylation procedure described by IUPAC 2.301 (Mossoba et al., 1996). Fatty acid methyl esters were quantified using a gas chromatograph (Agilent Technologies 6890, Palo Alto, CA, USA) and a mass spectrometer (Agilent Technologies 5973) in electron ionisation mode at 200°C with an electron current of 70 electron volts, acquire spectra over the mass 35–600 daltons at a rate of 0.7 s/scan with an interscan delay of 0.5 ms. The column chromatography was a SP 2560 (Supelco), highly polar biscyanopropyl capillary column (100 m · 0.25 mm i.d. with 0.2-lm film thickness; Supelco, Bellefonte, PA, USA). Gas chromatograhy, column, oven, gas variables and FA identification were performed as previously described (Moore, Kay, VanBaale, Collier, & Baumgard, 2005; Moore et al., 2004). 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 FA. The intra- and interassay CVs for each analyte measured were in average 3% and 6% respectively. The FA composition in milk fat is expressed as grams of each individual FA per 100 g of total FA. The CLA isomers are reported as the sum of all linoleic acid conjugated isomers *cis* and *trans*.

2.5 | Statistical analyses

Data were analysed using the SAS statistical package (SAS Institute, Cary, NC, USA). Milk production and composition and FA profile were analysed in a randomised block design using repeated measures (when corresponding) with the MIXED procedure. For the analysis of milk FA composition at 45 DIM, the model included the fixed effect of treatment, while for the analysis of Post-G0 vs. G1 cows included treatment, DIM (45 vs. 65) and their interaction as fixed effects. Block was included as a random effect, and the Kenward-Rogers method was used to adjust the degree of freedom of the denominator. The unrestricted (UN) covariance structure was specified for the repeated measure analysis. The Tukey–Kramer tests were conducted for the separation of means. Means are reported as least squares measure with their standard errors and are considered to differ when $p \leq .05$ and trends were identified when $.05 < p \leq .10$.

3 | RESULTS

3.1 | Effect of feeding strategy on fatty acid profile at 45 days in milk

Milk production, fat and lactose showed no differences between treatments, although protein content was greater in G0 than grazing treatments at 45 DIM (Table 2).

De novo FA was greater for G0 and G1 than G2 cows (Table 3). Indeed, capric and myristic acid (C10:0, C14:0 respectively; Table 4), representing approximately 65% of total de novo FA, showed no differences between G0 and G1 cows but were reduced in G2 cows. Lauric acid (C12:0; 14% of total de novo FA) was different among treatments with greatest amounts for G0 and lowest for G2. Pentadecylic acid (C15:0) tended to be greater in G0 than G2 cows, while caproic and caprylic acids (C6:0 and C8:0 respectively; Table 4) tended to be greater in G1 than G2.

The proportion of mixed-origin FA in milk fat did not differ between treatments (Table 3). However, palmitoleic acid (C16:1*cis*) tended ($p = .067$) to be greater in milk of G0 and G1 than G2 cows. Preformed FA in milk fat was greater (+27%) for G2 than G0 cows ($p = .045$), while no differences between these groups and G1 were found. Stearic acid (C18:0, representing 25% of preformed FA in milk) was approximately 30% greater for G2 than both, G0 and G1 cows.

Feeding strategy in early lactation did not affect saturated FA, MUFA and PUFA nor the saturated to unsaturated FA ratio in milk fat (Table 3). Conjugated linoleic acid content in milk (0.75, 0.65 and 0.39 g/100 g; G2, G1 and G0 respectively) did not statistically differ

TABLE 2 Milk production and composition of cows fed with 100% total mixed ration (TMR; G0), 50% TMR plus 6 hr access to pasture in one grazing session (G1/Post-G0), 50% TMR plus 9 hr access to pasture in two grazing sessions (G2) at 45 and 65 days in milk (DIM)

	45 DIM					65 DIM			
Feeding strategy	G0	G1	G2	*SEM*	*p*-Value	Post-G0	G1	*SEM*	*p*-Value
Milk, L/day	38.46	33.60	36.19	1.60	.123	36.72	37.08	1.83	.900
Solids*, g/100 g	12.45	11.79	11.25	0.39	.161	12.81	12.10	0.33	.191
Fat, g/100 g	4.14	3.73	3.40	0.31	.299	4.58	3.80	0.30	.123
Protein, g/100 g	3.37[a]	3.11[b]	2.99[b]	0.07	.011	3.34	3.26	0.07	.424
Lactose, g/100 g	4.93	4.94	4.86	0.08	.751	4.99	5.01	0.08	.850

[a,b]Means within a row with different superscripts differ ($p < .05$).
*Solids = Fat + Protein + Lactose.

TABLE 3 Milk FA components of cows fed with 100% TMR (G0), 50% TMR plus 6 hr access to pasture in one grazing session (G1) and 50% TMR plus 9 hr access to pasture in two grazing sessions (G2) at 45 days in milk (DIM)

	Treatments				
	G0	G1	G2	*SEM*	*p*-Value
FA origin, g/100 g of FA					
De novo (C4:0–C15:1)	23.09[a]	20.07[a]	15.34[b]	1.38	.004
Mixed origin (C16:0 + C16:1)	37.3	34.91	34.66	1.83	.561
Preformed (>C17:0)	39.49[b]	45.10[a,b]	50.09[a]	2.68	.045
FA saturation, g/100 g of FA					
Saturated	68.73	64.67	64.45	2.15	.335
Monounsaturated	27.09	30.91	31.16	1.95	.301
Polyunsaturated	4.12	4.41	4.43	0.23	.588
Saturated/Unsaturated	2.35	1.93	1.84	0.21	.235
n-3	0.41[b]	0.66[a]	0.79[a]	0.06	.005
n-6	2.75[a]	2.05[b]	1.99[b]	0.07	<.001
n-6/n-3	8.31[b]	3.48[a]	2.64[a]	0.67	<.001
Trans	2.79[b]	5.19[a]	5.94[a]	0.42	.001
C14:1/C14:0	0.08[a]	0.08[a]	0.05[b]	0.01	.013
C16:1/C16:0	0.06	0.06	0.04	0.01	.205
C18:1/C18:0	2.60	2.73	2.02	0.27	.162
Δ9I*	0.30	0.31	0.30	0.02	.783
RCLA†	0.23	0.21	0.15	0.03	.200

CLA, conjugated linoleic acid; FA, fatty acid; TMR, total mixed ration
[a,b]Means within a row with different superscripts differ ($p < .05$).
*Δ9I: desaturase activity; (C16:1*cis* + C18:*cis* + C18:2 CLA + C14:1*cis*)/(C14:0 + C16:0 + C 18:0 + C18:1*trans* + C16:1*cis* + C18:1*cis* + C18:2 CLA).
†RCLA: C18:2 CLA/C18:1*trans*.

according to treatments ($p = .12$). *Trans* FA in milk fat was greater ($p = .001$) for grazing treatments (G1 and G2) than G0 cows which was attributable to differences in vaccenic acid (C18:1*trans*; $p < .001$) and linoleic acid (C18:2*trans*). Linolenic acid [C18:3(n-3)] that represented 84% of total n-3 FA in milk fat was greatest ($p < .001$) for G2 cows and lowest for G0 cows (Table 4). Total n-3 FA was greater ($p = .005$) for both, G1 and G2 cows than G0 cows. In contrast, omega 6 (n-6) FA was greater for G0 than grazing (G1 and G2) cows, mainly due to 18:2*cis*(n-6), 89% of n-6 FA fraction in milk. This FA was greater ($p < .001$) in G0 than in G1 and G2 cows (Table 4). These results determined that n-6/n-3 FA ratio in milk was almost three times greater ($p < .001$) for G0 than G1 and G2 cows (Table 3).

The ratio of 14:1/14:0 was less ($p < .013$) for G2 than G0 and G1 cows, while neither 16:1/16:0, 18:1/18:0 nor delta-9-desaturase ratios differed between treatments.

3.2 | Effect of turn out to 6 hr of grazing after TMR feeding on fatty acid profile at 65 days in milk

Milk production, fat, protein and lactose showed no differences between treatments at 65 DIM (Table 2).

De novo FA tended to be affected by DIM ($p < .10$) and by treatment and DIM interaction ($p = .102$). While de novo FA in G1 cows was maintained from 45 to 65 DIM, cows that turn out to pasture after TMR feeding in early lactation (Post-G0) had a reduction in de novo FA in milk fat (Table 5). This was mainly due to a decrease in C12:0 (3.77 vs. 2.63 g/100 g) and C14:0 (11.97 vs. 10.00 g/100 g) from 45 to 65 DIM in these cows and to a lesser extent in C15:0 and myristoleic acid (C14:1*cis*) (2.34 vs. 1.64 and 0.99 vs. 0.73 g/100 g respectively).

Preformed FA was affected by the interaction between treatments and DIM (Table 5); while this fraction increased in Post-G0 cows, no differences were detected in G1 cows (45 vs. 65 DIM). At the same time, C18:0 and C18:1*trans* increased ($p < .05$) with the switch from G0 to Post-G0 (9.5 vs. 11.76 g/100 g and 2.01 vs. 3.56 g/100 g respectively), while content of the latter FA in G1 cows was maintained from 45 to 65 DIM.

Conjugated linoleic acid was affected by treatment ($p = .017$) and tended to be affected by DIM ($p = .063$). The CLA content tended to increase in Post-G0 cows (from 0.35 to 0.77 g/100 g from 45 to 65 DIM), not differing from G1 cows at 65 DIM.

No difference in n-3 fraction was found between G0 and Post-G0 (Table 5). However, C18:3(n-3) tended to increase (from 0.30 to 0.48 g/100 g, G0 and Post-G0; respectively; $p = .09$) determining that no differences were found between treatments in this FA at 65 DIM. The n-6 fraction decreased in Post-G0 cows (Table 5), mainly explained by the decrease of C18:2*cis*(n-6) (2.67 vs. 2.36 g/100 g; G0 and Post-G0; respectively) and arachidonic [C20:4(n-6); 0.16 vs. 0.09 g/100 g; G0 and Post-G0; respectively]. The ratio of n-6/n-3 FA

TABLE 4 Milk FA profile of cows fed with 100% TMR (G0), 50% TMR plus 6 hr access to pasture in one grazing session (G1) and 50% TMR plus 9 hr access to pasture in two grazing sessions (G2) at 45 days in milk (DIM)

	Treatments				
	G0	G1	G2	*SEM*	*p*-Value
FA (g/100 g)					
C6:0	0.44^{x,y}	0.47^{x}	0.30^{y}	0.05	.076
C8:0	0.58^{x,y}	0.53^{x}	0.36^{y}	0.06	.057
C10:0	2.23^{a}	1.82^{a}	1.16^{b}	0.19	.003
C11:0	0.10^{a}	0.06^{a,b}	<0.01^{b}	0.02	.027
C12:0	3.69^{a}	2.74^{b}	1.87^{c}	0.26	.001
C12:1*cis*	0.14^{a}	0.08^{b}	<0.01^{c}	0.01	<.001
C13:0	0.24	0.17	0.16	0.03	.119
C14:0	11.98^{a}	11.09^{a}	9.39^{b}	0.63	.031
C14:1*cis*	0.98^{a}	0.85^{a}	0.49^{b}	0.08	.001
C15:0	2.29^{x}	1.93^{x,y}	1.57^{y}	0.21	.089
C16:0	35.28	32.98	33.22	1.82	.634
C16:1*cis*	1.78^{x}	1.72^{x}	1.26^{y}	0.16	.067
C16:1*trans*	0.25	0.2	0.18	0.08	.812
C17:0	1.66	1.81	1.88	0.16	.659
C18:0	9.60^{b}	10.51^{b}	13.95^{a}	0.92	.009
C18:1*cis*	21.09	23.23	23.65	1.69	.552
C18:1*trans*	2.04^{b}	4.01^{a}	4.71^{a}	0.34	<.001
C18:2*cis*(n-6)	2.49^{a}	1.71^{b}	1.83^{b}	0.1	<.001
C18:2(CLA)	0.39	0.75	0.65	0.12	.118
C18:2*trans*	0.47^{b}	0.86^{a}	1.00^{a}	0.07	<.001
C18:3(n-3)	0.28^{c}	0.54^{b}	0.74^{a}	0.05	<.001
C19:0	0.14^{y}	0.22^{x,y}	0.39^{x}	0.07	.072
C19:1	0.11	0.09	0.05	0.02	.17
C20:0	0.15^{b}	0.14^{b}	0.18^{a}	0.01	.016
C20:4(n-6)	0.15^{a}	0.11^{b}	0.10^{b}	0.01	.002
Others*	1.16	1.34	0.95	0.13	.115

CLA, conjugated linoleic acid; FA, fatty acid; TMR, total mixed ration.
^{a,b,c}Means within a row with different superscripts differ ($p < .05$).
^{x,y}Means within a row with different superscripts differ ($.05 < p \leq .10$).
*Others: C4:0 + C15:1 + C16:2 + C17: 1*cis* + C18:2*cis* + C18:2*trans*(n-6) + C18:3*cis* + C18:3(n-6) + C20:1*cis* + C20:1*trans* + C20:2*cis* (n-3) + C20:2 *cis* (n-6) + C20:3 *cis* (n-3) + C20:3 (n-6) + C20:4 (n-3) + C20:5 (n-3) + C21:0 + C22:0 + C22:1*cis* + C22:3 + C22:4 + C22:5 (n-3) + C22:5 (n-6) + C23:0 + C24:0 + C24:1*cis* + C25:0 + C26:0.

showed a significant interaction between treatment and DIM (Table 5), as it decreased in Post-G0 cows while no difference was observed in G1 cows during the experiment.

Trans FA was affected by the interaction of treatment and DIM ($p = .004$) increasing in Post-G0 cows while no changes were observed in G1 cows during this period (Table 5). This increase in Post-G0 was mainly explained by C18:1*trans* (2.01–3.56 g/100 g; G0 and Post-G0; respectively; $p < .01$).

4 | DISCUSSION

4.1 | Effect of feeding strategy on fatty acid profile at 45 days in milk

Milk production and composition at 45 DIM were in agreement with results reported for the first 60 DIM in the same experiment but using a larger number of cows (Fajardo et al., 2015). As reported before, although G0 cows had greater milk yield (37.2, 33.7 and 33.9 L/day for G0, G1 and G2 cows respectively; Fajardo et al., 2015) and higher DMI than grazing cows (G1 and G2), no differences were found among treatments in fat yield.

Contents of de novo and preformed FA in milk fat at 45 DIM were affected by the feeding strategy. Indeed, greater preformed FA in milk fat of G2 than G0 cows was found, and results are consistent with other reports that compared TMR vs. TMR plus grazing treatments (Bargo, Delahoy, Schroeder, Baumgard, & Muller, 2006; Loor et al., 2003; Morales-Almaraz et al., 2010). The greater contents of preformed FA can be explained by increased utilisation of consumed lipids, increased body fat mobilisation or both (Palmquist et al., 1993). Pasture-based systems are characterised by higher concentrations of unsaturated long-chain FA intake, as in the pasture, C18:3 is the most important in quantity (50%–75%; Dewhurst et al., 2006). Interestingly, in our study, the pasture presented a lower proportion of C18:3 (20%) and a higher proportion of very long-chain saturated lipids (C20–C28) than that reported elsewhere (Dewhurst et al., 2006). The later lipids are considered waxes localised in the cuticle leaf that are increased under climatological drought (Millar, Wrischer, & Kunst, 1998), as it occurred in the present study (Fajardo et al., 2015), to prevent water loss. Besides this particular FA composition, other physicochemical modifications such as an increase in secondary plant metabolites such as phenols occur in response to drought stress and have the ability to decrease lipolysis and biohydrogenation in rumen (Buccioni, Decandia, Minieri, Molle, & Cabiddu, 2012). Thus, it can be postulated that the greater amount of milk preformed PUFA in grazing cows is the result of both, increased PUFA from dietary origin and decreased rumen biohydrogenation.

Lipid mobilisation can also explain the greater amount of preformed FA in milk fat as it occurs mainly during early lactation (Artegoitía et al., 2013). Stearic acid (C18:0) and oleic acid (C18:1*cis*) are predominant in adipocytes and are released during lipolysis (Rukkwamsuk, Geelen, Druip, & Wensing, 2000). Although C18:1*cis* represented almost 23% of milk FA in our study, its content did not differ among treatments, while C18:0 was greater in G2 than G0 cows. Rukkwamsuk et al. (2000) suggested that circulating FA from lipolysis (including C18:1*cis)* is accumulated in the liver, but not C18:0 which is secreted in a large proportion in milk. Thus, the greater content of preformed FA in milk fat (and mainly C18:0) of G2 cows joined with their loss of BCS compared with G0, support a greater negative energy balance in G2 cows (Fajardo et al., 2015). Moreover, de novo FA was reduced in G2 cows (compared to G0 cows); indeed, long-chain FA has an inhibitory effect on mammary gland synthesis (Dhiman, Anand, Satter, & Pariza, 1999), and the inhibition tends to be higher

TABLE 5 Effect of turn out to 50% TMR plus 6 hr access to pasture in one grazing session (Post-G0) after 100% TMR (G0) and 50% TMR plus 6 hr access to pasture in one grazing session (G1; as control) on milk FA profile of multiparous Holstein cows at 65 days in milk (DIM)

		Treatments			*p*-Value		
Variable	DIM	G0/Post G0	G1	*SEM*	*T*	*P*	*P* × *T*
FA origin, g/100 g of FA							
De novo (C4:0–C15:1)	45	23.17^{a}	19.69^{ab}	1.56	.502	.094	.102
	65	18.68^{b}	19.64^{ab}				
Mixed origin (C16:0 + C16:1)	45	36.27	34.8	2.1	.763	.168	.166
	65	31.77	34.82				
Preformed (>C17:0)	45	40.50^{b}	45.50^{ab}	2.92	.921	.026	.021
	65	49.54^{a}	45.30^{ab}				
FA saturation, g/100 g of FA							
Saturated	45	67.43	64.63	2.56	.772	.070	.331
	65	61.94	62.87				
Monounsaturated	45	28.21	31.01	2.30	.771	.046	.222
	65	33.47	32.40				
Polyunsaturated	45	4.04	4.32	0.35	.494	.224	.840
	65	4.59	4.72				
Sat/Unsat	45	2.26	1.92	0.24	.821	.088	.124
	65	1.69	1.89				
n-3	45	0.42^{b}	0.67^{a}	0.08	.079	.611	.192
	65	0.55^{ab}	0.61^{a}				
n-6	45	2.93^{a}	2.03^{b}	0.16	.013	.398	.032
	65	2.52^{c}	2.23^{bc}				
n-6/n-3	45	8.52^{a}	3.36^{b}	0.75	.009	.018	.001
	65	4.69^{b}	4.35^{b}				
Trans	45	2.85^{b}	5.27^{a}	0.46	.022	.053	.004
	65	4.50^{a}	4.87^{a}				
C14:1/C14:0	45	0.08^{xy}	0.07^{y}	0.01	.975	.645	.071
	65	0.08^{xy}	0.08^{x}				
C16:1/C16:0	45	0.06	0.06	0.01	.910	.482	.730
	65	0.06	0.07				
C18:1/C18:0	45	2.65	2.69	0.32	.459	.389	.455
	65	2.69	3.19				
Δ9I*	45	0.31	0.31	0.03	.871	.098	.595
	65	0.35	0.34				
RCLA†	45	0.23	0.18	0.04	.617	.457	.510
	65	0.24	0.25				

CLA, conjugated linoleic acid; FA, fatty acid; TMR, total mixed ration.

^{a,b,c}Means within a row with different superscripts differ ($p < .05$).

^{x,y}Means within a row with different superscripts differ ($.05 < p \leq .10$).

*Δ9I: desaturase activity; (C16:1*cis* + C18:*cis* + C18:2 CLA + C14:1*cis*)/(C14:0 + C16:0 + C 18:0 + C18 :1*trans* + C16:1*cis* + C18:1*cis* + C18:2 CLA).

†RCLA: C18:2 CLA/C18:1*trans*.

when the number of carbon atoms and/or the degree of unsaturation increase (Chilliard, Ferlay, Mansbridge, & Doreau, 2000). Specifically, C18:1*trans* isomers and CLA could be very potent inhibitors of fat synthesis (Chilliard et al., 2000).

Cows with only one grazing session (G1) presented intermediate contents of de novo and preformed FA when compared to no grazing cows (G0) or with cows with two grazing sessions (G2), thus the results are linked with the proportion of pasture in the diet.

In contrast to several reports (Bargo et al., 2006; Loor et al., 2003; Morales-Almaraz et al., 2010), CLA content did not reach significant difference ($p = .12$) between treatments. Loor et al. (2003) and Morales-Almaraz et al. (2010) reported increased contents of

CLA comparing 100% TMR vs. TMR plus grazing (both experiments had a minimum of 21.5% daily DMI of pasture). It should be taken into account that these studies were performed later in the lactation (185 or 94 DIM respectively) than the present work (45 DIM). The CLA content increases and it stabilises over lactation (Stoop, Bovenhuis, Heck, & van Arendonk, 2009). The vast majority of CLA in milk is synthesised in the mammary gland via desaturation of C18:1*trans* through delta-9 desaturase (Bauman et al., 2006). Although CLA content in milk fat did not differ among treatments, its main precursor C18:1*trans* was twofold greater in milk of grazing cows (G1 and G2) when compared to G0 cows as has been described before (Morales-Almaraz et al., 2010). Vaccenic acid is a FA originated from the incomplete ruminal biohydrogenation of dietary C18:3 and C18:2 (Griinari & Bauman, 1999). Thus, when feed supply increases the amount of these FA, the flux of C18:1*trans* that escapes the biohydrogenation process is absorbed post-ruminally (Bauman & Griinari, 2001). Besides the feed supply, others factors (i.e., structural characteristics of grass, plant enzymes activity, animal intake and digestive process) have an effect on the biohydrogenation rate and ruminal passage rate (Buccioni et al., 2012), modifying the FA profile reaching the duodenum and contributing to explain our results.

Similarly, n-3 fraction was greater in grazing (G1 and G2) than G0 cows. Linolenic acid in milk fat was the greatest for G2 and the lowest for G0 cows, consistent with Morales-Almaraz et al. (2010) that found 44% greater content of C18:3(n-3) with 12 vs. 6 hr of grazing (differing 12.7% of daily pasture DMI) and the lowest content for 100% TMR. The ruminant tissue cannot synthesise this FA (Chilliard et al., 2000), and the increase found in our study may be explained by higher herbage intake that supplied n-3 PUFA that escaped ruminal biohydrogenation (Dewhurst et al., 2006). Chilliard et al. (2000) suggested that up to 20% of C18:3(n-3) escapes ruminal biohydrogenation. In the present study, G2 cows presented almost 40% more C18:3(n-3) than G1 cows with pasture DMI of 34% and 27% of total DMI for G2 and G1 respectively (Fajardo et al., 2015). On the other hand, n-6 FA in milk fat was greater in G0 than grazing cows, being C18:2*cis*(n-6) the most important in quantity, that is consistent with Morales-Almaraz et al. (2010). In the present study, C18:2*cis*(n-6) content in TMR was almost three times higher than in pasture. Chilliard et al. (2000) suggested that up to 8% of C18:2*cis*(n-6) escapes ruminal biohydrogenation. Overall, n-3 and n-6 FA differences found in milk fat were clearly reflected in the milk n-6/n-3 FA ratio as reported previously (Petit, Germiquet, & Lebel, 2004).

The ratio C14:1/C14:0 indicated minor desaturase activity in G2 than G0 and G1 cows which is consistent with the lower de novo FA synthesis of the former cows compared to G0 and G1 cows. The lower FA synthesis and desaturation in G2 cows suggest a less active mammary gland and are consistent with the long-chain FA released from adipose tissue and/or increased amounts of PUFA influx reaching the duodenum in G2 cows that reduce mammary gland FA synthesis via downregulation of specific enzymes (delta-9 desaturase, acetyl-coA carboxylase and FA synthase; Chilliard et al., 2000).

4.2 | Effect of turn out to 6 hr of grazing after TMR feeding on fatty acid profile at 65 days in milk

The switch from TMR diet to a mixed system (TMR with 6 hr of access to paddocks in one grazing session a.m.) modified the milk FA profile showing the effect of grazing. An increase in the content of C18:3(n-3), C18:1*trans* and CLA in Post-G0 is consistent with inclusion of pasture in the diet. Moreover, the content of these FA did not differ between Post-G0 and G1 cows (65 DIM), which is aligned with the similar TMR and pasture of both treatments (Fajardo et al., 2015). These findings suggest that the ruminal process (specifically the biohydrogenation process) and the mammary gland synthesis were not different in Post-G0 and G1 cows. Although Khanal et al. (2008) reported that CLA increase took 23 days to reach the maximum after turning cows out to pasture, in the present study contents were twofold greater (0.35 vs. 0.77 g/100 g) 4 days after turning out G0 cows to pasture. Our data are consistent with the report of Kuzdal-Savoie and Kuzdal (1961) that showed a sharply increase of CLA content reaching a maximum effect 5 days after the switch. Moreover, while these studies used a switch-over design between contrasting diets (no grazing vs. 100% grazing), in our study, the increase in CLA content was the result of the switch from no grazing to 6 hr of grazing (20% of daily DMI of pasture; Fajardo et al., 2015). It can be suggested that delta-9 desaturase in the mammary gland responded actively to the arrival of its precursor (C18:1*trans*).

Linoleic acid [C18:2*cis*(n-6)] content was decreased when cows started to graze as has been discussed previously and taking into account the increase in C18:3(n-3), the n-6/n-3 ratio decreased in Post-G0 showing no differences with G1 at 65 DIM. As was mentioned, PUFA has an inhibitory effect on the de novo synthesis (Chilliard et al., 2000) and this was evident in the turn out to grazing, where de novo FA in Post-G0 decreased (compared with G0). An increase in C18:0 content was found when TMR-fed cows were turned out to pasture being even greater in these cows than in G1 at 65 DIM. As mentioned before, the increase in this FA may reflect lipid mobilisation (Rukkwamsuk et al., 2000), which may indicate the increase in energy requirements due to grazing and walking in Post-G0 cows (Fajardo et al., 2015).

In summary, the inclusion of one-third of total DMI as pasture enabled to capture the pasture benefits (postulated to produce a more "desirable" milk FA composition for human health) without productive losses during early lactation. In addition, cows that did not graze during the first 2 months of lactation when turned out to grazing pasture (20% of total DMI) supplemented with TMR presented a similar milk FA composition to cows that had been grazing pasture since calving.

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