Pasture access times and milk fatty acid profile of dairy cows from central highland of Mexico

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Abstract

The objective was to assess the effect of different access times to pasture of grazing dairy cows on milk yield and fatty acid (FA) profile. Six multiparous Holstein cows were used in a repeated 3x3 Latin Square design. Treatments were 1) Eight hours of grazing (8h), 2) Eight hours of grazing divided in two periods (4+4h), and 3) Twelve hours of grazing (12h). Cows were fed maize silage and concentrate when confined in a free pen. There was no effect (P>0.05) on milk FA profile between 8h and 4+4h treatments. The 12h treatment showed a higher (P<0.05) concentration of rumenic acid (C18:2 c9t11) and higher total polyunsaturated FA in milk than milk from the other treatments. Cows grazing 8h and 4+4h had 36.3 and 27.9% higher (P<0.05) intake of maize silage compared to cows grazing 12h, but total DM intake was not affected (17.3 ± 1.9; P>0.05). There were no differences (P>0.05) in milk yield and chemical composition with different access times to pasture. The conclusion is that allowing 12h of grazing to dairy cows increased the concentration of C18:2 c9t11 in milk without affecting milk yield.

Keywords: conjugated linoleic acid; vaccenic acid; milk fat; Holstein; dairy cows; grazing.

Introduction

There has been an increasing interest over the past years in the content of polyunsaturated fatty acids (FA) of cow milk due to their beneficial effects on human health. Food products derived from ruminants are naturally rich in conjugated linoleic (especially the isomer C18:2 c9t11 or rumenic acid) and vaccenic (C18:1 t11) acids; the proportional variation of these acids is closely linked to the diet (Buccioni et al., 2012). Linolenic acid (C18:3 c9c12c15), which is abundant in fresh herbage (Loor et al., 2003), goes through a process of bio-hydrogenation in the rumen that, among other intermediaries, produces C18:1 t11 (Buccioni et al., 2012), this FA is absorbed in the small intestine and desaturated in the mammary tissue by delta-9-desaturase to synthesis of C18:2 c9t11. This route represents up to 90% of total C18:2 c9t11 in milk (Griinari and Bauman, 1999).

Milk from grazing cows fed fresh herbages has a higher concentration of C18:2 c9t11 and C18:3 c9c12c15, compared to cows in confinement (Morales-Almaráz et al., 2011; Buccioni et al., 2012); the increase in grazing times leads to an increase in the content of these acids in milk (Kelly et al., 1998; Morales-Almaráz et al., 2010). Dairy cows are able to adapt their behaviour at pasture according to grazing conditions (Pérez-Prieto et al., 2011). Grazing animals partially compensate a reduction in grazing time owed to restricting time at the pasture by an increased intake rate (Kennedy et al., 2009; Pérez-Ramírez et al., 2009), or modifying rumination time to compensate for the reduction available grazing time (Gregorini et al., 2012). Pérez-Ramírez et al. (2009) affirm that daily access time to pasture may be used as a grazing management tool. Soriano et al. (2000) allowed 8 h access time to pasture after morning or afternoon milking reporting a higher intake in the afternoon, due to a higher time actually spent grazing (4 *vs.* 2 h). When access time to pasture was reduced from 8 to 4 h, there was a decrease in herbage DM intake, milk yield, and milk fat content (Pérez-Ramírez et al., 2008; Mattiauda et al., 2013). In addition, dividing the 8h access time to pasture in two periods (4+4h), resulted in differences in protein and lactose yield (Mattiauda et al., 2013). Other authors have observed a decreasing of milk yield by reduction of access time to pasture from 16h to 8h (Chilibroste et al., 2004) and from 21h to 8h (Delaby et al., 2008). Contrary, Kennedy et al. (2009) did not observe effect on milk yield with the restriction of access of dairy cows to pasture from 22h to 8h.

Information about the FA profile in milk by reducing or splitting the access time to pasture is lacking. Loor et al. (2003) reported higher concentration of C18:2 c9t11 and C18:1 t11 in milk from cows given the higher intake of herbage during the afternoon grazing compared to morning grazing with 8h at pasture after milking. In a previous study (Castro-Hernández et al., 2014), 12 h at pasture with maize silage *ad libitum* and concentrates (2.5 kg DM/cow/day) during evening confinement resulted in milk with higher levels of unsaturated fatty acids with low yielding dairy cows. When maize silage is limited during confinement of grazing dairy cows affects the intake of herbage dry matter at pasture rather than milk yield (Pérez-Prieto et al., 2011). Not restricting the offer of maize silage during confinement may result in lower herbage intake, but would be an important source of C18:2, a fatty acid that in high amounts leads to an increase in C18:c9t11.

The hypothesis was that offering a mixed ration *ad libitum* during confinement, and reducing access time to pasture (12 *vs*. 8h), or splitting this time in two (8h vs. 4+4h) does not affect herbage and fatty acid intake, mainly from C18:3, under similar PA; and a complete mixed ration during confinement will provide C18:2. Both acids are important substrates for the formation of vaccenic acid and C18:2 c9t11 in the rumen. The objective was to assess the effect of different access times to pasture on the yield, chemical composition and fatty acid profile of milk in grazing dairy cows.

Materials and methods

Study area

Work was carried out between April and June 2012, at the farm of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of the State of Mexico. This experiment was carried out under the supervision and approval of the Academic Ethical Committee of the Faculty.

Animals, treatments and experimental design

Six lactating multiparous Holstein cows (615.0 ± 36.0 kg live-weight; 22.7 ± 4.8 kg/d milk yield) were allotted to a repeated 3 x 3 Latin Square design. The treatments consisted of three times of access to pasture: 1) the cows had access to the pasture only between two milking in one session of eight hours, from 07:00 to 15:00h (8h). 2) The cows had access during two sessions per day, from 07:00 to 11:00 h after the morning milking (4h) and from 16:00 to 20:00 h after the afternoon milking (4h) (4+4h). 3) The cows had access to the pasture as 8h treatment, from 07:00 to 15:00 h, and four hours more from 16:00 to 20:00 h after afternoon milking (12h).

When they were not at pasture or at milking, the cows were indoors in free stalls, with free access to water, maize silage *ad libitum* and 4.48 kg DM/cow/d of concentrate, offered individually in two meals. The study comprised three experimental periods of 15 days each one (10 days for adaptation and 5 days for samples collection). Cows were milked twice a day (06:00 and 15:00 h), and were weighed at the beginning and end of each experimental period.

Pasture and grazing management

Two paddocks of *Festuca arundinacea*, *Dactylis glomerata*, *Lolium perenne*, and *Trifolium repens* sown seven years previously were used under a rotational grazing system. Pre-grazing herbage mass was measured at ground level one day before to each one days of measurement stage per period, by cutting of ten squares (0.5 x 0.5m) with manual shears. Before cutting, sward heights were recorded on each square with an ascending plate grass meter to homogenize the height sward at area allocated daily and to obtain homogeneous regrowth. Pre-grazing herbage mass in pasture was used for adjusting the size of grazing plots daily to meet assigned pasture allowances of 22 kg DM/cow/d with support of an electric fence. A strip of fresh pasture was assigned every day.

The cows in 8h and 12h grazing were managed as one group in the same pasture, according to time of access to pasture described previously and another pasture for cows in 4+4h treatment only. In the latter treatment, the size of grazing plot was allowed to meet the PA since first session grazing, was not offer a new strip in the pasture for second session grazing after to afternoon milking. Similarly, the cows in 12h treatment came back to the same pasture allocated after the afternoon milking. Water was always available at the pasture.

Measurements and sampling

Grass intake was estimated by the method described by Macoon et al. (2003) based on net energy requirements for dairy cattle (NE_L) (NRC 2001), and the net energy supplied by the indoor feed intake. Net energy content of feeds (herbage, maize silage and concentrate) was calculated from equations described by Menke and Steingass (1988), from acid detergent fibre (ADF) content. Maize silage and concentrate intake was measured individually by difference between offered and refusal, and milk yield was individually recorded daily.

Maize silage was sampled daily at the time of feeding from individual troughs during the collection phase, while the concentrate was sampled once per period. Grass was sampled by simulated grazing (hand plucking) (Wayne, 1964), before the beginning of pastures grazing cows. All samples were kept at -20ºC until their laboratory analysis. Milk was sampled individually at each milking (50 ml) and an aliquot was taken for chemical analysis.

Chemical analysis

Feed samples (maize silage, concentrate and herbage) were dried in a draught oven at 60°C for 24 h, ground with a 2 mm sieve and conserved in plastic bags. DM and ash content were determined by loss weight after drying at 100 ± 1°C in a draught oven for 24 h, followed by incineration at 600°C for 4 h in a furnace. Crude protein content was determined by the Kjeldahl method (AOAC 2012); ADF, NDF and ADL were determined according to Van Soest et al. (1991). Fatty acid content in feeds was determined on freeze-dried samples and following the technique described by Sukhija and Palmquist (1988), modified by Palmquist and Jenkins (2003), using methanolic hydrochloric acid at 10% for esterifying FA, and using hexane as an organic solvent. Methyl esters of FA were separated and quantified by gas chromatography (Perkin Elmer Clarus 500), with a capillary column 100 m x 0.25 mm x 0.2 µm (SUPELCO TM-2560), using nitrogen as a carrier gas. The detector and injector were kept at 260°C, and the initial oven temperature was 140°C for five minutes, increasing 4°C per minute up to 240°C. Each peak was identified from retention times of standard methyled esters (Supelco 37, FAME MIX analytical SIGMA USA). Fatty acids are reported in g 100 g^{-1} of total FA.

Milk composition (fat, protein and lactose) was determined with the ultrasound analyser Lactoscan (Milkotronic, LTD). Milk fat was isolated before FA determination following the method of Feng et al. (2004); methylation was carried out according to the methodology described by Christie (1982), modified by Chouinard et al. (1999). Methyled esters of FA were quantified in the same way as for feeds.

Statistical analysis

Data were analysed with a Latin Square design using PROC MIXED procedure (SAS 2006). The statistical model was: $Y_{ijkl} = \mu + S_i + P_{(i)j} + C_{(i)k} + TP_l + E_{ijkl,}$ where: Y_{ijkl} = response variable; μ = general mean; S_i = is the random effect of square (i: 1, 2); $P_{(i)j}$ = is the fixed effect of period (j = 1, 2, 3) within square; $C_{(i)k}$ = is the random effect of cow (k = 1, 2, 3) within square; TP_l = is the fixed effect of time of access to pasture (l = 1, 2, 3); and E_{ijkl} = random error term. Orthogonal contrasts were carried out to determine differences due to grazing times. Differences were designated as significant at $P \leq 0.05$. Trends were considered present when $0.05 < P < 0.10$.

Results

Fatty acid content of concentrate and maize silage showed as main fatty acids C18:1 c9 and C18:2 c9c12, with around 65% of total FA; while in herbage, the most abundant acid was C18:3 c9c12c15 (Table 1).

Animal performance

Treatments 8h and 4+4h showed ($P<0.05$) 36.3 and 27.9% more DM intake of maize silage, compared to 12h of grazing (Table 2). Herbage DM intake by dairy cows was 2.5 times higher ($P<0.05$) in 12h compared to 8h of grazing. However, total DM intake was not affected ($P>0.05$) by treatments (17.29 ± 1.86 kg DM/cow/d).

There were no differences ($P>0.05$) in pasture intake between treatments 8h and 4+4h at the same pasture allowance (22 kg). In our study, there were not differences ($P>0.05$) in milk yield and its chemical composition among treatments (Table 2).

Milk fatty acid profile

Access time to grazing did not affect the milk FA profile (Table 3). Concentration of C18:2 c9t11 acid was increased ($P<0.05$) when cows had maximal access time to pasture (12h) compared with 8h and 4+4h treatments (19.5 and 14.6%, respectively).

Table 1. Ingredients, chemical composition and fatty acid content of feeds.

Item	Concentrate	Maize silage	Herbage
Ingredients [g/100 g DM]			
Sorghum	48.7		
Soy bean meal	20.0		
Canola meal	14.8		
Wheat bran	14.7		
Minerals [*]	1.8		
Chemical composition [%]			
Dry matter (DM)	89.6	31.0	28.7
Organic matter (OM)	91.3	93.9	88.8
Crude protein (CP)	20.4	6.7	16.2
Neutral detergent fibre (NDF)	20.5	55.1	53.6
Acid detergent fibre (ADF)	7.0	31.1	25.0
Acid detergent lignin (ADL)	2.2	4.1	2.7
Net energy (NE_L) [MJ/kg DM][¶]	8.4	6.0	6.6
Fatty acids [g/100 g FA]			
Lauric (C12:0)	0.05	0.35	1.26
Tridecanoic (C13:0)	0.78	0.06	2.39
Myristic (C14:0)	0.14	0.65	0.58
Palmitic (C16:0)	18.75	17.48	16.26
Palmitoleic (C16:1)	0.38	0.38	2.45
Estearic (C18:0)	3.50	4.82	1.94
Oleic (C18:1n9c)	31.49	29.04	2.36
Linoleic (C18:2n6c)	36.96	36.18	10.38
Linolenic (C18:3n3)	2.64	3.99	52.28
Other	5.31	7.05	10.10

[*] Multitec, lechero bovino® , Vitamin A: 231 UI, Vitamin D_3: 58.5 UI, Vitamin E: 566 mg, Copper: 400 mg, Iron: 2,560 mg, Manganese: 1,860 mg, Cobalt: 5.85 mg, Iodine: 19.84 mg, Zinc: 2,000.16 mg, Selenium: 12 mg, Phosphorus: 38,220 mg, Magnesium: 39,959.92, Calcium carbonate: 194 g, Salt: 236.621 g, Sodium bicarbonate: 150 g, Sodium: 1,851.60 mg, Potassium: 2,439 mg.

[¶] Estimated by equation proposed by Menke and Steingass (1988), NEL, (9.07- 0.0097*ADF [g/kg DM]).

Discussion

Animal performance

Differences in pasture intake in 12h treatment in comparison with the other two treatments was due to pasture access time, since there are reports that show a lineal increase in pasture intake as access time to pasture is increased, even when offered a total mixed ration during confinement (Morales-Almaráz et al., 2010; 2011), *ad libitum* silage (Castro-Hernández et al., 2014; Rego et al., 2008) or restricted silage (Dhiman et al., 1999; Pérez-Ramírez et al., 2009). The higher herbage intake in treatment 12h means that cows were able to meet their nutritional requirements, compensating the reduced intake of maize silage due to reduced time indoor.

Table 2. Daily feed intake, milk yield and its chemical composition of Holstein cows with different access time to pasture.

Item	Time at pasture*			SEM†	Contrasts ($p \leq$)	
	8h	4+4h	12h		12h *vs.* others	8h *vs.* 4+4
DMI [kg/d]						
Maize silage	10.11	8.94	6.44	0.7364	0.0037	0.2776
Concentrate	4.48	4.48	4.48	-	-	-
Herbage	2.64	3.70	6.58	1.0299	0.0163	0.4780
Total intake	17.24	17.13	17.50	0.0855	0.7501	0.9244
Intake [% of LW]	2.81	2.84	2.85	0.1237	0.8839	0.8882
Milk yield and chemical composition						
Milk yield [kg/d]	21.48	21.96	21.86	0.7748	0.8789	0.6646
Fat [g/kg]	42.2	41.5	41.9	0.7459	0.9811	0.5027
Protein [g/kg]	31.4	31.2	31.1	0.2989	0.5464	0.6770
Lactose [g/kg]	46.3	46.6	46.3	0.3463	0.7899	0.5239

*Time at pasture, 8h = 0700 to 1500 h; 4+4h = 0700 to 1100 h and 1600 to 2000 h; 12h = 0700 to 1500 h and 1600 to 2000 h.
†SEM, standard error of means.

Table 3. Fatty acid profile in milk of Holstein cows with different access time to pasture.

Fatty acid [g/ 100g]	Time at pasture*			SEM†	Contrast ($p \leq$)	
	8h	4+4h	12h		12h *vs.* others	8h *vs.* 4+4
C4:0	3.53	3.61	3.57	0.1263	0.9835	0.7218
C6:0	2.48	2.44	2.40	0.0574	0.6209	0.4881
C8:0	1.48	1.47	1.43	0.0420	0.7571	0.6695
C10:0	3.14	3.10	2.99	0.1499	0.8011	0.7300
C11:0	0.49	0.46	0.47	0.0276	0.5769	0.3464
C12:0	3.63	3.52	3.45	0.1835	0.9320	0.6601
C13:0	0.21	0.21	0.21	0.0219	0.6374	0.5431
C14:0	12.07	11.99	11.82	0.3502	0.9524	0.8770
C14:1	1.34	1.24	1.33	0.0573	0.2717	0.3096
C15:0	1.21	1.17	1.20	0.1022	0.7989	0.7913
C16:0	32.56	31.33	31.13	1.1068	0.6593	0.4783
C16:1	1.99	1.95	1.95	0.1197	0.8788	0.8504
C17:0	0.72	0.72	0.72	0.0300	0.8620	0.7640
C17:1	0.22	0.22	0.21	0.0129	0.7625	0.6036
C18:0	10.76	11.59	11.51	0.8695	0.9438	0.5985
C18:1t11	1.26	1.40	1.60	0.0885	0.0661	0.2861
C18:1n9c	19.54	20.15	20.42	1.1046	0.8539	0.6467
C18:2n6t	0.14	0.13	0.14	0.0091	0.8942	0.4983
C18:2n6c	1.32	1.34	1.32	0.1042	0.6549	0.8624
C18:3n3	0.36	0.40	0.45	0.0371	0.1153	0.3847
C18:2c9t11	0.66	0.70	0.82	0.0537	0.0390	0.5094
C20:0	0.15	0.15	0.15	0.0110	0.8137	0.6847
C20:1c11	0.16	0.13	0.14	0.0288	0.8922	0.4901
C23:0	0.10	0.12	0.10	0.0292	0.7225	0.7581
Others	0.46	0.45	0.48	0.0194	0.3341	0.7285
Categories¶						
SFA	72.95	72.11	71.71	1.1005	0.5660	0.6102
MUFA	24.29	25.05	25.27	1.0324	0.6510	0.6228
PUFA	2.76	2.84	3.02	0.0726	0.0494	0.4480
SFA/UFA	2.72	2.60	2.56	0.1439	0.5850	0.5873

*Time at pasture, 8h = 0700 to 1500 h; 4+4h = 0700 to 1100 h and 1600 to 2000 h; 12h = 0700 to 1500 h and 1600 to 2000 h.
† SEM, standard error of means.
¶SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. SFA/UFA, ration SFA/unsaturated fatty acids.

Contrary to our results of pasture intake, Pérez-Ramírez et al. (2008) and Mattiauda et al. (2013) observed a decrease in herbage intake of 18.6 and 19.9% when access time to pasture was restricted from eight to four hours per day. Soriano et al. (2000) observed differences in pasture intake of milking cows with 6h access time during the day or 7h during the afternoon, supplemented with maize grain. Cows spent more time grazing after the afternoon milking (4.1h/d). Lower day time pasture intake lead to a higher TMR consumption during confinement compared to cows grazing in the evening (Soriano et al., 2001). A higher intake of pasture was expected for cows that spent four hours at pasture after milkings, since hunger indicators are increased post-milking (Gregorini et al., 2009). However, the option that cows on the 4+4h treatment had to return to confinement after 4h to *ad libitum* total mixed ration may have represented a lower stimulus for grazing, and therefore, lower intakes. Contrary, when the fed in indoor was fixed (Soca et al., 2014), the modulation of grazing behaviour for dairy cows in response to restricting time at pasture (from 24 to 20 and 15.5h) maintained herbage dry matter intake. Pérez-Ramírez et al. (2008) and Mattiauda et al. (2013) reported in dairy cows a decrease in milk yields of 4.9 and 5.1%, when grazing time was reduced from 8 to 4h. Soca et al., (2014) concluded that changes in grazing behaviour in response to restricting time at pasture plus supplementation counteract restrictions of time at pasture, and thereby help to maintain herbage intake without negative effects on milk production.

Milk fatty acid profile

There are several ways to increase the concentration of desired FA, PUFA and functional components, in ruminant food products. These are increasing the amount (i.e. intake) or concentration of their precursors in the diet, reducing the extent of bio hydrogenation in the rumen, and/or enhancing activity of the delta 9 desaturase enzyme that converts C18:1 t11 into C18:2 c9t11 in the udder of cow. Considering this, it is most important to optimise the synthesis of C18:1 t11 in the rumen. Certainly, out of the total content of C18:2 c9t11 acid in cow milk, a small amount comes from the isomerisation of C18:2 c9c12 in the rumen, and most of it comes from *de novo* synthesis in the mammary gland by the action of the delta 9 desaturase enzyme, using C18:1 t11 acid as a substrate (Griinari and Bauman, 1999). In the work herein reported, there was a trend (P=0.066) in the C18:1 t11 acid increasing in milk in the 12h grazing time, being 12.5 and 21.3% higher than 4+4h and 8h treatments, respectively (Table 3). This is associated to a higher content of C18:2 c9t11 in milk fat that would indicate a higher availability of C18:1 t11 acid as a substrate for the synthesis of C18:2 c9t11 in the mammary gland tissue, or a lower capability or efficiency in cows with less access time to pasture.

As mentioned before, the action of the delta 9 desaturase enzyme in the mammary gland seems to be the main route for C18:2 c9t11 acid synthesis in milk (Kay et al., 2004). In the process, this enzyme uses C18:1 t11 acid, produced from bio hydrogenation of C18:2 c9c12 and C18:3 c9c12c15 acids as a substrates, which tended to be higher in milk in treatment 12h, probably derived from a higher pasture intake and therefore more C18:3 c9c12c15 acid ingested. Palladino et al. (2014) found a higher concentration of C18:1 t11 in rumen when pasture allowance was 20 kg DM/cow/d, which was attributed to a higher intake of C18:3 c9c12c15, precursor of this fatty acid. The higher concentration of C18:1 t11 acid in rumen might have improved its flow into the small intestine, and its absorption and desaturation in the mammary gland for the synthesis of C18:2 c9t11 acid.

When dairy cows intakes herbage of high nutritional quality, the presence of a high content of intermediate products (C18:2 and C18:1) and a low concentration of C18:0 in the ruminal fluid, it could indicates that the last step of bio hydrogenation is inhibited (Sun and Gibbs, 2012). In the rumen of dairy cows grazing high quality pastures, there is a greater production of C18:1 t11 acid due to a decrease in ruminal pH, compared with cows grazing at low quality pasture (Palladino et al., 2014).

Conclusion

The concentration of C18:2 c9t11 was increased in milk fat of cows with more grazing access time (12h). Giving access of grazing time of eight or two periods of four hours did not modify milk fatty acid profile in dairy cows. The higher intake of C18:2 c9c12 from maize silage did not increase the content of C18:2 c9t11 in milk in the treatments with lower pasture access times (8h and 4+4h). This was due to lower pasture intakes, and therefore, lower intake of C18:3 c9c12c15 due to lower time at pasture, resulting in low contents of C18:2 c9t11 in milk due to reducing pasture time from 12 to 8h, irrespective if these eight hours are continuous or split in two 4+4h periods.

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