Short communication: Intake, milk production, and milk fatty acid profile of dairy cows fed diets combining fresh forage with a total mixed ration

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ABSTRACT

To establish the effects of access time to high-quality temperate fresh forage (FF) on intake and performance of cows fed a total mixed ration (TMR), 9 Holstein cows were assigned to three 3 × 3 Latin squares with 20-d periods with sampling in the last 10 d. The following treatments were evaluated: 0 (T0), 4 (T4), and 8 (T8) h of daily access to FF. Forage (*Lolium multiflorum*) was cut daily and offered ad libitum beginning at 0800 h in individual stalls, and a TMR was offered ad libitum during the period when cows had no access to FF. Fresh forage dry matter intake (DMI) increased and TMR DMI decreased with the access time to FF, whereas total DMI was 3.0 kg higher for T4 than for T8; no differences were detected with T0. Compared with T0, 8 h/d of access to FF decreased milk, protein, and casein yields, and tended to decrease fat and lactose yields, but treatment had no effect on milk composition or feed efficiency. The milk fatty acid profile in T8 had a higher content of rumenic, vaccenic, and linolenic acids compared with T0. We concluded that cows with 4 h of access to high-quality FF had similar DMI and milk yields as cows fed only the TMR, but more than 4 h of access reduced DMI and performance without changes in feed efficiency, although milk fat had enhanced levels of beneficial fatty acids.

Key words: total mixed ration, fresh forage, rumenic acid

Short Communication

In recent years, there has been renewed interest in the inclusion of fresh forage (FF) in the diet of cows worldwide. This interest was triggered by increasing

housing, labor, and feeding costs related to TMR-fed cows, which are usually managed in indoor housing systems. Feeding cows with FF also increases the proportion of certain fatty acids [e.g., rumenic acid (RA) and vaccenic acid (VA)] that may have nutritional benefits by promoting good health and preventing disease (Elgersma et al., 2006). However, one limitation when feeding FF to dairy cows is the difficulty of achieving high levels of DMI. Compared with that in TMR-fed cows, DMI is lower in grazing cows, which partially explains their lower milk yield (Kolver and Muller, 1998; Bargo et al., 2002). However, recent studies (Vibart et al., 2008; Chapinal et al., 2010) have not reported decreases in intake or milk yield in grazing cows supplemented with a TMR compared with TMR-fed cows. Although this finding may be explained by the actual proportion of FF and TMR in the diet or its nutritive value, the relative contribution of each factor is unknown. Thus, it may be possible to find a combination of FF and TMR that provides a high level of milk yield while retaining the advantages that FF confers in terms of obtaining milk with a higher content of beneficial fatty acids. Restricting the number of hours of access to FF may be an easy tool that could be used to design better feeding strategies, but research on this topic is limited. Morales-Almaráz et al. (2010) reported no differences in total DMI or milk yields of dairy cows fed TMR only or with 6 or 12 h of access to a mixed pasture of grasses and legumes, but they observed linear increases in rumenic and linolenic acids in milk fat with increased grazing time. Kristensen et al. (2007) compared 3 different grazing times (4, 6.5, or 9 h/d) in TMR-fed cows and reported that milk yield increased as time on pasture increased from 4 to 9 h. Because pasture intake was not directly measured in those experiments, it is unclear whether these discrepancies reflect differences in total DMI or differences in the actual proportion of FF in the diet. It could be hypothesized that an excessive time of access to FF would reduce DMI and milk yield but increase the content of beneficial fatty acids in milk fat. Therefore, the objectives of this experiment were to determine changes in intake, milk yield, and

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milk composition of TMR-fed cows with varying times of access to FF.

The experiment was performed from June to August 2011 at the Experimental Station of the Veterinary Faculty (Universidad de la República, Uruguay) in San José, Uruguay (34°40' S, 56°32' W), and complied with regulations of the Bioethics Committee of the Veterinary Faculty. Nine multiparous Holstein cows with previous grazing experience were selected from the station herd (a total of 91) and were blocked into 3 squares according to BW [572 ± 76 (SD) kg], previous 305-d milk yield ($7,079 \pm 1226$ kg), DIM (100 ± 25 d), and parity (4.3 ± 1.2). Within each square, the cows were randomly assigned to treatment sequences according to a replicated 3×3 Latin square design. Each experimental period lasted 20 d, and consisted of 10 d for adaptation and 10 d for data and sample collection. A single TMR (Table 1) was formulated to meet the requirements of a 600-kg cow producing 30 kg/d of milk (NRC, 2001) and was fed to all cows. The following treatments were evaluated: 0 h of access to FF plus 24 h of access to TMR (T0), 4 h of access to FF plus 20 h of access to TMR (T4), or 8 h of access to FF plus 16 h of access to TMR (T8). The cows were housed in individual tiestalls (2.0×1.3 m) with sand bedding, located in a barn with natural light, and were milked at 0700 and 1800 h. The forage (*Lolium multiflorum* var. INIA Bakarat) was obtained from the same pastureland. It was seeded (15 kg/ha) on March 3, 2011, and was fertilized with 27 kg of N/ha and 69 kg of P/ha as diammonium phosphate. The pastureland was divided in 3 paddocks, with 1 paddock being used for each experimental period. Each paddock was cut to a height of 5 cm 30 d before the beginning of each period to allow for similar regrowth periods; 28 kg of N as urea was applied after each cut. The average herbage mass for the 3 periods was $2,413 \pm 552$ kg of DM/ha. All forage used was in a vegetative stage, with a height between 20 and 25 cm. Herbage was harvested daily at 0700 h with a rotary drum mower, with a residual height of 10 cm. The FF was offered, without chopping, to animals in the T4 and T8 treatments from 0800 to 1200 h and from 0800 to 1600 h, respectively. To ensure that the amount of feed was not limiting at any time, the feed trough was observed each 30 min and more feed was added if necessary. From 1200 to 0700 h and from 1700 to 0700 h, the T4 and T8 cows, respectively, had access to TMR, and it was offered as previously described for the FF. Cows in the T0 group had ad libitum access to TMR all day. Each day at 0800 h (hereafter, h 0), refusals from the previous 24 h were removed from the feed trough and new feed was offered. Meteorological data were obtained from a meteorological station located 18

Table 1. Ingredients and nutrient composition (SD in parentheses) of TMR and fresh forage (% of DM, unless otherwise stated)

Item	TMR	Fresh forage
Ingredients of TMR		
Corn silage	45.2	—
Ground corn grain	31.6	—
Solvent-extracted soybean meal	21.3	—
Additives premix ¹	1.94	—
Vitamin and mineral premix ²	0.04	—
Nutrient composition		
DM, % of amount fed	35.8 (2.9)	15.3 (0.7)
OM	92.0 (0.3)	90.1 (0.2)
NDF	40.3 (3.3)	47.1 (2.6)
ADF	22.9 (0.8)	26.5 (2.2)
ADL	2.7 (0.3)	4.4 (0.7)
NFC	34.0 (3.0)	23.7 (2.1)
Ether extract	1.8 (0.3)	2.3 (0.1)
CP	16.1 (0.3)	17.1 (0.9)
NDIN	0.55 (0.07)	1.32 (0.07)
ADIN	0.19 (0.02)	0.23 (0.04)
NE _L , Mcal/kg of DM	1.55 (0.04)	1.46 (0.01)
Particle size distribution, % as fed		
>19 mm	2.8 (0.9)	—
8 to 19 mm	50.5 (0.3)	—
1.8 to 8 mm	40.4 (1.5)	—
<1.8 mm	6.3 (0.3)	—

¹Provided (per kg of DM): 6 g of sodium bicarbonate, 4 g of dicalcium phosphate, 3 g of urea, 2 g of calcium carbonate, 2 g of salt, 2 g of magnesium oxide, and 0.4 g of a mycotoxin adsorbent.

²Provided (per kg of DM): 0.85 g of Cu, 2.6 g of Zn, 0.9 g of Se, 1.0 g of Mn, 23 mg of I, 3 mg of Co, 63,700 IU of vitamin A, 12,700 IU of vitamin D, and 250 IU of vitamin E.

km from the experimental site. The mean temperatures at a 2-m height in periods 1, 2, and 3 were 8.5, 9.5, and 11.0°C, respectively. Sunrise occurred at 0753 h, and sunset occurred at 1742 h on the first day of period 1, and at 0717 h and 1819 h, respectively, during the last day of period 3. Lights in the barn were on from sunset (approximately 1800 h) until 2100 h.

In each period, daily intake was measured between d 13 and 20 by weighing the amounts of feed offered and refused. Before 0800 h, the amount of TMR refused by the cows (i.e., all treatments) was weighed, and at 1200 h and 1600 h, the amount of fresh forage refused by the T4 and T8 cows was weighed. Samples of TMR and fresh forage were taken at 0800, 1200, and 1600 h and composited to obtain one sample per day. For the TMR samples, particle size distributions were assessed using the modified Penn State Particle Size separator (Kononoff et al., 2003). Approximately 20% of feed refusals from each cow were sampled. All samples were kept frozen at -20°C until analysis. Drinking water intake was measured between d 11 and 13 in each period by weighing the amounts of water offered and refused. Total water intake was calculated as the sum of drinking water intake plus water intake from each feed. All feed samples were dried in a forced-air oven at 60°C and then

ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA). Samples were analyzed for DM, OM, total N, and ether extract (AOAC, 1990; methods 934.01, 942.05, 955.04, and 920.39, respectively), NDF (using heat-stable α -amylase and sodium sulfite), ADF, and ADL (Van Soest et al., 1991), expressed exclusive of residual ash, and NDIN and ADIN (Licitra et al., 1996). The concentration of NFC was estimated as $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ ash})$. The NE_L concentration for each feed was calculated according to NRC (2001) from feed analyses, actual DMI, and individual cow characteristics during each period. Milk yield was recorded daily during each period. Yield of 3.5% FCM was calculated according to Tyrrell and Reid (1965), and feed efficiency was calculated as 3.5% FCM yield/DMI. Individual milk samples with bronopol preservative were obtained from each milking from d 14 to 19 during each period, and were used to determine fat, protein, total casein, lactose, and MUN by infrared analysis (model 2000, Bentley Instruments Inc., Chaska, MN). Two additional individual milk samples were taken without preservatives on d 14 from each milking and stored at -20°C until analyzed for fatty acid composition. For fatty acid analysis, frozen milk samples were thawed at room temperature, and milk lipids were separated according to Feng et al. (2004). An aliquot of 50 mg of milk fat was dissolved in 100 μL of hexane, followed by esterification with 100 μL of 2 *N* potassium hydroxide in methanol to obtain FAME, which were separated and quantified using a GC-MS (Agilent 7890A GC System, Agilent Technologies Inc., Santa Clara, CA) equipped with a 60-m column (250- μm i.d., 0.25- μm film thickness; Thermo Scientific Inc., Marietta, OH). Helium was used as the carrier gas, with a flow rate of 1.0 mL/min. The injector temperature (split ratio of 100:1) was set at 250°C . The initial column temperature (40°C) was held for 0.5 min, increased at $25^\circ\text{C}/\text{min}$ to 175°C , held for 10 min, increased at $5^\circ\text{C}/\text{min}$ to 210°C , held for 5 min, increased at $5^\circ\text{C}/\text{min}$ to 230°C , and held for 5 min. Fatty acids were identified by comparing their retention times with the following FAME standards: 37 Component FAME Mix (47885, Supelco, Bellefonte, PA), *trans*-11-octadecenoic methyl ester (46905-U, Supelco), and octadecadienoic acid, conjugated methyl ester (O5632, Sigma-Aldrich, St. Louis, MO), and those stored in the National Institute of Standards and Technology (US Government library). The Δ^9 -desaturase index and the atherogenicity index were calculated as described by Kelsey et al. (2003) and Ulbricht and Southgate (1991), respectively. Data were averaged per period and analyzed using PROC MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC) with a model that included

the random effect of square, the random effect of cow nested in square, the random effect of period, and the fixed effect of treatment (Kaps and Lamberson, 2004). Residuals were tested for normal distribution. Means were separated with the Tukey test; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Although FF DMI increased as the access time to FF increased, total DMI was higher in T4 than T8, but T4 did not differ from T0, whereas intakes of OM, CP, and NFC were higher in T0 and T4 than in T8 (Table 2). The intakes of NDF and ether extract tended to be higher in T4 than in T8 but did not differ from that of T0. It is remarkable that FF represented only 11 and 16% of total DMI in T4 and T8, respectively. For T8 cows, DMI during the first 8 h after the beginning of the feeding bout, as a percentage of daily total DMI, was only 9.7%, which suggests that these animals may have faced constraints that prevented them from achieving a high DMI rate when offered the FF. One possible explanation for these findings could be related to the low DM content of the FF. Cabrera Estrada et al. (2004) observed that a reduction in FF intake of dairy cows due to a high content of internal water intake was associated with a decrease in DMI rate, but modifications on rumen fill or a more difficult prehension of feed particles could also be involved. Although the T4 cows may have experienced this constraint, they had 4 h more time for eating the TMR than the T8 cows. It is probable that, because of the higher DM content of the TMR, a higher DMI rate was achieved when the cows were offered this feed, which may have allowed the T4 cows to maintain a similar nutrient intake as in T0. Morales-Almaráz et al. (2010) fed cows with a TMR offered ad libitum in combination with 0, 6, or 12 h of grazing time of a high-quality pasture and they reported no depression in total DMI, even when pasture contributed up to 37% of the DM in the diet. However, Vibart et al. (2008) reported linear decreases in total DMI with increasing inclusion of a high-quality pasture in the diets of TMR-fed cows (up to a level of 35% of total DM), although CP intake did not differ among treatments. When Vibart et al. (2008) used the same pasture but in a more mature stage and with a lower CP content than the TMR, DMI decreased as the proportion of pasture in the diet increased. This finding highlights the influence of FF quality on the overall response of dairy cows, suggesting that, in our experiment, the high quality of the FF may also have contributed, at least for T4, to maintaining a similar nutrient intake as in T0. Drinking water intake was higher in T0 and T4 than in T8, and water intake from feeds was higher in T4 and T8 than in T0. Total water intake was higher in T4 than in T8 (Table 2).

Table 2. Dry matter, nutrient, and water intakes

Item	Treatment ¹			SEM	P-value
	T0	T4	T8		
DMI, kg/d					
Fresh forage	0 ^a	2.8 ^b	3.6 ^c	0.48	<0.001
TMR	24.5 ^a	22.8 ^a	19.0 ^b	1.54	0.002
Total	24.5 ^{ab}	25.6 ^a	22.6 ^b	1.58	0.048
Fresh forage in total DM, %	0 ^a	10.9 ^b	16.4 ^c	1.54	<0.001
Nutrient intake, kg/d					
OM	22.9 ^a	23.8 ^a	20.9 ^b	1.52	0.045
CP	4.2 ^a	4.3 ^a	3.8 ^b	0.24	0.046
NDF	9.7 ^{xy}	10.4 ^x	9.2 ^y	0.44	0.079
ADF	5.6 ^{ab}	5.9 ^a	5.3 ^b	0.38	0.040
NFC	8.6 ^a	8.6 ^a	7.5 ^b	0.88	0.015
Ether extract	0.43 ^{xy}	0.46 ^x	0.42 ^y	0.07	0.065
Water intake, L/d					
Drinking	65.0 ^a	63.5 ^a	48.5 ^b	5.40	0.008
Fresh forage	0 ^c	15.1 ^b	19.4 ^a	2.43	<0.001
TMR	41.5 ^a	39.5 ^a	33.5 ^b	3.60	0.003
Feeds	41.5 ^b	54.5 ^a	52.9 ^a	3.86	<0.001
Total	106.5 ^{ab}	118.0 ^a	101.4 ^b	8.94	0.044

^{a-c}Within a row, means with different superscripts are different ($P < 0.05$).

^{x,y}Within a row, means with different superscripts are different ($P < 0.10$).

¹T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

Previous studies have reported that the inclusion of FF in the diet of TMR-fed cows is accompanied by reductions in milk and solids yields, which is mainly explained by a reduction in DMI (Kolver and Muller, 1998; Bargo et al., 2002). However, in our study, decreases in 3.5% FCM, protein, and total casein yields were observed only when cows were fed FF for more than 4 h/d, but there were no differences in milk yield or composition between T0 and T4 (Table 3). Previous reports that did not detect differences in milk or FCM yield among cows fed a TMR with or without access to FF also failed to detect any differences in DM or nutrient intake (Chapinal et al., 2010; Morales-Almaráz et al., 2010). Vibart et al. (2008) reported a reduction in milk yield of cows only when the inclusion of FF led to a reduction in total DMI, which occurred when a pasture of lower quality than the TMR was used. Treatments did not affect milk composition (Table 3), which is coincident with results reported by Bargo et al. (2002). As a result, solids yield followed the same trend as milk yield, reflecting the different ingestion of precursors of milk components. Although MUN values were expected to be higher in T0 than T8 because of the higher CP intake in the former, we observed no differences among treatments (Table 3). High CP degradability of FF (NRC, 2001) probably led to a similar ingestion of RDP among treatments despite the lower CP intake of T8 cows and, because the level of RDP intake affects MUN values (Reynal and Broderick, 2005), this could explain

the lack of effect of treatments on MUN. The effects of including FF in the diet of TMR-fed cows on feed efficiency are inconsistent, with experiments reporting either an increase or a decrease in feed efficiency as the proportion of FF in the diet is increased (Wales

Table 3. Milk yield and composition

Item	Treatment ¹			SEM	P-value
	T0	T4	T8		
Milk, kg/d	34.4 ^a	34.9 ^a	32.7 ^b	1.50	0.027
3.5% FCM, kg/d	37.6 ^a	37.3 ^a	35.1 ^b	1.17	0.012
Fat, kg/d	1.40 ^x	1.37 ^{xy}	1.29 ^y	0.048	0.068
Fat, %	4.10	3.95	3.99	0.224	0.604
Protein, kg/d	1.13 ^a	1.16 ^a	1.06 ^b	0.039	0.022
Protein, %	3.32	3.35	3.25	0.125	0.118
Total casein, kg/d	0.86 ^a	0.88 ^a	0.80 ^b	0.032	0.026
Total casein, %	2.51	2.54	2.46	0.112	0.195
Casein:protein ratio	0.76	0.76	0.76	0.005	0.827
Lactose, kg/d	1.70 ^{xy}	1.72 ^x	1.62 ^y	0.079	0.058
Lactose, %	4.95	4.93	4.95	0.057	0.674
MUN, mg/dL	21.2	19.8	18.9	0.97	0.140
Feed efficiency ²	1.56	1.46	1.57	0.072	0.372

^{a,b}Within a row, means with different superscripts are different ($P < 0.05$).

^{x,y}Within a row, means with different superscripts are different ($P < 0.10$).

¹T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

²3.5% FCM yield (kg/d)/DMI (kg/d).

et al., 2013). In our trial, the similar feed efficiencies among treatments suggest that the observed differences in milk yield were primarily due to differences in nutrient intake rather than changes in nutrient digestion or utilization (Table 3), as described by Kolver and Muller (1998). This highlights the quality of the FF used in the present experiment, which permitted a nutrient digestion comparable to that of T0, a result also reported by Bargo et al. (2002). It should be noted that in our study

the cows had no energy expenditure due to walking or grazing, as would occur in a pasture-based system, and were likely able to divert more energy to milk synthesis than if they actually had grazed. Nevertheless, this is a promising result because it suggests the possibility of including a low-cost feed such as FF (provided it is of high quality) in the diet of dairy cows and achieving a feed efficiency similar to that of cows fed only a TMR. Moreover, it highlights the need for additional research

Table 4. Milk fatty acid (FA) profile and components

Item	Treatment ¹			SEM	P-value
	T0	T4	T8		
FA content (g/100 g of total FA)					
Selected individual FA					
4:0	1.95 ^x	1.66 ^{xy}	1.48 ^y	0.130	0.080
6:0	2.03 ^a	1.76 ^{ab}	1.56 ^b	0.112	0.029
8:0	1.66 ^a	1.50 ^{ab}	1.32 ^b	0.089	0.022
10:0	4.14 ^x	3.91 ^{xy}	3.42 ^y	0.245	0.056
12:0	4.95	4.91	4.38	0.304	0.120
14:0	13.60	13.58	12.83	0.403	0.221
14:1 <i>cis</i> -9	1.45	1.47	1.43	0.121	0.870
16:0	32.53	32.18	31.74	1.452	0.653
16:1 <i>cis</i> -9	1.81 ^a	1.90 ^{ab}	2.07 ^b	0.129	0.047
18:0	9.39	9.40	9.96	0.960	0.569
18:1 <i>cis</i> -9 ²	19.00 ^a	19.51 ^a	21.14 ^b	0.595	0.031
18:1 <i>trans</i> -9	0.30	0.35	0.35	0.028	0.330
18:1 <i>trans</i> -11 ³	1.24 ^a	1.52 ^{ab}	1.69 ^b	0.145	0.019
18:2 <i>cis</i> -9, <i>cis</i> -12	2.63	2.68	2.64	0.154	0.881
18:2 <i>cis</i> -9, <i>trans</i> -11 ⁴	0.49 ^a	0.65 ^{ab}	0.77 ^b	0.047	0.003
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 ⁵	0.34 ^x	0.41 ^{xy}	0.49 ^y	0.048	0.062
Summation by origin					
De novo (4:0–15:0)	31.59 ^x	30.63 ^{xy}	28.20 ^y	1.214	0.097
Mixed origin (16:0 + 16:1)	34.34	34.08	33.81	1.452	0.807
Preformed (>17:0)	34.07 ^x	35.29 ^{xy}	37.88 ^y	1.877	0.065
Summation by saturation					
SFA	72.16 ^a	70.90 ^{ab}	68.71 ^b	0.734	0.020
MUFA	24.39 ^a	25.36 ^{ab}	27.29 ^b	0.659	0.025
PUFA	3.46	3.74	3.90	0.190	0.133
Saturated:unsaturated ratio	2.61 ^a	2.44 ^{ab}	2.21 ^b	0.086	0.023
n-6:n-3 ratio	8.03 ^a	6.77 ^{ab}	5.64 ^b	0.735	0.035
Δ^9 -desaturase ratio					
14:1/14:0	0.106	0.108	0.111	0.0077	0.655
16:1/16:0	0.056 ^x	0.059 ^{xy}	0.066 ^y	0.0036	0.069
18:1/18:0	2.219	2.309	2.343	0.1363	0.383
18:2 <i>cis</i> -9, <i>trans</i> -11/18:1 <i>trans</i> -11	0.400 ^a	0.431 ^b	0.453 ^b	0.0226	0.002
Δ^9 -desaturase index ⁶	0.29 ^a	0.29 ^a	0.31 ^b	0.006	0.015
Atherogenicity index ⁷	3.33 ^a	3.15 ^{ab}	2.81 ^b	0.147	0.043

^{a,b}Within a row, means with different superscripts are different ($P < 0.05$).

^{xy}Within a row, means with different superscripts are different ($P < 0.10$).

¹T0 = 24 h of access to TMR; T4 = 4 h of access to fresh forage plus 20 h of access to TMR; T8 = 8 h of access to fresh forage plus 16 h of access to TMR.

²Oleic acid.

³Vaccenic acid.

⁴Rumenic acid.

⁵Linolenic acid.

⁶Calculated as (14:1 *cis*-9 + 16:1 *cis*-9 + 18:1 *cis*-9 + 18:2 *cis*-9,*trans*-11)/(14:0 + 16:0 + 18:0 + 18:1 *trans*-11 + 14:1 *cis*-9 + 16:1 *cis*-9 + 18:1 *cis*-9 + 18:2 *cis*-9,*trans*-11).

⁷Calculated as (12:0 + 4 × 14:0 + 16:0)/(MUFA + PUFA).

to quantify whether comparable feed efficiencies could be obtained when more high-quality FF is included in the diet of the cows.

The milk fatty acid profile in T8 had healthier characteristics than that of T0, including higher contents of VA and RA (Table 4), the latter of which potentially has beneficial effects in reducing the incidence of cancer, among other effects (Elgersma et al., 2006). Our fatty acid profile results align with those reported by Vibart et al. (2008) and Morales-Almaráz et al. (2010), who observed that supplementing TMR-fed cows with increasing amounts of FF increased the content of RA and VA in milk fat. It is remarkable that a level of inclusion of DM of FF as low as 16% in the diet of the TMR-fed cows could exert positive effects in terms of increasing the content of beneficial fatty acids. These changes in the milk fatty acids profile of cows with an increased proportion of FF in the diet may be explained by the high proportion of linolenic acid (LA) in fresh grass forage (Elgersma et al., 2006). The ingestion of LA increases VA synthesis in rumen, which in turn is converted to CLA in the mammary gland by action of Δ^9 -desaturase (Bauman et al., 2008). Part of LA is not biohydrogenated in the rumen but is absorbed intact in the gut and secreted into milk, which may explain the tendency for a higher LA content in T8 than in T0. The higher product-to-substrate ratio of RA to VA may also partially explain its higher RA content. Lock and Garnsworthy (2003) have also reported an increase in the activity of Δ^9 -desaturase with increasing pasture in the diet of dairy cows; however, the reasons for this finding are unclear. The milk fat in T8 had a lower atherogenicity index than that of T0, which is explained by the higher content of MUFA, particularly oleic acid. Likely, the higher LA uptake by the mammary gland explains the lower saturated:unsaturated ratio in T8 compared with T0, as well as the lower content of de novo FA. Although the PUFA content was not affected by treatment, the n-6:n-3 ratio was lower in T8 than in T0, which may have some health-related benefits (Ruxton et al., 2005). Overall, it is possible to include high-quality FF in the diet of cows fed a TMR and achieve a level of milk yield similar to that of cows fed only a TMR. However, more than 4 h of access to high-quality FF resulted in reduced DMI and milk and solids yields without changes in feed efficiency, and resulted in enhanced contents of RA, VA, and LA. Given that FF usually has a lower cost than a TMR per unit of DM, future research should focus on identifying feeding strategies that increase the proportion of FF in a TMR to achieve a higher content of beneficial FA without depressing DMI, milk yield, or feed efficiency.

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