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Fat Profiles of Milk and Butter Obtained from Different Dairy Systems (High and Low Pasture) and Seasons (Spring and Fall): Focus on Healthy Fatty Acids and Technological Properties of Butter

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Abstract: This study aimed to evaluate the fatty acid (FA) profile in milk from commercial farms with varying pasture levels in the diet during spring and fall, and to investigate the physical and chemical properties of butter to assess the impact of FAs on technological and nutritional properties. Milk sampling was conducted biweekly from six farms, categorized into high (HP) and low (LP) pasture treatments based on pasture intake: >60% and <35%, respectively. Butter was made from a pasture-based system (GRZ) and a confined system (C). No differences were observed in milk fat percentage between HP and LP in either season. High pasture had 85–66% more conjugated linoleic acid (CLA, $p = 0.01$), 74–48% more trans-vaccenic acid (TVA, $p = 0.01$), and 21–15% more branched-chain FAs (BCFAs, $p = 0.006$) than LP in spring and fall, respectively. In fall, butter from C had lower saturated FAs (SFAs, $p = 0.005$), higher unsaturated FAs (UFA, $p = 0.008$), and a lower spreadability index (SI, $p = 0.005$) than GRZ, resulting in softer butter. In conclusion, HP in both seasons had higher contents of FAs considered healthy for consumers compared to LP. Contrary to expectations, in fall, C showed higher UFAs and lower SFAs in butter, leading to better technological characteristics than GRZ.

Keywords: feeding systems; season; milk; dairy products; conjugated linoleic acid

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1. Introduction

Milk and dairy products hold significant nutritional value in the human diet, supplying essential energy, protein, vitamins, and minerals [1–4]. Various factors influence milk composition, including diet, breed, parity, environmental conditions, feeding and management practices, season, and lactation state [5,6]. Among these factors, lipids constitute the most variable fraction of milk and are highly responsive to dietary modifications in terms of composition and concentration [1,3]. The chain length and degree of unsaturation of fatty acids (FAs) are critical determinants of milk fat quality concerning human health. These factors are closely linked to lipid digestion in bovine species. Lipids in the diet undergo lipolysis and biohydrogenation processes mediated by rumen bacteria, leading to the saturation of most consumed unsaturated FAs (UFAs). The progression of this process is influenced by the characteristics (type and quantity) of lipids and the type of

diet [1,4,7]. Moreover, it should be noted that some natural fatty materials, such as fat from meat and ruminant milk, contain trans FAs, including vaccenic acid (TVA) and conjugated linoleic acid (CLA). Conjugated linoleic acid comprises a family of positional and geometric isomers, all of which are conjugated dienes of linoleic acid (18:2). These FAs have been reported to offer beneficial health effects, including hypocholesterolemic, anticarcinogenic, antiatherogenic properties, modulation of the immune response, and improvement of bone mineralization [8–10]. It has been reported that the consumed amount of CLA needed to produce observable health benefits ranges from 0.8 to 3.0 g/d [11]. TVA, an intermediate in CLA formation, also possesses beneficial health properties, including the reduction of cardiovascular disease risk and potential inhibition of tumor growth [12]. Additionally, oleic acid and TVA found in milk have also been shown to have beneficial effects on human health, similar to CLA. Oleic acid reduces total cholesterol and low-density lipoprotein (LDL) [13], while TVA is desaturated to CLA in the human body at a rate of 19% [14]. Furthermore, odd- and branched-chain FAs (OBCFAs) are unique components of ruminant fat, representing the primary contribution of BFAs to human nutrition. The quantification of milk BFAs has garnered significant interest in recent years. Due to their origin and the correlations observed between diet and ruminal microbial population, OBCFAs are considered potential biological indicators of ruminal function [15]. The potential inhibitory effects on tumor cells [16] and the reduced risk of cardiovascular disease, associated with the consumption of these FAs further underscore their beneficial effects on human health, including a lower risk of developing type 2 diabetes [17]. Specifically, 15:0 and 17:0 iso and anteiso have been found to enhance the fluidity of cell membranes [18]. The concentration and composition of milk fat can be readily modified through dietary adjustments [19]. For example, increasing the proportion of forage in the diet compared to concentrate results in higher concentrations of OBCFAs in the milk. Similarly, a diet rich in grass silage could elevate the total content of OBCFAs in milk. The profile of OBCFAs in cow's milk is primarily influenced by the FAs in the diet and FA metabolism in the rumen [20]. Therefore, understanding the origin of OBCFAs in milk and manipulating the diet of dairy cows to produce milk enriched with odd- and branched-chain FAs can be important both scientifically and industrially.

Prior studies have investigated milk FAs and their correlation with human health using certain indices. The atherogenic index (AI) is indicative of the risk impact on cardiovascular diseases. A higher AI suggests a greater risk of such diseases [21]. Additionally, the ratio between hypocholesterolemic (18:1, 18:2, and 18:3) and hypercholesterolemic FAs (12:0; 14:0, and 16:0), denoted as H/H, is associated with AI [22]. Another index associated with human health is the n-6/n-3 ratio [23]. Excessive levels of n-6, commonly found in Western diets, can hinder human enzymatic systems, contributing to the development of certain diseases. Conversely, higher levels of n-3 can have the opposite effects [23,24]. Furthermore, the technological potential of milk for butter production was assessed using the spreadability index (SI) [25].

Moreover, pasture-based systems are regarded as more environmentally friendly, animal welfare-conscious, and sustainable, compared to confinement systems [26]. Additionally, milk and dairy products from these systems offer potential nutritional benefits and market opportunities due to their improved composition, compared to those derived from total mixed ration (TMR) systems [27,28]. Pasture-based systems are commonly employed in regions with mild climates like South America and Oceania, owing to their low production costs, which are favored by climate conditions and forage accessibility [29]. However, even in these systems, the use of reserves and concentrates is necessary to ensure the fulfillment of energy requirements and nutrient quality [29,30]. In Uruguay, pasture utilization is crucial for reducing production costs and maintaining satisfactory production levels [29]. Furthermore, it has been widely reported that including pasture improves the FA profile (FAP) in milk and dairy products [31,32]. For instance, milk and dairy products from systems with a high proportion of pasture exhibit higher levels of UFAs and CLA, and lower proportions of saturated fatty acids (SFAs), compared to systems with low levels

or no access to pasture [25,33–38]. Therefore, the ability to modify the composition of milk fat through pasture utilization and strategic supplementation can serve as a tool to differentiate dairy products, resulting in milk and dairy products with a healthier FAP for human consumption. It is important to consider that this management approach has its limitations. For instance, the addition of UFAs to the diet increases CLA production, but excessive amounts can result in a lower percentage of milk fat by reducing the production of new FAs in the udder [7,39]. Moreover, seasonality, particularly in pasture-based systems, affects the FAP in milk and dairy products. Climate factors influencing the thermal comfort of cows, primarily heat stress in summer, impact the lipid catabolism of the animals [40]. Additionally, seasonality impacts the quality of pastures [41], thus significantly influencing the quality of milk fat. In this regard, higher levels of monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), and CLA, along with a lower n-6/n-3 ratio and palmitic acid content, have been reported in milk and cheeses in spring compared to fall [42–44]. This may result from lower pasture availability and increased utilization of preserved forages in temperate countries during fall, leading to decreased FA quality in the cows' diet [29,43].

In the past, the consumption of dairy fat (such as milk cream, butter, or cheese) raised concerns among consumers due to its high levels of SFAs, which had been linked to elevated cholesterol levels, arteriosclerosis, and cardiovascular diseases [21,45]. However, recent reviews and meta-analyses have concluded that milk consumption has at least a neutral effect on various health outcomes, and cow's milk consumption may even be beneficial for osteoporosis, cardiovascular disease, stroke, type II diabetes, and certain cancers [46–48]. Regarding organoleptic characteristics, it has been reported that modifications in the FAP result in changes in the texture, sensory, and nutritional quality of butter [49]. In this regard, butter from pasture-based systems has shown better nutritional and rheological quality, compared to butter from confinement systems using TMR [50]. Additionally, higher levels of PUFAs, including CLA, and a higher yellow index have been reported in milk from pasture-based systems compared to TMR systems [51].

This study aimed to evaluate the FAP (mainly OBCFAs) in milk obtained from farms located in the northwest region of Uruguay, using feeding strategies with varying pasture content (classified as high and low pasture in the diet) during two seasons (spring and fall). The physical and chemical properties of butter were investigated to assess the impact of varying FAs profiles on the technological and nutritional properties of high-fat dairy products.

2. Materials and Methods

2.1. Experimental Design, Localization, and Sample Analysis

Six dairy farms located in the northwest region of Uruguay (Salto, Paysandú and Río Negro provinces) were selected according to the pasture intake, milk production, and somatic cell count from the previous year. Two treatments were created according to pasture intake: High Pasture (HP) (>65% pasture of total dry matter intake: DMI) and Low Pasture (LP) (<35% pasture of total DMI).

The study was conducted during two seasons: spring 2021 and fall 2022. Milk samples were collected from bulk tanks (if there were multiple tanks, a proportional mixture was prepared based on the volumes of each tank), considering that the milk originated from two or four milkings. Sampling was conducted fortnightly, completing a total of five periods for each season. The samples were extracted in 15 mL Falcon tubes, frozen for transfer, and subsequently analyzed in the laboratory of Food Technology at the School of Chemistry, Universidad de la República. Concurrently, feed samples (provided to dairy cows), including pasture and supplements (concentrate and reserves), were collected. In each dairy farm, the following records were kept: herd management, milk production, feeding routine (type of supplement, quantity, and composition), type of pasture (allocation, availability, and species), milk production, and number of milking cows.

During the spring, the HP treatment had an average of 74% pasture and 26% supplement, with a forage/concentrate ratio of 79:21, while the LP treatment had 10% pasture

and 90% supplement, with a forage/concentrate ratio of 68:32. In the fall, the HP had an average of 45% pasture and 55% supplement, with a forage/concentrate ratio of 76:24, while the LP had 3% pasture and 97% supplement, with a forage/concentrate ratio of 61:39.

Pastures consisted of a variety of grass species (*Festuca arundinacea*, *Avena sativa*) and legumes (*Medicago sativa*, *Lotus corniculatus*, *Trifolium pretense*, *Trifolium repens*), as well as natural grassland. The ingredients used in commercial dairies varied over time, depending on available conserved forage and the market availability of grains and by-products for concentrate. The most commonly used conserved forages were whole-plant maize or sorghum silage, as well as grass hay, silage, or haylage (such as oat, lucerne, and moha). Concentrate mixes could include ground corn grain, rice bran, soybean expeller, soybean meal, barley rootlets, urea, yeast, and minerals.

Daily pasture DMI (kg DM/cow) was estimated using the energy balance method according to National Research Council (NRC) [52] guidelines, as the amount of pasture required to provide the remaining energy needed to meet the cow's net energy (NE) requirement, not supplied by supplements (reserves and concentrates). Cow NE requirements were estimated as the sum of maintenance and milk production requirements [53]. The average data for neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract (EE) of each group (pasture and supplement) are presented in Table 1.

Table 1. Average values of neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract (EE) for each group (pasture and supplement) in Spring and Fall.

	Spring						Fall					
	HP			LP			HP			LP		
	Past	Suppl	Total Diet **	Past *	Suppl	Total Diet **	Past	Suppl	Total Diet **	Past *	Suppl	Total Diet **
[1] NDF	47.3 ± 4.8	30.3 ± 7.3	43.1 ± 3.5	46.3 ± 1.7	36.2 ± 1.8	37.2 ± 2.6	36.7 ± 6.9	43.9 ± 7.6	40.1 ± 5.7	36.9 ± 2.4	44.7 ± 5.8	44.5 ± 5.9
[1] ADF	26.2 ± 2.3	14.3 ± 6.1	23.4 ± 1.5	26.3 ± 1.5	19.8 ± 2.5	20.6 ± 2.2	20.1 ± 5.0	24.1 ± 6.5	22.0 ± 3.6	20.9 ± 1.7	26.5 ± 7.2	26.5 ± 7.2
[2] Lipids	3.7 ± 0.9	4.8 ± 1.6	4.1 ± 1.1	2.6 ± 0.06	3.9 ± 0.3	4.2 ± 0.5	6.6 ± 4.1	3.9 ± 1.2	5.4 ± 2.5	6.9 ± 0.1	5.8 ± 1.5	5.8 ± 1.5

Past: Pasture; Suppl: Supplement. HP: High Pasture; LP: Low Pasture. NDF: Neutral Detergent Fiber. ADF: Acid Detergent Fiber. * The "LP pasture" data in both seasons corresponds to the only producer of that treatment offering fresh pasture in the diet. ** The total diet chemical composition value was calculated by weighting the inclusion level of pasture and supplement. [1] Expressed as % of dry matter (DM); [2] expressed as g/100 g of pasture and supplement.

Table 2 shows the FAP of pasture and supplement in each treatment (HP and LP) (averaged over the experimental period).

Table 2. Fatty acid profiles of pasture and supplement in both treatments: HP and LP in Spring and Fall.

Fatty Acids (g/100 g Fat)	Spring				Fall			
	HP		LP		HP		LP	
	Past	Suppl	Past	Suppl	Past	Suppl	Past	Suppl
10:0	0.36 ± 0.20	0.11 ± 0.03	0.82 ± 0.44	0.41 ± 0.22	0.06 ± 0.05	0.03 ± 0.05	0.20 ± 0	0.29 ± 0.18
12:0	0.74 ± 0.21	0.14 ± 0.05	1.3 ± 0.55	0.61 ± 0.27	0.36 ± 0.08	0.34 ± 0.27	0.30 ± 0	0.45 ± 0.42
14:0	2.09 ± 0.79	0.50 ± 0.15	4.62 ± 1.53	1.69 ± 0.70	0.95 ± 0.31	0.56 ± 0.23	0.63 ± 0.05	1.29 ± 1.08
16:0	18.25 ± 2.91	15.23 ± 2.19	24.82 ± 2.36	16.49 ± 1.73	12.6 ± 1.5	15.3 ± 1.4	12.4 ± 0.1	14 ± 3.1
16:1 n7 cis	0.37 ± 0.19	0.17 ± 0.06	0.60 ± 0.27	0.33 ± 0.08	0.03 ± 0.08	0.29 ± 0.21	0.18 ± 0.05	0.29 ± 0.37
17:0	0.36 ± 0.27	0.11 ± 0.03	0.44 ± 0.05	0.13 ± 0.12	0 ± 0	0 ± 0	0.05 ± 0.10	0.03 ± 0.05
18:0	3.1 ± 0.97	2.06 ± 0.46	5.24 ± 0.33	3.49 ± 0.42	2.21 ± 0.32	2.49 ± 0.48	2.08 ± 0.05	4.35 ± 1.60
18:1 n9 cis	7.21 ± 4.19	28.81 ± 6.01	11.44 ± 1.15	22.8 ± 2.76	5.00 ± 1.57	27.68 ± 5.71	3.90 ± 0.60	22.66 ± 2.50
18:2 n6 cis	13.41 ± 5.05	46.43 ± 4.86	10.18 ± 0.38	41.37 ± 4.35	13.77 ±3.04	41.81 ± 5.82	14.18 ± 1.35	37.90 ± 10.02
20:0	0.79 ± 0.39	0.43 ± 0.05	0.58 ± 0.11	0.49 ± 0.14	0.38 ± 0.08	0.62 ± 0.14	0.40 ± 0	0.53 ± 0.21
20:1	0.04 ± 0.08	0.39 ± 0.14	0 ± 0	0.11 ± 0.09	0.10 ± 0.10	0.49 ± 0.09	0 ± 0	0.19 ± 0.18
18:3 n3 cis	42.42 ± 12.93	3.41 ± 1.66	31.72 ± 11.61	8.36 ± 4.11	58.66 ± 7.61	6.84 ± 5.47	57.60 ± 2.40	13.36 ± 9.32
22:0	0.51 ± 0.12	0.24 ± 0.06	0.54 ± 0.05	0.33 ± 0.14	0.66 ± 0.24	0.53 ± 0.13	0.55 ± 0.10	0.48 ± 0.16
24:0	0.47 ± 0.12	0.3 ± 0.08	0.32 ± 0.16	0.27 ± 0.12	0.40 ± 0.07	0.55 ± 0.25	0.53 ± 0.05	0.38 ± 0.11
SFA	27.21 ± 5.52	19.17 ± 2.64	40.22 ± 7.28	24.73 ± 3.65	18.77 ± 3.60	18.36 ± 1.57	19.25 ± 2.10	18.76 ± 2.01
MUFAs cis	7.63 ± 4.31	29.37 ± 5.89	12.4 ± 1.37	23.25 ± 2.76	19.56 ± 8.65	22.14 ± 15.97	6.68 ± 7.15	8.47 ± 6.18
PUFAs cis	55.83 ± 9.16	49.84 ± 5.56	41.9 ± 11.23	49.73 ± 3.95	56.80 ± 8.91	56.47 ± 16.42	66.80 ± 6.80	67.38 ± 6.93
Total fat (g/100 g DM)	3.73 ± 1.00	4.86 ± 1.63	2.62 ± 0.06	3.95 ± 0.30	5.96 ± 1.17	9.03 ± 5.52	5.78 ± 2.00	4.41 ± 0.68

Past: Pasture; Suppl: Supplement. HP: High Pasture; LP: Low Pasture. SFA: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids. DM: dry matter.

Butter Production

Butter was produced from two contrasting dairy farms based on diet and management: grazing + supplement (GRZ) and confinement cows with total mixed ration (TMR) (C: confined). At the time of milk extraction for butter production, a sample of feed (pasture and supplement) was obtained from each producer in each season. For the C dairy farm in spring and fall, the total mixed ration consisted of ground corn grain and soybean expeller for concentrate, haylage (*Medicago sativa*) as conserved forage, urea, and minerals. For the GRZ dairy farm in spring and fall, the supplement comprised corn grain and barley rootlet. Pastures consisted of *Avena sativa* and *Medicago sativa* in the fall, and *Trifolium pretense* and *Lolium multiflorum*. The FAP in the diet of cows from each farm (GRZ and C) is shown in Table 3.

Table 3. Fatty acid profiles of the total diet (pasture and supplement) from two selected dairy farms for butter production (C and GRZ).

Fatty Acids (g/100 g Fat)	Spring		Fall	
	GRZ	C	GRZ	C
16:0	18.5 ± 2.5	17.0 ± 2.1	15.4 ± 1.4	12.6 ± 1.6
18:0	3.7 ± 0.7	3.5 ± 0.3	2.6 ± 0.4	4.0 ± 0.6
18:1 n9 cis	11.3 ± 5.1	18.2 ± 1.9	14.0 ± 3.3	26.2 ± 1.5
18:2 n6 cis	21.7 ± 4.9	34.6 ± 2.3	25.5 ± 4.4	47 ± 5.2
18:3 n3 cis	29.2 ± 7.2	15.1 ± 2.8	32.2 ± 6.5	4.2 ± 5.8

GRZ: pasture-based system (grazing + supplement); C: confined (total mixed ration, TMR).

For both producers (contrasting conditions: GRZ and C), in each season (spring and fall), two butter productions were carried out on consecutive days in triplicate. For each butter production, 60 L of bulk milk (MilkB) was obtained and immediately transported under refrigerated conditions (4 °C) to the dairy pilot plant of the Universidad Tecnológica del Uruguay (UTEC) (La Paz, Colonia, Uruguay) for processing. Two batches of fresh cream (5 L each; 40–42% fat) were separated at 45 °C from pre-pasteurized milk. The cream was then stored overnight at 10 °C for maturation. Subsequently, both cream samples were churned in a rotary churn (Edibon España) at 26 rpm and 13 °C. After grain formation, the butter was washed with 1.7 L of water at 4 °C, and finally, it was kneaded until it formed into butter. Portions of butter were clarified for further analysis using the British Standards Method 769 (BSI 1961 54) [54].

2.2. Sample Analysis

2.2.1. Fatty Acid Profile in Milk and Butter

Milk and butter samples (3 g each) were extracted using the Rose-Gottlieb technique [55]. Analyses were conducted in triplicate, and FAs methyl esters were prepared following the IUPAC 2.301 protocol, [56] and analyzed by gas chromatography, according to the AOCS Ce 1c-89 and AOCS Ce 1f-96 protocols [57]. The gas chromatograph was a Shimadzu (Kyoto, Japan) model 2014 equipped with a Supelco (Bellefonte, PA, USA) SP 2560 (100 m × 0.25 mm × 0.2 mm) capillary column and a flame ionization detector (FID). The injection volume of the samples was 1 µL. The temperature program used was as follows: an initial temperature of 90 °C for 2 min, then an increase to 175 °C at a rate of 20 °C/min, maintained for 35 min, followed by an increase to 240 °C at a rate of 15 °C/min, maintained for 25 min. Peak identification was achieved through the analysis of authentic standards. Standards and reagents used for the analysis were supplied by Sigma-Aldrich (Burlington, MA, USA). The milk fat compositions were expressed in grams of each individual FA per 100 g of total fat. The atherogenicity index (AI) was calculated as (12:0 + 4 × 14:0 + 16:0)/(MUFAs + PUFAs) [21], and the hypocholesterolemic/hypercholesterolemic FA (H/H) ratio was calculated as (18:1 cis + 18:2 cis + 18:3 cis)/(12:0 + 14:0 + 16:0) [22]. The spreadability index (SI) was calculated as the ratio of 16:0 to 18:1, as proposed by O'Callaghan et al. [25].

2.2.2. Fatty Acid Profile in Pasture and Supplement

Fat was extracted from the different samples (1 g each) using the Hara and Radin [58] technique, with a mixture of hexane and isopropanol (3:2). Initially, approximately 1 g of milled samples was weighed into a tube, and 20 mL of the solvent mixture was added. Extraction was conducted at room temperature (20 °C) with magnetic stirring for 90 min. Subsequently, centrifugation was performed (Hermle, model Z 200 A, Gosheim, Germany) at 3000 rpm for 15 min. Following centrifugation, 5 mL of the solvent mixture was added for complete lipid extraction. Finally, the total solvent mixture was removed using a nitrogen flow at 40 °C until the weight of the lipids remained constant. Analyses were conducted in triplicate, and the FAP was determined as previously described for milk and butter.

2.2.3. Butter Firmness

The firmness of butter was measured at 10 °C using a Texture Analyzer (Brookfield CT3 50k, MA, USA) following the procedure previously described [59], with modifications. The procedure consisted of testing the cutting force at a depth of 16 mm applied to a given sample, which was stored at 10 °C for 12 h, at a speed of 2 mm/s using a TA53 cutting wire probe. The force of cutting between 8 and 16 mm was reported as the firmness of the sample. Each sample was measured six times.

2.2.4. Differential Scanning Calorimeter (DSC)

A Shimadzu differential scanning calorimeter (DSC-60A plus, Shimadzu Co., Kyoto, Japan) was used to examine the melting and crystallization properties of milk fat from butter. Oxygen (99.999% purity) was used as the purge gas. The DSC was calibrated using high-purity indium (m.p. 156.6 °C, ΔHf = 28.45 J g^{-1}), according to standard DSC procedures. Clarified butter samples of about 10 mg were placed in open aluminum pans and inserted into the heating chamber of the DSC cell, with an empty aluminum pan as the reference. Prior to analysis, the samples were heated at 50 °C for 5 min to melt all crystals and nuclei. Subsequently, they were tempered in a freezer at −20 °C for 48 h. The final crystallization was carried out in the DSC by cooling from 2 °C to −50 °C and left at −50 °C for 30 min. After that time, the melting was studied by heating the sample at 5 °C/min from −50 °C to 60 °C. At least duplicate determinations were carried out. Characteristic data were obtained using the Shimadzu DSC-TA 60A plus Version 2.21 software. The thermograms were integrated using the TA60 Version 2.21 software. From this integration, the fraction of liquid formed for each temperature was determined, and the curve of solids percentage vs. temperature was constructed.

2.2.5. Color

Clarified butter was measured at 50 °C using a Minolta Chroma-Meter CR-400 colorimeter. The parameters L*, a*, and b* were recorded in triplicate (Chiyodaku, Tokyo, Japan).

2.3. Statistical Analysis

For each season, data on fat content, FAP, and nutritional quality indices (AI, H/H, n-6/n-3) in milk were analyzed using repeated measures ANOVA with PROC GLIMMIX of SAS 9.04 (SAS Institute Inc., Cary, NC, USA). The statistical models included treatment (HP and LP), period (1 to 5), and the interaction between treatment and period. Farms within each treatment were considered as random effects. The Kenward–Roger approximation was utilized to calculate the denominator degrees of freedom for the fixed-effects tests of the model. Least squares (LS) means were generated using the LSMEANS/DIFF option, and post hoc comparisons were conducted with the Tukey test.

For butter variables (FAP, SI, firmness, and color), analyses were performed using one-way ANOVA, followed by post hoc Tukey tests, to compare the two contrasting farms (GRZ and C) in each season. Results were considered significant at $p \leq 0.05$. Data are presented as mean ± SEM (standard error of the mean). The temperature and fusion enthalpy variables obtained from the DSC were analyzed using descriptive statistics. The mean ± SD data for both variables are shown. All variables were performed using SAS software 9.04. (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Milk Fat and Fatty Acid Profile in Commercial Dairy Farms

In spring, the HP treatment had a milk fat content of 3.71 ± 0.06%, and the LP had 3.78 ± 0.06%. In fall, the HP had fat values of 3.84 ± 0.08%, and LP had fat values of 3.89 ± 0.08%. In spring, there were no treatment or period effects, and no interaction between the treatments and period was observed. In fall, there was no treatment effect and no interaction between treatments and period, but there was a period effect. In period 1, the milk fat content was 4.08 ± 0.10%, whereas in period 2, it was 3.65 ± 0.10% ($p = 0.037$).

There were no differences between treatments (HP and LP) in total SFAs or the total sum (Table 4) in both seasons. Additionally, the presence of branched-chain FAs (BCFAs) was noted, with the most prevalent being the 15:0 anteiso and 17:0 anteiso (odd-chain FAs; Table 5). On the other hand, SFAs had the highest percentage (Table 4) compared to MUFAs and PUFAs (Tables 6 and 7). Regarding SFAs, it was observed that about 10% were short-chain (especially butyric acid 4:0 and caproic acid 6:0), and the three most abundant FAs in this lipid fraction of milk were palmitic acid 16:0, myristic acid 14:0, and stearic acid 18:0.

There was a treatment effect on most of the iso and anteiso BCFAs ($p < 0.05$; Table 5) in both seasons (spring and fall). In fall, the BCFAs were higher in HP compared to LP ($p = 0.007$). Among the BCFAs, the OBCFAs 15:0 anteiso, 17:0 iso, and 17:0 anteiso were in higher proportions (Table 5). The HP treatment showed a higher proportion of iso FAs compared to the LP ($p < 0.05$), particularly in spring, while in fall this was observed only for 15:0 iso ($p = 0.003$; Table 5).

There were no significant differences in MUFA content between treatments in either season (Table 6). Oleic acid (18:1 cis) was the most abundant of the MUFAs, with a content ranging from 19.68% to 21.74% in both HP and LP, across both seasons. For TVA, there was a difference between treatments in spring ($p = 0.012$). The HP treatment had a higher proportion of TVA (4.11% vs. 2.36%) compared to LP (Table 6). Regarding total PUFAs, in spring, there were no differences in linoleic acid (18:2 cis) content between treatments (HP and LP); however, in fall, LP had higher total PUFAs than HP and 18:2 cis tended to be higher than HP (Table 7). The content of α-linolenic acid (18:3 n-3 cis) was higher in HP compared to LP in spring ($p = 0.01$). Regarding CLA content, milk from HP was higher than LP in both spring ($p = 0.014$) and fall ($p = 0.033$, Table 7).

Regarding the indicators of nutritional quality, the atherogenic index (AI) showed no significant differences between the different treatments (HP vs. LP) in either spring or fall. The values obtained for HP were 3.1 ± 0.2 and 3.3 ± 0.3 in spring and fall, respectively, while LP presented values of 2.9 ± 0.2 and 2.7 ± 0.3 in spring and fall, respectively. For the H/H index, we also found no differences between treatments in either season. The values obtained for HP were 0.5 ± 0.03 in both spring and fall, while LP presented values of 0.5 ± 0.03 and 0.6 ± 0.07 in spring and fall, respectively. Regarding the n-6/n-3 ratio, in fall, the HP was lower than the LP treatment (2.8 ± 0.4 vs. 5.8 ± 0.4; $p = 0.05$), while in spring, there were no differences between treatments.

Table 4. Saturated Fatty Acid Profile of Milk in Spring and Fall.

Fatty Acid (g/100 g Fat)	Spring											Fall										
	T		P					SEM	*p*-Value			T		P					SEM	*p*-Value		
			1	2	3	4	5		T	P	T×P			1	2	3	4	5		T	P	T×P
4:0	HP	3.03	2.92	3.54	3.43	2.51	2.76	0.09	ns	0.022	ns	HP	3.16	3.20	3.31	2.94	3.49	2.87	0.13	ns	ns	ns
	LP	2.86	2.58	3.18	2.61	2.55	3.37					LP	3.30	3.81	3.13	3.26	3.28	3.04				
6:0	HP	1.82	1.93 [a]	2.12 [a]	1.91 [a]	1.49 [a]	1.63 [b]	0.05	ns	0.050	0.045	HP	2.00	1.86	2.15	1.92	2.25	1.82	0.10	ns	ns	ns
	LP	1.86	1.73 [a]	2.04 [a]	1.64 [a]	1.70 [a]	2.21 [a]					LP	2.13	2.48	2.04	2.13	2.09	1.90				
8:0	HP	1.06	1.18	1.23	1.01	0.87	1.00	0.05	ns	ns	ns	HP	1.23	1.07	1.35	1.24	1.40	1.09	0.08	ns	ns	ns
	LP	1.13	1.10	1.19	1.00	1.04	1.34					LP	1.30	1.47	1.30	1.28	1.31	1.12				
10:0	HP	2.37	2.77	2.78	2.15	2.04	2.12	0.16	ns	ns	ns	HP	2.78	2.75	2.93	2.73	3.10	2.39	0.29	ns	0.015	ns
	LP	2.64	2.64	2.71	2.41	2.52	2.89					LP	2.62	2.65	2.54	2.69	2.83	2.39				
11:0	HP	0.30	0.35	0.34	0.26	0.26	0.28	0.02	ns	ns	ns	HP	0.36	0.31	0.42	0.36	0.41	0.29	0.03	ns	ns	ns
	LP	0.32	0.30	0.33	0.29	0.32	0.39					LP	0.31	0.27	0.35	0.33	0.32	0.27				
12:0	HP	2.78	3.26	3.20	2.47	2.50	2.49	0.10	ns	ns	ns	HP	3.22	2.93	3.49	3.23	3.63	2.81	0.42	ns	ns	ns
	LP	3.08	3.07	3.19	2.91	2.89	3.32					LP	2.95	2.91	2.82	3.03	3.23	2.75				
14:0	HP	10.61	11.56	11.50	10.08	10.20	9.72	0.42	ns	ns	ns	HP	10.98	11.01	11.16	11.03	11.50	10.21	0.85	ns	ns	ns
	LP	10.86	10.84	10.88	10.75	10.66	11.17					LP	10.15	10.61	9.89	10.21	10.54	9.51				
15:0	HP	1.37	1.42	1.36	1.35	1.34	1.38	0.14	ns	ns	ns	HP	1.20	1.17	1.26	1.21	1.23	1.11	0.06	ns	ns	ns
	LP	1.21	1.23	1.19	1.19	1.13	1.29					LP	1.04	1.03	1.04	1.08	1.01	1.02				
16:0	HP	29.46	29.75	29.69	29.14	30.00	28.71	1.00	ns	ns	ns	HP	30.75	31.40	30.59	30.43	31.07	30.26	1.16	ns	ns	ns
	LP	29.94	29.30	29.65	30.29	29.73	30.71					LP	28.79	30.00	28.41	28.06	29.19	28.28				
17:0	HP	0.82	0.79	0.81	0.82	0.83	0.87	0.06	ns	ns	ns	HP	0.71	0.64	0.68	0.76	0.73	0.76	0.04	ns	ns	ns
	LP	0.69	0.71	0.69	0.69	0.65	0.71					LP	0.67	0.62	0.70	0.68	0.64	0.72				
18:0	HP	10.08	9.27	9.73	10.72	10.24	10.42	0.45	ns	ns	ns	HP	9.15	9.12	8.52	9.59	8.56	9.95	0.85	ns	ns	ns
	LP	10.10	11.03	10.49	9.92	10.12	8.92					LP	10.93	11.19	11.44	10.57	10.37	11.09				
Total SFA	HP	63.88	65.36	66.46	63.55	62.45	61.59	1.08	ns	ns	ns	HP	65.55	64.94	66.02	65.59	67.51	63.71	2.13	ns	ns	ns
	LP	64.91	64.71	66.01	63.87	63.49	66.46					LP	64.34	67.19	63.84	63.46	64.95	62.24				

HP = High pasture; LP = Low pasture. T: treatments; P: periods, T×P: interaction between treatments and periods. SFA: saturated fatty acid. SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance.

Table 5. Branched-chain Fatty Acid Profile of Milk in Spring and Fall.

Fatty Acid (g/100 g Fat)	Spring											Fall									
	T		P					SEM	*p*-Value			T		P				SEM	*p*-Value		
			1	2	3	4	5		T	P	T×P			1	2	3	4	5		T	P	T×P
14:0 iso	HP	0.17	0.16	0.15	0.18	0.17	0.19	0.01	ns	ns	ns	HP	0.13	0.13	0.12	0.14	0.12	0.12	0.01	ns	ns	ns
	LP	0.13	0.14	0.12	0.13	0.11	0.13					LP	0.11	0.10	0.12	0.11	0.11	0.10				
15:0 iso	HP	0.36 [a]	0.36	0.35	0.36	0.33	0.37	0.02	0.029	ns	ns	HP	0.30 [a]	0.27	0.31	0.32	0.29	0.30	0.01	0.003	ns	ns
	LP	0.28 [b]	0.30	0.32	0.25	0.24	0.27					LP	0.24 [b]	0.25	0.24	0.23	0.22	0.24				
15:0 anteiso	HP	0.69 [a]	0.74	0.71	0.70	0.64	0.68	0.03	0.008	ns	ns	HP	0.56 [a]	0.52	0.57	0.58	0.56	0.56	0.01	0.0004	ns	ns
	LP	0.48 [b]	0.49	0.48	0.48	0.46	0.51					LP	0.43 [b]	0.45	0.43	0.44	0.43	0.42				
16:0 iso	HP	0.34 [a]	0.35	0.34	0.34	0.33	0.35	0.01	0.003	ns	ns	HP	0.29	0.26	0.27	0.31	0.30	0.30	0.02	ns	ns	ns
	LP	0.30 [b]	0.33	0.29	0.30	0.29	0.31					LP	0.28	0.25	0.30	0.30	0.28	0.28				
17:0 iso	HP	0.43 [a]	0.37	0.46	0.45	0.44	0.44	0.02	0.005	ns	ns	HP	0.38	0.34	0.38	0.40	0.37	0.44	0.02	ns	0.003	ns
	LP	0.34 [b]	0.36	0.35	0.34	0.33	0.33					LP	0.33	0.29	0.35	0.34	0.30	0.37				
17:0 anteiso	HP	0.53	0.55	0.53	0.52	0.50	0.55	0.03	ns	ns	ns	HP	0.48	0.43	0.48	0.50	0.50	0.48	0.02	ns	ns	ns
	LP	0.44	0.46	0.43	0.44	0.43	0.47					LP	0.43	0.38	0.46	0.45	0.41	0.46				
18:0 iso	HP	0.054 [a]	0.057	0.053	0.052	0.052	0.057	0.001	0.025	0.040	ns	HP	0.063	0.059	0.053	0.060	0.067	0.077	0.01	ns	ns	ns
	LP	0.048 [b]	0.051	0.048	0.046	0.046	0.050					LP	0.045	0.045	0.048	0.043	0.039	0.050				
Total BCFAs	HP	2.57 [a]	2.58	2.59	2.59	2.46	2.64	0.09	0.006	ns	ns	HP	2.19 [a]	2.01	2.18	2.30	2.21	2.27	0.06	0.007	0.030	ns
	LP	2.02 [b]	2.13	2.03	1.98	1.91	2.06					LP	1.86 [b]	1.77	1.95	1.92	1.78	1.91				

HP = High pasture; LP = Low pasture. T: treatments; P: periods, T×P: interaction between treatments and periods. BCFA: branched-chain fatty acid. SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance.

Table 6. Monounsaturated Fatty Acid Profile of Milk in Spring and Fall.

Fatty Acid (g/100 g Fat)	Spring											Fall									
	T		P					SEM	*p*-Value			T		P				SEM	*p*-Value		
			1	2	3	4	5		T	P	T×P			1	2	3	4	5		T	P	T×P
14:1 cis	HP	0.85	0.99	0.92	0.79	0.81	0.76	0.05	ns	ns	ns	HP	1.01	1.00	1.17	0.99	1.04	0.88	0.11	ns	ns	ns
	LP	0.92	0.89	0.88	0.92	0.84	1.05					LP	0.84	0.76	0.91	0.86	0.87	0.79				
16:1 cis	HP	1.29	1.34	1.27	1.28	1.35	1.23	0.12	ns	ns	ns	HP	1.59 [a]	1.58	1.71	1.53	1.59	1.54	0.05	0.022	ns	ns
	LP	1.36	1.39	1.31	1.41	1.23	1.44					LP	1.31 [b]	1.17	1.42	1.33	1.26	1.39				
18:1 elaidic	HP	0.23	0.26	0.17	0.22	0.26	0.26	0.04	ns	ns	ns	HP	0.22 [b]	0.31	0.22	0.24	0.20	0.11	0.04	0.015	ns	ns
	LP	0.34	0.32	0.34	0.39	0.41	0.25					LP	0.38 [a]	0.33	0.34	0.41	0.36	0.46				
18:1 trans-vaccenic	HP	4.11 [a]	4.14	3.84	4.22	4.24	4.10	0.28	0.012	ns	ns	HP	3.55	3.80	3.45	3.44	3.39	3.70	0.41	ns	ns	ns
	LP	2.36 [b]	2.28	2.35	2.58	2.38	2.20					LP	2.40	1.91	2.41	2.58	2.34	2.73				
18:1 cis	HP	19.68	18.00	18.04	19.97	21.69	20.68	0.73	ns	ns	ns	HP	19.68	20.68	18.65	19.43	18.01	21.62	1.83	ns	ns	ns
	LP	21.04	21.32	20.54	21.81	22.23	19.29					LP	21.75	20.77	22.78	21.27	21.01	22.94				
Total MUFAs cis	HP	21.82	20.34	20.23	22.04	23.84	22.67	0.68	ns	ns	ns	HP	22.28	23.26	21.52	21.94	20.65	24.04	0.73	ns	ns	ns
	LP	23.32	23.61	22.73	24.14	24.30	21.79					LP	23.90	22.70	25.11	23.45	23.13	25.12				

HP = High pasture, LP = Low pasture. T: treatments, P: periods, T×P: interaction between treatments and periods. MUFAs: monounsaturated fatty acids. SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance.

Table 7. Polyunsaturated Fatty Acid Profile of Milk in Spring and Fall.

Fatty Acid (g/100 g Fat)	Spring											Fall									
	T		P					SEM	*p*-Value			T		P				SEM	*p*-Value		
			1	2	3	4	5		T	P	T×P			1	2	3	4	5		T	P	T×P
18:2 trans	HP	1.12 [a]	1.07	1.05	1.09	1.19	1.19	0.04	0.003	ns	ns	HP	1.27 [a]	1.22	1.21	1.22	1.24	1.45	0.04	0.031	ns	ns
	LP	0.89 [b]	0.79	0.79	1.17	0.86	0.82					LP	1.12 [b]	1.00	1.13	1.09	1.04	1.33				
18:2 n6 cis	HP	1.72	1.44	1.54	1.91	1.80	1.91	0.32	ns	ns	ns	HP	1.48	1.33	1.51	1.55	1.33	1.69	0.08	<0.0001	ns	ns
	LP	2.54	2.42	2.53	2.55	2.64	2.58					LP	2.86	2.45	2.62	2.99	3.07	3.19				
18:2 CLA	HP	1.65 [a]	1.86	1.51	1.58	1.70	1.62	0.11	0.014	ns	ns	HP	1.43 [a]	1.53	1.53	1.47	1.37	1.29	0.15	0.033	ns	ns
	LP	0.89 [b]	0.79	0.83	0.96	0.93	0.94					LP	0.86 [b]	0.66	0.97	0.90	0.85	0.90				
18:3 n3 cis	HP	0.89 [a]	0.82	0.89	0.97	0.87	0.90	0.08	0.033	ns	ns	HP	0.53	0.47	0.52	0.56	0.55	0.57	0.03	ns	0.03	ns
	LP	0.54 [b]	0.36	0.50	0.63	0.60	0.63					LP	0.50	0.37	0.54	0.54	0.54	0.53				
Total PUFAs cis	HP	2.61	2.27	2.43	2.88	2.67	2.82	0.24	ns	ns	ns	HP	2.02 [b]	1.80	2.03	2.11	1.87	2.26	0.08	<0.0001	ns	ns
	LP	3.09	2.78	3.03	3.17	3.24	3.21					LP	3.37 [a]	2.82	3.16	3.53	3.61	3.72				

HP = High pasture, LP = Low pasture. T: treatments, P: periods, T×P: interaction between treatments and periods. PUFAs: polyunsaturated fatty acids. SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance.

3.2. Butter Production from Two Farms (GRZ and C)

3.2.1. Fatty Acid Profile in Butter

In spring, the butter made from GRZ had higher content of CLA, 18:2 cis, and TVA, and lower total MUFA content, primarily represented by 14:1, 16:1 compared to C (Table 8). Regarding SFA, although there were no differences in total content, GRZ butter had a higher proportion of 11:0, 12:0 and BCFAs 14:0 iso, 15:0 anteiso compared to C (Table 8). In fall, GRZ butter showed a higher proportion of total SFA and BCFA compared to C, showing differences in almost all individual SFAs (Table 8). Regarding UFAs, GRZ butter had lower MUFAs and PUFAs compared to C, primarily represented by TVA, 18:1 cis, and 18:2 cis (Table 8). The FAs that showed differences between treatments (GRZ vs. C) in butter, both in spring and fall, were consistent with those observed in MilkB. Table 8 shows a transfer in CLA, 18:1 trans-vaccenic, total PUFAs cis and total BCFAs content from MilkB to butter, demonstrating that the butter processing procedure did not negatively affect the content of these beneficial FAs.

Table 8. Fatty Acid Profile of Butter and Bulk Milk Elaborated in Spring and Fall.

Fatty Acid (g/100 g Fat)	Spring								Fall							
	Butter				MilkB				Butter				MilkB			
	GRZ	C	SEM	*p*-Value	GRZ	C	SEM	*p*-Value	GRZ	C	SEM	*p*-Value	GRZ	C	SEM	*p*-Value
4:0	3.84	2.61	0.58	ns	2.36	2.97	0.44	ns	2.75 ^{a}	2.62 ^{b}	0.02	0.043	2.74	2.78	0.22	ns
6:0	2.33	1.70	0.25	ns	1.59	1.82	0.27	ns	1.86 ^{a}	1.59 ^{b}	0.01	0.002	2.00	1.56	0.13	ns
8:0	1.28	1.87	0.57	ns	1.04	1.03	0.13	ns	1.12 ^{a}	0.92 ^{b}	0.01	0.003	1.30	0.91	0.12	ns
10:0	2.8	2.78	0.25	ns	2.52	2.33	0.11	ns	2.71 ^{a}	1.99 ^{b}	0.04	0.005	3.13	1.86	0.32	ns
11:0	0.34 ^{a}	0.32 ^{b}	0.002	0.040	0.33	0.34	0.01	ns	0.29 ^{a}	0.23 ^{b}	0.01	0.040	0.38	0.22	0.05	ns
12:0	3.20 ^{a}	3.00 ^{b}	0.01	0.006	3.13	2.65	0.25	ns	3.27 ^{a}	2.33 ^{b}	0.02	0.001	3.73	2.16	0.41	ns
14:0 iso	0.18 ^{a}	0.16 ^{b}	0.002	0.046	0.14	0.15	0.02	ns	0.11 ^{a}	0.09 ^{b}	0.002	0.020	0.12	0.10	0.01	ns
14:0	11.37	11.06	0.17	ns	11.56	10.14	0.43	ns	11.36 ^{a}	8.86 ^{b}	0.05	0.001	11.83	7.92	0.73	ns
15:0 iso	0.36	0.40	0.01	ns	0.32	0.31	0.03	ns	0.25 ^{a}	0.19 ^{b}	0.004	0.009	0.28	0.23	0.01	ns
15:0 anteiso	0.76 ^{a}	0.67 ^{b}	0.01	0.012	0.66	0.59	0.04	ns	0.48 ^{a}	0.39 ^{b}	0.01	0.010	0.57 ^{a}	0.43 ^{b}	0.01	0.004
14:1	0.93 ^{b}	1.03 ^{a}	0.01	0.027	0.96	0.95	0.14	ns	0.83	0.72	0.03	ns	0.95	0.66	0.06	ns
15:0	1.55 ^{b}	1.62 ^{a}	0.01	0.025	1.50	1.60	0.09	ns	1.16 ^{a}	0.99 ^{b}	0.004	0.001	1.34	1.07	0.05	ns
16:0 iso	0.38	0.40	0.01	ns	0.32	0.32	0.03	ns	0.27	0.27	0.003	ns	0.30	0.27	0.01	ns
16:0	29.79	31.14	0.45	ns	33.25	30.99	1.94	ns	34.23 ^{a}	28.29 ^{b}	0.43	0.010	32.61 ^{a}	25.81 ^{b}	0.58	0.014
17:0 iso	0.48	0.5	0.02	ns	0.43	0.36	0.03	ns	0.45 ^{a}	0.33 ^{b}	0.01	0.008	0.38	0.42	0.04	ns
17:0 anteiso	0.63	0.69	0.01	ns	0.53	0.56	0.05	ns	0.42 ^{a}	0.19 ^{b}	0.01	0.006	0.48	0.51	0.03	ns
16:1	1.34 ^{b}	1.71 ^{a}	0.03	0.016	1.49	1.57	0.17	ns	1.47	1.35	0.02	ns	1.50	1.36	0.10	ns
17:0	0.94 ^{b}	1.00 ^{a}	0.01	0.030	0.88	0.86	0.04	ns	0.75 ^{a}	0.69 ^{b}	0.01	0.022	0.80	0.82	0.05	ns
18:0 iso	0.1	0.08	0.03	ns	0.06	0.05	0.003	ns	0.06	0.06	0.01	ns	0.06	0.06	0.01	ns
18:0	9.06	8.97	0.26	ns	8.30	9.06	1.27	ns	9.36 ^{b}	11.16 ^{a}	0.10	0.006	8.65 ^{b}	11.81 ^{a}	0.51	0.048
18:1 elaidic	0.22	0.19	0.03	ns	0.20	0.29	0.04	ns	0.22 ^{b}	0.41 ^{a}	0.03	0.035	0.17 ^{b}	0.50 ^{a}	0.04	0.035
18:1 trans-vaccenic	3.22 ^{a}	1.89 ^{b}	0.15	0.024	3.65	2.33	0.11	0.015	2.89 ^{b}	2.97 ^{a}	0.01	0.009	3.14	2.57	0.26	ns
18:1 cis	17.65 ^{b}	19.95 ^{a}	0.35	0.043	17.33	20.80	1.85	ns	18.10 ^{b}	25.73 ^{a}	0.22	0.002	17.72	27.28	1.83	ns
18:2 trans	0.81	0.90	0.08	ns	1.10	0.88	0.16	ns	1.01 ^{b}	1.86 ^{a}	0.05	0.008	1.19	1.34	0.08	ns
18:2 cis	2.00 ^{a}	1.77 ^{b}	0.02	0.010	1.90	1.53	0.15	ns	1.70 ^{b}	2.84 ^{a}	0.06	0.005	1.74 ^{b}	3.23 ^{a}	0.24	0.048
18:2 CLA	1.44 ^{a}	0.98 ^{b}	0.07	0.042	1.57	1.21	0.12	ns	0.92	0.91	0.005	ns	1.06	0.97	0.08	ns
18:3 n3 cis	1.09	1.02	0.07	ns	1.07 ^{a}	0.96 ^{b}	0.02	0.050	0.56	0.55	0.02	ns	0.60	0.58	0.02	ns
20:0	0.18	0.18	0.003	ns	0.18	0.17	0.03	ns	0.15 ^{a}	0.14 ^{b}	0.001	0.011	0.15	0.17	0.01	ns
Total SFAs	66.67	66.25	1.40	ns	66.65	63.95	2.01	ns	69.02 ^{a}	59.79 ^{b}	0.45	0.005	68.66 ^{a}	57.09 ^{b}	1.68	0.040
Total MUFAs cis	19.92 ^{b}	22.68 ^{a}	0.39	0.037	19.78	23.31	1.54	ns	20.76 ^{b}	28.17 ^{a}	0.22	0.002	20.17	29.30	1.86	ns
Total PUFAs cis	3.1	2.78	0.08	ns	2.97	2.50	0.15	ns	2.27 ^{b}	3.38 ^{a}	0.07	0.008	2.34	3.81	0.26	ns
Total BCFAs	2.87	2.90	0.03	ns	2.46	2.34	0.18	ns	2.04 ^{a}	1.51 ^{b}	0.02	0.002	2.19	2.01	0.08	ns
Total Trans	4.26 ^{a}	2.98 ^{b}	0.20	0.046	4.95	3.51	0.29	ns	4.12 ^{b}	5.24 ^{a}	0.08	0.009	4.26	5.20	0.21	ns
Spreadability index (C16:0/C18:1)	1.69	1.56	0.05	ns	1.93	1.52	0.25	ns	1.89 ^{b}	1.10 ^{a}	0.04	0.005	1.85	0.95	0.13	0.037

GRZ: pasture-based system (grazing + supplement); C: confined (total mixed ration, TMR). Milk B: milk to butter. SFAs: saturated fatty acids. BCFAs: branched-chain fatty acids. MUFAs cis: monounsaturated fatty acids. PUFAs cis: polyunsaturated fatty acids. SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance. MilkB: bulk milk.

3.2.2. Techno-Functional Characteristics of Butter from Two Farms (GRZ and C)

- Butter Firmness

There were no differences in firmness between GRZ and C in spring. However, in fall, butter from C showed lower firmness compared to GRZ butter ($p = 0.008$; Figure 1).

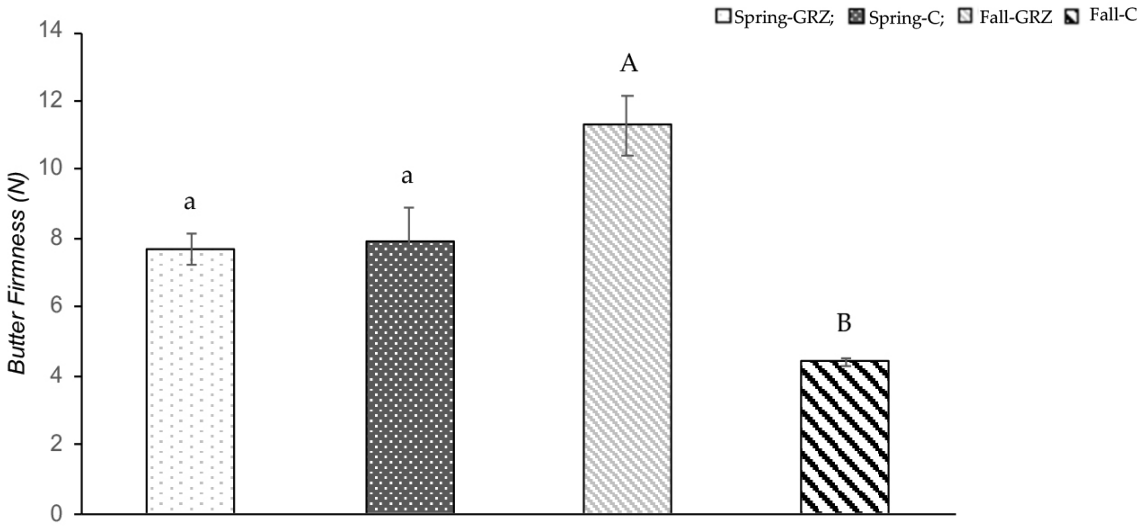

Figure 1. Butter firmness elaborated from GRZ: pasture-based system (grazing + supplement); C: confined (total mixed ration, TMR). Difference between farms (GRZ and C) in spring and fall are shown with small letter and capital letter, respectively ($p < 0.05$). N: Newton.

- Differential Scanning Calorimetry (DSC)

The thermograms obtained from the Differential Scanning Calorimeter (DSC) are shown in Figure 2, for anhydrous milk fat from GRZ and C. The thermal behaviors are very similar, exhibiting two peaks that can be associated with groups of triglycerides with different melting ranges [60]. These are represented by the two peaks observed in the respective thermograms.

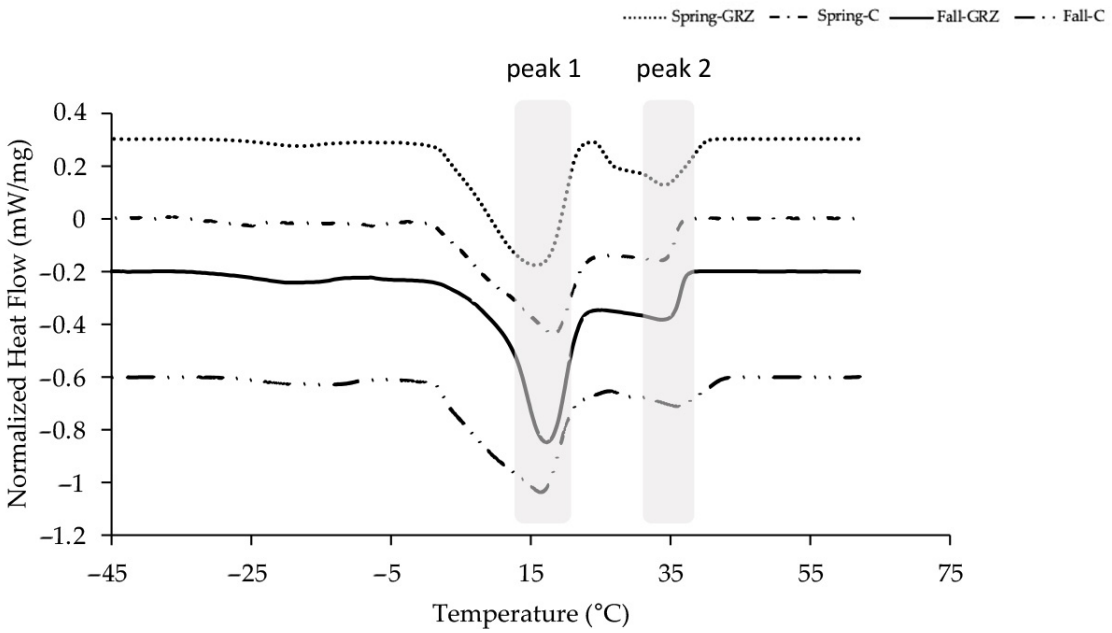

Figure 2. Thermograms of anhydrous milk fat from the prepared butter from GRZ: pasture-based system (grazing + supplement); C: confined (total mixed ration, TMR), in spring and fall. Soft grey bars indicate triglyceride groups with the lower melting point (peak 1) or the higher melting point (peak 2) for the four anhydrous milk fat samples.

The temperature for the peak corresponding to the triglyceride group with the lower melting point (peak 1) ranged from 14.9 to 16.7 °C, while for the triglycerides with the higher melting point (peak 2), the temperature ranged from 32.7 to 34.1 °C (Table 9).

Additionally, the enthalpies of fusion for the anhydrous milk fat ranged between 89.4 J/g and 112.9 J/g, as shown in Table 9.

Table 9. Peak temperature (Tpeak), enthalpies of fusion (ΔH) and final melting temperature (Tendset) for anhydrous milk fats from butter elaborated in Spring and Fall.

	Tpeak (°C)		ΔH (J/g)	Tendset (°C)
	1	2		
Spring-GRZ	15.7 ± 0.4	34.1 ± 0.9	96.9 ± 2.9	39.9 ± 0.3
Spring-C	18.2 ± 0.5	33.1 ± 0.8	97.9 ± 3.0	36.4 ± 0.4
Fall-GRZ	17.2 ± 0.8	33.7 ± 0.9	112.9 ± 3.3	37.1 ± 0.3
Fall-C	16.4 ± 0.7	35.9 ± 0.9	89.4 ± 2.6	42.4 ± 0.5

GRZ: pasture-based system (grazing + supplement) and C: confined (total mixed ration, TMR) in spring and fall.

The curves of percentage of solids for each of the anhydrous milk fats (Figure 3A) show very little difference between them. However, significant differences were observed at −5 °C and between 9 °C and 20 °C, where the percentage solids decreased rapidly (Figure 3B). It is worth noting that firmness and the percentage solids values at 10 °C, (the temperature at which firmness was determined), showed the same trend. Anhydrous milk fat Fall-C, which exhibited the lowest firmness at 10 °C, also had the lowest percentage of solids at that temperature. Conversely, Fall-GRZ anhydrous milk fat, which showed the highest firmness, had the highest percentage of solids compared to the other samples (Figure 3B), consistent with its higher firmness (Figure 1).

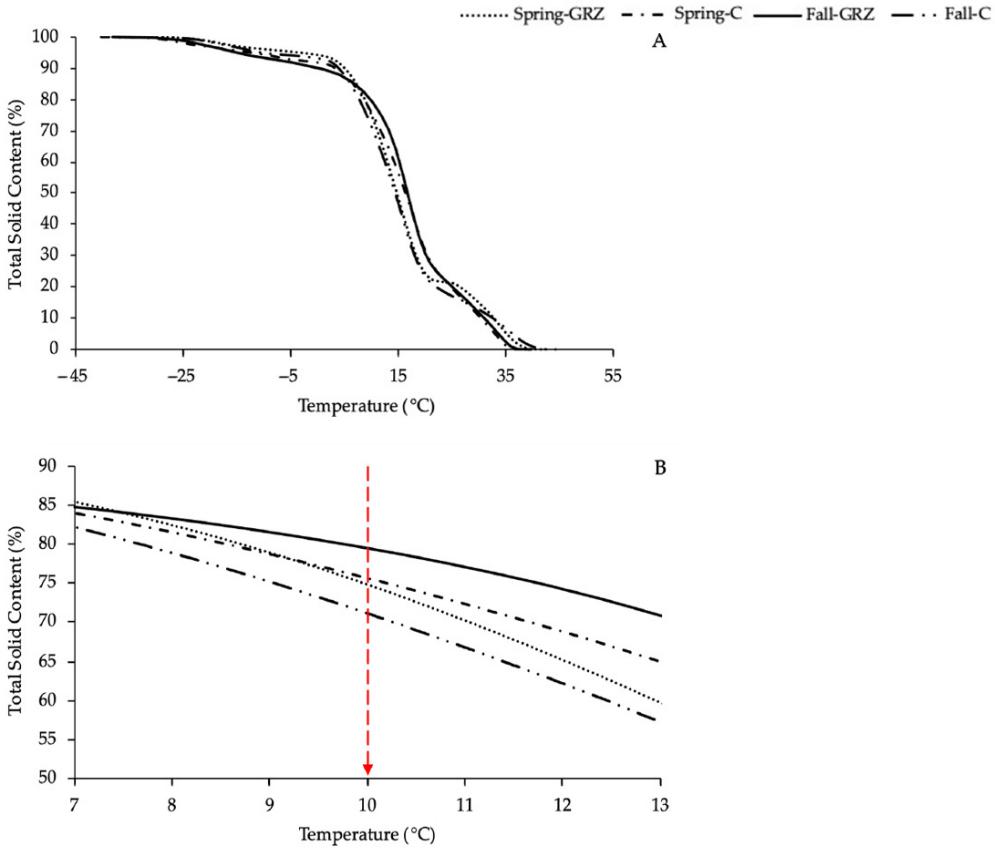

Figure 3. (**A**) Percentage of solids in anhydrous milk fat from butter produced under GRZ: pasture-based system (grazing + supplement) and C: confined (total mixed ration, TMR) in spring and fall. (**B**): Percentage of solids in anhydrous milk fat: magnification in the range between 8 and 13 °C, with an arrow indicating the temperature at which firmness was determined (10 °C).

- Color

Regarding color parameters, no differences were observed between GRZ and C butter in spring. In fall, there were no differences in L and a* parameters. Nevertheless, butter from GRZ had higher b* values than those from C (p = 0.003; Table 10).

Table 10. Evaluation of color from butter elaborated in Spring and Fall.

	Spring				Fall			
	GRZ	**C**	**SEM**	***p*-Value**	**GRZ**	**C**	**SEM**	***p*-Value**
L*	55.08	57.16	0.58	ns	59.24	58.95	0.14	ns
a*	−5.05	−5.09	0.04	ns	−4.57	−4.57	0.13	ns
b*	22.88	23.26	1.00	ns	28.39 ^{a}	15.71 ^{b}	0.46	0.003

GRZ: pasture-based system (grazing + supplement); C: confined (total mixed ration, TMR). SEM: standard error of the mean. Different letters indicate significant difference ($p < 0.05$). ns: no significance.

4. Discussion

4.1. Fatty Acid Profile in Milk

When it comes to the proportion of the main groups of FAs composing milk fat, our results align with findings reported by other authors, where 60–70% corresponds to SFAs, 20–25% to MUFAs, and 3–4% to PUFAs [36,61,62]. Among the SFAs, the most abundant found in this study were palmitic acid (16:0), myristic acid (14:0), and stearic acid (18:0), which also coincides with reports from other authors in similar dairy cow production systems [63,64]. The fact that these are the most abundant SFAs in cow's milk is related to the biosynthesis mechanisms of fat in the dairy cow. Lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0) are primarily synthesized in the mammary gland from precursors originating in the rumen [65]. Particularly, palmitic acid (16:0) is one of the most abundant, partly due to its mixed origin, as it can be synthesized in the gland or come from body reserves [66]. On the other hand, long-chain saturated FAs such as stearic acid (18:0) reach the milk primarily from the rumen, originating from FAs in the diet that undergo biohydrogenation by ruminal microbiota or from body reserves [65].

While no differences were found in the total amount of SFAs between treatments, we observed that milk from HP had a higher proportion of BCFAs compared to LP treatment. Within BCFAs, OBCFAs such as 15:0 and 17:0 iso and anteiso were the most abundant, consistent with findings reported by Ran Ressler et al. [67] for bovine milk. The fact that HP had a higher proportion of these FAs compared to LP treatment (both in spring and fall) could be explained by the higher forage-to-concentrate ratio (F/C ratio) in the diet of HP. This higher F/C ratio could increase the concentration of cellulolytic bacteria in the rumen, which are associated with higher proportions of OBCFAs in milk (mainly iso forms), as reported by Vlaeminch et al. [20]. Xin et al. [68] also reported that cellulolytic bacteria had a stronger correlation with OBCFAs compared to amylolytic microbiota. Specifically, these authors found a positive correlation between the population of *Ruminococcus flavefaciens* and the concentrations of 15:0 and 15:0 iso. Based on the reported benefits of OBCFAs for human health and the limited knowledge available to date on their levels in commercial dairy systems, this study provides relevant information on the positive association between higher pasture inclusion levels in the diet and OBCFAs concentrations in milk, consistent with the authors cited above. This reinforces the importance of including pasture in cows' diets and the resulting benefits on the quality of milk fat.

Furthermore, the higher proportion of TVA, 18:3 n-3 cis, and CLA (main rumenic FA: 18:2 cis 9 trans 11) in milk from the HP treatment compared to LP, particularly in spring, is consistent with findings reported by Elgersma et al. [69], Alothman et al. [36], and Moscovici Joubran et al. [62], and could be associated with the higher proportion of pasture (percentage of total diet) in HP compared to LP treatment during this season (Table 2). As widely reported, fresh pastures contain a high proportion of 18:3 n-3 cis (the main precursor of TVA and CLA in milk) [69] and exhibit maximum digestibility between August to October

(spring) [70–72], which could explain these differences between systems, although no differences were observed in the total MUFAs and PUFAs. Taking into account the fact that these FAs constitute the main components of dairy fat considered beneficial for consumers due to their anti-atherogenic, anti-carcinogenic, anti-inflammatory, immunostimulant, and insulin resistance-modulating effects [73], and that current nutritional trends promote an increase in PUFAs and CLA consumption [74], milk from HP could be considered healthier than milk from LP. Moreover, regarding the content of TVA and CLA in spring, it is observed that the trends in their variation are similar: increases in their content correspond to increases in the other. This confirms the metabolic dependence for the formation of both FAs, due to the stages of biohydrogenation in the rumen and dehydrogenation in the mammary gland [75]. Regarding the indices related to the risk of foods causing cardiovascular problems, the HP treatment showed lower n-6/n-3 ratios compared to LP, both in fall and spring, suggesting that farms with higher pasture inclusion in the diet produce healthier milk (values below 4/1) [23]. While this study did not find differences in AI and H/H between treatment or seasons in milk, the values were similar to those reported by other authors [64,76–78].

Overall, we could suggest that milk from farms with a higher proportion of pasture may have health benefits compared to milk from farms with a lower proportion of pasture, as indicated by the higher levels of TVA, CLA and 18:3 n-3 cis in milk fat, together with the higher levels of OBCFAs mentioned above. Furthermore, the higher content of OBCFAs (which have demonstrated beneficial effects on human health [17,18], contributes to this suggestion. Therefore, quantifying these FAs in milk (especially the latter) from commercial farms in contrasting seasons based on dietary structure is crucial for valorizing attributes related to the nutritional quality of the milk produced.

4.2. Fatty Acid Profile in Butter

Based on the FAs profiles obtained from the butter, the higher proportion of CLA and TVA in the GRZ during spring indicates a healthier nutritional profile compared to C. This could be attributed to the higher percentage of pasture (grazing) in the total diet of GRZ cows compared to C. Although there was no difference in 18:3 n-3 cis in the butter, it was observed that this FAs was higher in MilkB from GRZ compared to C, which aligns with Elgersma [69], who reported that pasture contributes significantly to 18:2 and 18:3, precursors of major n-3, CLA, and TVA in milk and dairy products.

In the case of fall butter, contrary to expectations, both MilkB and butter from GRZ had a higher proportion of SFA (mainly represented by 14:0, which is considered one of the main atherogenic FAs). However, the concentration of OBCFAs, such as 15:0 and 17:0 iso and anteiso, especially 17:0 anteiso, was more than twice as high in GRZ, compared to C. This is interesting, because although GRZ butter had high SFAs content, a significant portion consists of OBCFAs known for its bioactive properties [79]. These compounds could potentially counteract the adverse effects associated with consuming atherogenic FAs found in this dairy product.

4.3. Physical Properties of Butter

The lower firmness observed in butter made from C compared to GRZ in fall aligns with the FAP in MilkB and the butter made. The C treatment had higher PUFAs than GRZ, and showed a tendency to be higher in MilkB. This increase was mainly explained by high values of 18:2, both in MilkB and butter. Additionally, the C treatment had lower SFA than GRZ in MilkB and butter. While a higher concentration of PUFAs was expected in the GRZ treatment compared to C (as per the design with and without fresh pasture in the diet), analysis of the FAP of both treatment' diets and ingredients revealed that the C treatment, despite lacking grazing, had more than double the 18:2 n-6 and 18:1 n-9 in the diet, compared to GRZ. These results could be explained by the high proportion of maize grain and soybean expeller (as concentrate) in the C diet during the butter production periods, as these ingredients provide a relatively high proportion of fat [80], specifically a

high proportion of 18:2 n-6 in the diet. In this regard, Roy et al. [81] reported that maize silage generally contains between 30% and 40% grain, which is rich in 18:2 n-6, 18:1 n-9, and low in 18:3 n-3 cis. Regarding the addition of soybean in pasture-based systems, it has been reported to decrease FAs from 6:0 to 14:0 and increase 18:0, 18:1, 18:2, and 18:3 in milk [82,83]. Therefore, the C producer's diet, composed of maize grain and soybean expeller, had a high contribution of 18:2 in the diet. On one hand, this may have increased the passage rate and resulted in higher concentrations of 18:2 in milk [84]. On the other hand, the high concentrations of these FAs in the rumen may have inhibited ruminal biohydrogenation, resulting in lower levels of SFA and higher levels of MUFAs and PUFAs in MilkB and butter from the C farm. Furthermore, the higher levels of MUFAs and PUFAs could explain the lower firmness of C butter, compared to those from GRZ [25,85].

The high firmness of butter from GRZ in fall could be explained by the higher proportion of SFAs compared to C, considering that short-chain FAs (with low melting points) did not vary significantly between samples. This is observed not only in total SFAs, but also in most individual SFAs. It has been reported that butter made from creams with a high proportion of SFAs are firmer and less spreadable than those made from creams with a higher proportion of UFAs [25,86], determined by the higher melting point of SFAs [87]. Among SFAs, palmitic acid (16:0), which has a high melting point (62.9 °C), was higher in GRZ butter than in C butter, leading to a higher spreadability index, consistent with findings reported by Techeira et al. [85], Marangoni and Ghazani [86] and Chamberlain et al. [88], and therefore to higher firmness. In spring, no differences in butter firmness were observed between GRZ and C. These results could be explained because MilkB and butter from the two farms (GRZ and C) had a similar FAP in this season, with no differences found in most FAs (mainly SFAs and PUFAs).

Through DSC analysis, the melting behavior, melting temperatures, enthalpies of fusion, and solid content at different temperatures was investigated. Table 9 shows the temperatures corresponding to the peak for triglycerides with lower melting points (ranging between 14.9 and 16.7 °C) and for those with higher melting points (ranging between 32.7 and 34.1 °C). In this study, differences in the feeding regimes between GRZ and C, in fall, led to different enthalpy of fusion values for the anhydrous milk fats used in the butter made from GRZ versus C. This differed from what had occurred in spring, where changes in SFA, MUFA, and PUFA content were not as significant between treatments. The values obtained ranged between 89.4 J/g (Fall-C) and 112.9 J/g (Fall-GRZ). Tomaszewska-Gras [89] reported enthalpy of fusion values for anhydrous milk fat of 80.3 J/g. Although this value is lower than that found in the present study, it is likely due to differences in its composition. The differences in the enthalpy of fusion values found may be attributed to variations in the triacylglycerol composition among different anhydrous milk fats. Additionally, the enthalpy of fusion of fat materials not only depends on the triacylglycerol composition, but also on the minor components present in them [90]. The final melting temperature (Tendset) for anhydrous milk fat ranged from 36.4 °C to 42.4 °C. Additionally, DSC analysis revealed that GRZ anhydrous milk fat in fall had the highest solid content (Figure 3B), which correlates with it being the sample with the greatest firmness (Figure 1). The results indicated that most of the variation in composition and thermal properties occurred in the fall samples, likely due to the feeding regimes, specifically, GRZ versus C. For example, in fall, GRZ had a higher SFA content compared to C (21.6% vs. 18.1%) and a lower MUFA content (14.4% vs. 26.5%). Therefore, variations in feeding should be considered, as they can affect the physicochemical properties of the butter produced. Consequently, the results shown in Figure 3 were expected, because a higher solid content should be reflected in a fatty material with greater firmness.

Regarding the color of the butter, there were differences between GRZ and C in those made in fall. The higher intensity of the yellow color in the butter made from GRZ compared to C could be attributed to pastures having a higher concentration of β-carotene than ensiled feeds, as the ensiling process negatively impacts the concentration of this compound in the feed provided [91]. Since β-carotene is one of the main components

influencing butter color, a higher intake of β-carotene in the diet could have resulted in a greater intensity of yellow color in the GRZ butter, consistent with findings reported by O'Callaghan et al. [25].

The predominant use of pastures (fresh pasture: grazing or conserved forage) promotes an increase in UFAs and BCFAs of nutritional and technological interest (CLA, TVA in spring, 15:0 iso and anteiso: 17:0 iso) in milk. This not only enhances the added value of milk produced in pasture-based systems, but also the products, such as butter, made from it. In this study, samples from actual producers supplying the industry were analyzed, representing commercial production systems and, collectively, including variations in herd management, pasture availability, and the nature of supplementation. This wide variability in sources makes analyzing the results a complex task, but it also provides a truthful view of the production conditions present in the western coastal systems of Uruguay. Observing the results for butter produced on a pilot scale highlights the importance of the availability and management of different supplements, pasture levels and season. In the butter made in fall, the reported 18:2 cis values in the feed from farm C doubled those of GRZ, likely due to the nature of the lipids provided by corn grains and the presence of soybean expeller in the supplement. Consequently, in fall, the butter produced from C had lower firmness than those manufactured from GRZ, paradoxically with respect to the inclusion of pasture. In this case, for C, the richness in lipids from the supplement with soybean expeller combined with corn grain in the TMR promoted functional and technological properties associated with high-pasture conditions, although it did not increase the yellow coloration or the content of OBCFAs. The texture and crystallization profile of the fats present in the butter, then, highlight the importance of selecting the overall feed based on the available sources and the possibility of obtaining suitable technological properties, even in the absence of pastures.

5. Conclusions

The results obtained in this study, conducted on commercial farms, underscore the benefits of high-pasture inclusion (in both spring and fall) in the diet of dairy cows, for producing healthier milk for consumers. This is supported by higher concentrations of TVA, CLA, and 18:3 n-3 cis, and a lower n-6/n-3 ratio. Additionally, higher levels of some SFAs, recently reported beneficial for human health, such as OBCFAs 15:0 and 17:0, were observed. Moreover, butter derived from milk of the pasture-based system (GRZ) also exhibited a healthier profile, characterized by higher proportions of CLA and TVA in spring butter, and higher proportions of 15:0 and 17:0 iso and anteiso in fall butter. In addition, the yellow color of GRZ butter was stronger. However, contrary to expectations, GRZ butter in fall presented greater firmness, possibly related to the higher palmitic/oleic ratio, influenced by the FA composition of the diet. This demonstrates the potential benefits of adjusting the management of feed for confined cows with oils, and highlights the need for further studies considering supply and cost constraints.

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