

Communication

# Summer Pasture in Mountainous Area Affects Milk Fatty Acid Profile of Dual-Purpose Cows

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**Abstract:** The change in feeding regime of dairy herds using summer grazing in mountainous areas has several implications on cows' productivity and milk composition. The present study quantified the effect of summer grazing on the milk fatty acids (FA) profile of Simmental and crossbred cows in an alpine holding. A total of 616 milk samples of 71 cows were collected 3 days before the onset of the grazing season until 91 days of grazing. Individual and groups of FA were quantified through mid-infrared spectroscopy. Data were analysed using a linear mixed model including the fixed effects of breed, stage of lactation, parity, sampling period, and the random effects of cows nested within breed and the residual. The stage of lactation and the sampling period were the most significant factors associated with milk FA. The variance explained by the random cow effect ranged from 15.10% (C18:1) to 25.31% (medium-chain FA). The concentration of C14:0, C16:0, and short- and medium-chain FA decreased across the summer season. Long-chain FA and polyunsaturated FA concentrations were greater in milk obtained at pasture compared with milk obtained indoors. Given these outcomes, the present study demonstrated the positive effect of grazing on milk FA composition from a nutraceutical point of view.

**Keywords:** cattle; polyunsaturated fatty acid; milk quality; pasture



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## 1. Introduction

A common practice of dairy farmers in mountainous areas is to move cows to pasture, typically from late spring until the end of summer. This management strategy may have positive implications not only on farm profitability but also on landscape maintenance, touristic appeal of the territory, and the quality of animal-derived products [1,2]. The use of pasture is perceived as a practice to improve animal welfare compared to indoors housing, and possible benefits of this practice are related to both animal health and behaviour [3]. However, lactating cows may experience a period of negative energy balance in grazing conditions due to insufficient energy intake, which is reflected in a loss of productivity and body condition. Therefore, pasture supplementation with some concentrates is generally advised for lactating cows [4,5]. The risk of energy deficit is more common in high-producing cows regardless of their farming conditions; therefore, cows of specialized dairy breeds, such as Holstein, are generally more difficult to be kept in good health when grazing in the mountains [6]. Outdoor grazing requires robust cattle breeds that are well-adapted to low-input feeding and farming systems. Although alpine, and generally mountainous

areas, have been a pool of cattle biodiversity for the number of breeds present today, many farmers have directed their choice towards Simmental cows [7]. Simmental is a dual-purpose cattle breed known for its robustness and for the capacity to adapt to extensive farming conditions, including summer grazing in mountain areas [8]. In Italy, the breeding goal of Simmental sets an emphasis of 24% and 44% on beef and milk traits, respectively [9]. The remaining emphasis is divided into conformation (19.5%, including feet and legs and udder conformation) and fitness (12.5%, including milkability and somatic cell count).

In Western countries, milk and dairy products are important sources of a plethora of nutrients, including proteins of high biological value, minerals, vitamins, and fatty acids (FA) [10]. Bovine milk fat is generally composed of about 70% saturated FA (SFA), 25% monounsaturated FA (MUFA), and 5% polyunsaturated FA (PUFA) [11]. The milk FA profile is affected by several genetic and non-genetic factors, including individual genotype, breed, lactation stage, parity, metabolic status of the animal, and feeding [12–14]. The measurement of milk FA at the population level has been hampered by expensive and time-consuming gold standard analysis, i.e., gas chromatography. However, mid-infrared (MIR) spectroscopy is an effective high-throughput tool to collect several animal characteristics and milk quality traits on a large scale with moderate to high accuracy, including the FA profile [15].

Given the importance of milk FA intake from a nutritional point of view, the hypothesis of the present study was to test the effect of summer grazing on the concentration of individual FA and groups of FA, predicted through MIR spectroscopy, in the milk of dual-purpose cows farmed in a mountain holding. The hypothesis was that the milk FA profile obtained from grazing cows is closer to consumer's needs, and, given the importance of FA intake from a nutritional point of view, this could be a strategy to valorize milk production in mountain areas.

## 2. Materials and Methods

### 2.1. Herd

The commercial dairy farm involved in this study was located in the Veneto Region (Cansiglio, BL, Italy) at 1100 m above sea level and comprised 55 Simmental (SI), 3 Holstein (HO), and 22 HOxSI crossbreed (CR) cows. During autumn, winter, and spring (i.e., between October and late May), animals were housed indoors, fed a total mixed ration based on meadow hay (9.5 kg as fed), alfalfa hay (3 kg), high moisture corn (7 kg), cereals meal (3 kg), and protein-mineral-vitamin premix (3.5 kg). Milking took place in the morning (5.00 to 7.30 a.m.) and in the evening (5.00 to 7.30 p.m.). During late-spring and summer (i.e., between late May and September), animals had access to pasture adjacent to the barn. Cows grazed fresh herbage following the Voisin rotational grazing system [16], implying that animals utilised the entire herbage production of a paddock and moved to a different paddock every day. This grazing management scheme is based on a short grazing period, a high stock density, and sufficient resting time for plants to recover after grazing. In the present study, lactating cows grazed 24 h in each paddock with a stocking rate between 50 and 70 animals per hectare over one day. During milking, which took place twice per day, cows received 2 kg of an energy supplement based on high-moisture corn and cereal meals. A comprehensive description of farm management and diets' chemical composition can be retrieved from Niero et al. [17].

### 2.2. Sample Collection and Chemical Analysis

Repeated milk samples were collected on all cows from late May to late August 2020, exclusively during evening milking through an automatic sampler installed in the milking parlour. The experiment was designed to characterise milk production before the onset and during the grazing season. Milk sampling was performed 3 days and 1 day prior to the beginning of the grazing period to characterise milk quality under indoors farming conditions, hereinafter called barn farming (BF). Similarly, milk samples were collected 2, 3, 7, 10, and 14 days after the beginning of the grazing period to characterise milk quality

in the early grazing (EG) season. Further milk samples were collected 21, 49, and 91 days after the beginning of the grazing period to characterise milk quality in the mid-late grazing (MLG) season.

Immediately after sampling, 200 µL of preservative (Bronopol; 2-bromo-2-nitropropan-1,3-diol) was added to 40 mL of milk and transferred at 4 °C to the laboratory of the Breeders Association of Veneto Region (ARAV, Padova, Italy) for the determination of gross chemical composition (fat, protein, casein, and lactose), individual FA, and groups of FA content using the MilkoScan FT6000 (FOSS Analytical A/S, Hillerød, Denmark). The somatic cell count (SCC, cells/µL) was determined using the Fossomatic 7 DC (FOSS Analytical A/S, Hillerød, Denmark). Values of SCC were transformed to the somatic cell score (SCS) to achieve the normality and homogeneity of variances using the formula  $SCS = 3 + \log_2(SCC/100)$  [18]. The accuracies of MIR prediction models to determine individual and groups of FA are reported in Gottardo et al. [13]. Briefly, the coefficient of determination in validation of individual FA ranged from 0.55 (C16:0) to 0.81 (C18:1), varying from 0.72 (PUFA) to 0.98 (SCFA) for FA groups.

### 2.3. Data Editing and Statistical Analysis

The initial dataset comprised 658 test day records of 55 SI, 3 HO, and 22 CR (first-generation HOxSI). Records of HO cows were discarded due to the very few test days available. Moreover, cows with only one test day record were removed from the dataset. Days in milk (DIM) were restricted to be between 5 and 560 d; parity was between 1 and 8, and parity numbers greater than 5 were grouped into a unique class.

Records with milk yield (MY) < 2 kg/milking were also discarded. Within breed, samples exceeding three standard deviations from the mean of each trait were considered as missing values. After editing, the dataset comprised 616 test days of 52 SI and 19 CR cows. The number of records, before and after edits, for each parity number is reported in Table 1. To assess sources of variation of the studied traits, the following linear mixed model was implemented in the GLIMMIX procedure of SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA):

$$y_{ijklm} = \mu + \text{period}_i + \text{breed}_j + \text{stage}_k + \text{parity}_l + \text{cow}_m(\text{breed}_j) + e_{ijklm}$$

where  $y_{ijklm}$  is the dependent variable (MY, composition trait, individual FA or group of FA);  $\mu$  is the overall intercept of the model;  $\text{period}_i$  is the fixed effect of the  $i$ th period of sampling ( $i = \text{BF, EG, and MLG}$ );  $\text{breed}_j$  is the fixed effect of the  $j$ th cow breed ( $j = \text{SI and CR}$ );  $\text{stage}_k$  is the fixed effect of the  $k$ th class of stage of lactation of the cow ( $k = 1$  to 8, the first being a class from 5 to 45 DIM, followed by 7 classes of 45 DIM each, and the last class including DIM > 315 days);  $\text{parity}_l$  is the fixed effect of the  $l$ th class of parity of the cow ( $l = 1$  to 6, with the last class including parities from 6 to 8);  $\text{cow}_m(\text{breed}_j)$  is the random effect of the  $m$ th cow nested within breed; and  $e_{ijklm}$  is the random residual. Differences between least-squares means of the fixed effects were tested using the Tukey *post-hoc* test ( $p < 0.05$ ). Pearson correlation coefficients ( $r$ ) between the residuals of milk composition, the SCS, individual FA, and groups of FA were estimated.

**Table 1.** Number of records for each parity number prior and post editing.

Parity Number	N, Prior Editing	N, Post Editing
1	120	118
2	194	192
3	96	83
4	80	79
5	104	92
6	32	21
7	10	10
8	22	21

### 3. Results and Discussion

#### 3.1. Descriptive Statistics and Analysis of Variance

Descriptive statistics of MY, gross composition, and SCS are reported in Table 2. Detailed discussion of such traits can be retrieved from Niero et al. [17], who analysed changes in health-related milk indicators during summer grazing in the same herd. Among individual FA, C14:0 and C18:1 had the lowest and the greatest concentration, averaging 0.32 and 1.01 g/100 mL of milk, respectively (Table 2). The content of C18:0 ranged from 0.02 to 0.66 g/100 mL milk and had the greatest coefficient of variation (30.69%), followed by C18:1 with a coefficient of variation of 29.22%. On the other hand, C14:0 and C16:0 had the lowest coefficients of variation (23.03% and 22.18%, respectively). Two recent studies on the milk FA profile of dual-purpose Pinzgauer [19] and SI [20] cattle breeds predicted through MIR spectroscopy reported greater proportions of C14:0 (+26 and +27%, respectively), C16:0 (+23 and +28%, respectively), and C18:0 (+8 and +3%, respectively) and a lower proportion of C18:1 (−25 and −27%, respectively). Saturated FA were the most abundant among FA groups, averaging 2.31 g/100 mL of milk, followed by MUFA (0.97 g/100 mL of milk) and PUFA (0.11 g/100 mL of milk; Table 2). Compared to the results of the present experiment, Bobbo et al. [21] reported a greater concentration of SFA (+13%) and lower concentrations of PUFA (−10%) in a study that aimed at estimating genetic parameters of the milk FA profile of Italian HO. Similar considerations can be resumed by comparing the findings of the present study with those of Manuelian et al. [19,20], who again observed a greater content of SFA and a lower content of MUFA and PUFA in milk of dual-purpose Pinzgauer and SI cattle breeds farmed in the northeast of Italy. Such a lower proportion of SFA in turn of a greater amount of MUFA and PUFA may be explained by two reasons. The first may rely on different genetic backgrounds, due to the specific breeds involved in the present experiment and those considered in previous studies. The second is likely due to different feeding regimes, with particular regard to the fresh herbage grazed by cows enrolled in the present study, which is known to reduce SFA in favour of unsaturated FA [22,23].

**Table 2.** Descriptive statistics of milk yield, gross composition, somatic cell score (SCS), individual fatty acids (FA), and groups of FA <sup>1</sup>.

Trait	N	Mean	SD	CV <sup>2</sup> , %	Minimum	Maximum
Milk yield, kg/milking	608	13.53	4.63	34.18	2.00	27.50
Milk gross composition, %						
Fat	584	3.58	0.75	21.10	2.05	6.36
Protein	607	3.46	0.34	9.76	2.54	4.44
Casein	606	2.73	0.27	10.04	1.95	3.46
Lactose	610	4.85	0.19	4.01	3.76	5.30
SCS, units	605	2.81	1.70	60.42	−1.06	8.76
Individual FA, g/100 mL milk						
C14:0	610	0.32	0.07	23.03	0.13	0.66
C16:0	604	0.86	0.19	22.18	0.35	1.40
C18:0	605	0.33	0.10	30.69	0.02	0.66
C18:1	601	1.01	0.30	29.22	0.06	2.12
Groups of FA, g/100 mL milk						
SCFA	604	0.46	0.12	26.56	0.09	0.86
MCFA	606	1.34	0.29	21.65	0.54	2.23
LCFA	601	1.23	0.40	32.77	0.01	2.66
SFA	605	2.31	0.54	23.63	0.62	4.13
MUFA	601	0.97	0.28	29.19	0.10	1.86
PUFA	607	0.11	0.03	25.19	0.03	0.25
TFA	576	0.11	0.04	40.20	0.00	0.25

<sup>1</sup> SCFA: short-chain fatty acids, MCFA: medium-chain fatty acids, LCFA: long-chain fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TFA: trans fatty acids. <sup>2</sup> CV: coefficient of variation.

Results from the analysis of variance for the concentration of milk FA are summarized in Table 3. The stage of lactation was significant in explaining the variation of individual FA and groups of FA ( $p < 0.001$ ), with only the exception of short-chain FA (SCFA). The sampling period was significant for C14:0, C16:0, and the majority of groups of FA ( $p < 0.05$ ). Among individual FA, only C14:0 exhibited significant variation due to the parity effect ( $p < 0.001$ ). Individual FA and groups of FA were not significantly affected by breed. The random effect of the cow nested within breed accounted for a relatively low proportion of variance of the studied traits, namely, 15.10 to 21.78% for individual FA and 17.02 to 25.31% for groups of FA.

**Table 3.** Results from analysis of variance ( $F$ -values and significance) of individual fatty acids (FA) and groups of FA <sup>1</sup>.

Trait	Fixed Effects				Cow Variance, %	RSD <sup>2</sup>
	Sampling Period	Breed	Days in Milk	Parity		
Individual FA, g/100 mL milk						
C14:0	56.15 ***	0.05	11.58 ***	4.18 ***	21.78	0.06
C16:0	71.27 ***	0.07	11.07 ***	1.30	21.50	0.14
C18:0	2.21	0.01	14.60 ***	1.31	21.55	0.08
C18:1	0.56	0.03	16.87 ***	1.59	1.50	0.23
Groups of FA, g/100 mL milk						
SCFA	99.88 ***	0.46	1.90	1.33	22.72	0.09
MCFA	100.15 ***	0.20	9.19 ***	2.17	25.31	0.21
LCFA	8.48 ***	0.03	16.51 ***	1.53	17.97	0.31
SFA	70.55 ***	0.15	9.83 ***	2.07	17.53	0.41
MUFA	1.56	0.01	20.77 ***	2.42 *	17.02	0.21
PUFA	3.99 *	0.07	11.70 ***	1.60	22.48	0.02
TFA	28.17 ***	1.01	7.81 ***	2.29 *	21.12	0.03

<sup>1</sup> SCFA: short-chain fatty acids, MCFA: medium-chain fatty acids, LCFA: long-chain fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TFA: trans fatty acids. <sup>2</sup> RSD: residual standard deviation. \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

### 3.2. Phenotypic Correlations

Pearson correlation coefficients of individual FA and groups of FA with milk chemical composition and SCS, and between FA, all adjusted for the systematic effects of the statistical model, are presented in Table 4. As expected, all FA groups were strongly correlated to fat concentration ( $r > 0.75$ ), except for trans FA (TFA;  $r = 0.57$ ). Correlations of individual FA and groups of FA with other composition traits were generally weak and, in some cases, did not differ from zero.

The associations between individual FA and groups of FA, as well as those between groups of FA, were significant, positive, and generally greater than 0.50. Moreover, the correlation between TFA and groups of FA increased with the degree of unsaturation of FA, ranging from 0.57 (TFA and SFA) to 0.75 (TFA and PUFA). These results agreed with the findings of Soyeurt et al. [24] and Petrini et al. [25] who estimated phenotypic and genetic correlations between milk FA predicted by MIR spectroscopy and other milk quality traits (i.e., fat, protein, lactose concentration, and SCS) in the Holstein breed in Belgium and under tropical conditions, respectively. Management and breeding strategies aiming at increasing total milk fat concentration are expected to increase not only the unsaturated fraction of FA but also SFA, with different implications. Indeed, a reduction in the dietary ingestion of SFA and in medium-chain FA (MCFA) is encouraged in humans to reduce the risk of cardiovascular diseases, atherosclerosis, and hyperlipidaemia [14]. Moreover, Vanrobays et al. [26] quantified both the phenotypic and genetic correlations between milk FA and methane emissions throughout the lactation using random regression models, demonstrating that methane production (g/d) was positively correlated to C14:0 (average phenotypic and genetic  $r$  values of 0.14 and 0.24, respectively), C16:0 (average phenotypic and genetic  $r$  values of 0.16 and 0.31, respectively), C18:0 (average phenotypic and genetic  $r$  values of 0.11 and 0.32, respectively), and SFA (average phenotypic and genetic  $r$  values

of 0.16 and 0.30, respectively) concentration in milk. These correlations, however, had a different magnitude and pattern depending on the lactation stage. Chillard et al. [27] reported that the association between milk FA and methane production could be due to the common biochemical pathways between methane, acetate, and butyrate in the rumen but also due to the effect of some dietary FA (SCFA, MCFA, and polyunsaturated C18) on the reduction in archaea methanogens in the rumen. Given the correlations quantified in the present study between individual and groups of FA, strategies to increase milk fat concentration per se should also consider the milk FA profile, which is now predictable with moderate to high accuracy on a large-scale using MIR spectroscopy.

**Table 4.** Pearson correlation coefficients between milk yield and composition, somatic cell score (SCS), individual fatty acids (FA), and groups of FA <sup>1</sup>.

Trait	Individual FA					Groups of FA					
	C14:0	C16:0	C18:0	C18:1	SCFA	MCFA	LCFA	SFA	MUFA	PUFA	TFA
Milk yield	0.01	0.01	0.03	0.03	0.14 ***	0.01	0.05	0.06	0.03	0.05	0.10 *
Milk gross composition											
Fat	0.63 ***	0.79 ***	0.87 ***	0.85 ***	0.85 ***	0.77 ***	0.88 ***	0.95 ***	0.85 ***	0.79 ***	0.57 ***
Protein	0.22 ***	0.13 ***	0.01	−0.01	0.11 ***	0.21 ***	−0.05	0.12 ***	−0.02	0.03	0.00
Casein	0.26 ***	0.19 ***	0.11 *	0.08 *	0.19 ***	0.26 ***	0.05	0.20 ***	0.07	0.09 *	0.07
Lactose	−0.18 ***	−0.17 ***	0.01	−0.01	−0.02	−0.22 ***	0.00	−0.13 **	−0.03	−0.01	−0.02
SCS	0.26 ***	0.30 ***	0.22 ***	0.16 ***	0.15 ***	0.30 ***	0.19 ***	0.26 ***	0.21 ***	0.17 ***	0.09 *
Individual FA											
C14:0		0.86 ***	0.47 ***	0.31 ***	0.63 ***	0.93 ***	0.42 ***	0.76 ***	0.37 ***	0.36 ***	0.19 ***
C16:0			0.69 ***	0.51 ***	0.73 ***	0.96 ***	0.59 ***	0.89 ***	0.55 ***	0.47 ***	0.30 ***
C18:0				0.89 ***	0.67 ***	0.61 ***	0.94 ***	0.81 ***	0.91 ***	0.81 ***	0.57 ***
C18:1					0.68 ***	0.45 ***	0.96 ***	0.76 ***	0.98 ***	0.88 ***	0.73 ***
Groups of FA											
SCFA						0.76 ***	0.66 ***	0.93 ***	0.64 ***	0.71 ***	0.64 ***
MCFA							0.54 ***	0.89 ***	0.48 ***	0.44 ***	0.29 ***
LCFA								0.78 ***	0.98 ***	0.86 ***	0.63 ***
SFA									0.75 ***	0.73 ***	0.57 ***
MUFA										0.86 ***	0.66 ***
PUFA											0.75 ***

<sup>1</sup> SCFA: short-chain fatty acids, MCFA: medium-chain fatty acids, LCFA: long-chain fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TFA: trans fatty acids. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.3. Effect of Grazing on Milk Fatty Acid Composition

Least-squares means of FA profiles across different sampling periods are displayed in Table 5. Stoop et al. [28] reported that milk FA composition is mainly determined by four factors, which may act independently or together: (i) animal diet, (ii) de novo synthesis in mammary gland, (iii) rumen biohydrogenation, and (iv) mobilization of body fat reserves. It is likely that variations observed in the present study were due to the change of animal diet and to the mobilization of body fat in response to metabolic stress that animals might have experienced while moving from BF to grazing. Among individual FA, C14:0 and C16:0 exhibited a progressive decline, with the greatest values in the BF period (0.36 and 0.95 g/100 mL milk, respectively) and the lowest in the MLG period (0.29 and 0.76 g/100 mL milk, respectively). Such a decrease mirrors the decrease in groups of FA, with particular regard to MCFA and SFA concentration towards late summer. The concentration of PUFA increased across the grazing season compared with BF. Similar results were reported by Gottardo et al. [13], who observed lower MCFA and SFA in favour of greater unsaturated FA proportion in milk produced during the summer season. From a nutraceutical perspective, a lower proportion of MCFA and SFA in favour of PUFA is desirable in human diet to reduce the risk of cardiovascular diseases and atherosclerosis [14]. Therefore, the outcomes of the present study suggest that, considering the degree of unsaturation and carbon chain length, milk produced by grazing cows could better fulfil human dietary recommendations in terms of the FA profile. The content of C18:1 did not change between indoors and grazing periods; again, among groups of FA,

this was reflected in the MUFA content, which did not vary significantly across BF, EG, and MLG. The greatest concentration of TFA was recorded in the EG period, when cows were more susceptible to metabolic stress entailed by augmented motility and swing in feeding regime and farming conditions. Such results agree with those of Manuelian et al. [19], who reported that negative energy balance hampers the biohydrogenation capacity of the rumen, leading to a greater production of TFA.

**Table 5.** Least-squares means (with standard errors) of individual and groups of fatty acids (FA) <sup>1</sup>.

Trait	Barn Farming	Early Grazing	Mid-Late Grazing
Individual FA, g/100 mL milk			
C14:0	0.36 (0.007) <sup>a</sup>	0.33 (0.006) <sup>b</sup>	0.29 (0.006) <sup>c</sup>
C16:0	0.95 (0.02) <sup>a</sup>	0.89 (0.01) <sup>b</sup>	0.76 (0.02) <sup>c</sup>
C18:0	0.32 (0.01)	0.34 (0.01)	0.34 (0.01)
C18:1	1.00 (0.03)	1.03 (0.02)	1.02 (0.02)
Groups of FA, g/100 mL milk			
SCFA	0.51 (0.01) <sup>a</sup>	0.49 (0.01) <sup>a</sup>	0.37 (0.01) <sup>b</sup>
MCFA	1.49 (0.03) <sup>a</sup>	1.40 (0.02) <sup>b</sup>	1.16 (0.02) <sup>c</sup>
LCFA	1.18 (0.04) <sup>b</sup>	1.23 (0.03) <sup>b</sup>	1.33 (0.03) <sup>a</sup>
SFA	2.53 (0.05) <sup>a</sup>	2.40 (0.04) <sup>b</sup>	2.00 (0.04) <sup>c</sup>
MUFA	0.96 (0.03)	0.98 (0.02)	1.01 (0.02)
PUFA	0.105 (0.003) <sup>b</sup>	0.111 (0.002) <sup>a</sup>	0.106 (0.002) <sup>ab</sup>
TFA	0.108 (0.004) <sup>b</sup>	0.117 (0.003) <sup>a</sup>	0.091 (0.004) <sup>c</sup>

<sup>1</sup> SCFA: short-chain fatty acids, MCFA: medium-chain fatty acids, LCFA: long-chain fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TFA: trans fatty acids. Least-squares means with different letters within a row are significantly different ( $p < 0.05$ ).

#### 4. Conclusions

The present study aimed at investigating the effect of grazing on the concentration of individual FA and groups of FA in milk of SI and CR cows. The effect of breed was not significant in explaining the variation of the studied traits. Milk produced during EG and MLG periods was characterized by lower concentration of MCFA and SFA, and partially a greater amount of PUFA, compared to milk obtained indoors. The results from the present study suggest that, in general, pasture grazing has a positive impact on milk FA composition, especially in the view of human nutrition and health. This information, which needs to be deepened by future large-scale studies, could be considered for market segmentation and to promote and facilitate the selling of milk produced by grazing cows, which potentially has positive implications also on the promotion of the territory and on the warranty of a right and proper income to farmers operating in low-input dairy systems.

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