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Changes in milk characteristics and fatty acid profile during the estrous cycle in dairy cows

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ABSTRACT

The relationship of the estrous cycle to milk composition and milk physical properties was assessed on Holstein (n = 10,696), Brown Swiss (n = 20,501), Simmental (n = 17,837), and Alpine Grey (n = 8,595) cows reared in northeastern Italy. The first insemination after calving for each cow was chosen to be the day of estrus and insemination. Test days surrounding the insemination date (from 10 d before to 10 d after the day of the estrus) were selected and categorized in phases relative to estrus as diestrus high-progesterone, proestrus, estrus, metestrus, and diestrus increasing-progesterone phases. Milk components and physical properties were predicted on the basis of Fourier-transform infrared spectra of milk samples and were analyzed using a linear mixed model, which included the random effects of herd, the fixed classification effects of year-month, parity number, breed, estrous cycle phase, day nested within the estrous cycle phase, conception, partial regressions on linear and quadratic effects of days in milk nested within parity number, as well as the interactions between conception outcome with estrous cycle phase and breed with estrous cycle phase. Milk composition, particularly fat, protein, and lactose, showed clear differences among the estrous cycle phases. Fat increased by 0.14% from diestrus high-progesterone to estrous phase, whereas protein concomitantly decreased by 0.03%. Lactose appeared to remain relatively constant over diestrus high-progesterone, rising 1 d before the day of estrus followed by a gradual reduction over the subsequent phases. Specific fatty acids were also affected across the estrous cycle phases: C14:0 and C16:0 decreased (−0.34 and −0.48%) from proestrus to estrus with a concomitant increase in C18:0 and C18:1 *cis*-9 (0.40 and 0.73%). More general categories of fatty acids showed a similar behavior; that is, unsaturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, *trans* fatty acids, and long-chain fatty acids increased, whereas the saturated fatty acids, medium-chain fatty acids, and short-chain fatty acids decreased during the estrous phase. Finally, urea, somatic cell score, freezing point, pH, and homogenization index were also affected indicating variation associated with the hormonal and behavioral changes of cows in standing estrus. Hence, the variation in milk profiles of cows showing estrus should potentially be taken into account for precision dairy farming management.

Key words: mammary gland activity, de novo fat synthesis, heat detection, milk quality, saturated fatty acid

INTRODUCTION

The estrous cycle in dairy cattle has been widely studied given its importance for reproductive performance in dairy cattle. Opportune heat detection and correct insemination timing and techniques are fundamental to a good reproductive management program (Kaproth and Foote, 2011; Nebel et al., 2011). Inferences of negative genetic correlations between milk production and fertility (Lucy, 2001; Pryce et al., 2004) have led to the inclusion of fertility traits in genetic evaluation and selection index programs (VanRaden et al., 2004; Huang et al., 2007). That, along with genomic selection, has led to positive genetic gains in pregnancy rates over the last decade, at least in North America (García-Ruiz et al., 2016). Reproductive improvement in dairy cattle continues to be a priority with estrus detection being a particular concern (Roelofs et al., 2010; Fricke et al., 2014). The goal of a good heat detection program should be to accurately detect estrus, differentiating

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between cycling cows and cows with irregular cycles (Nebel et al., 2011). Estrus is typically detected using behavioral signs, such as standing to be mounted; however, several innovative and automated tools have been developed to detect estrus such as neck-mounted collars to detect physical activity, pedometers, pressure sensing devices, and tail temperature detectors. Nevertheless, these technologies may require potentially burdensome investments in management and equipment (Roelofs et al., 2015; Miura et al., 2017).

Some studies have indicated a reduction of milk yield during the day of standing estrus (Lopez et al., 2004, 2005; Akdag et al., 2010). However, studies on the variation of milk yield and characteristics in relation to the various phases of the estrous cycle have been scarce and often contradictory with many of these studies being rather old. For example, some studies have reported an increase of fat content (Copeland, 1929; Erb et al., 1952), or a reduction in protein content (King, 1977) on the day of estrus, whereas other researchers have not detected any such effects in Holsteins and Jerseys (Cowan and Larson, 1979; Akdag et al., 2010). Horrell et al. (1985) estimated small increases in lactose content in Holsteins during estrus, whereas Akdag et al. (2010) did not find any such effects in Jerseys. Some studies have reported no changes in SCC for cows showing estrus (Anderson et al., 1983; Horrell et al., 1985), whereas others have inferred an increase in SCC during estrus (King, 1977). In other dairy livestock, increases in SCC have been found for Nili-Ravi buffaloes in proestrus/estrus phase compared with metestrus and diestrus stages (Akhtar et al., 2008).

As scientific evidence is scarce, contradictory, and often originating from dairy populations not representative of modern dairy breeds and farming conditions, a better understanding of the associations of milk characteristics with the phases of the estrous cycle is needed. Moreover, this study could lead to new on-farm indicators of reproductive changes of the cow. Therefore, the aim of this study was to investigate the effects of various estrous phases on milk yield, composition, physical traits, and fatty acid composition in Holsteins, Brown Swiss, Simmental, and Alpine Grey cows.

MATERIALS AND METHODS

Data

Milk recording data were collected on dairy cows between January 2011 and December 2016 from the Breeders Federation of Alto Adige/Südtirol (Associazione Provinciale delle Organizzazioni Zootecniche Altoatesine/Vereinigung der Südtiroler Tierzuchtverbände, Bolzano/Bozen, Italy) within the northeastern region of Bolzano/Bozen province in Italy. We extracted a total of 85,329 test-day (**TD**) records related to inseminations on 20,501 Brown Swiss, 10,696 Holsteins, 17,837 Simmentals, and 8,595 Alpine Grey cows distributed across 4,071 herds. Parity numbers were grouped into first (n = 25,820), second (n = 20,358), third (n = 15,114), and ≥ fourth (n = 24,037). Only records ranging from 30 to 200 DIM were used for analysis. Furthermore, subsequent gestation lengths for successful conceptions were required to be within 30 d of the average for each breed and subsequent calving intervals for successful conceptions were required to be between 300 and 700 d.

Milk Characteristics

Milk data included TD production and characteristics routinely obtained from milk samples by the laboratory of the Federazione Latterie Alto Adige/Sennereiverband Südtirol (Bolzano/Bozen). All milk samples were collected and processed according to the International Committee for Animal Recording procedures (ICAR, 2016). The SCC was analyzed using a Fossomatic (Foss Electric, Hillerød, Denmark) and logarithmically transformed to SCS. All the other milk characteristics were predicted on the basis of Fourier-transform infrared (**FTIR**) spectra. The milk samples were analyzed by a MilkoScan (Foss Electric) using the calibration equations preinstalled by the company; further details on infrared spectrometry are given in Toledo-Alvarado et al. (2018). The milk components analyzed were lactose, fat, protein, casein, and urea. The fat:protein (**F:P**) ratio was also calculated. The milk physical traits were freezing point depression (**FPD**) (Arnvidarson et al., 1998) expressed in 10^{-2} °C, and the homogenization index (**HI**) reflecting the fat globule size (Sjaunja et al., 1994). Milk samples were analyzed for the following primary fatty acids: myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1 *cis*-9), expressed as a percentage of fat. Furthermore, analysis of the following fatty acid categories were determined: free fatty acids (**FFA**), SFA, MUFA, PUFA, UFA, short-chain fatty acids (**SCFA**), medium-chain fatty acids (**MCFA**), long-chain fatty acids (**LCFA**), and *trans* fatty acids (**TFA**). For all milk traits, only data within the range of mean ± 5 SD for each trait were kept (~1% of records discarded).

Estrous Cycle Definition

All insemination dates were available as well as the calving date for each cow. The first insemination or service after calving for each cow was considered to be the day when the cow was in estrus. The mean interval [± standard deviation (**SD**)] between the previous calving and first service dates was 85.3 ± 33 d. The closest TD to the first service date was selected to be within a range from −10 to +10 d with the day of estrus as being d 0 for each cow. We selected only the first insemination to avoid overlapping of the same TD with 2 inseminations when the difference between the 2 insemination dates was less than the 21-d range. The number of observations available for each one of the 21 d (−10 to +10 d) varied from 3,828 to 4,232. With this range reference, we created 5 phase categories: diestrus high-progesterone (**diestrus-HP**) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (**diestrus-IP**) ranging from 3 to 10 d (n = 33,052). Figure 1 further clarifies the relative importance of key hormones during these various estrous phases as defined above. Conception at first service was confirmed if the cow had not been serviced with a second insemination within 90 d after first service (n = 47,606) along with a subsequent calving and the edits for gestation lengths and calving intervals as previously noted; otherwise, the cow was deemed to be nonpregnant (n = 37,723; ICAR, 2016).

Statistical Analysis

A univariate mixed effects model was used for the analysis of milk yield, milk components, other traits, and individual fatty acids and fatty acid categories, for a total of 24 different response variables. Data were analyzed using PROC MIXED of SAS (release 9.4, SAS Institute Inc., Cary, NC). For each trait, the model was defined as follows:

$$
\begin{aligned}
y_{ijklmnrt} &= \mu + YM_i + parity_j + breed_k + conception_l \\
&+ estrous_m + day_n\left(estrous\right)_m + \beta_{1j}\left(dim_t\right) + \beta_{1j}\left(dim_t^2\right) \\
&+ estrous_m \times conception_l + estrous_m \times breed_k + herd_r \\
&+ \varepsilon_{ijklmnrt},
\end{aligned}
$$

where $y_{ijklmnrt}$ is the response on the trait (milk, lactose, fat, protein, casein, F:P, urea, SCS, pH, FPD, HI, C14:0, C14:16, C18:0, C18:1 *cis*-9, SFA, UFA, MUFA, PUFA, TFA, SCFA, MCFA, LCFA, FFA); μ is the general mean; YM_i is the effect of year-month date i ($i = 1$ to 72) for the TD t; $parity_j$ is the effect of parity number j ($j = 1,2,3,4$), $breed_k$ is the effect of breed k ($k =$ Brown Swiss, Holstein, Simmental, Alpine Grey), $conception_l$

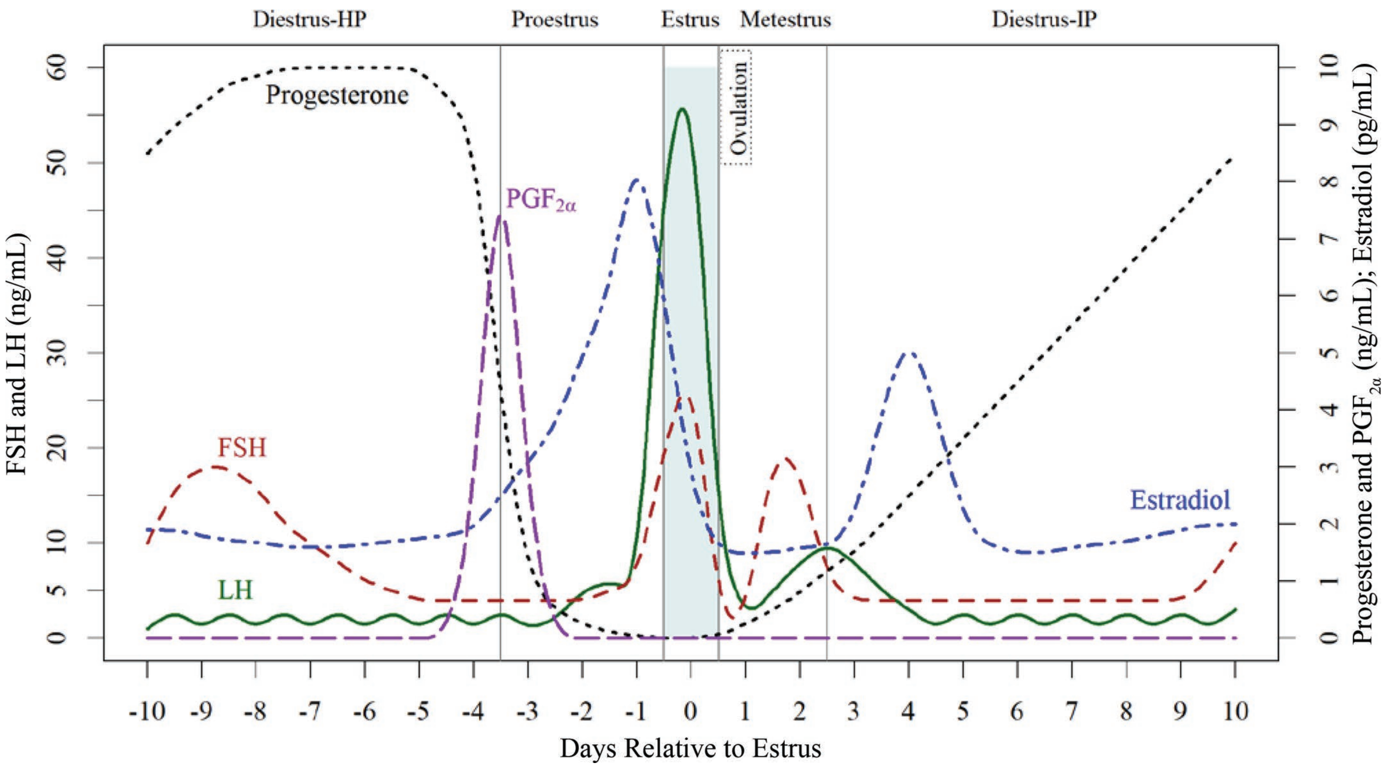

Figure 1. Schematic depiction of the pattern of secretion of FSH, LH, progesterone, $PGF_{2\alpha}$, and estradiol during the estrous cycle in cattle. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). Color version available online.

is the effect of pregnancy success l ($l = 0, 1$) for the first insemination, $estrous_m$ is the effect of estrous cycle phase m (m = diestrus-HP, proestrus, estrus, metestrus, diestrus-IP), $day_n(estrous)_m$ is the effect of the distance in days n ($n = -10$ to 10) of the TD relative to estrus nested within the estrous phase m; dim_t is the number of DIM (ranging from 30 to 200 d) at TD t, β_{1j} and β_{2j} are the partial linear and quadratic regression coefficients on DIM nested within corresponding parity j, $estrous_m \times conception_l$ is the effect of the interaction between the estrous phase m and the conception success l, $estrous_m \times breed_k$ is the effect of the interaction between the estrous phase m and the breed k of the cow, $herd_r \sim NIID\left(0, \sigma_h^2\right)$ is the random effect of herd r ($r = 1$ to 4,071), and $\varepsilon_{ijklmnrt} \sim NIID\left(0, \sigma_e^2\right)$ is the random residual. Contrasts were estimated between least squares means (**LSM**) of each trait for the effect of (1) estrous cycle phases: (a) estrus vs. diestrus-HP, (b) estrus vs. proestrus, (c) estrus vs. metestrus, and (d) estrus vs. diestrus-IP; and (2) breed: (a) Brown Swiss and Holstein (dairy breeds) vs. Simmental and Alpine Grey (dual-purpose breeds), (b) Brown Swiss vs. Holstein, and (c) Simmental vs. Alpine Grey.

RESULTS AND DISCUSSION

Descriptive Statistics and Main Causes of Variation of Milk Characteristics

Descriptive statistics for milk yield, milk components, other milk traits, and the fatty acid profile are reported in Table 1. The overall mean milk yield was 26.87 ± 7.19 kg/d with a coefficient of variation (**CV**) of 26%, the latter being somewhat inflated by differences among production systems and breeds. All milk components showed small to medium variability (CV <20%). Fat showed large variability (CV = 18%) relative to protein and casein (CV = 9%), whereas lactose had relatively low variability (CV = 3%). Urea averaged 20.92 ± 7.56 mg/100 g (CV = 36%), and ranged from 4.90 to 41.20 mg/100 g, thereby indicating the high variability in the feeding systems of the breeds, particularly with respect to the amount of CP in the diet. The SCS also had relatively large variability (CV = 33%) suggesting intrinsic variability of all the factors involved in the milking process. On the other hand, pH (CV = 0.9) and FPD (CV = 1.5), both normally used as quality indicator traits for freshness and adulteration of the milk, showed relatively small variability implying that there is a finely tuned system to ensure the quality of the milk. The homogenization index of the sample (**HI**) given by the Foss spectrometers is proportional to the mean diameter of the fat globules and is calculated as volume, ranging from 0 (bigger size of fat globules) to 1 (smaller size of the fat globules; Sjaunja et al., 1994). The average HI was 0.67 (CV = 13%) with values ranging from 0.49 to 0.93. The mean values of the composition of major fatty acids and fatty acid categories were similar to that previously reported for FTIR predicted fatty acid profiles (Rutten et al., 2009; Gottardo et al., 2017). The CV for the fatty acids ranged from 9 to 17%, whereas the variability of the fatty acids categories ranged from 5 to 27%, showing similar variability in comparison with other studies in the same region of Italy for the same breeds (Gottardo et al., 2017). The FFA showed a high CV (54%).

The results of the statistical analysis of the 24 milk characteristics considered are summarized in Table 2. This large database allowed inference on the effects of several sources of variation such as breed, parity, and days in milk of cows, the phase of the estrous cycle, the day within estrous cycle, and the subsequent pregnancy, and also the year-month and the herd of cows. Moreover, the interactions perceived to be most important were included in the statistical model. The majority of these factors of variation and of their interactions were statistically significant for all or large part of the traits analyzed. Several studies have assessed the variation accounted for by some of these factors (Cowan and Larson, 1979; Akdag et al., 2010). However, none of them have accounted for all these factors simultaneously; therefore, direct comparisons of our study with these other studies are not possible.

The estrous phase and the day nested in the estrous phases influenced all the traits included in the study (Table 2). The conception outcome was important for milk yield and almost all the major components of milk, with the exception of fat. For the rest of the traits, the effect of conception outcome had variable results, being important for SCS, TFA, MCFA, and FFA but with no evidence of association with any individual fatty acids or for the rest of the fatty acid categories. The interaction between estrous × conception outcome did not have an important effect for the majority of the traits (Table 2), with the exception of lactose and the FPD, and a low effect for F:P, HI, and some fatty acids categories (UFA, MUFA, LCFA, and FFA).

Breed had a significant effect ($P < 0.001$) for all traits evaluated, as a result of the natural differences in milk composition of the 4 breeds involved in the study. Also, a statistically significant interaction between estrous and breed was observed for milk yield and for the main milk components (except lactose). These differences in milk and milk components among breeds and estrous phases indicate that estrous phases differently affect the activity of mammary glands of cows of different breeds.

The effects of other factors such as parity number, DIM nested in parity, and year-month of the TD were also important (Table 2), showing high variability of milk composition across lactations, within lactation, and in different seasonal conditions. These effects of these sources of variation are not the specific focus of this study but were previously discussed in research conducted within the same region (Bittante et al., 2011; Cecchinato et al., 2015).

Effect of Estrous Cycle on Milk Yield and Quality

The LSM for the milk production and quality traits in the different estrous cycle phases and for the conception outcome are presented in Table 3. There was a small but significant increment of estrus day on milk yield relative to the other previous or subsequent phases of the estrous cycle.

The effect of the estrous cycle on the main components of milk (fat, protein, casein, lactose, and F:P) is evident comparing the LSM of the different phases of the estrous cycle (Table 3) together with the results of the day by day variation within the estrous phases (Figure 2). We observed a clear effect of the estrous cycle on milk fat content that, with respect to diestrus-HP, started to increase during proestrus, reached its zenith the day of estrus, began to decrease during metestrus, before returning to the basal value during the following diestrus-IP. An increment of the fat content during estrus has been described in other studies involving Jersey (Copeland, 1929) and Holstein cows (Erb et al., 1952).

The effect of the estrous phase in protein and casein showed an opposite pattern with respect to the one observed with fat, with a small decrease around the day of estrus. The F:P had similar tendencies with fat in all the phases of the estrous cycle. Our results agree with those obtained by King (1977) who reported a positive effect of estrus on fat together with a negative effect of protein on the estrous day. Lactose remained practically constant over the diestrus-HP, rising significantly 1 d before estrus, then remaining constant on the metestrus phase, followed by a gradual reduction over the diestrus-IP. Conversely, Akdag et al. (2010) found no differences for lactose at estrus; nevertheless, the breed (Jerseys) was different and the sample sizes were substantially smaller in that study.

The LSM estimates for day (which was nested in the estrous phase) for SCS, pH, urea, FPD, and HI are plotted in Figure 3. The urea content of milk showed

Table 1. Descriptive statistics of milk yield, main components, other traits, and fatty acid profile[1]

Trait	n	Cows	Mean	SD	P1	P99
Milk yield (kg/d)	85,329	57,629	26.87	7.19	12.40	45.70
Main component (%)						
Fat	85,329	57,629	3.88	0.70	2.28	5.96
Protein	85,329	57,629	3.26	0.32	2.61	4.07
Casein	85,329	57,629	2.57	0.25	2.04	3.19
Lactose	85,329	57,629	4.83	0.17	4.36	5.19
Fat:protein	85,329	57,629	1.20	0.22	0.70	1.89
Other milk trait						
SCS (ln)	85,329	57,629	4.07	1.35	1.61	7.62
pH	85,329	57,629	6.60	0.06	6.44	6.75
Urea (mg/100 mL)	85,315	57,620	20.92	7.56	4.90	41.10
FPD (10^{-2} °C)	85,329	57,629	−52.60	0.79	−54.40	−50.70
HI	85,329	57,629	0.67	0.09	0.49	0.93
Individual fatty acid (%)						
Myristic acid (C14:0)	62,833	37,089	12.31	1.27	8.58	14.90
Palmitic acid (C16:0)	62,833	37,089	31.71	3.07	23.42	37.99
Stearic acid (C18:0)	62,833	37,089	10.41	1.55	7.02	14.46
Oleic acid (C18:1 *cis*-9)	62,833	37,089	21.27	3.62	14.58	31.88
Fatty acid category (%)						
SFA	62,833	37,089	70.09	3.46	59.92	76.52
UFA	62,833	37,089	29.09	3.87	22.29	40.87
MUFA	62,833	37,089	24.79	3.56	18.23	35.22
PUFA	62,833	37,089	3.09	0.62	1.83	4.77
TFA	62,833	37,089	2.17	0.59	0.84	3.61
SCFA	62,833	37,089	10.53	1.27	7.12	13.12
MCFA	62,833	37,089	42.85	7.14	25.07	59.01
LCFA	62,833	37,089	31.86	4.80	23.12	45.90
FFA	78,772	53,376	0.64	0.35	0.03	1.71

[1] P1 = 1st percentile; P99 = 99th percentile; FPD = freezing point depression; HI = homogenization index; TFA = *trans*-unsaturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; FFA = free fatty acids.

Table 2. Results from ANOVA (*F*-value and significance) of milk yield, main components, other traits, and fatty acid profile[1]

Trait	Estrous	Day (Estrous)	Conception	Estrous × conception	Breed	Estrous × breed	Parity	DIM (parity)	DIM^2 (parity)	Year-month	RMSE
Milk yield (kg/d)	3.9**	3.1***	157.0***	1.0	1,676.6***	2.6**	433.2***	156.6***	12.6***	50.9***	4.36
Main component (%)											
Fat	93.8***	13.6***	0.7	2.0	341.9***	2.4**	6.4***	2.6*	6.4***	24.5	0.62
Protein	31.0***	3.9***	47.9***	1.3	1,391.5***	2.7**	11.7***	264.9***	14.5***	73.0***	0.24
Casein	38.4***	3.7***	46.5***	1.6	1,378.8***	3.1***	14.4***	233.3***	12.2***	92.5***	0.18
Lactose	273.7***	34.9***	17.4***	4.7***	176.2***	1.8	155.2***	31.4***	4.7***	33.7***	0.14
Fat:protein	144.7***	16.4***	6.2*	2.6*	142.7***	4.0***	11.5***	49.5***	19.8***	5.9***	0.19
Other milk trait											
SCS (ln)	41.8***	3.4***	18.3***	0.1	131.4***	0.7	30.2***	31.3***	4.2**	3.6***	1.22
pH	43.6***	4.9***	0.3	0.9	218.7***	0.9	0.3	92.8***	18.3***	181.5***	0.05
Urea (mg/100 mL)	11.0***	3.9***	1.4	0.4	240.7***	1.4	16.1***	30.7***	14.2***	96.4***	6.51
FPD (10^{-2} °C)	339.8***	29.8***	0.2	7.2***	149.5***	2.6**	31.7***	17.4***	2.9*	43.6***	0.67
HI	181.7***	16.7***	0.9	4.3**	264.0***	3.0***	56.3***	28.3***	12.3***	629.8***	0.07
Individual fatty acid (%)											
Myristic acid (C14:0)	119.1***	3.6***	0.3	2.3	64.7***	1.3	20.7***	436.8***	281.2***	56.1***	1.08
Palmitic acid (C16:0)	76.1***	3.2***	0.9	1.6	202.2***	1.8*	16.0***	559.3***	300.5***	171.0***	2.51
Stearic acid (C18:0)	165.8***	8.4***	2.3	1.7	345.9***	0.8	28.3***	1,086.4***	480.0***	59.6***	1.24
Oleic acid (C18:1 *cis*-9)	71.8***	4.6***	2.2	2.0	402.6***	2.0*	26.9***	461.3***	264.3***	172.1***	2.93
Fatty acid category (%)											
SFA	97.7***	3.9***	0.0	2.2	92.5***	1.5	15.3***	360.9***	222.9***	146.1***	2.83
UFA	89.1***	3.2***	1.5	2.5**	100.7***	1.5	14.7***	483.5***	293.7***	108.9***	3.26
MUFA	90.2***	4.5***	1.8	2.8*	228.9***	2.6**	31.5***	315.5***	191.3***	195.2***	2.87
PUFA	13.8***	1.6*	0.0	0.7	217.2***	2.4**	36.1***	152.2***	73.4***	366.0***	0.47
TFA	103.6***	17.9***	7.5**	2.1	832.4***	2.3**	92.4***	27.6***	0.5	122.2***	0.45
SCFA	11.7***	2.8***	0.4	1.1	914.1***	1.7	17.8***	3.7**	4.2**	46.6***	1.03
MCFA	22.1***	6.5***	9.9**	1.6	592.6***	2.4**	8.4***	132.7***	37.2***	104.5***	6.06
LCFA	165.7***	9.2***	1.7	2.8*	184.6***	1.0	37.7***	735.6***	394.2***	287.3***	3.71
FFA	58.3***	6.9***	10.0**	2.6*	112.9***	3.1***	7.3***	79.6***	13.4***	80.9***	0.30

[1] FPD = freezing point depression; HI = homogenization index; TFA = *trans*-unsaturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; FFA = free fatty acids. RMSE = root mean square error.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3. Least squares means and SE range of milk yield, main components, other traits, and fatty acid profile for the estrous cycle phases and for conception success averaged across all breeds[1]

	Estrous cycle phase[2]					Conception		
Trait	Diestrus-HP	Proestrus	Estrus	Metestrus	Diestrus-IP	No	Yes	SE
Milk yield (kg/d)	25.78**	25.81**	26.03	25.82*	25.73***	26.09	25.57	0.06–0.10
Main component (%)								
Fat	3.82***	3.87***	3.95	3.87***	3.78***	3.85	3.86	0.01–0.01
Protein	3.19***	3.18***	3.15	3.19***	3.20***	3.17	3.19	0.0–0.0
Casein	2.50***	2.50*	2.49	2.52***	2.52***	2.49	2.51	0.0–0.0
Lactose	4.81***	4.81***	4.86	4.86	4.83***	4.84	4.83	0.0–0.0
Fat:protein	1.20***	1.22***	1.26	1.21***	1.19***	1.22	1.21	0.0–0.0
Other milk trait								
SCS (ln)	4.04***	4.17	4.17	4.14	4.02***	4.08	4.13	0.01–0.02
pH	6.59***	6.59***	6.60	6.60	6.59***	6.59	6.59	0.0–0.0
Urea (mg/100 mL)	20.56	20.45	20.42	20.05**	20.60	20.45	20.38	0.07–0.12
FPD (10^{-2} °C)	−52.47***	−52.58***	−52.83	−52.73***	−52.57***	−52.64	−52.63	0.01–0.01
HI	0.69***	0.69***	0.67	0.67	0.68***	0.68	0.68	0.0–0.0
Individual fatty acid (%)								
Myristic acid (C14:0)	12.41***	12.37***	12.02	12.13***	12.31***	12.24	12.25	0.01–0.02
Palmitic acid (C16:0)	31.75***	31.88***	31.40	31.48	31.40	31.56	31.59	0.03–0.05
Stearic acid (C18:0)	10.27***	10.30***	10.71	10.63*	10.49***	10.49	10.47	0.01–0.03
Oleic acid (C18:1 *cis*-9)	21.50***	21.58***	22.31	22.09**	21.65***	21.85	21.80	0.03–0.06
Fatty acid category (%)								
SFA	69.98***	69.99***	69.15	69.40***	69.61***	69.62	69.63	0.03–0.06
UFA	29.21***	29.23***	30.15	29.90**	29.56***	29.63	29.58	0.03–0.07
MUFA	24.94***	24.99***	25.72	25.63	25.21***	25.32	25.27	0.03–0.06
PUFA	3.12***	3.11***	3.17	3.15	3.14**	3.13	3.13	0.01–0.01
TFA	2.23***	2.24***	2.38	2.33***	2.29***	2.30	2.28	0.01–0.01
SCFA	10.31***	10.28***	10.18	10.32***	10.32***	10.28	10.28	0.01–0.02
MCFA	41.98**	42.41***	41.47	41.62	41.77*	41.74	41.95	0.06–0.13
LCFA	31.99***	32.08***	33.32	33.16*	32.58***	32.65	32.59	0.04–0.08
FFA	0.64**	0.65	0.65	0.63***	0.60***	0.63	0.62	0.0–0.0

[1] FPD = freezing point depression; HI = homogenization index; TFA = *trans*-unsaturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; FFA = free fatty acids.

[2] Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052).

Asterisks indicate the significance of the contrast of this phase with estrus: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

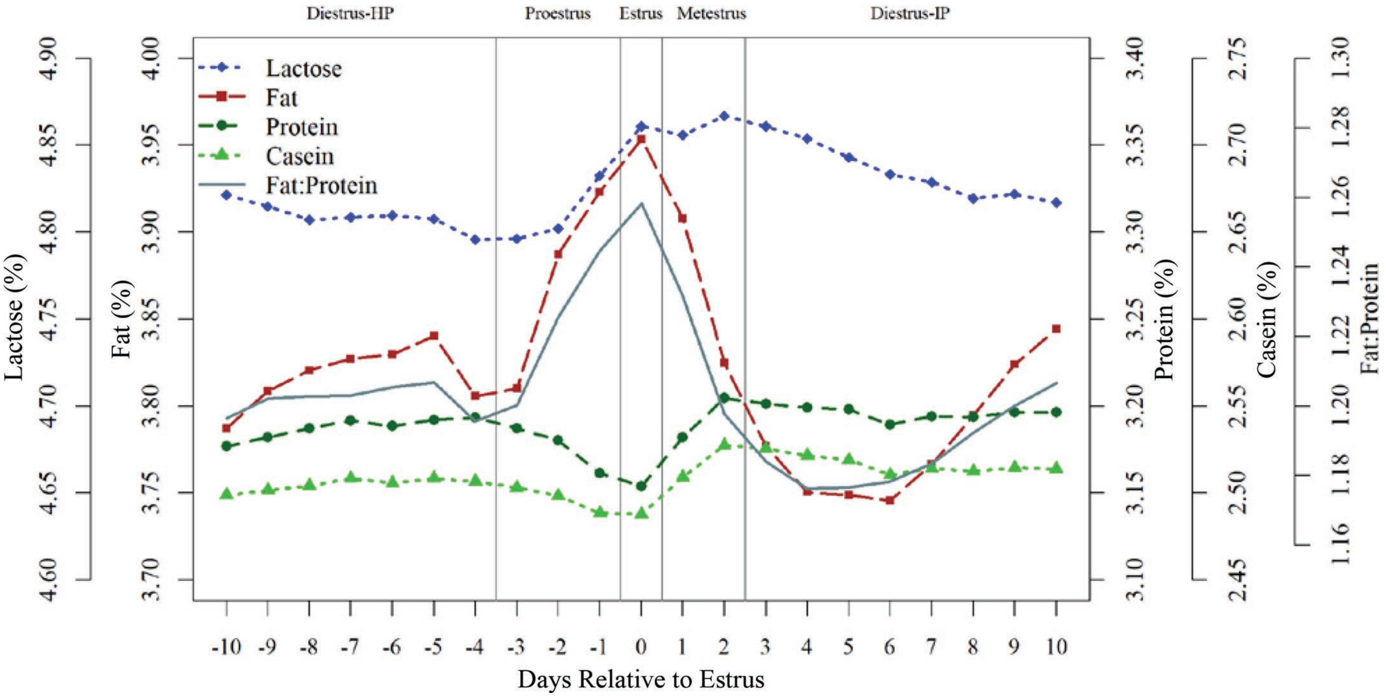

Figure 2. Least squares means of fat (±0.011 SE), protein (±0.005 SE), casein (±0.004 SE), lactose (±0.003 SE), and fat:protein (±0.004 SE) ratio for each day nested in the estrous cycle phases. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). Color version available online.

an erratic pattern, decreasing from diestrus-HP to the estrus and then to the nadir value reached at the end of metestrus and recovering rapidly during the diestrus-IP. This tendency could reflect the variability in the nutrition and a reduction in feed consumption around the estrous cycle and its carry-over effects in subsequent days. In dairy goats, the stress associated with estrus has been related to variation in milk urea content and correlated with feeding and metabolism changes, in spite of a nonsignificant association with cycle stage (Moroni et al., 2007). Conclusions regarding the variation of milk urea around the insemination day have not been consistent in previous studies, but extremely high or low values of milk urea have been associated as a risk factor for conception failure in Holstein cows (Melendez et al., 2000; Albaaj et al., 2017).

The SCS increased gradually from the diestrus-HP to the proestrus phase and then gradually decreased during the estrus, metestrus, and initial diestrus-IP phases. No significant differences were found between the day of estrus versus either proestrus or metestrus phases. The increase from diestrus-HP to proestrus could be due to the increase of estrogens in the udder, leading to the highest value of SCS 1 d before estrus coinciding with the estradiol peak (Zdunczyk et al., 2003). In addition, an increase in SCS has been associated with an increase of FFA (discussed later), which is also characterized as having its highest content on the same day (Bachman et al., 1988). King (1977) reported a significant increase of SCS on estrus day compared with metestrus in Ayrshire and Holstein cows. On the other hand, other researchers have reported no evidence of SCS differences for cows showing estrus (Anderson et al., 1983; Horrell et al., 1985) relative to cows not showing estrus, independent of the phase of the estrous cycle. In other species, estrous cycle variation in SCS has been found. Akhtar et al. (2008) reported an increase of SCS in Nili-Ravi buffaloes in proestrus and estrus associated with high plasma estradiol. In addition, a significant increase in SCC has been reported in cows with exogenous estrogen implants (Lammers et al., 1999).

The FPD showed a stable pattern from the diestrus-HP until the proestrus where it dropped, reaching the nadir value on the day of estrus and recovering gradually in the metestrus and diestrus-IP phases. This quality indicator is used normally to detect adulterated milk; however, it is also affected by several environmental and physiological factors intrinsic to milk composition. The principal factors affecting FPD are pH, lactose concentration, and to a lesser degree, potassium, chloride, sodium, citrates, and urea (Zagorska and Ciprovica, 2013). So the depression of FPD at estrus, in comparison with other estrous phases, could be explained by the concomitant increase of the lactose content in combination with the increase in the pH of milk.

Milk pH is commonly associated with bacterial deterioration (low values) and mastitis (high values), and also adulterations with alkali such as detergents (Vassen, 2003). The pH trend over the estrous phases (Figure 3), independent from mastitis, adulteration, or microbial deterioration of milk, involves a rapid increase during estrus and metestrus phases with a gradual reduction during the diestrus-IP. Variation in pH of milk during the different estrous phases can be attributed to the casein and protein variation (Rose, 1961; Ma and Barbano, 2003); nonetheless, contents of several milk constituents as chloride, sodium, potassium, lactose, calcium, and magnesium can also directly affect the pH of milk (Luck and Smith, 1975; Vassen, 2003).

The HI markedly decreased until 1 d before estrus and remained low during estrus and metestrus before nearly recovering at diestrus value (IP and HP). This indicated that the fat globule sizes increased during the estrus and metestrus phases. The concomitant increase in fat content can explain this increase of fat globule size in milk samples of cows showing estrus since high-fat content has been related to an increase of average globule size (Goulden and Phipps, 1964; Wiking et al., 2004). Nevertheless, changes in the feeding behavior have also been associated with changing fat globule sizes (Abeni et al., 2005; Couvreur and Hurtaud, 2007).

Figure 4 shows the LSM for the most important fatty acids of the milk: myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1 *cis*-9) for each day nested in the estrous cycle phases. It seemed that myristic acid was relatively constant value during all of the diestrus (IP and HP) and proestrus, with a sudden reduction on the day of estrus followed by a gradual recovery during metestrus. Palmitic acid also decreased sharply on the day of estrus, remaining stable at low values during metestrus and diestrus-IP, before increasing progressively during diestrus-HP close to the zenith value reached at proestrus (d −2). On the other hand, the 2 long-chain fatty acids, stearic and oleic acids, showed an opposite trend with a rapid increase during proestrus and reaching the zenith values on the day of estrus. Subsequently, during metestrus, oleic acid rapidly decreased to baseline values, whereas stearic acid showed a smooth reduction during the diestrus-IP phase. As is well known, there are 2 main routes for the production of even-chain milk fatty acids. First, the C4:0 to C14:0 saturated even fatty acids and about half of the C16:0 are synthesized de novo in

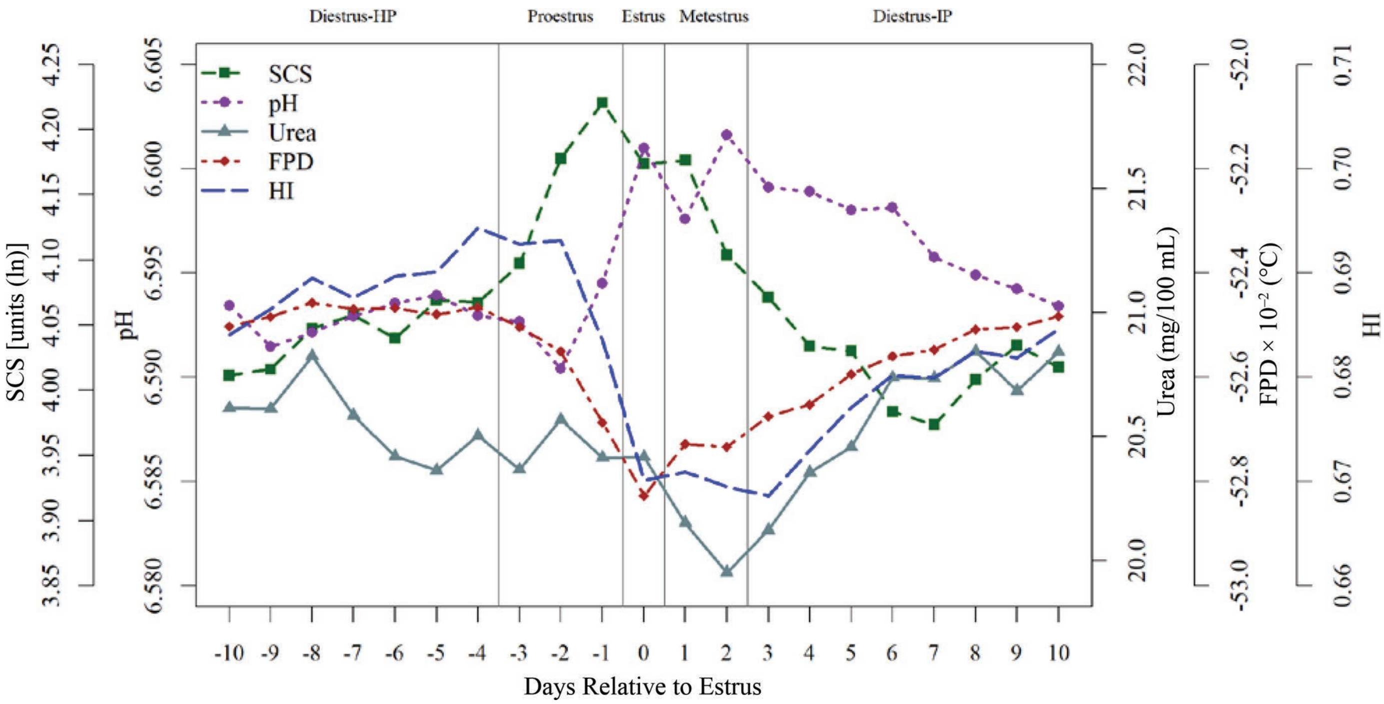

Figure 3. Least squares means of SCS (±0.021 SE), pH (±0.001 SE), urea (±0.121 SE), freezing point depression (FPD; ±0.012 SE), and homogenization index (HI; ±0.001 SE) for each day nested in the estrous cycle phases. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). Color version available online.

the udder mainly from acetate and BHB. Acetate and butyric acid are, in turn, produced in the rumen by fermentation of feed components, upon which butyric acid is converted to BHB during absorption through the rumen epithelium. Second, the rest of the C16:0 and almost all of the LCFA originate from dietary lipids absorbed by the small intestine and from lipolysis of adipose tissue triacylglycerols (Grummer, 1991; Månsson, 2008). Therefore, the increase of stearic and oleic acids in the estrous phase indicates the release in the mammary gland of LCFA from the mobilization of body fat reserves, possibly due to reduced feed intake at the onset of estrus. Consequently, the contents of myristic and palmitic fatty acids tended to be lower because the high uptake of LCFA inhibits the de novo synthesis of fatty acids by the mammary gland tissue (Gross et al., 2011; Mele et al., 2016).

This dynamic relationship is more evident for the fatty acid groups (Figure 5), where the SFA increased progressively until the proestrus phase with a sudden decrease on the day of estrus, and a subsequent increase gradually throughout the metestrus and diestrus-IP phases. An opposite pattern was observed for the UFA, MUFA, PUFA, and also for TFA, gradually decreasing during the diestrus-HP with a rapid increase from proestrus to estrus, and then progressively decreasing throughout the metestrus and diestrus-IP phases. The TFA are produced during biohydrogenation of PUFA and isomerization of MUFA in the rumen, with the most common in ruminant fat being vaccenic acid (C18:1 *trans*-11), accounting for 60 to 80% of total TFA (Vargas-Bello-Pérez and Garnsworthy, 2013).

Another way to represent the different proportions between mammary gland de novo synthesis on one hand and dietary sources combined with fat mobilization, on the other hand, is to observe the fatty acids grouped according to their carbon-chain length (Figure 6). Again, for SCFA, MCFA, and LCFA, the antagonistic pattern was obvious. Both SCFA and the MCFA showed an increase during the proestrus followed by a significant reduction in the estrous phase. Subsequently, the SCFA increased in the metestrus and stabilized during diestrus, whereas the MCFA remained low during metestrus and increased gradually during diestrus-HP. Furthermore, the LCFA decreased progressively during the diestrus-HP and substantially increased from proestrus to the day of estrus; LCFA then decreased progressively in the metestrus and diestrus-IP phases.

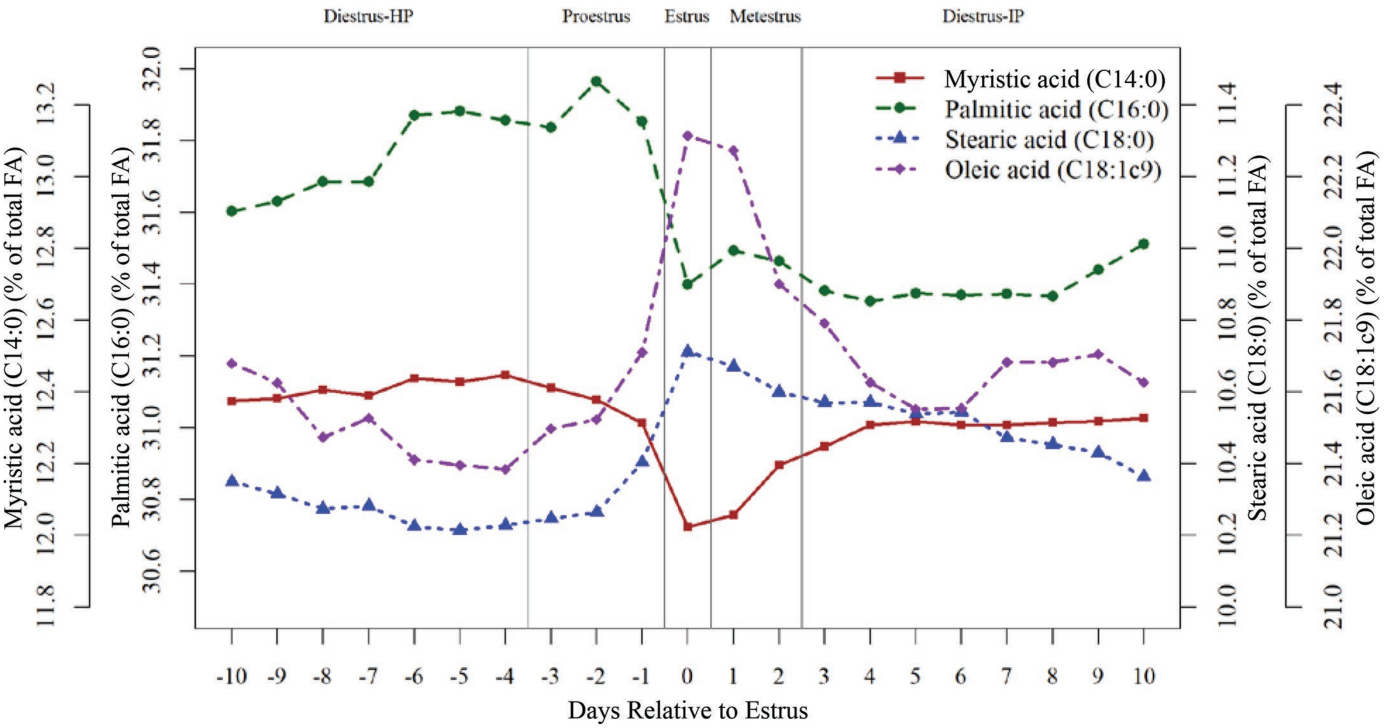

Figure 4. Least squares means of myristic acid (C14:0 ± 0.023 SE), palmitic acid (C16:0 ± 0.052 SE), stearic acid (C18:0 ± 0.026 SE), and oleic acid (C18:1 *cis*-9 ± 0.061 SE) for each day nested in the estrous cycle phases. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). FA = fatty acids. Color version available online.

The FFA, or nonesterified fatty acids, were at their peak levels during proestrus (Figure 6), exactly 1 d before estrus, then slowly decreased during the estrus and the diestrus-IP phases. This effect may be associated with the estradiol peak in the proestrus, since it has been associated with an increase of FFA, due to a shift of lipoprotein lipase activity (Bachman et al., 1988). An elevated level of FFA is one of the indicators of negative energy balance because the energy requirements of the cows are compensated by intensive lipolysis, and releasing fatty acids in the blood (Adewuyi et al., 2005). This increment of FFA mobilization has been described particularly in periparturient cows and can be explained by (1) the suppression of de novo synthesis or uptake, and then esterification of fatty acids, (2) promotion of lipolysis, (3) reduction of the intracellular re-esterification of fatty acids released by lipolysis, and (4) some combination among these possibilities (Bell, 1995).

It should be realized that some of these milk components would be highly correlated with each other (positively or negatively). Therefore, the variation in one trait affects directly variation in another (i.e., SFA vs. UFA), and potentially drives indirect associations between various milk components with the factors studied in this paper.

Differences in Milk Yield and Quality Between Cows that Conceived or Did Not Conceive at Estrus

The differences between the milk traits of cows that conceived or did not conceive at the insemination carried out the day of estrus (estrus was defined as the day of insemination) during diestrus-HP and proestrus (i.e., during the 10 d before insemination) do not obviously depend on future pregnancy but may reflect the differences between the cows that are initially more versus less fertile.

A first scenario involves traits with differences among the estrous cycle phases but without any evidence of differences due to eventual conception and no evidence of any interaction between estrous phases and conception. This could be interpreted as absence of both effects of both initial fertility of the cows and of the following conception of these cows. This seems to be the case for milk fat content and for the proportion of the 4 major fatty acids, of SFA, of PUFA, and of SCFA, but also of milk pH and urea content (Table 2).

A second scenario involves trait differences between cows that conceived and those that did not conceive, with no evidence of interaction between estrous phases and pregnancy. This could be interpreted as traits reflecting the initial differences in cows' fertility levels, but

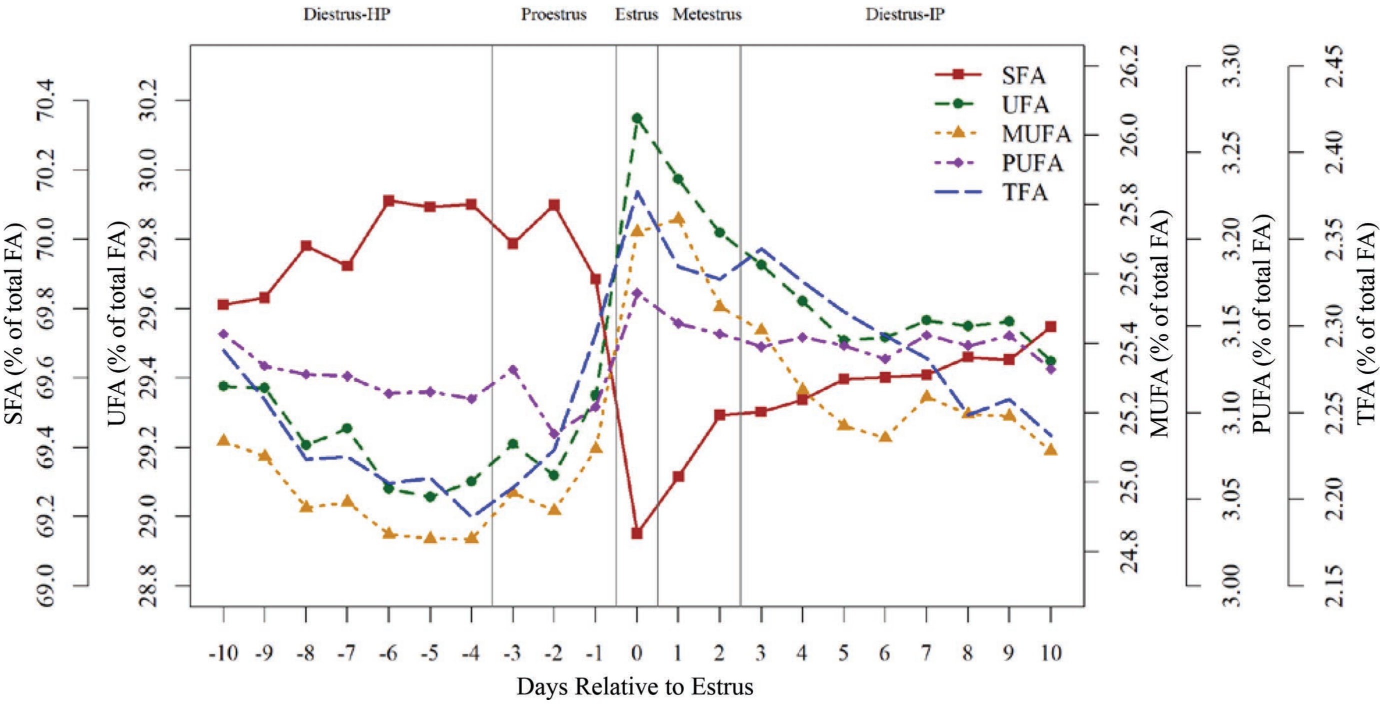

Figure 5. Least squares means of SFA (±0.060 SE), UFA (±0.068 SE), MUFA (±0.060 SE), PUFA (±0.010 SE), and *trans*-unsaturated fatty acids (TFA; ±0.010 SE) for each day nested in the estrous cycle phases. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). FA = fatty acids. Color version available online.

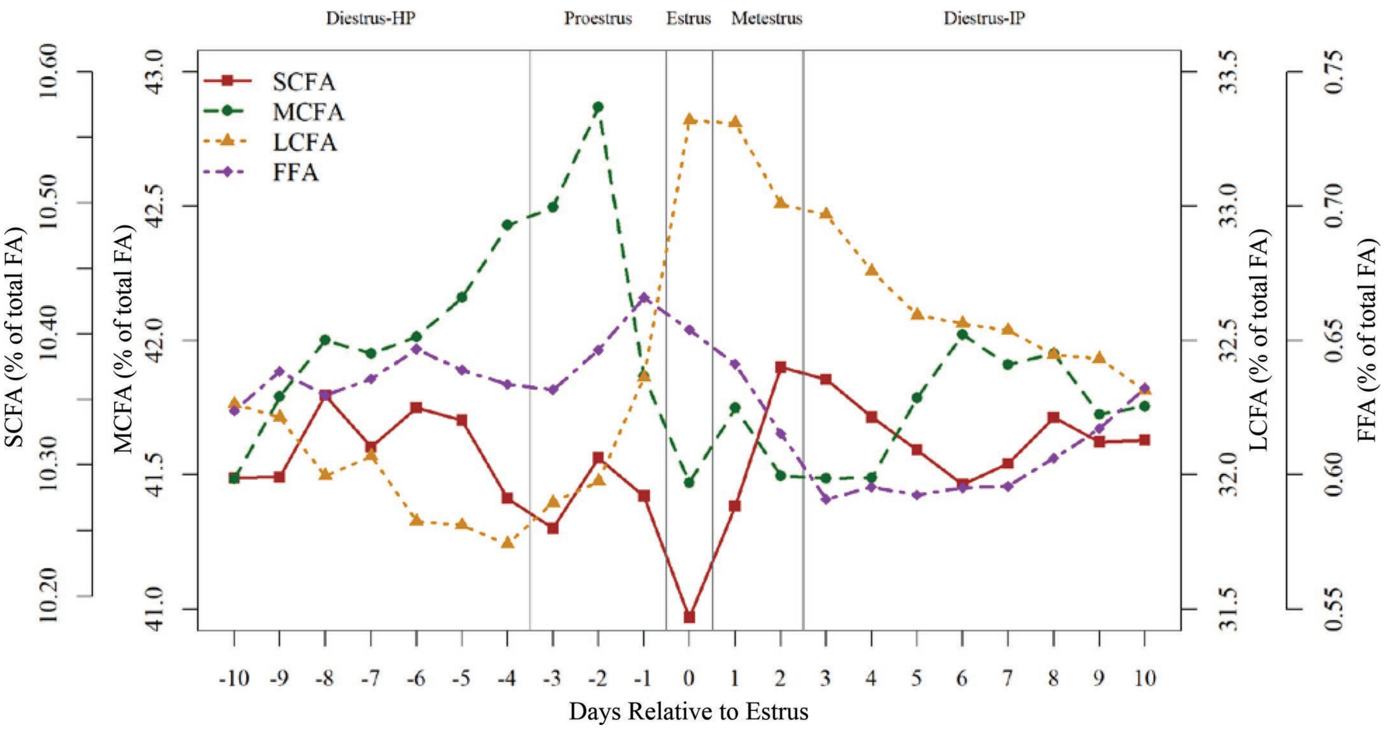

Figure 6. Least squares means of short-chain fatty acids (SCFA ± 0.022 SE), medium-chain fatty acids (MCFA ± 0.123 SE), long-chain fatty acids (LCFA ± 0.077 SE), and free fatty acids (FFA ± 0.006 SE) for each day nested in the estrous cycle phases. Diestrus high-progesterone (diestrus-HP) ranging from −10 to −4 d (n = 27,574); proestrus ranging from −3 to −1 d (n = 12,302); estrus at d 0 (n = 4,144); metestrus from 1 to 2 d (n = 8,275); and diestrus increasing-progesterone (diestrus-IP) ranging from 3 to 10 d (n = 33,052). FA = fatty acids. Color version available online.

probably not affected by the initial stage of pregnancy. This was the case for milk yield, milk content of protein and casein, SCS, and fatty acid groups TFA and MCFA (Table 2). In fact, even though the differences are not quantitatively important, the cows that conceived had slightly lower milk yield and slightly greater protein and casein contents (Table 3). Both of these differences could be interpreted as a slightly more favorable energy balance for the more fertile cows (Patton et al., 2007; Olson et al., 2011), noting that these differences are based on an analysis that corrects the LSM for the herd, year and season, breed, parity, and DIM of the cows. The small difference in SCS could probably depend on a greater concentration per unit milk due to the lower milk produced daily than to a greater production of somatic cells per day by the udder. Lastly, a greater MCFA and lower TFA are both consistent with the hypothesis of a more favorable energy balance.

A third scenario is represented by traits not seemingly affected by the main effect of conception, but presenting evidence of an interaction of conception with estrous phases. One interpretation is a possible effect induced by conception without evidence of initial differences due to fertility level of the cows before the day of estrus. The traits in this situation are the 2 physical characteristics of milk (FPD and HI), and the MUFA, UFA, and LCFA, among the fatty acid categories. Further studies are needed for the interpretation of these results.

Finally, the fourth scenario is represented by traits affected by both the effects of conception and its interaction with estrous phase, which could be interpreted as traits with initial differences in fertility level but also influenced by the establishment of pregnancy. However, it is also possible that this phenomena could be simply the result of the different effects of initial fertility levels in the different phases of the estrous cycle, independent of the effect of conception. This scenario appeared to characterize the content of lactose, F:P, and FFA (Table 2).

Breed Effects

Breed effects in milk yield and milk quality traits, as well as their significance, are presented in Table 4. For most traits, except milk content, oleic acid and SCFA, the 2 specialized dairy breeds (Holstein and Brown Swiss) were different from the dual-purpose

Table 4. Least squares means and SE range of milk yield, main components, other traits, and fatty acid profile for each breed: Holstein (Ho), Brown Swiss (BS), Simmental (Si), and Alpine Grey (AG)[1]

	Breed LSM				Contrast[1]			
Trait	Ho	BS	Si	AG	Ho+BS vs. Si+AG	Ho vs. BS	Si vs. AG	SE
Milk yield (kg/d)	30.06	25.80	26.46	21.01	***	***	***	0.08–0.12
Main component (%)								
Fat	3.81	4.05	3.92	3.66	***	***	***	0.01–0.01
Protein	3.03	3.33	3.20	3.18	*	***	**	0.00–0.01
Casein	2.39	2.62	2.51	2.50	*	***	*	0.00–0.00
Lactose	4.81	4.83	4.82	4.88	***	***	***	0.00–0.00
Fat:protein	1.26	1.22	1.23	1.16	***	***	***	0.00–0.00
Other milk trait								
SCS (ln)	4.31	4.22	3.91	3.99	***	***	**	0.01–0.02
pH	6.58	6.60	6.60	6.61	***	***	***	0.0–0.0
Urea (mg/100 mL)	18.76	21.86	19.86	21.18	*	***	***	0.09–0.14
FPD (10^{-2} °C)	−52.49	−52.75	−52.64	−52.65	*	***		0.01–0.01
HI	0.70	0.67	0.69	0.66	***	***	***	0.0–0.0
Individual fatty acid (%)								
Myristic acid (C14:0)	12.29	12.39	12.10	12.21	***	***	***	0.02–0.02
Palmitic acid (C16:0)	32.05	31.40	32.11	30.76	***	***	***	0.04–0.05
Stearic acid (C18:0)	9.91	10.50	10.69	10.83	***	***	***	0.02–0.03
Oleic acid (C18:1 *cis*-9)	22.87	20.76	21.78	21.90		***		0.04–0.06
Fatty acid category (%)								
SFA	69.64	70.03	69.96	68.88	***	***	***	0.04–0.06
UFA	29.60	29.12	29.24	30.47	***	***	***	0.05–0.06
MUFA	25.77	24.41	25.25	25.77	***	***	***	0.04–0.06
PUFA	3.09	3.14	3.00	3.32	***	***	***	0.01–0.01
TFA	2.52	2.05	2.24	2.37	*	***	***	0.01–0.01
SCFA	9.72	10.84	10.08	10.50		***	***	0.02–0.02
MCFA	40.49	42.61	44.74	39.56	***	***	***	0.08–0.12
LCFA	32.99	31.55	32.68	33.28	***	***	***	0.05–0.08
FFA	0.70	0.65	0.63	0.55	***	***	***	0.0–0.01

[1] FPD = freezing point depression; HI = homogenization index; TFA = *trans*-unsaturated fatty acids; SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; FFA = free fatty acids.

Asterisks indicate the significance of the contrast: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

breeds (Simmental and Alpine Grey), whereas the 2 dairy breeds were different from each other for all traits considered. Moreover, the 2 dual-purpose breeds were different from each other for all traits considered with the exception of FPD and oleic acid.

Milk yields were greater for Holsteins, followed by the large-framed Alpine breeds (Brown Swiss and Simmental) and lastly by the medium-framed Alpine Grey. The variation across breeds can be explained by the different genetic potential characterizing each breed, but also by environmental differences, especially related to the farming systems (Toledo-Alvarado et al., 2017). The breed differences found in this study are not fully representative of the entire lactation, but they are very similar to those found in studies considering all the production phases of cows of the same breeds reared in the same area (Stocco et al., 2017, 2018). The Alpine Grey cows are mainly raised in small traditional farms with tied animals fed hay and some concentrate, whereas the Holstein cows are mainly managed in more modern dairy systems with loose housing, milking parlors, and TMR including silages, especially corn silage, cereals, protein meals, hay, and some straws. Brown Swiss and Simmental cows are raised in both types of farming systems. A better description of the dairy systems in the Alps is given by Sturaro et al. (2013) and the different contribution of breed and farming systems is given by (Stocco et al., 2017). It should also be considered that almost all Alpine Grey, part of Simmental and Brown Swiss, and only few Holstein cows are moved during the summer to the highland pastures (Zendri et al., 2016); nevertheless, the majority of cows moved are pregnant so that inseminations are rarely done at pasture.

The Brown Swiss had the highest contents of the major components of milk (except lactose, higher in Alpine Grey), with Holstein having the lowest values (except fat and F:P, lower in Alpine Grey), and dual-purpose breeds being intermediate in agreement with other recent studies (Stocco et al., 2017, 2018). Similar rankings characterizing the 4 breeds were also determined for urea and FPD of milk. We also observed a lower SCS value of milk from Simmental cows and modest differences in terms of milk pH between breeds. We also determined that HI was greater in Holsteins and lower in Alpine Grey cows.

Holstein and Alpine Grey were the extreme breeds for major fatty acids and fatty acid categories (Table 4) in the large majority of cases. The Holsteins were, in fact, generally characterized by highest proportion of the most represented milk fatty acids: palmitic and oleic acids, and conversely, by the lowest proportion of SCFA. These results are similar to those of Gottardo et al. (2017) who analyzed the same database but considered the entire lactation and not just the TD closest to the first insemination (early lactation). Taking into consideration that Holsteins and Alpine Grey are the extreme breeds for milk yield, whereas their milk fat content is not much different, we could argue that these differences are probably a consequence of the different proportions among fatty acids supplied by intestinal absorption, by fatty depot mobilization, and by de novo udder synthesis. If a greater proportion of oleic acid characterize Holstein also in comparison with other breeds and different farming systems (Kelsey et al., 2003; Vanbergue et al., 2017), caution should be used when large differences in milk-fat content characterizes the breeds compared, especially in the case of Jerseys (Poulsen et al., 2012; Maurice-Van Eijndhoven et al., 2013).

The interactions between breed and estrous phases were significant for the majority of the traits analyzed (Table 2), but the differences among breeds did not seem relevant in terms of the general pattern during the subsequent phases of the estrous cycle. The results are presented in Appendix Figures A1, A2, and A3) but are not further discussed here.

Possible Use of Estrous Cycle Modification of Milk Traits

The results obtained here permit a better understanding of the effect of estrous cycle on milk characteristics and consequently on udder functions. The modifications in milk components are not specific for each estrous cycle phase, and they cannot be used as specific biomarkers; however, the variation observed suggests a possible use for diagnoses of cows in relation to their reproductive activity. All the quality traits analyzed in this study, except SCS, were obtained from milk spectra through proper calibration. This means that absorbance of many wavelengths of the FTIR spectrum are affected by the estrous cycle of the cows and potentially could be used as diagnostic aid. In particular, FTIR spectrum could be used for diagnosis of cows in heat or, even better, approaching heat. It is clear that this use cannot be based on milk samples collected periodically from conventional milk recording services and analyzed by some centralized laboratory, but could be useful if infrared devices are eventually installed in the milking parlor for real-time (milking to milking) analyses of milk. If a single milk sample is not sufficiently reliable for the diagnosis of estrus, the evolution of FTIR spectrum analysis of samples at each milking could be much more valuable taking into account variability in baseline levels between cows and estrous stage variability for milk composition within cows. This could be integrated with other farming devices for monitoring the performance and wellbeing of dairy cows (Caja et al., 2016).

For a possible diagnostic use of incoming or actual estrus, the choice of the traits (or corresponding FTIR wavelengths, or both), perhaps more than the absolute level of differences with the previous days, should be based on the level of significance (F-value) of "estrous" effect, or also of "days within estrous" effect, observing changes in lactose content, F:P, FPD, HI, myristic and stearic fatty acids, and LCFA. Another criterion of evaluation of candidate traits/wavelengths for estrus diagnosis could be the time of maximum differentiation. From the figures plotting the day within estrous phase patterns of different traits, we note that MCFA exhibited a zenithal value 2 d before estrus and FFA 1 d before (Figure 6). We also note that TFA reached their nadir value 4 d and PUFA 2 d before estrus (Figure 5). Any anticipation of incoming estrus based on milk component analyses could facilitate a more efficient planning of direct observation of cows for estrus behavior and their subsequent and timely insemination.

CONCLUSIONS

Milk composition showed variability among the estrous phases: all of the 24 milk traits studied were significantly affected. Among these traits, the fat, protein, casein, and lactose were most affected by the reproduction cycle of the cow. Furthermore, the milk's fatty acid profile also showed important differences over the estrous cycle, likely induced by the hormonal and behavioral changes of the cows (DMI, rumination time, and activity). Moreover, the estrous cycle also affected the urea, SCS, FPD, pH, and HI. Breed and environmental effects were also responsible for variation in milk composition. Assessments of the relationship of milk composition with estrous phases could lead to new on-farm low-cost indicators of reproductive changes of the cow. Milk profiles could be useful in automated management-systems to identify cows in estrus or predict cows with incoming estrus and should be taken into account for breeding purposes. However, further research is needed to study the prediction capabilities of milk composition to discriminate between cows approaching versus not approaching estrus.

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APPENDIX

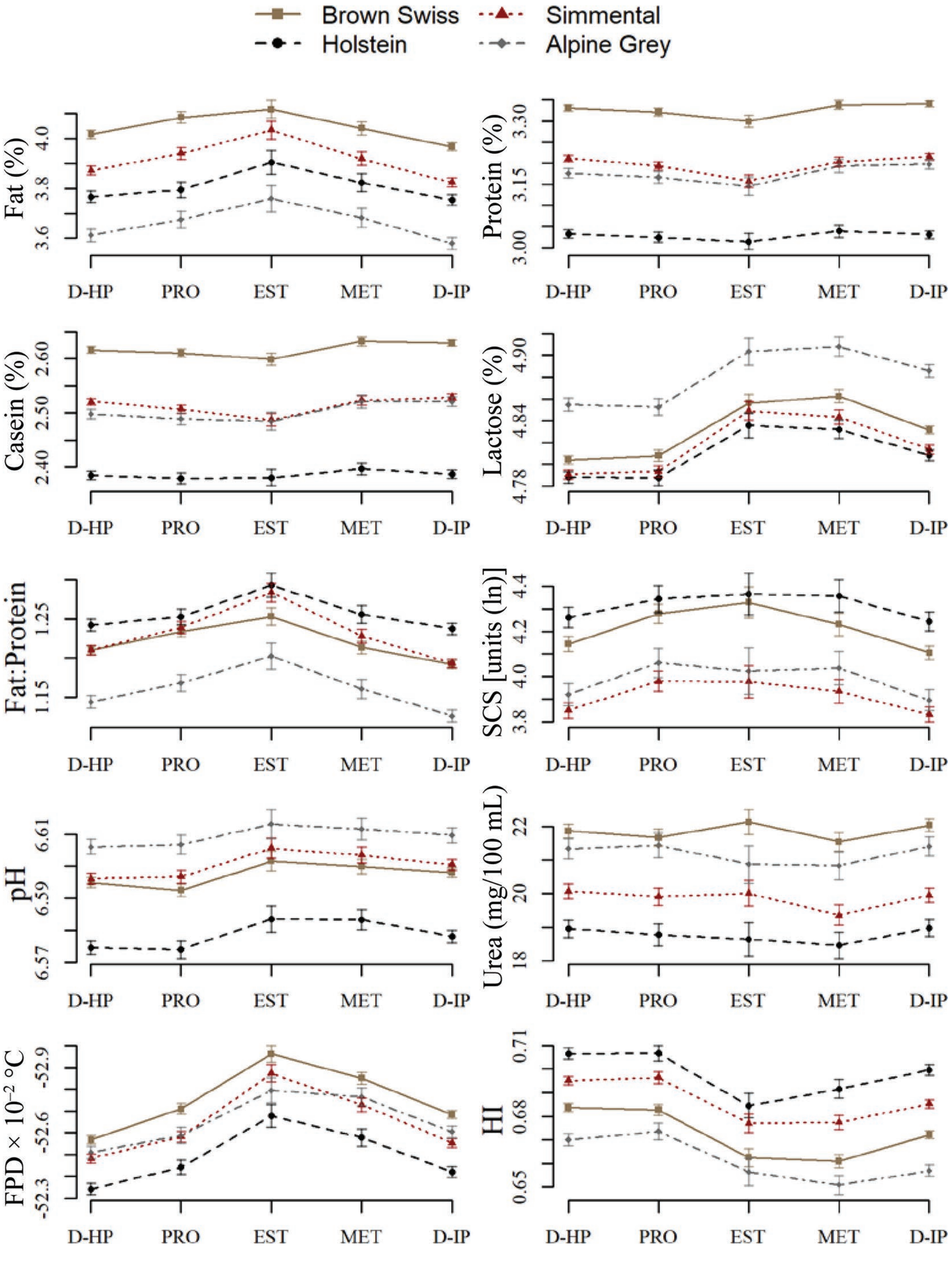

Figure A1. Least squares means (±SE) of fat, protein, casein, lactose, fat:protein ratio, SCS, pH, urea, freezing point depression (FPD), and homogenization index (HI) for the estrous cycle phases by breed. D-HP = diestrus high progesterone; PRO = proestrus; EST = estrus; MET = metestrus; D-IP = diestrus increasing progesterone. Color version available online.

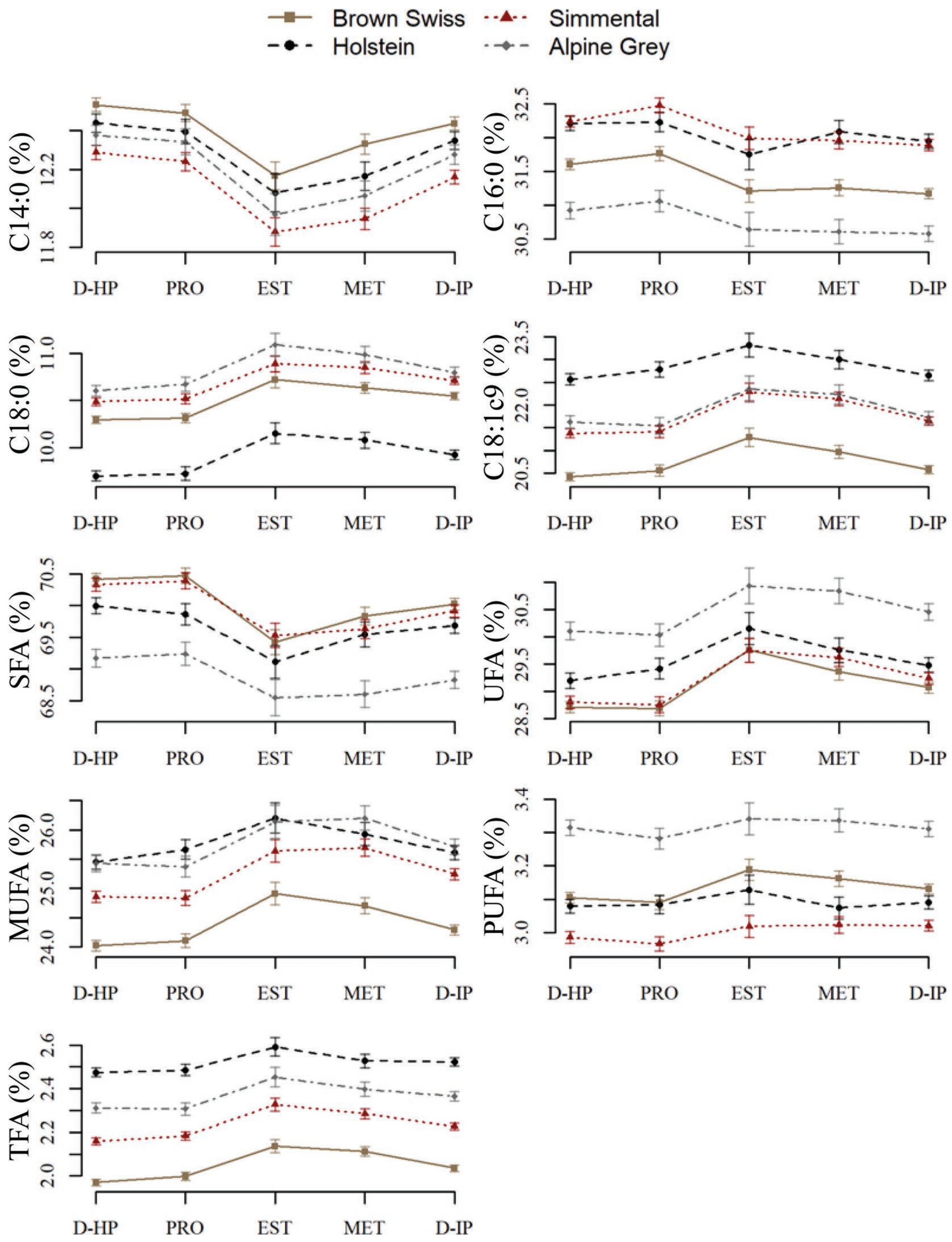

Figure A2. Least squares means (±SE) of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1 *cis*-9), SFA, UFA, MUFA, PUFA, and *trans*-unsaturated fatty acids (TFA), for the estrous cycle phases by breed. D-HP = diestrus high progesterone; PRO = proestrus; EST = estrus; MET = metestrus; D-IP = diestrus increasing progesterone. Color version available online.

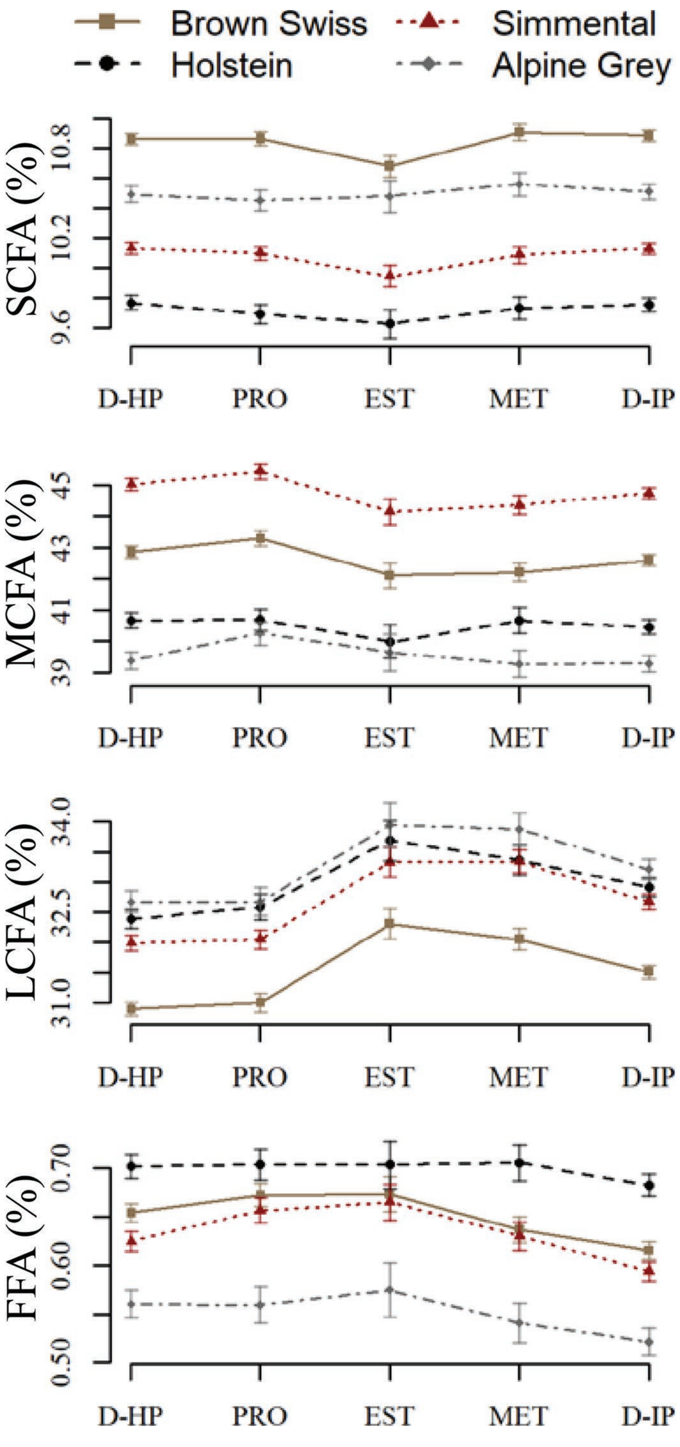

Figure A3. Least squares means (±SE) of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), and free fatty acids (FFA) for the estrous cycle phases by breed. D-HP = diestrus high progesterone; PRO = proestrus; EST = estrus; MET = metestrus; D-IP = diestrus increasing progesterone. Color version available online.