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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 1st (n = 25,820), 2nd (n = 20,358), 3rd (n = 15,114), and  $\geq$ 4th (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  $(\mathbf{F}:\mathbf{P})$ 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  $\pm$  5 SD for each trait were kept ( $\sim 1\%$  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  $[\pm$  standard deviation (**SD**)] between the previous

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