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Effect of total mixed ration composition and daily grazing pattern on milk production, composition and fatty acids profile of dairy cows

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The possibilities of using high quality pastures in conjunction with total mixed ration (TMR) during the grazing season have been examined. An experiment with sixteen Holstein cows blocked and randomly assigned to four treatments in a factorial arrangement was conducted in order to evaluate the influence of grazing time of day (day or night) and type of silage (maize or Italian ryegrass) included in the TMR of dairy cows grazing 12 h daily on milk yield, composition and fatty acid profile. The silage type had no effect on the dry matter intake, milk yield and fat and protein proportions. However, cows grazing during the night ate more grass than cows grazing during the day (8·53 *vs.* 5·65 kg DM/d; $P<0·05$). No differences were seen between grazing-time with respect to milk production, fat and protein contents. However, the proportion of polyunsaturated fatty acid was higher in milk of dairy cows grazing at night-time than grazing at day-time, especially 18:2*n*-6 (2·37 *vs.* 2·12 g/100 g FA respectively, $P<0·05$) and 18:2cis9trans11 (2·08 *vs.* 1·74 g/100 g FA respectively, $P<0·05$).

Keywords: Dairy cow, grazing time of day, dry matter intake, milk, fatty acid profile.

Pasture provides a natural environment for ruminants, and the access to pasture improves the animal welfare. However, many lactating dairy cows have no access to pasture in continental Western Europe. Lack of access to pasture has been linked with higher rates of lameness among other diseases (Hernandez-Mendo et al. 2007). Increasing the proportion of grazed grass in the diet during the grazing season as part of the diet could reduce feed costs and increase herd health. Grazing has been proposed as an essential strategy for the efficient use of pastoral resources (Capper et al. 2009). However, grazing alone cannot meet the nutritional requirements of high yielding dairy cows, particularly in early lactation (Kennedy et al. 2011). Dairy cows permanently on pasture have more weight loss and produced less milk than dairy cows in free-stalls (Hernandez-Mendo et al. 2007), due to lower dry matter intake than cows housed indoors. In addition, the lower nutrient density of grass than compound feeds provided indoors is likely to reduce milk production for pasture-kept animals (Soriano et al. 2001). Thus, supplementation is required to provide the energy requirements to fully reach the animal's genetic potential. The possibilities of using high-quality pastures and total mixed rations (TMR) feeding during the grazing season have been examined (Bargo et al. 2002). It could help to produce high yields of milk enhancing protein and fat contents (Morales-Almaráz et al. 2010), and save feeding costs (Vibart et al. 2008). Grazing cows produce milk with a fatty acid (FA) profile more favourable to human health, with higher proportions of CLA and 18:3*n*-3 (Stockdale et al. 2003; Bargo et al. 2006; Dewhurst et al. 2006; Morales-Almaráz et al. 2011), which have health benefits for consumers (Belury, 2002). Similar results have been reported in cows feeding fresh cut forage (Ferlay et al. 2006; Auldist et al. 2013) and feeding fish and plant oils (AbuGhazaleh et al. 2007). Moreover, pasture feeding imparts grassy flavours that can be recognised by some consumers in milk (Bendall, 2001) and in cheese (Martin et al. 2009) and improves the softness and spreadability of butter (Couvreur et al. 2006) and the body texture cheese (Martin et al. 2009).

Previous studies have shown that herbage chemical composition varies throughout the day, with change in the fatty acid profile, crude protein, water soluble carbohydrates, starch and digestible organic matter (Avondo et al. 2008; Gregorini et al. 2009; Vasta et al. 2012). The 18:3*n*-3 represents the major component of herbage fatty acids

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(Elgersma et al. 2003), and could play a key-role in photosynthesis (Browse et al. 1981), and therefore this would justify its greater content in leaf in the afternoon than in the morning. Other authors (Gregorini et al. 2008) have found no differences in 18:3*n*-3 concentration in herbages sampled either in the morning or in the afternoon. However, the grazing time of day can affect the fatty acid composition of milk in goats (Avondo et al. 2008). More than 80% of the total dry matter intake (DMI) by dairy cows continuously is consumed between 07·00 to 20·00 h (Huzzey et al. 2007), with a maximum intake rate occurring twice daily after both milkings (Cuadrado et al. 2011). Dairy cows with free access to pasture in damp temperate areas spend the night outdoors preferably (Legrand et al. 2009), although they use the meadow more as resting area than feeding area (Chapinal et al. 2010).

Therefore, the objective was to study the effect on the dry matter intake, production and composition of milk, and fatty acid profile of milk fat of dairy cows in a regime involving different 12 h feeding periods. These periods were grazing for 12 h during day or night-time and the remaining 12 h spent indoors two different TMR, based on maize silage or Italian ryegrass silage.

Materials and methods

This work was undertaken between March and May of 2009 at the SERIDA experimental farm (43°28′50″N, 5°26′27″W, 10 masl). Daily average temperature was 12 °C (range: 04–22 °C) and the total rainfall was 262 mm on 51 rainy days. Sunrise ranged from 06·50 to 08·15 h and sunset from 19·10 to 21·55 h throughout the experimental period. All animals were cared for according to the standards of the European Union Animal Welfare Directive Number 86/609/EEC.

Sixteen Holstein-Frisian dairy cows were assigned to the assay, balancing for body weight (mean ± SD; 631 ± 81 kg), parity (1·9 ± 0·9; range: 1–4) and days in milk (106 ± 45). Daily average milk yield of these cows was 31·2 ± 2·0 l/d in the week prior to the start of the experiment. Cows were kept in a free stall barn with rubber mat bedding and the exercise areas had concrete floor and a scraper system for manure removal. The animals were allocated into four groups and randomly assigned to four experimental treatments. Each trial period lasted 21 d, including 14 for adaptation and seven for data collection. The treatments were: (1) feeding a maize silage-based TMR and grazing after the morning milking (08·00 to 19·00 h); (2) feeding a ryegrass silage-based TMR and grazing after the morning milking (08·00 to 19·00 h); (3) feeding a maize silage-based TMR and grazing after the afternoon milking (20·00 to 07·00 h), and (4) feeding a ryegrass silage-based TMR and grazing after the afternoon milking (20·00 to 07·00 h). The animals were randomly assigned in a change-over design.

All cows were weighed on the first and last day of each period after morning milking. On the day prior to the start of data collection, pasture dry matter yield was measured to determine pasture availability. All cows were milked twice daily from 07·00–08·00 h and 19·00–20·00 h. Both TMR were formulated according to NRC (2001) requirements for dairy cattle including maize (MS) or Italian ryegrass (IRS) silages (Table 1). Fresh TMR were supplied twice daily at 08·00 and 20·00 h, so there was always enough fresh TMR available. All animals also received, during both milking sessions, two concentrates, L and S, as energy source and in order for the cows to remain calm during milking session. Table 1 shows the percentage ingredients of both concentrates. Concentrate L was provided at 2 kg/d per cow, and concentrate S was supplied at 0·2 kg by litre of milk produced above 30 l/d in the multiparous cows and above 25 l/d in the primiparous cows. A rotational grazing system was established on a multi-species grassland with a prevalence of gramineae, 77%, legumes, 19%, and other species in an area of 4·75 ha divided into 4 plots. The cows on treatments with grazing at day-time were moved to a fresh paddock after morning milking and kept indoors at night, while the cows on treatments with grazing at night-time were moved to a fresh paddock after evening milking and kept indoors between morning and evening milking. All animals had free access to water both in the barn and in the pasture plots.

The TMR intake of individual animals was automatically recorded at each time by an electronic weighing system integrated to the scale pans using a computerised system and concentrates by an automatic feeder. Grass intake was estimated using NRC prediction equations (Macoon et al. 2003). Briefly, energy requirements were recorded as net

Table 1. Ingredients (% DM) included in the Total Mixed Rations based on maize silage (MS) or Italian ryegrass silage (IRS) and concentrates L and S

	MS	IRS	L	S
Maize Silage	34·9	—	—	—
Italian Ryegrass Silage	—	25·2	—	—
Barley Straw	12·3	11·5	—	—
Lucerne	10·0	11·2	—	—
Maize grain	2·0	3·9	50·0	40·0
Barley grain	0·9	9·6	3·0	15·0
Rye grain	—	2·4	2·0	9·1
Maize flakes	18·9	13·2	—	—
Wheat bran	—	—	—	2·0
Roasted soybean	14·6	10·9	35·0	19·0
Cotton seed	0·9	0·8	—	—
Sunflower seed meal	—	4·3	1·8	4·2
Soybean hulls	—	1·6	2·0	2·0
Beet pulp	2·7	0·8	—	—
Salts of FA by-pass	1·1	1·7	2·6	2·2
Sodium bicarbonate	0·8	0·9	1·0	2·0
Calcium carbonate	0·3	1·5	—	0·8
Dicalcium phosphate	0·3	0·1	—	0·9
Sodium chloride	0·3	0·3	—	0·6
Magnesium oxide	—	—	1·0	—
Molasses	—	—	1·0	2·0
Mineral-vitamin premix	0·1	0·1	0·7	0·3

energy (NE Mcal/d) requirements for maintenance, lactation, body weight changes, walking and grazing. The NE from pasture intake was estimated as total NE requirements minus the NE supplied by the TMR and concentrate intakes.

TMR samples were taken daily during the data collection period in each experimental period. Concentrates and pasture were sampled at the beginning of each trial period. Pasture was sampled in both grazing times. Milk yield was recorded daily at both milking times over the 7 d of data collection. Milk samples were also taken daily from each individual animal throughout the 7 d of data collection in the four experimental periods. Morning and evening samples from individual cow were mixed according to its milk yield to get a representative sample for day and cow. Samples were divided into two subsamples: One of them for chemical analysis was preserved with 0·13 ml of a bacteriostatic agent, and the other one was immediately analysed for fatty acids.

TMR and forage samples were dried (60 °C, 24 h) and milled (0·75 mm); concentrate samples were milled through a 1 mm screen. Feed samples were analysed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and starch by near infra-red spectroscopy (FOSS NIRSystem 5000, Silver Springs, MD, USA). The water soluble carbohydrates (WSC) of grass samples were analysed according to Hoffman (1937) using a spectrophotometer Varian Cary 50 UV/Vis (Varian, Inc. Palo Alto, CA, USA). The FA content of the feeds was analysed according to Palmquist & Jenkins (2003), using a Varian 3800 GC- 4000 mass spectrometer (Varian, Inc., Palo Alto, CA, USA) with a CP-Sil 88 column (100 m × 0·25 mm, 0·20 μm i.d.; Varian, Inc.). Helium was used as carrier gas at a flow rate of 1 ml/min. The temperature of injector and detector were 250 °C. The column temperature was held at 40 °C during 1·20 min; from 40 to 140 °C at 30°/min and held during 25 min; from 140 to 190 °C at 1 °C/min and held during 15 min; from 190 to 215 °C at 1 °C/min and held during 1 min and finally, from 215 to 240 °C at 30 °C/min and held during 1 min. Individual FA peaks were identified by comparison of their retention times and mass spectra between samples and pure methyl ester standards (52 fatty acid methyl esters mix, ref. GLC-463, and 18:2 trans10cis12, ref. UC-61-M of Nu-Chek Prep, Inc. (Elysian, MN,USA) and methyl 9(Z),11(E)-octadecadienoate, ref. 1245-10 of Matreya LLC (Pleasant Gap, PA, USA).

Milk samples were analysed for fat, protein, lactose and urea contents (MilkoScan FT 6000). For milk FA analysis, fat was isolated as described by Feng et al. (2004) and transesterified as described by Christie (1982) with the modifications of Chouinard et al. (1999). Peak quantification was performed in the same conditions as the feeds.

Statistical analyses were performed with SAS (1999) in a randomised block design with the animals considered as a random variable. Data were subjected to a split plot as follows. Main plot effects: Grazing time (G) and Silage (S); Sub-plot effects Period within every grazing time (P). Data were analysed using PROC MIXED and means were separated using the Tukey test.

Results

Chemical composition of the MS and IRS TMRs, the concentrates L and S and pasture, sampled at day and at night grazing times, used are shown in Table 2. Both TMR had similar protein and energy contents, around 143 g CP/kg DM and 1·58 Mcal NE_l/kg DM respectively. The ratio 18:2*n*-6/18:3*n*-3 of MS was 4 : 1, and 2 : 1 in IRS. The grass was similar between pasture grazing at day and at night, except in WSC content. The pasture grazing at night had a higher content of WSC than pasture grazing at day (85 *vs.* 57 g WSC/kg DM, respectively). The average nutritive value of both grass was 203 g CP/kg DM, 434 g NDF/kg DM, and 1·67 Mcal EN_l/kg DM.

The animals remained in good health throughout the experiment. The diets were well accepted by the animals, the TMR refusals amounted to <10% of the feed offered. Table 3 shows the body weight and dry matter intake (DMI) results. The average body weight was 648 kg, and the average daily weight variation (ADWV) during the experiment were − 0·02 ± 0·304 and + 1·27 ± 0·382 kg/d for animals grazing at day-time or night-time respectively ($P<0·01$). Growth rates were 0·58 ± 0·375 and 0·68 ± 0·382 kg/d for animals feeding MS and IRS, respectively, and did not differ significantly between diets.

The DMI decreased significantly in the cows grazing at day-time (23·53 *vs.* 25·89 kg DM/d for day-time and night-time respectively, $P<0·05$). No significant differences in the DMI associated with TMR, concentrate L or concentrate S were observed between any treatments. The DMI from grazing was 51% higher in the night-time grazing treatments (8·53 kg DM/d) than in the day-time grazing treatments (5·65 kg DM/d, $P<0·05$). However, for cows feeding the TMR based on IRS and grazed during the day, the grass intake was lower than those of other treatments, whereas in cows feeding with TMR based on IRS and grazed during night, their intake of grass was higher than the other treatments.

Table 4 shows the milk yield and composition for the four treatments. No differences were observed between treatments with respect to milk production, ranging from 29·6 kg/d until 32·5 kg/d. The milk fat content tended to be higher for the cows feeding TMR based on IRS and grazed at night-time (34·7 g/kg) than the others treatments (32·0 g/kg; $P<0·1$). No differences were found between treatments with respect to protein, lactose and urea contents.

The fatty acid profiles of milk collected from cows under the experimental treatments are shown in Table 5. The 18:1cis11 content was higher ($P<0·05$), and the 20:0 concentration was lower ($P<0·01$) in MS than IRS. The *n*-6 : *n*-3 ratio in dairy cows feeding TMR based on MS was 30% higher than cows feeding TMR based on IRS ($P<0·001$), without differences between grazing pattern.

Table 2. Chemical composition (g/kg DM) of the Total Mixed Rations based on maize silage (MS) or Italian ryegrass silage (IRS), concentrates L and S, and pasture. Values are means for $n = 28$

	MS	IRS	L	S	Pasture day	Pasture night
Dry matter	522	496	881	871	119	106
Organic matter	913	894	922	921	885	886
Crude protein	141	144	203	173	214	191
Neutral detergent acid	403	434	153	192	413	454
Acid detergent acid	251	263	79	100	208	230
Starch	196	150	414	408	NA†	NA†
Water soluble carbohydrate	NA†	NA†	NA†	NA†	56·9	84·6
NE_l‡ (Mcal/kg DM)	1·57	1·59	1·97	1·84	1·66	1·67
Fatty acids (g/100 g FA)						
C6:0	1·22	0·62	1·01	2·59	0·31	0·16
C7:0	BQL§	BQL	BQL	BQL	0·01	BQL
C8:0	0·06	0·04	BQL	BQL	0·06	0·04
C10:0	0·10	0·05	BQL	BQL	0·03	0·02
C11:0	0·01	0·01	BQL	BQL	BQL	BQL
C12:0	0·31	0·32	5·97	BQL	0·36	0·20
C14:0	0·56	0·64	1·51	BQL	0·54	0·37
C15:0	0·03	BQL	BQL	BQL	0·15	0·13
C16:0	25·39	25·83	40·6	48·9	16·98	16·58
C16:1	0·03	0·21	BQL	BQL	0·17	0·09
C17:0	0·02	0·01	BQL	BQL	BQL	BQL
C18:0	2·06	1·62	3·42	4·03	1·18	0·92
C18:1 cis9	12·16	9·37	25·5	30·6	1·32	1·46
C18:1 cis11	0·14	0·20	BQL	BQL	BQL	BQL
C18:2	45·94	38·60	21·6	13·9	14·00	15·77
C18:3	11·98	22·48	0·40	0·01	64·85	64·24
C20:0	BQL	BQL	BQL	BQL	0·04	0·03

† NA, Not analysed
‡ NE_l, Net energy of lactation
§ BQL, Below the limit of quantification

Table 3. Body weight and dry matter intake (DMI) for the four treatments. Values are means for $n = 64$ for body weight values, $n = 448$ for the dry matter intake variables

Grazing (G)	Day grazing		Night grazing					
Silage (S)	MS	IRS	MS	IRS	SEM	G	S	G × S
Body weight (kg)	646	646	652	649	29·8	NS‡	NS	NS
ADWV† (kg/d)	−0·1[a]	0·1[a]	1·3[b]	1·3[b]	0·75	**	NS	NS
DMI (kg/d)								
TMR	13·3	15·7	14·1	13·7	0·70	NS	NS	NS
Concentrate L	1·7	1·8	1·7	1·7	0·14	NS	NS	NS
Concentrate S	1·7	1·7	2·1	1·5	0·28	NS	NS	NS
Pasture	7·7[ab]	3·6[a]	7·8[ab]	9·2[b]	1·93	*	NS	*
Total	24·3[ab]	22·7[a]	25·7[b]	26·0[b]	1·94	*	NS	NS

† ADWV, Average daily weight variation
‡ NS, Not significant
[a,b]Values in the same row with different letters differ significantly
* $P < 0·05$; ** $P < 0·01$

The content of polyunsaturated fatty acid (PUFA) was higher in milk of dairy cows grazing at night-time than dairy cows grazing at day-time ($P < 0·01$). This higher proportion of PUFA is mainly due to the higher ($P < 0·05$) proportion of 18:2cis9trans11 and 18:2*n*-6. The milk of dairy cows grazing at night-time had higher concentrations of 18:1trans9, 16:1cis9 and 15:0 than the milk of dairy cows grazing at day-time ($P < 0·05$). The proportion of 18:0 was lower for

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Table 4. Milk yield and composition of milk for the four treatments. Values are means for $n=448$

Grazing (G)	Day grazing		Night grazing					
Silage (S)	MS	IRS	MS	IRS	SEM	G	S	G×S
Milk (kg/d)	29·6	31·4	32·5	30·4	2·59	NS[†]	NS	NS
Composition (g/kg)								
Fat	31·7	32·0	32·3	34·7	0·72	0·1	NS	NS
Protein	31·7	32·5	31·6	32·3	0·40	NS	NS	NS
Lactose	49·9	49·4	50·0	49·1	0·46	NS	NS	NS
Urea (mmol/L)	313	319	311	329	6·02	NS	NS	NS

† NS, Not significant

Table 5. Milk FA profile (g/100 gFA) for the four treatments. Values are means for $n=448$

Grazing (G)	Day grazing		Night grazing					
Silage (S)	MS	IRS	MS	IRS	SEM	G	S	G×S
6:0	2·39	2·28	2·11	2·31	0·066	NS[‡]	NS	NS
8:0	1·28	1·13	1·15	1·21	0·055	NS	NS	NS
10:0	2·66	2·19	2·37	2·43	0·164	NS	NS	NS
12:0	3·78	3·26	3·70	3·56	0·193	NS	NS	NS
13:1cis2	0·03	0·03	0·05	0·04	0·030	NS	NS	NS
14:0	12·85	12·53	13·05	13·00	0·377	NS	NS	NS
15:0	1·34[ab]	1·26[b]	1·53[a]	1·33[ab]	0·038	*	NS	NS
16:0	32·41	31·53	31·76	31·47	0·525	NS	NS	NS
16:1cis9	1·07[b]	1·11[ab]	1·49[a]	1·27[a]	0·085	*	NS	NS
16:1trans9	0·03	0·02	0·02	0·03	0·009	NS	NS	NS
17:0	0·59	0·56	0·63	0·57	0·018	NS	NS	NS
17:1cis10	0·15	0·15	0·18	0·14	0·013	NS	NS	NS
18:0	11·01[a]	11·45[a]	9·27[b]	10·41[ab]	0·441	*	NS	NS
18:1cis9	22·67	23·75	22·90	23·03	0·536	NS	NS	NS
18:1trans9	0·46[ab]	0·41[b]	0·54[a]	0·50[a]	0·023	*	NS	NS
18:1cis11	0·93[ab]	0·86[b]	1·06[a]	0·87[b]	0·039	NS	*	NS
18:1trans11	1·86	2·26	1·96	2·16	0·136	NS	NS	NS
18:1cis12	BQL[†]	BQL	0·07	0·06	0·268	NS	NS	NS
18:2*n*-6	2·07[b]	2·17[ab]	2·30[a]	2·44[a]	0·071	*	NS	NS
18:2cis9trans11	1·61[b]	1·88[ab]	2·14[a]	2·03[a]	0·104	*	NS	NS
18:2trans9trans12	BQL	0·04	0·01	0·04	0·022	NS	NS	NS
18:3*n*-3	0·31	0·45	0·32	0·45	0·018	NS	NS	NS
18:3*n*-6	0·03	0·03	0·03	0·03	0·002	NS	NS	NS
20:0	0·24	0·27	0·14	0·28	0·022	NS	**	NS
20:3*n*-6	0·02	0·01	BQL	BQL	0·007	NS	NS	NS
20:3*n*-3	0·05	0·04	0·05	0·05	0·028	NS	NS	NS
20:4*n*-6	0·02	0·03	0·04	0·04	0·011	NS	NS	NS
>20 carbons	0·26	0·30	0·28	0·25	0·059	NS	NS	NS
SFA	68·57	66·49	65·73	66·61	1·011	NS	NS	NS
MUFA	27·32	28·87	29·30	28·30	0·903	NS	NS	NS
PUFA	4·11[b]	4·64[b]	4·97[ab]	5·09[a]	0·236	**	NS	NS
SFA : UFA ratio	2·21	2·03	1·96	2·03	0·089	NS	NS	NS
n-6 : *n*-3 ratio	6·94[a]	4·92[b]	7·99[a]	5·59[b]	0·499	NS	***	NS
Trans-fatty acids	0·43[b]	0·45[b]	0·64[a]	0·54[a]	0·056	*	NS	NS

† BQL, below the limit of quantification
‡ NS, Not significant
[a,b]Values in the same row with different letters differ significantly
* $P<0·05$; ** $P<0·01$; *** $P<0·001$

dairy cows grazing during the night-time than for dairy cows were grazing at day-time (9·84 *vs.* 11·23 g/100 g FA respectively, $P<0·05$). The *trans* fatty acids content of the milk from dairy cows grazing at night-time was higher than that of cows grazing at day-time (0·59 *vs.* 0·44 g/100 g FA respectively, $P<0·05$), without differences between both silages.

Discussion

The food intake pattern is related to the photoperiod. Some authors have described patterns of behaviour in cattle where cows spend up to 75% of the time eating between 6·00 and 18·00 h (Ingvartsen, 1994). When the cows have access to food throughout the day, the highest eating activity is during the day-time, and the early evening and the lowest eating activity is during the late evening and early morning hours (DeVries et al. 2003). There is an increase in the eating activity immediately following the delivery of fresh feed (DeVries et al. 2003) and fresh grass (Abrahamse et al. 2009), and after milking sessions, especially after the afternoon milking event (Cuadrado et al. 2011). On average in our study, dairy cows ate more grass at the night-time grazing. Similar results were reported for dairy goats (Avondo et al. 2008), lambs (Vasta et al. 2012), and beef heifers (Gregorini et al. 2006). Abrahamse et al. (2009) did not find differences between grazing after morning or afternoon milking, while other authors (Loor et al. 2003) have observed a decrease in DMI at night in dairy cows. However, in the study of these authors, cows offset the lower intake of grass with an increased intake of TMR, while our animals consumed the same amount of TMR in all treatments. Traditionally, cattle have been thought to exhibit diurnal feeding patterns whereby they consume the majority of their daily DMI between dawn and dusk (Hafez & Boissou, 1975), and more specifically, with their largest and most extensive meals occurring at sunrise and sunset (Ruckebusch & Bueno, 1978). However, the longest and most intense grazing events occur at dusk, because the dairy cows are more motivated to access pasture at night (Charlton et al. 2013), and are often associated with a greater intake rate at this time of the day (Orr et al. 2001). This may be due to the increase in WSC of the grass at night (Avondo et al. 2008), making it more palatable to be sweeter the grass, as seen in the alfalfa hay (Burns et al. 2005) and grass silage (Huntington & Burns, 2007). The higher DMI at night-time grazing also justifies the higher ADWV observed for this treatment compared with the day-time grazing.

Despite the higher DMI of the night-time grazing cows, the different treatments had no effect on milk yield and the proportions of protein, lactose and urea in milk. These results agree with those reported by other authors who examined the feeding of cows with TMR plus the grazing of pasture (Morales-Almaráz et al. 2010), and different grazing time (Abrahamse et al. 2009). This absence of any difference in milk yield might be explained by the adequate net energy intake of the grazing cows. Differences in grass chemical composition within a single day have been recorded. These differences are due to the accumulation of simple sugars of photosynthetic origin (Orr et al. 1997), and thereby probably caused higher intake of sugar in night-time grazing than day-time grazing (Abrahamse et al. 2009) resulting in a higher milk fat content. The slight reduction in milk fat content observed in cows grazing at day-time may also be related with the lower NDF content of grass at night than at day (Vasta et al. 2012).

The fat of grasses is dominated by 18:3*n*-3 (Van Ranst et al. 2009), whereas lipids of maize are dominated by 18:2*n*-6 (Chilliard et al. 2001). According to these authors, in our study, the content of PUFA in milk did not differ due to the different proportions of 18:2*n*-6 and 18:3*n*-3 between both types of total mixed rations. Chilliard et al. (2001) reported differences in milk fat from maize silage or grass silage diets, because maize silage diets are richer in short-chain FA and 18:2*n*-6 than grass silage diets. Nevertheless, the maize silage could change ruminal fermentation towards propionate production because of the presence of maize grain (Latham et al. 1974), and decreasing the proportion of acetate and β-hydroxybutyrate, that are necessary for the *de novo* synthesis of short chain FAs in the mammary gland (Bauman & Griinari, 2003). However, we found no differences in the fatty acid profile between types of silages, except in 18:1cis11 and 20:0. Therefore, it is possible to speculate that FA of both silages could have had similar biohydrogenation pathways by microbial action in the rumen.

Fresh herbage is often the major source of 18:3*n*-3 and 18:2*n*-6 in grazing dairy cows, despite their relatively low FA content. Grazing dairy cows ingest higher quantities of these PUFA than cows feeding only TMR (Schroeder et al. 2003). A high content of PUFA in rumen could affect the biohydrogenation of 18:2*n*-6 (Troegeler-Meynadier et al. 2006) and, therefore, could also affect the presence of intermediate products in milk, with higher content of 18:1trans11, 18:2cis9trans11 and 18:3*n*-3 in milk. Regardless of the type of silage, in the present study, the cows with access to pasture at night ate more grass on average than dairy cows grazing at day-time. Consequently, the milk fat from those cows had higher contents of PUFA, especially 18:2*n*-6 and 18:2cis9trans11, than the cows with access to pasture at day-time. When the animals are allowed to graze in the afternoon compared with those grazing in the morning, a lower degree of biohydrogenation of PUFA has been described (Avondo et al. 2008). These authors attributed this effect to lower nitrogen availability, due to the higher ratio between water soluble carbohydrates and crude protein, in the pasture during the afternoon. However, the highest pasture intake was observed in cows grazing at night-time on IRS treatment, while the pasture DMI in cows on MS treatment was similar in both grazing sessions. This could be due to the higher degradation rate of IRS than MS (González et al. 2009, 2010), which would increase the rumen passage rate, and hence, implies higher intake of grass. In spite of what has just been said, the lowest pasture intake was observed in cows on IRS treatment and grazing at day-time. A lower palatability due to less pasture sweetness because of the lower WSC content of grass sampled at day-time could explain these results (Avondo et al. 2008).

Avondo et al. (2008) did not observe differences in PUFA and SFA in milk of goats grazing in the morning or in the afternoon, while in our study a higher content of PUFA in milk of cows grazing at night-time was observed. The increase of grass intake should also have led a higher response in concentrations of 18:3*n*-3 and 18:1trans11 in dairy cows grazing at night-time compared with grazing at day-time. However, milk fatty acid composition arises not only from the fatty acids ingested but also from the depletion of adipose tissues, and this could have masked or mitigated the effect of the different grazing times (day or night), in spite of our animals not being in negative balance energy, hence that contribution was very low.

In conclusion, cows grazing mixed pastures increased DMI during night. The milk yield was similar in both grazing time and type of silage used, however, an incomplete biohydrogenation of herbage fatty acids increases concentrations of 18:2*n*-6 and 18:2cis9trans11 in milk fat from cows grazing at night-time.

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References

Abrahamse PA, Tamminga S & Dijkstra J 2009 Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. *Journal of Agricultural Science* **147** 721–730

AbuGhazaleh AA, Felton DO & Ibrahim SA 2007 Milk conjugated linoleic acid response to fish oil and sunflower oil supplementation to dairy cows managed under two feeding systems. *Journal of Dairy Science* **90** 4763–4769

Auldist MJ, Marett LC, Greenwood JS, Hannah M, Jacobs JL & Wales WJ 2013 Effects of different strategies for feeding supplements on milk production responses in cows grazing a restricted pasture allowance. *Journal of Dairy Science* **96** 1218–1231

Avondo M, Bonanno M, Pagano RI, Valenti B, Di Grigoli A, Alicata ML, Galofaro V & Pennisi P 2008 Milk quality as affected by grazing time of day in Mediterranean goats. *Journal of Dairy Research* **75** 48–54

Bargo F, Muller LD, Delahoy JE & Cassidy TW 2002 Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science* **85** 2948–2963

Bargo F, Delahoy JE, Schroeder GF & Muller LD 2006 Milk fatty acid composition of dairy cows grazing at two pasture allowances and supplemented with different levels and sources of concentrate. *Animal Feed Science and Technology* **125** 17–31

Bauman DE & Griinari JM 2003 Nutritional regulation of milk fat synthesis. *Annual Review of Nutrition* **23** 203–227

Belury MA 2002 Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annual Review of Nutrition* **22** 505–531

Bendall JG 2001 Aroma compounds of fresh milk from New Zealand cows fed different diets. *Journal of Agricultural and Food Chemistry* **49** 4825–4832

Browse J, Roughan PG & Slack CR 1981 Light control of fatty acid synthesis and diurnal fluctuations of fatty acid composition in leaves. *Biochemical Journal* **196** 347–354

Burns JC, Mayland HF & Fisher DS 2005 Dry matter intake and digestion of alfalfa harvested at sunset and sunrise. *Journal of Animal Science* **83** 262–270

Capper JL, Cady RA & Bauman DE 2009 The environmental impact of dairy production: 1944 compared with 2007. *Journal of Animal Science* **87** 160–2167

Chapinal N, Goldhawk C, de Passillé AM, von Keyserlingk MAG, Weary DM & Rushen J 2010 Overnight access to pasture does not reduce milk production or feed intake in dairy cattle. *Livestock Science* **129** 104–110

Charlton GL, Rutter SM, East M & Sinclair LA 2013 The motivation of dairy cows for access to pasture. *Journal of Dairy Science* **96** 4387–4396

Chilliard Y, Ferlay A & Doreau M 2001 Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science* **70** 31–48

Chouinard PY, Corneau L., Barbano DM, Metzger LE & Bauman DE 1999 Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. *Journal of Nutrition* **129** 1579–1584

Christie WW 1982 A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. *Journal of Lipid Research* **23** 1072–1075

Couvreur S, Hurtaud C, Lopez C, Delaby L & Peyraud JL 2006 The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *Journal of Dairy Science* **89** 1956–1969

Cuadrado F, Morales-Almaráz E, de la Roza-Delgado B, Martínez-Fernández A & Vicente F 2011 Voluntary unifeed ration intake by grazing dairy cows according their physiologic state. In *Proceedings of the 15th ESVCN Congress* 153

DeVries TJ, von Keyserlingk MAG & Beauchemin KA 2003 Diurnal feeding pattern of lactating dairy cows. *Journal of Dairy Science* **86** 4079–4082

Dewhurst RJ, Shingfield KJ, Lee MRF & Scollan ND 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

Elgersma A, Ellen G, Bekker PR, van der Horst H, Boer H & Tamminga S 2003 Effects of perennial ryegrass (*Lolium perenne*) cultivars with different linolenic acid contents on milk fatty acid composition. *Aspects of Applied Biology* **70** 107–114

Feng S, Lock AL & Garnsworthy PC 2004 Technical note: a rapid lipid separation method for determining fatty acid composition of milk. *Journal of Dairy Science* **87** 3785–3788

Ferlay A, Martin B, Pradel Ph, Coulon JB & Chilliard Y 2006 Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. *Journal of Dairy Science* **89** 4026–4041

González J, Centeno C, Moruji N, Faria-Mármol J & Martínez A 2009 In situ ruminal amino acid degradability of green and ensiled Italian rye-grass according to particle transit model and microbial contamination correction. *Livestock Science* **123** 209–214

González J, Faria-Mármol J, Arroyo JM, Centeno C & Martínez A 2010 Effects of ensiling on in situ ruminal degradability and intestinal digestibility of corn forage. *Archives of Animal Nutrition* **64** 204–220

Gregorini P, Eirin M, Refi R, Ursino M, Ansin O & Gunter SA 2006 Timing of herbage allocation: effect on beef heifers daily grazing pattern and performance. *Journal of Animal Science* **84** 1943–1950

Gregorini P, Soder KJ & Sanderson MA 2008 Case study: a snapshot in time of fatty acids composition of grass herbage as affected by time of day. *The Professional Animal Scientist* **24** 1–6

Gregorini P, Soder KJ, Sanderson MA & Ziegler GR 2009 Toughness, particle size and chemical composition of meadow fescue (*Festuca pratensis* Hud.) herbage as affected by time of day. *Animal Feed Science and Technology* **151** 330–336

Hafez ESE & Boissou MF 1975 The behaviour of cattle. In *The Behaviour of Domestic Animals*, pp. 203–245 (Ed. ESE Hafez). London, UK: Bailliere Tindall

Hernandez-Mendo O, von Keyserlingk MAG, Veira DM & Weary DM 2007 Effects of pasture on lameness in dairy cows. *Journal of Dairy Science* **90** 1209–1214

Hoffman WS 1937 A rapid photoelectric method for the determination of glucose in blood and urine. *Journal of Biological Chemistry* **120** 51–55

Huntington GB & Burns JC 2007 Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switchgrass baleage by beef steers. *Journal of Animal Science* **85** 276–284

Huzzey JM, Veira DM, Weary DM & von Keyserlingk MAG 2007 Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. *Journal of Dairy Science* **90** 3220–3230

Ingvartsen KL 1994 Models of voluntary food intake in cattle. *Livestock Production Science* **39** 19–38

Kennedy E, Curran J, Mayes B, McEvoy M, Murphy JP & O'Donovan M 2011 Restricting dairy cow access time to pasture in early lactation: the effects on milk production, grazing behaviour and dry matter intake. *Animal* **5** 1805–1813

Latham MJ, Sutton JD & Sharpe ME 1974 Fermentation and microorganisms in the rumen and the content of fat in the milk of cows given low roughage rations. *Journal of Dairy Science* **57** 803–810

Legrand AL, von Keyserlingk MAG & Weary DM 2009 Preference and usage of pasture *versus* free-stall housing by lactating dairy cattle. *Journal of Dairy Science* **92** 3651–3658

Loor JJ, Soriano FD, Lin X, Hirbein JH & Polan CE 2003 Grazing allowance after the morning or afternoon milking for lactating cows fed a total mixed ration (TMR) enhances trans11–18:1 and cis9,trans11–18:2 (rumenic acid) in milk fat to different extents. *Animal Feed Science and Technology* **109** 105–119

Macoon B, Sollenberger LE, Moore JE, Staples CR, Fike JH & Portier KM 2003 Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. *Journal of Animal Science* **81** 2357–2366

Martin B, Hurtaud C, Graulet B, Ferlay A, Chilliard Y & Coulon JB 2009 Grass and the nutritional and organoleptic qualities of dairy products. *Fourrages* **199** 291–310

Morales-Almaráz E, Soldado A, González A, Martínez-Fernández A, Domínguez-Vara I, de la Roza-Delgado B & Vicente F 2010 Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. *Journal of Dairy Research* **77** 225–230

Morales-Almaráz E, de la Roza-Delgado B, González A, Soldado A, Rodríguez ML, Peláez M & Vicente F 2011 Effect of feeding system on unsaturated fatty acid level in milk of dairy cows. *Renewable Agriculture and Food Systems* **26** 224–229

National Research Council 2001 *Nutrient Requirements of Dairy Cattle*, 7th rev. edition. Washington, DC: National Academy Press

Orr RJ, Penning PD, Harvey A & Champion RA 1997 Diurnal patterns of intake rate by sheep grazing monocultures of ryegrass or white clover. *Applied Animal Behaviour Science* **52** 65–77

Orr RJ, Rutter SM, Penning PD & Rook AJ 2001 Matching grass supply to grazing patterns for dairy cows. *Grass and Forage Science* **56** 352–361

Palmquist DL & Jenkins TC 2003 Challenges with fats and fatty acid methods. *Journal of Animal Science* **81** 3250–3254

Ruckebusch Y & Bueno L 1978 An analysis of ingestive behavior and activity of cattle under field conditions. *Applied Animal Ethology* **4** 301–313

SAS 1999 *SAS/STATTM. User's Guide*. Cary, NC: Statistical Analysis System Inst

Schroeder GF, Delahoy JE, Vidaurret I, Bargo F, Gagliostro GA & Muller LD 2003 Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *Journal of Dairy Science* **86** 3237–3248

Soriano FD, Polan CE & Miller CN 2001 Supplementing pasture to lactating holsteins fed a total mixed ration diet. *Journal of Dairy Science* **84** 2460–2468

Stockdale CR, Waler GP, Wales WJ, Dalley DE, Birkett A, Shen Z & Doyle PT 2003 Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. *Journal of Dairy Research* **70** 267–276

Troegeler-Meynadier A, Bret-Bennis L & Enjalbert F 2006 Rates and efficiencies of reactions of ruminal biohydrogenation of linoleic acid according to pH and polyunsaturated fatty acids concentrations. *Reproduction Nutrition Development* **46** 713–724

Van Ranst G, Fievez V, De Riek J & Van Bockstaele E 2009 Influence of ensiling forages at different dry matters and silage additives on lipid metabolism and fatty acid composition. *Animal Feed Science and Technology* **150** 62–74

Vasta V, Pagano RI, Luciano G, Scerra M, Caparra P, Foti F, Cilione C, Biondi L, Priolo A & Avondo M 2012 Effect of morning *vs.* afternoon grazing on intramuscular fatty acid composition in lamb. *Meat Science* **90** 93–98

Vibart RE, Fellner V, Burns JC, Huntington GB & Green JT Jr 2008 Performance of lactating dairy cows fed varying levels of total mixed ration and pasture. *Journal of Dairy Research* **75** 471–480