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Comparative evaluation of major milk quality parameters of Holstein and Simmental cows at different lactation stages under similar environmental conditions*

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Summary

The aim of this study was to evaluate the composition and quality parameters of Simmental and Holstein cows' milk with different lactation stages under the same environmental conditions. Multiparous Holstein and Simmental cows from different lactation stages (n = 210) were included in the present study. MDA, SCC, composition, and fatty acid analyzes were performed from the collected milk samples. To determine the effect of breed, lactation stage and their interactions, linear mixed models were applied to these parameters. Among the breeds, only milk fat and pH were determined statistically significant as composition parameters. While C15:0 and C17:1 n8 were observed to be statistically significant for the breed factor, C18:2 n6 trans were statistically significant only for the lactation stage factor. Moreover, the fatty acids of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C16:0, C18:1 n9, C20:0 and C22:6 n3 were determined to be statistically significant in terms of interactions. For the fatty acid indices, SCFA, MCFA, n3 and n6/n3 were statistically significant in terms of interactions while SFA, MUFA, UFA and AI were statistically significant in terms of breeds. On the other hand, new studies are needed to investigate the differences between these breeds at the molecular level for milk quality and fatty acid synthesis.

Keywords: Cow milk, lactation stage, milk fatty acids, milk quality, mixed model

Animal breeding is one of the most critical sectors in the agriculture industry. Milk and dairy products are important components of livestock production. Milk obtained from farm animals is a highly nutritious biological fluid (12, 31). On the other hand, cow milk, which accounts for more than 80% of total milk production, is the most produced and preferred farm animal milk in the world.

With the increasing global population, the demand for food is gradually increasing. According to the Food and Organization statistics between 1988 and 2018, the total global milk production has increased by approximately 60%. In addition to necessity, the need for milk increases with the rise in nutritional awareness. Quality, which is the state of conformity

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with international standards, has become one of the most important parameters of nutritional awareness. Fat, protein and lactose composition, fatty acid profile, and other ingredients are responsible for the quality of milk (41, 59).

Milk, which contains essential amino acids, vitamins, and minerals, is a highly valuable product for human consumption. Milk composition is influenced by many factors such as season, lactation stage, feeding, and age. It has been reported that environmental factors are the primary responsible reasons for the differences in milk composition (60). Moreover, the breed is another important factor that affects milk composition (31). Holstein and Simmental breeds are the highly preferred cattle breeds for milk production. Although there are many studies on the milk composition of various cattle breeds, the number of studies comparing the composition and quality of milk of different breeds under the same environmental conditions is limited (17, 30, 31, 46).

In this study, the composition and quality parameters of milk obtained from Holstein and Simmental breeds in all lactation stages were evaluated under the same environmental conditions.

Material and methods

Animals and milk collection. The study was carried out on a private dairy farm in Kayseri province, Turkey. The study material consisted of two hundred and ten (n = 210) milk samples collected from multiparous and healthy Holstein (n = 13, early lactation, n = 41, mid lactation and n = 67, late lactation) and Simmental cows (n = 16, early lactation, n = 41, mid lactation and n = 32, late lactation) (lactation number of the cows = 2.5 ± 0.85) at different lactation stages. The lactation stage of cows was classified as early lactation (ELS) (< 100 days in milk), mid lactation (MLS) (100-200 days in milk) and late lactation (LLS) (> 200 days in milk) (61). All animals in the study were housed under the same environmental conditions and feeding program. The barns were naturally ventilated. All the cows had *ad libitum* access to water. Cows were fed a total mixed ration (TMR) according to the NRC for the nutritional requirements (40). The chemical composition and ingredients of the TMR are shown in Table 1. Moreover, the fatty acid content of the ration is given in Table 2.

Milk samples were collected from each cow included in the study at morning milking (07:00) using the automatic milking system (AfiMilk, Afkim, Israel). All mammary lobes of the cows were checked for mastitis using the California Mastitis Test (CMT) before the collection of milk samples. First, the udder was cleaned and then, after the first squeezed milk was expelled, approximately 100 ml of samples were collected from each animal in 50 ml sterile falcons. The quality parameters of the milk samples were analyzed in the laboratory located right next to the milking unit.

Somatic cell count (SCC) and milk composition parameters. To determine the milk somatic cell counts, the samples were prepared according to the protocol of the Lactoscan SCC kit (18.05.2021/R) and measured using a milk somatic cell counter (Lactoscan SCC 6010, Bulgaria). For determination of the milk pH, approximately 5 ml of milk was collected and transferred into glass tubes. The pH of the milk was measured via a pH probe (Hanna pH meter, HI83141, USA). Fat, fat-free dry matter, protein, lactose, freezing point and electrical conductivity of milk quality parameters were measured with a milk analyzer (Milkotester Master Classic LM2-P1, Bulgaria).

Determination of malondialdehyde (MDA) levels. MDA levels were measured according to the method specified by Esterbauer and Cheeseman (21). Approximately

Tab. 1. The chemical composition and ingredients of TMR

Item	Ration
DM, %	57.81
CP, %	17.82
ADF, %	17.64
NDF, %	29.06
TDN, %	74.17
NEL, Mcal/kg	1.69
Component	Ingredient.% of DM
Corn	14.62
Soybean meal	6.39
Concentrated feed	13.30
Corn silage	50.10
Alfalfa hay	12.20
Vetch hay	0.67
Magnesium oxide	1.19
Sodium bicarbonate	0.54
Bypass fat	0.94
Antioxidant	0.05

Explanations: DM – dry matter, CP – crude protein, ADF – acid detergent fiber, NDF – neutral detergent fiber, TDN – total digestible nutrients, NEL – net energy lactation

Tab. 2. The fatty acid content of the ration

Dietary Fatty Acids	%	Dietary Fatty Acids	%
C10:0	0.05	C20:0	0.18
C12:0	0.35	C20:1	0.01
C13:0	0.01	C20:2	0.01
C14:0	0.71	C20:3 n6	0.06
C14:1	0.01	C20:4 n6	0.04
C15:0	0.04	C20:3 n3	0.01
C15:1	0.09	C20:5 n3	0.11
C16:0	52.47	C22:0	0.04
C16:1	0.39	C22:1 n9	0.01
C18:0	2.54	C22:2	0.04
C18:1	15.99	C22:6 n3	0.01
C18:2 n6	23.82	C24:0	0.05
C18:3 n3	0.03	C24:1	0.01
C18:3 n6	2.92		

500 µl of each milk sample was collectedin glass tubes. 2500 µl of 10% trichloroacetic acid (AC31301000, Spain) was added to the milk samples. The samples were then incubated at 95°C for 15 minutes. After incubation, the samples were centrifuged at 3000 × g for 10 minutes at 4°C. Afterwards, approximately 2000 µl of the supernatant was collected and transferred to a new tube. Then, 2000 µl of 0.675% 2-thiobarbituric acid (#BCBH3605V, Germany) was added and vortexed. The homogenized samples were incubated at 95°C for 15 minutes. After the incubation phase, the samples were kept in an ice pool and MDA levels were determined with a UV-Spectrophotometer at a wavelength of 532 nm (21).

Crema layer collection and fatty acid analysis. Approximately 50 ml of milk was collected from each milk sample and placed in falcon tubes. Milk samples in Falcon tubes were centrifuged at 1800 × g for 10 minutes at 4°C. The samples were then stored at –20°C for 10 minutes to collect the cream layer. The cream layer was then transferred to new glass tubes using a spatula. Cream layers were stored at –20°C until fatty acid analysis (43).

Approximately 500 µl of cream from each sample was used for fatty acid analysis. The cream layers were vortexed with 2 ml of 2N methanolic KOH for 4 minutes at room temperature until homogenized. Later, 4 ml of n-heptane (Merck, USA) was added to the homogenate. After homogenization for 2 minutes at room temperature, the samples were centrifuged at 200 × g for 5 minutes for phase separation. The methyl esters collected in the supernatant were collected in 1.5 ml vials and fatty acids were determined by Gas Chromatography (HP Agilent 6890, USA) using an HP Innowax column (60 m length, 0.25 mm i.d. × 0.25 µm film). The injector temperature was set to 250°C and the detector temperature to 270°C. Helium was used as the carrier gas. The injection was washed three times with n-heptane. The oven temperature was initially programmed at 90°C for 3 minutes and was increased to 250°C at a ramp rate of 3°C/min. For the verification of fatty acids, the determined peak retention times of the samples were compared with the internal standard (FAME Mix, Restek, USA). The results of fatty acids were calculated as "percentage (%)". The formulations of fatty acid indices (short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), saturated fatty acids (SFA), n6 (omega-6), n3 (omega-3), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), unsaturated fatty acids (UFA), atherogenic index (AI), and nutritive value (NV)) were calculated according to Özkan et al. (43).

Statistical analysis. Statistical analyses were performed using Stata 16.1 statistical software (StataCorp, College Station, TX, USA). The sample size was calculated with G*Power software version 3.1.9.2. The result of the sample size calculation showed that the minimum number of dairy cows (a total number of Simmental and Holstein cows) was 196, considering a medium prior effect size of 0.25, an alpha value of 0.05, and a power of 0.80. Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean (SEM)". Furthermore, MDA, SCC, milk quality parameters and fatty acid indices were displayed as a figure. The normality of all variables was confirmed using the Kolmogorov-Smirnov test.

All variables were analyzed using a linear mixed model. The effect of breed, lactation stage and their interaction on MDA, SCC, milk composition parameters, fatty acid profile and fatty acid indices was examined by using the following model:

$$Y_{ijkl} = \mu + B_i + L_j + (B \times L)_{ij} + C_k + e_{ijkl}$$

where, Y_{ijkl} is the observed value for each variable; µ is the overall mean; B_i is the effect of the breed (i = 2 classes; Simmental cows and Holstein cows); L_j is the effect of lactation stage (j = 3 classes; early lactation stage (ELS), mid lactation stage (MLS) and late lactation stage (LLS)); $(B \times L)_{ij}$ is the interaction between breed i and lactation stage j; C_k is the random effect of the cows and e_{ijkl} is the residual error.

In the model, cows were evaluated as a random effect, while breed, lactation stage and their interaction term were evaluated as a fixed effect. Variance components were used as the covariance structure in the established model since it resulted in the lowest Akaike information criterion (AIC). When a significant difference was observed in the model, significant terms were compared by simple effect analysis with Bonferroni correction. A probability value of less than 0.05 was considered significant.

Results and discussion

MDA, SCC, fat, fat-free dry matter, protein, lactose, freezing point, electrical conductivity, and pH, which are milk composition and quality parameters, are shown in Figure 1 and Figure 2. There was a statistical difference between breed, lactation stage and their interaction term in terms of MDA, SCC, fat-free dry matter, protein, lactose, freezing point, and electrical conductivity parameters. Milk fat and pH parameters were determined to be statistically significant only between breeds ($p = 0.005$ and $p < 0.001$, respectively). Moreover, fat and pH were determined at higher levels in Holstein cows than in Simmental cows ($p < 0.05$).

The fatty acid composition is shown in Table 3. The fatty acids C4:0, C6:0, C8:0, C10:0, C11:0, C12:0 and C13:0 were observed to be statistically significant in terms of interactions (B*L) ($p = 0.044$, $p = 0.004$, $p = 0.005$, $p = 0.003$, $p = 0.025$, $p = 0.005$ and $p = 0.049$, respectively). In Simmental cows, C4:0, C6:0, C8:0 and C10:0 fatty acids were determined to be higher in MLS compared to other stages ($p < 0.05$). Furthermore, Simmental cows had higher levels of these fatty acids in the MLS than Holstein cows ($p < 0.05$). Besides, C11:0 fatty acid was higher in Simmental cows in ELS, while it was higher in Holstein cows in MLS and LLS ($p < 0.05$). In Simmental cows, C12:0 fatty acid was observed to be lower in ELS compared to other stages ($p < 0.05$), while this situation was observed to be the opposite in Holstein cows ($p < 0.05$). Moreover, C12:0 fatty acid in MLS was lower in Holstein cows than in Simmental cows ($p < 0.05$). While C13:0 fatty acid was found to be indifferent in terms of lactation stage in Holstein cows ($p > 0.05$), it was observed at a lower level in ELS in Simmental cows compared to

Tab. 3. The result of the linear mixed model for milk fatty acids (Mean ± SEM)

Parameters	Breeds	Lactation Stages			P-value		
		ELS	MLS	LLS	B	L	B*L
C4:0	Simmental	0.539 ± 0.098^{b}	1.584 ± 0.341$^{a, A}$	1.204 ± 0.332^{ab}	0.589	0.716	0.044
	Holstein	1.138 ± 0.462	0.678 ± 0.172^{B}	1.064 ± 0.183			
C6:0	Simmental	1.489 ± 0.229^{b}	3.807 ± 0.493$^{a, A}$	2.488 ± 0.409^{ab}	0.234	0.379	0.004
	Holstein	2.281 ± 0.964	1.579 ± 0.280^{B}	2.394 ± 0.317			
C8:0	Simmental	1.673 ± 0.253^{b}	3.341 ± 0.386$^{a, A}$	2.806 ± 0.308^{ab}	0.387	0.588	0.005
	Holstein	2.723 ± 0.880	1.739 ± 0.230^{B}	2.507 ± 0.225			
C10:0	Simmental	5.120 ± 0.626^{b}	8.303 ± 0.793$^{a, A}$	7.585 ± 0.602^{a}	0.293	0.608	0.003
	Holstein	7.518 ± 1.280	4.960 ± 0.429^{B}	6.516 ± 0.414			
C11:0	Simmental	0.435 ± 0.055^{A}	0.270 ± 0.022^{B}	0.354 ± 0.041^{B}	0.426	0.170	0.025
	Holstein	0.272 ± 0.060^{B}	0.420 ± 0.031^{A}	0.468 ± 0.036^{A}			
C12:0	Simmental	6.246 ± 0.523^{b}	8.033 ± 0.481$^{a, A}$	7.914 ± 0.410^{a}	0.270	0.429	0.005
	Holstein	7.919 ± 0.859^{a}	5.898 ± 0.303$^{b, B}$	6.957 ± 0.283^{ab}			
C13:0	Simmental	0.198 ± 0.013$^{b, B}$	0.261 ± 0.016$^{a, A}$	0.253 ± 0.013^{ab}	0.905	0.740	0.049
	Holstein	0.259 ± 0.022^{A}	0.224 ± 0.012^{B}	0.234 ± 0.010			
C14:0	Simmental	16.942 ± 0.596	16.966 ± 0.267	17.745 ± 0.456	0.168	0.210	0.745
	Holstein	16.834 ± 0.810	16.296 ± 0.392	16.785 ± 0.285			
C14:1 n5	Simmental	1.591 ± 0.105	1.676 ± 0.117	1.736 ± 0.145	0.022	0.993	0.692
	Holstein	1.994 ± 0.188	1.942 ± 0.095	1.886 ± 0.076			
C15:0	Simmental	1.136 ± 0.077	1.232 ± 0.043	1.264 ± 0.068	0.014	0.282	0.223
	Holstein	1.310 ± 0.091	1.454 ± 0.053	1.312 ± 0.041			
C15:1 n5	Simmental	0.238 ± 0.042	0.177 ± 0.010	0.174 ± 0.016	0.459	0.664	0.742
	Holstein	0.223 ± 0.021	0.210 ± 0.024	0.222 ± 0.016			
C16:0	Simmental	41.728 ± 0.799$^{a, A}$	34.736 ± 1.557$^{b, B}$	37.053 ± 1.217^{ab}	0.815	0.771	0.002
	Holstein	35.835 ± 2.594^{B}	40.607 ± 0.879^{A}	37.980 ± 0.907			
C16:1 n7	Simmental	2.053 ± 0.231	2.057 ± 0.122	1.790 ± 0.122	0.210	0.202	0.559
	Holstein	1.983 ± 0.235	2.316 ± 0.126	2.145 ± 0.104			
C17:0	Simmental	0.406 ± 0.047	0.299 ± 0.029	0.303 ± 0.017	0.435	0.335	0.060
	Holstein	0.334 ± 0.045	0.385 ± 0.015	0.345 ± 0.016			
C17:1 n8	Simmental	0.197 ± 0.024	0.167 ± 0.017	0.139 ± 0.012	0.044	0.391	0.303
	Holstein	0.189 ± 0.057	0.215 ± 0.011	0.205 ± 0.013			
C18:0	Simmental	3.624 ± 0.295	2.859 ± 0.213	3.151 ± 0.265	0.131	0.491	0.155
	Holstein	3.607 ± 0.440	3.721 ± 0.194	3.341 ± 0.143			
C18:1 n9	Simmental	12.850 ± 1.040	10.999 ± 0.539^{B}	10.895 ± 0.683	0.118	0.210	0.047
	Holstein	11.648 ± 1.308^{b}	14.003 ± 0.559$^{a, A}$	12.159 ± 0.413^{b}			
C18:2 n6 trans	Simmental	0.142 ± 0.019	0.143 ± 0.011	0.118 ± 0.012	0.007	0.005	0.395
	Holstein	0.201 ± 0.028	0.192 ± 0.020	0.136 ± 0.007			
C18:2 n6 cis	Simmental	2.352 ± 0.217	1.752 ± 0.118	1.770 ± 0.089	0.542	0.141	0.134
	Holstein	2.033 ± 0.268	2.096 ± 0.087	1.965 ± 0.087			
C18:3 n3	Simmental	0.043 ± 0.010	0.111 ± 0.041	0.071 ± 0.015	0.949	0.909	0.163
	Holstein	0.095 ± 0.043	0.054 ± 0.013	0.081 ± 0.017			
C18:3 n6	Simmental	0.308 ± 0.034	0.257 ± 0.027	0.259 ± 0.019	0.221	0.463	0.862
	Holstein	0.317 ± 0.060	0.299 ± 0.015	0.291 ± 0.015			
C20:0	Simmental	0.035 ± 0.005^{B}	0.067 ± 0.015	0.130 ± 0.050 ^{A}	0.319	0.077	0.002
	Holstein	0.209 ± 0.051$^{a, A}$	0.042 ± 0.004^{b}	0.057 ± 0.009$^{b, B}$			
C20:1 n7	Simmental	0.012 ± 0.003	0.020 ± 0.003	0.037 ± 0.013	0.195	0.137	0.062
	Holstein	0.052 ± 0.023	0.019 ± 0.005	0.027 ± 0.005			

Parameters	Breeds	Lactation Stages			P-value		
		ELS	MLS	LLS	B	L	B*L
C20:2 n6	Simmental	0.021 ± 0.005	0.059 ± 0.022	0.045 ± 0.017	0.587	0.935	0.094
	Holstein	0.081 ± 0.031	0.027 ± 0.010	0.043 ± 0.010			
C20:3 n6	Simmental	0.023 ± 0.006	0.043 ± 0.013	0.045 ± 0.011	0.861	0.191	0.329
	Holstein	0.031 ± 0.012	0.021 ± 0.004	0.052 ± 0.011			
C20:4 n6	Simmental	0.096 ± 0.022	0.097 ± 0.023	0.060 ± 0.012	0.368	0.915	0.083
	Holstein	0.105 ± 0.040	0.082 ± 0.009	0.120 ± 0.016			
C20:3 n3	Simmental	0.030 ± 0.014	0.055 ± 0.017	0.038 ± 0.009	0.948	0.698	0.107
	Holstein	0.045 ± 0.017	0.022 ± 0.005	0.059 ± 0.014			
C20:5 n3	Simmental	0.036 ± 0.015	0.055 ± 0.014	0.049 ± 0.016	0.464	0.774	0.352
	Holstein	0.086 ± 0.038	0.036 ± 0.010	0.060 ± 0.017			
C21:0	Simmental	0.233 ± 0.024	0.139 ± 0.020	0.172 ± 0.016	0.455	0.266	0.059
	Holstein	0.190 ± 0.040	0.205 ± 0.013	0.189 ± 0.012			
C22:0	Simmental	0.034 ± 0.014	0.050 ± 0.011	0.047 ± 0.015	0.303	0.356	0.162
	Holstein	0.072 ± 0.029	0.033 ± 0.006	0.071 ± 0.012			
C22:1 n9	Simmental	0.043 ± 0.009	0.045 ± 0.006	0.052 ± 0.011	0.357	0.358	0.708
	Holstein	0.055 ± 0.014	0.049 ± 0.009	0.077 ± 0.015			
C22:2 n6	Simmental	0.016 ± 0.005	0.037 ± 0.011	0.031 ± 0.010	0.912	0.804	0.531
	Holstein	0.030 ± 0.008	0.023 ± 0.008	0.034 ± 0.008			
C22:6 n3	Simmental	0.013 ± 0.004^{B}	0.068 ± 0.014	0.047 ± 0.018	0.350	0.623	0.046
	Holstein	0.107 ± 0.036^{A}	0.038 ± 0.020	0.034 ± 0.008			
C23:0	Simmental	0.033 ± 0.012	0.091 ± 0.019	0.073 ± 0.016	0.594	0.928	0.124
	Holstein	0.101 ± 0.031	0.050 ± 0.012	0.079 ± 0.015			
C24:0	Simmental	0.033 ± 0.014	0.069 ± 0.029	0.037 ± 0.009	0.650	0.763	0.484
	Holstein	0.029 ± 0.007	0.034 ± 0.016	0.047 ± 0.017			
C24:1 n9	Simmental	0.032 ± 0.008	0.075 ± 0.020	0.063 ± 0.022	0.845	0.923	0.212
	Holstein	0.093 ± 0.034	0.032 ± 0.005	0.057 ± 0.012			

Explanations: ELS – early lactation stage; MLS – mid lactation stage; LLS – late lactation stage; B – breed; L – lactation stage; B * L – breed * lactation stage interaction term; a, b, c – lowercases in the same line represent the difference between lactation stages ($p < 0.05$); A, B, C – uppercases in the same row represent the difference between breeds ($p < 0.05$)

other periods ($p < 0.05$). Furthermore, Holstein cows had higher levels of C13:0 fatty acid in ELS, but lower levels in MLS ($p < 0.05$). Regarding the breed, C14:1, C15:0 and C17:1 fatty acids were generally higher in Holstein cows than in Simmental cows ($p = 0.022$, $p = 0.014$ and $p = 0.044$, respectively). C16:0, C18:1, C20:0 and C22:6 n3 fatty acids were observed to be statistically significant only for the interaction term ($p = 0.002$, $p = 0.047$, $p = 0.002$ and $p = 0.046$, respectively), while C18:2 n6 t fatty acids were statistically significant between breeds ($p = 0.007$) and lactation stages ($p = 0.005$). While C16:0 fatty acid was similar in Holstein cows in terms of lactation stages ($p > 0.05$), it was higher in ELS in Simmental cows compared to other stages ($p < 0.05$). In addition, the C16:0 fatty acid was determined to be higher in ELS in Simmental cows than in Holstein cows, while the opposite was observed in MLS ($p < 0.05$). C18:1 fatty acid was similar in Simmental cows at different lactation stages ($p > 0.05$) and, it was higher in MLS in Holstein cows compared to other stages ($p < 0.05$). C18:2 n6 t fatty acid was higher in Holstein cows than in Simmental cows ($p = 0.007$), but lower in LLS than in other periods ($p = 0.005$). As C20:0 fatty acid was similar in Simmental cows in terms of lactation stages ($p > 0.05$), it was higher in Holstein cows in ELS compared to other stages ($p < 0.05$). In addition, C20:0 fatty acid was higher in ELS in Holstein cows than in Simmental cows ($p < 0.05$); the opposite situation, however, was determined in LLS ($p < 0.05$). C22:6 n3 fatty acid was determined statistically higher in Holstein cows than in Simmental cows only in ELS ($p < 0.05$).

The fatty acid indices of the milk samples are shown in Figure 3 and Figure 4. The interaction term was observed as statistically significant in SCFA and MCFA indices ($p = 0.004$ and $p = 0.008$, respectively). In Simmental cows, SCFA was higher in MLS and LLS than in ELS ($p < 0.05$). Besides, SCFA in MLS was lower in Holstein cows than in Simmental cows ($p < 0.05$). MCFA was higher in ELS than in MLS in Simmental cows ($p < 0.05$). Furthermore, MCFA in MLS was higher in Holstein cows than in Simmental

cows ($p < 0.05$). While SFA was higher in Simmental cows than in Holstein cows ($p = 0.043$), MUFA and UFA were lower ($p = 0.039$ and $p = 0.043$, respectively). Additionally, n3 was higher in Holstein cows than in Simmental cows in ELS ($p < 0.05$) and lower in MLS ($p < 0.05$). AI was lower in Holstein cows than in Simmental cows ($p = 0.027$). The n6/n3 ratio was higher in Simmental cows in ELS compared to other stages ($p < 0.05$) and in Holstein cows in MLS compared to other stages ($p < 0.05$). Moreover, the n6/n3 ratio was higher in Simmental cows than in Holstein cows in ELS ($p < 0.05$) and the n6/n3 ratio in Holstein cows was higher than in Simmental cows in MLS ($p < 0.05$).

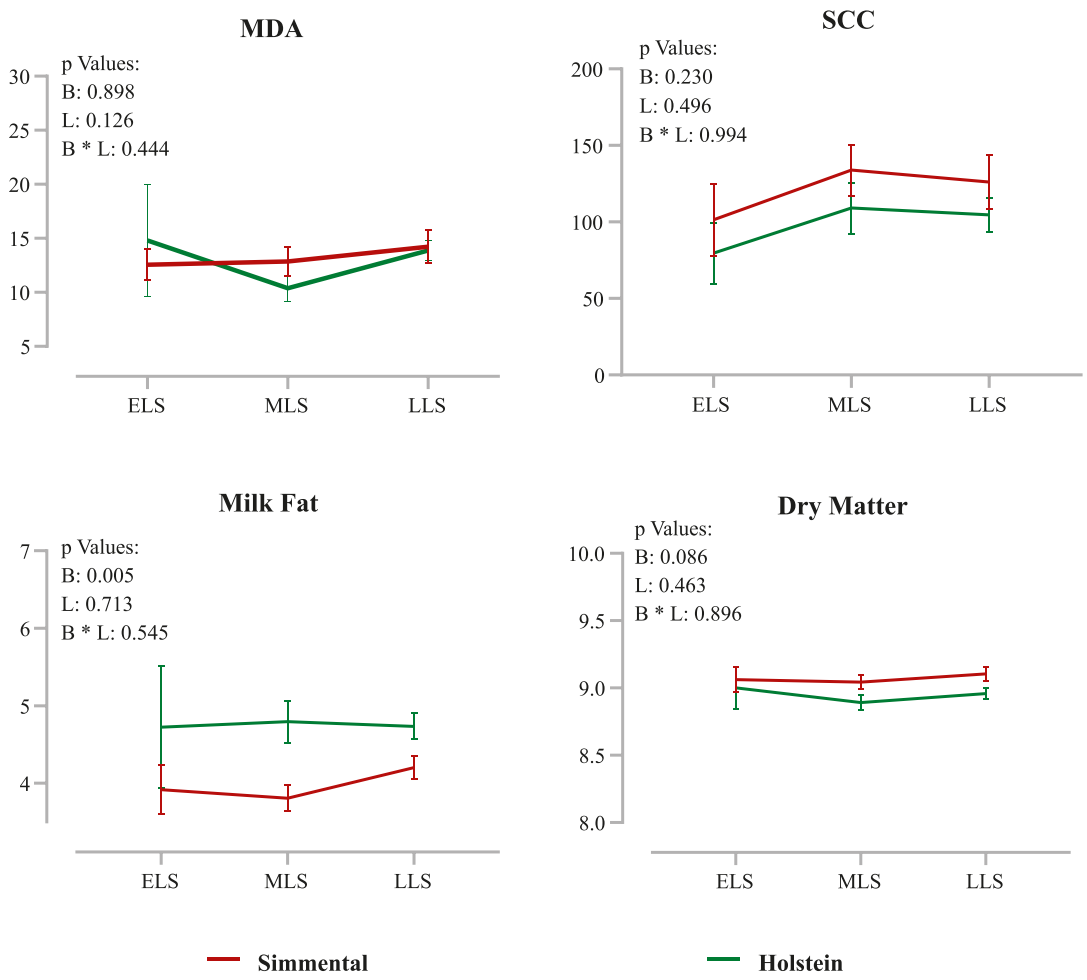

Fig. 1. The effect of the breed, lactation stage and their interaction for milk composition parameters (MDA, SCC, milk fat and dry matter)
Explanations: B – breed; L – lactation stage; B * L – breed * lactation stage interaction term

As a result of long term improvement studies on genetics and environmental factors in animal breeding over a long time, significant progress has been made in terms of various quantitative characteristics. Quantitative characteristics such as milk yield and milk quality are formed by the additive effects of many genes, and there may be significant differences at the breed level in terms of these characteristics (24, 35, 54). Substantial studies have been carried out to improve milk yield and quality in Holstein and Simmental cattle. Yield and quality parameters have been improved in these breeds with progeny testing and effective selection practices. Besides, significant progress has been made in terms of environmental factors such as ration and breeding conditions (39, 46, 56).

Milk secretion in mammals is a physiological property that occurs following birth and continues for different periods depending on the species and breeding strategies. Milk secretion begins as colostrum. After transitional milk, it transforms into a mature form called milk (59). Although many parameters make up milk quality, the most important quality parameters of milk are compositional parameters such as fat, protein, and lactose (43). In the present study, it was determined that there were significant differences in some parameters responsible for the formation of milk quality in different lactation periods in Holstein and Simmental cows reared under the same environmental conditions. While MDA, SCC, FFDM, protein, lactose, freezing point, and electrical conductivity parameters were observed to be similar in both breeds, milk fat and pH values of milk were higher in Holstein cows. MDA is the final oxidation product and is widely used as an oxidative stress indicator (59). In the present study, MDA levels were determined stable in different lactation stages and breeds. Besides, SCC, which is strongly related to mammary health, was observed at acceptable levels (22). Milk fat is one of the major quality parameters in milk (10). Milk fat percentages of the Holstein breed were determined higher as expected (44). Although they were under the same environmental conditions, it is thought that the difference in milk fat values is related to the breed factor. In general, higher levels in milk are related to the oxidative and inflammatory status of the mammary gland. On the other hand, it is thought that the pH differences between breeds might be related to the fat ratio of the milk samples (59). Since, different fractions of milk such as protein, fat, lactose, and others are also responsible for the levels of milk pH (1).

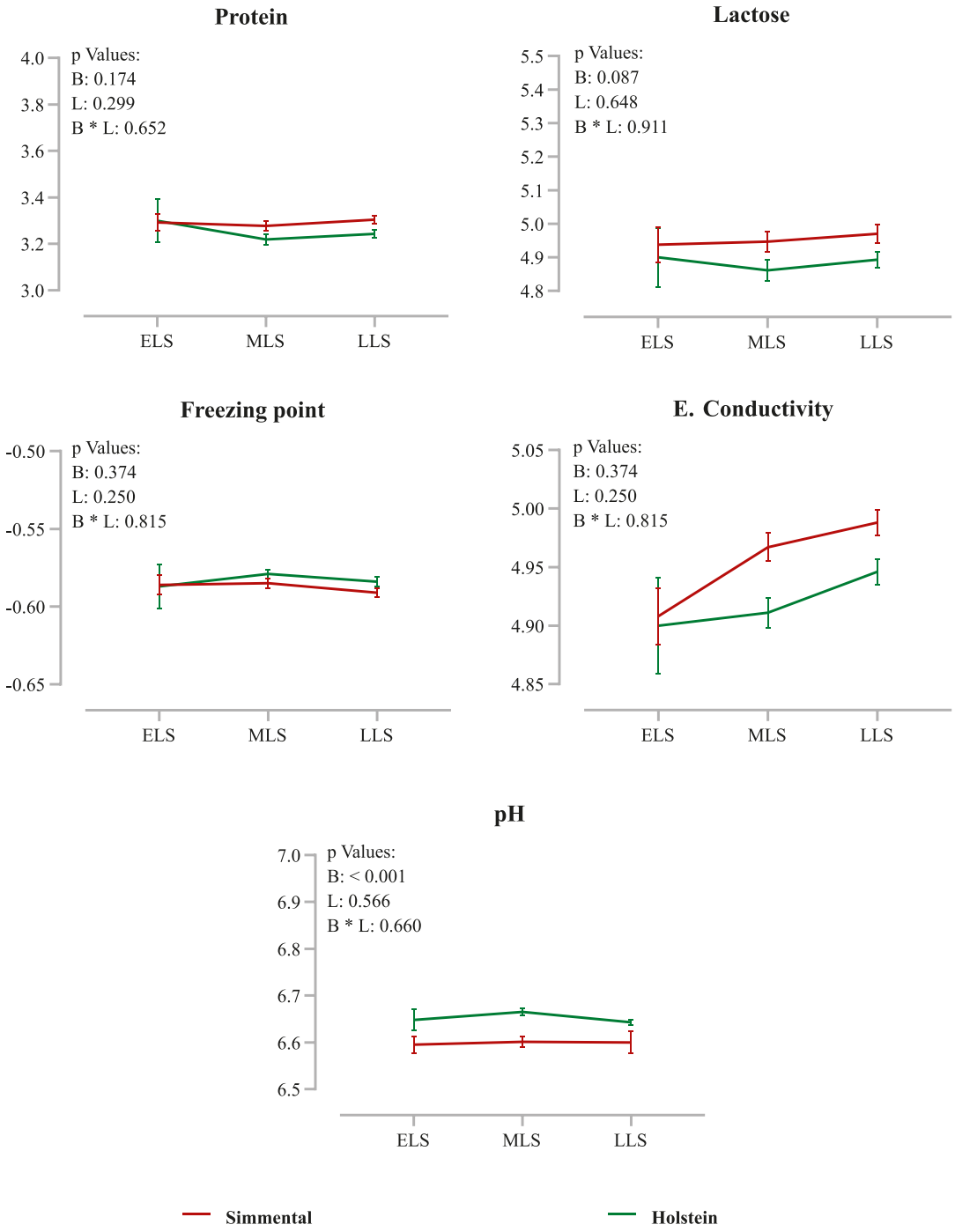

Fig. 2. The effect of the breed, lactation stage and their interaction for milk composition parameters (protein, lactose, freezing point, electrical conductivity and pH)
Explanations: B – breed; L – lactation stage; B * L – breed * lactation stage interaction term

The fatty acid profile of milk is one of the most important parameters of milk quality (4, 59). Debates on the healthiness of the fatty acid profile of milk have been ongoing (46). Significant differences have been detected between Simmental and Holstein breeds in terms of milk fatty acids. Particularly, most of the SCFA in the Simmental breed were found higher at the mid-lactation stage. C4:0, C6:0, C8:0 and C10:0 fatty acids were almost two times higher in MLS in Simmental. In addition, medium-chain fatty acids such as C10:0, C12:0, and C13:0 were determined higher in the Simmental breed in the MLS. On the other hand, C14:1 was lower while C14:0 was higher in Simmental milk. Even if the ration of the animals was similar, the C16:0 levels were different in Simmental and Holstein cows in ELS and MLS. Interestingly, C16:0 was higher in Simmental milk in ELS, however, it was lower than Holstein in later stages. It is known that parts of C16:0 fatty acids, C4:0-C14:0 fatty acids in the milk are synthesized de novo in the mammary gland. Moreover, fatty acids longer than C16:0 come into the milk from the bloodstream and originate from the ration (55). It has been thought that the main reason for the difference in C16:0 as well as short and medium-chain fatty acids may be related to the activity of mammary epithelial cells in the related breeds (16). Although it has been reported that fatty acids longer than C16:0 are associated with the diet, C17:1, C18:1 and C18:2, C20:0, C22:6 n3 were observed to be different between breeds. Considering that the two breeds were under similar environmental conditions, these results may be related to genotypic differences in Simmental and Holstein (11, 49, 55).

Fatty acid indices such as SFA, MUFA, PUFA, UFA, n3, and n6 also have substantial roles in determining milk quality. Gottardo et al. (26) reported similar levels of SFA, SCFA, MUFA, UFA and PUFA in the milk of randomly selected Holstein and Simmental cows. In this study, milk SFA contents were higher in Simmental milk, while MUFA and UFA values were found lower. Recent studies have reported that many parameters such as genotype, diet, and different milking methods

have an effect on the change of parameters related to fatty acids (5, 18, 47, 50). In addition, the amounts of SFA, MUFA and UFA may have varying effects on obesity depending on their source and endogenous synthesis (14). Many studies argue that the decrease in the amount of SFA causes an increase in the amount of MUFA and UFA, which has a protective effect against metabolic disorders such as cardiovascular diseases (25, 36, 38, 48, 57). Moreover, the amount of SFA is directly related to the AI (47). Similarly, in this study the AI was higher in the milk of Simmental cows. Scientific reports have indicated that the increase potential in the AI causes an increase in the amount of low-density lipoprotein (LDL) (2, 29). There is strong evidence that an increase in LDL leads to an increase in cholesterol, which increases cardiovascular risk (13, 23, 34, 51).

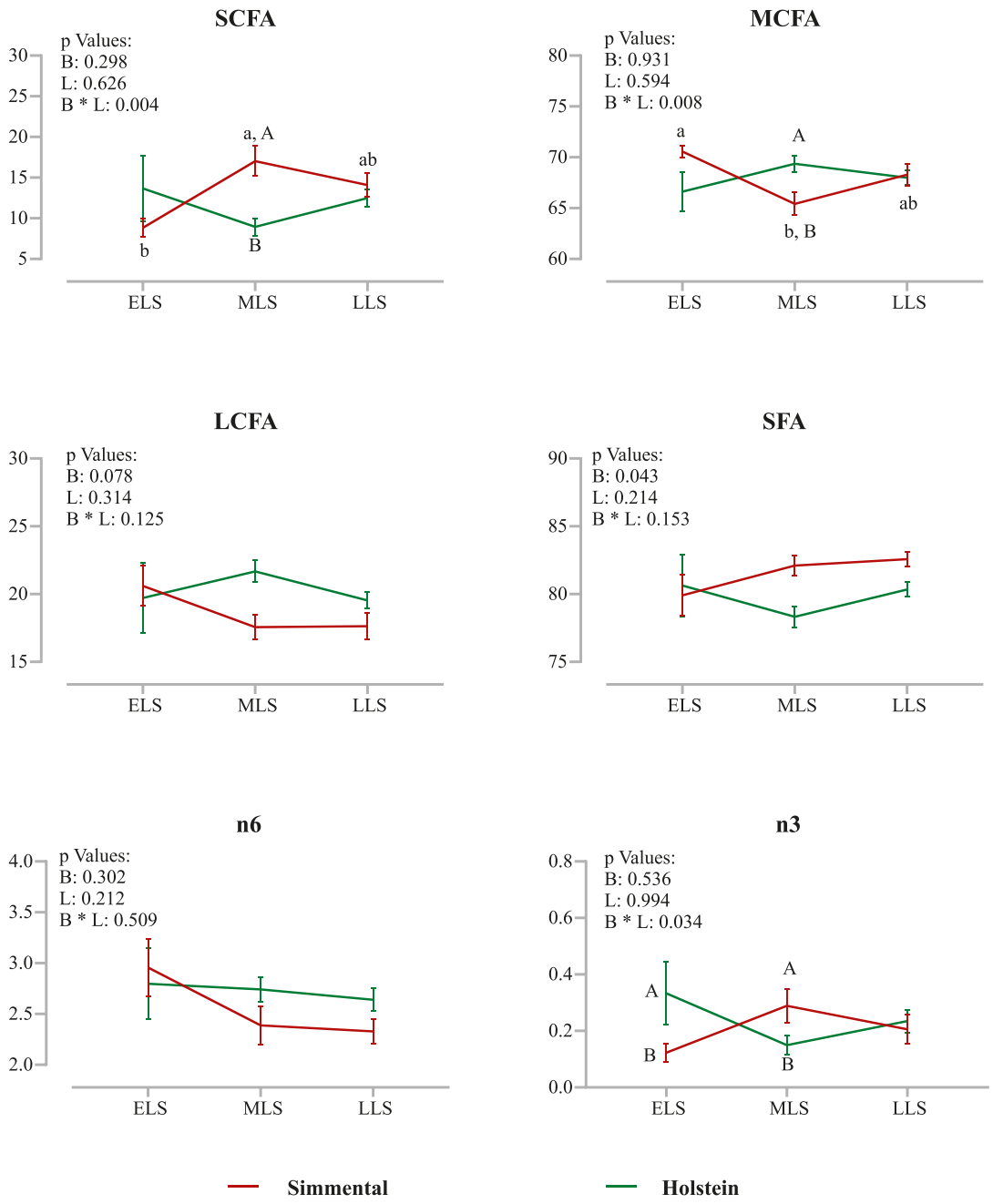

Fig. 3. The effect of the breed, lactation stage and their interaction for fatty acid indices (SCFA, MCFA, LCFA, SFA, n6 and n3)

Explanations: B – breed; L – lactation stage; B * L – breed * lactation stage interaction term; a, b, c – lowercases in the same line represent the difference between lactation stages ($p < 0.05$), A, B, C – uppercases in the same row represent the difference between breeds ($p < 0.05$)

In the present study, the amount of MCFA in Holstein milk increased in MLS. The variation in the amount of MCFA observed in animals under similar environmental conditions is thought to be due to interracial genotypic variation. On the other hand, SCFA was higher in MLS as expected (53). Martinez-Castillero et al. (37) reported that the amount of SCFA in Holstein milk was significantly higher than in Simmental milk. Recent studies have reported that SCFAs ingested with milk play an active role in the regulation of immune functions by affecting the intestinal flora (15, 58). SCFAs in milk are also known to be effective fatty acids in the initiation of gluconeogenesis in the liver of milk-consuming calves (32). SCFA and MCFA are crucial components of the fatty acids in milk. Even with the same environmental conditions, the reason for differences between the breeds might be the genotypes of the animals.

In terms of n3, which is one of the important parameters, Holstein milk was better in ELS, while Simmental milk was better in MLS. Similarly, in the study conducted by Bär et al. (8), it was reported that there was a significant change in the amount of n3 in different stages of lactation in Holstein milk. By playing a role in the inflammatory response, n3 found in milk has anti-inflammatory activity (28). The increase in the amount of n3 in milk shows a protective effect against diseases by increasing the ability of calves to respond

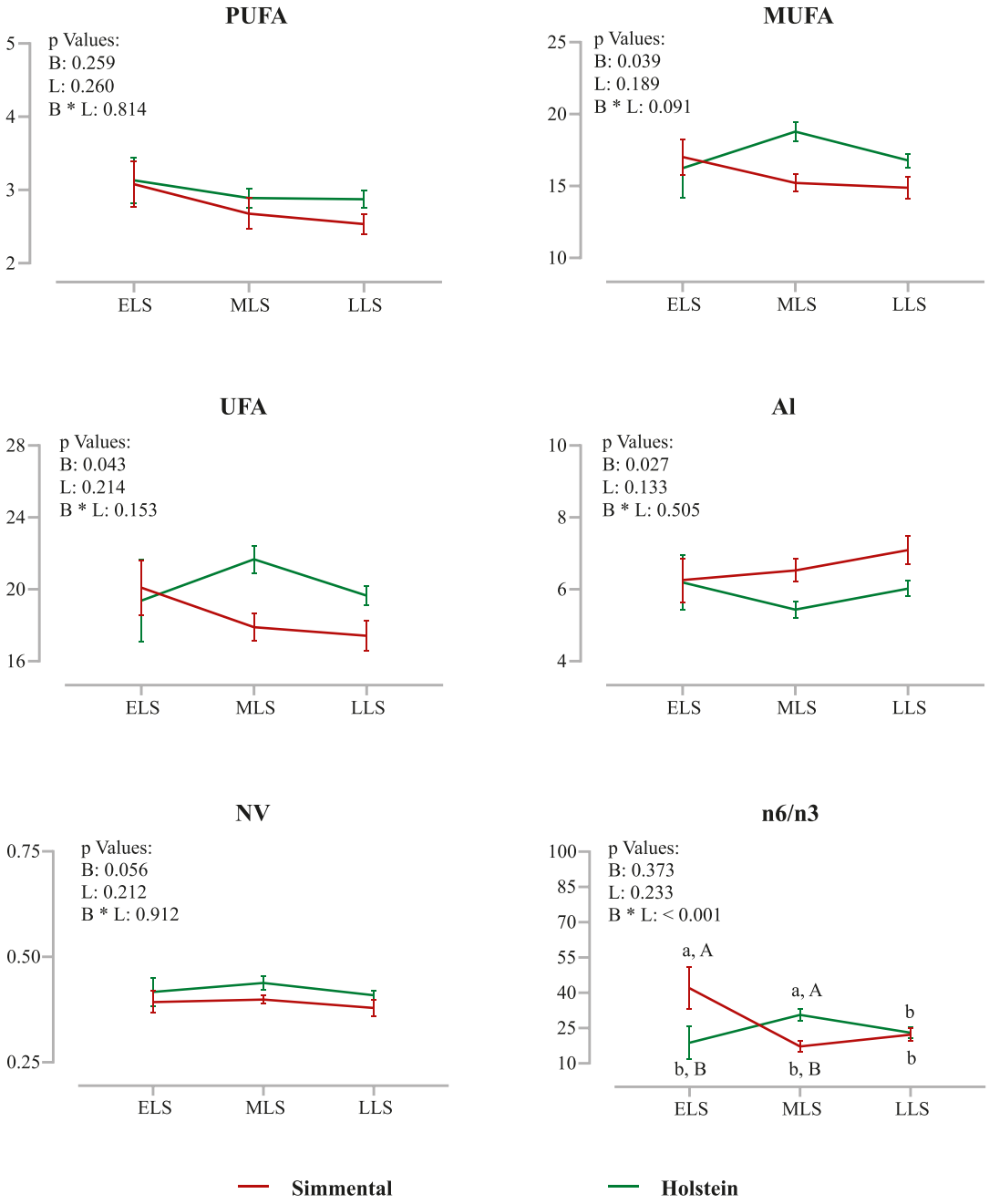

Fig. 4. The effect of the breed, lactation stage and their interaction for fatty acid indices (PUFA, MUFA, UFA, AI, NV and n6/n3)

Explanations: B – breed; L – lactation stage; B * L – breed * lactation stage interaction term; a, b, c – lowercases in the same line represent the difference between lactation stages ($p < 0.05$); A, B, C – uppercases in the same row represent the difference between breeds ($p < 0.05$)

to infection (45, 62). Furthermore, n3 fatty acid, which has an antioxidant effect, plays an important role in the immunity of newborn calves (42, 52). In parallel with the results of the present study, studies show that the survival rate of Holstein's calves is higher than that of Simmental calves (7, 19). Also, higher levels of n3 in the milk are the desired factor for the higher quality of milk for consumption (27).

The increase in the n6/n3 ratio triggers eicosanoid synthesis in the body and plays a role in the anti-inflammatory response in the calf (3, 20). Moreover, it is strongly related to cardiovascular health for consumers (9). In the present study, a significant decrease was observed in the n6/n3 ratio in the transition from ELS to MLS in Simmental milk, while a significant increase was observed in Holstein milk. Bainbridge et al. (6) reported that the n6/n3 ratio in cow's milk varies according to the breed and lactation period of the cow. Although there is a limitation of this study using the same farm animals for the evaluation of milk quality parameters, the obtained results of this study have led to the notion that Holstein milk quality is better than Simmental milk in this environment.

In conclusion, even if the Simmental breed is one of the most preferred cattle breeds for the production of high-quality milk in recent years (33), the data obtained in the present study show that Holstein milk is more consumable for both calves and consumers. On the other hand, considering the differences in fatty acids, which are mostly synthesized de novo in the mammary tissue, new studies are needed to investigate the differences between these breeds at the molecular level.

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