



# The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery

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## ABSTRACT

The aim of this study was to investigate the relationships between somatic cell count (SCC) in milk and several milk technological traits at the individual cow level. In particular, we determined the effects of very low to very high SCC on traits related to (1) milk yield and composition; (2) coagulation properties, including the traditional milk coagulation properties (MCP) and the new curd firming model parameters; and (3) cheese yield and recovery of milk nutrients in the curd (or loss in the whey). Milk samples from 1,271 Brown Swiss cows from 85 herds were used. Nine coagulation traits were measured: 3 traditional MCP [rennet coagulation time (RCT, min), curd firming rate ( $k_{20}$ , min), and curd firmness after 30 min ( $a_{30}$ , mm)] and 6 new curd firming and syneresis traits [potential asymptotic curd firmness at infinite time ( $CF_P$ , mm), curd firming instant rate constant ( $k_{CF}$ ,  $\% \times \text{min}^{-1}$ ), syneresis instant rate constant ( $k_{SR}$ ,  $\% \times \text{min}^{-1}$ ), rennet coagulation time estimated using the equation ( $RCT_{eq}$ , min), maximum curd firmness achieved within 45 min ( $CF_{max}$ , mm), and time at achievement of  $CF_{max}$  ( $t_{max}$ , min)]. The observed cheese-making traits included 3 cheese yield traits ( $\%CY_{CURD}$ ,  $\%CY_{SOLIDS}$ , and  $\%CY_{WATER}$ , which represented the weights of curd, total solids, and water, respectively, as a percentage of the weight of the processed milk) and 4 nutrient recoveries in the curd ( $REC_{FAT}$ ,  $REC_{PROTEIN}$ ,  $REC_{SOLIDS}$ , and  $REC_{ENERGY}$ , which each represented the percentage ratio between the nutrient in the curd and milk). Data were analyzed using a linear mixed model with the fixed effects of days in milk, parity, and somatic cell score (SCS), and the random effect of herd-date. Somatic cell score had strong influences on casein number and lactose, and also affected pH; these were traits characterized by a quadratic pattern of the data. The results also showed a negative linear relationship between SCS and milk

yield. Somatic cell score influenced almost all of the tested coagulation traits (both traditional and modeled), with the exceptions of  $k_{20}$ ,  $CF_P$ , and  $k_{SR}$ . Gelation was delayed when the SCS decreased (slightly) and when it increased (strongly) with respect to a value of 2, as confirmed by the quadratic patterns observed for both RCT and  $RCT_{eq}$ . The SCS effect on  $a_{30}$  showed a quadratic pattern almost opposite to that observed for RCT. With respect to the  $CF_t$  parameters,  $k_{CF}$  decreased linearly as SCS increased, resulting in a linear decrease of  $CF_{max}$  and a quadratic pattern for  $t_{max}$ . Milk SCS attained significance for  $\%CY_{CURD}$ ,  $\%CY_{WATER}$ , and  $REC_{PROTEIN}$ . As the SCS increased beyond 3, we observed a progressive quadratic decrease of the water retained in the curd ( $\%CY_{WATER}$ ), which caused a parallel decrease in  $\%CY_{CURD}$ . With respect to  $REC_{PROTEIN}$ , the negative effect of SCS was almost linear. Recovery of fat and (consequently)  $REC_{ENERGY}$  was characterized by a more evident quadratic trend, with the most favorable values associated with an intermediate SCS. Together, our results confirmed that high SCS has a negative effect on milk composition and technological traits, highlighting the nonlinear trends of some traits across the different classes of SCS. Moreover, we report that a very low SCS has a negative effect on some technological traits of milk.

**Key words:** somatic cell count, milk coagulation property, curd firming, cheese yield, whey loss

## INTRODUCTION

The consumption of milk and dairy products is growing worldwide (International Dairy Federation, 2013), making increased milk production a key dairy breeding goal in recent decades (VanRaden, 2004; Miglior et al., 2005). However, selection for higher milk production has led to deteriorations of milk quality and cow welfare (Oltenuacu and Broom, 2010). For instance, unfavorable genetic correlations between milk yield and diseases (e.g., mastitis and ketosis) have been reported (Ingvarsen et al., 2003). Bovine mastitis is one of the most economically important diseases in dairy herds;

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the consequent high SCC, which is measured as a standard indicator trait of udder health and milk quality, reduces the price paid for milk (Seegers et al., 2003; Viguier et al., 2009). Moreover, milk with a high cell count is reported to have lower casein (Haenlein et al., 1973; Auldism and Hubble, 1998) and lactose (Kitchen, 1981; Auldism and Hubble, 1998) contents, due to increased proteinase-mediated degradation and decreased biosynthesis, respectively. The influence of SCC on fat concentration is more controversial; although some authors (Harmon, 1994; Schallibaum, 2001) found lower values due to reduced synthetic activity of the mammary gland, others observed a higher fat content due to a reduced milk volume (Shuster et al., 1991; Bruckmaier et al., 2004).

Alterations in the chemical composition of high-SCC milk make it less suitable for consumption and cheese processing, with the latter issue reflecting slower coagulation, weak consistency of the curd, and reduced cheese yield (Barbano et al., 1991; Auldism and Hubble, 1998). The technological quality of the milk used in cheese making is commonly evaluated by measuring milk coagulation properties (MCP; Annibaldi et al., 1977; McMahon and Brown, 1982) with computerized renneting meters. The 3 traditional parameters that define the clotting ability of milk, and that can be measured by mechanical lactodynamograph (Formagraph; Foss Electric A/S, Hillerød, Denmark), are rennet coagulation time (RCT, min), curd-firming time ( $k_{20}$ , min), and firmness of the curd at 30 min after the addition of rennet ( $a_{30}$ , mm). An association between elevated SCC and an increase in RCT has been observed by different authors (Ng-Kwai-Hang et al., 1989; Barłowska et al., 2009).

The large majority of relevant published studies have included SCC as a linear covariate in the model, or compared the results obtained using milk with “normal” versus “high” SCC. In these studies, when several classes of SCC are used, they normally do not include classes <100,000 cells/mL, so the detailed effects of very low to very high SCC on milk technological traits have not been fully studied.

Recent studies introduced the strategy of prolonging the observation time and modeling curd firmness (CF) using new time (CF<sub>t</sub>) parameters (Bittante, 2011; Cipolat-Gotet et al., 2012; Bittante et al., 2013). Milk coagulation properties are of interest for 2 main reasons: first, they have technological value for optimizing the cheese-making process and predicting possible abnormalities both during the process and in the final product; and second, they may be used to indirectly predict cheese yield (CY) through their relationships with losses of fines in whey and with moisture retained in the curd. This second aspect is important because

MCP are relatively easy to measure in multiple samples at the laboratory level, whereas direct measurements of CY and nutrient recovery traits are expensive and time-consuming.

Given the complexity of cheese making, the fat and protein contents of milk have frequently been used as proxies for measuring CY. However, the efficiency of the cheese-making process is better defined by the recoveries of milk components in the curd and their losses in the whey (Banks, 2007). More recently, percentage cheese yield (%CY) and nutrient recovery (REC) of individual milk samples have been analyzed using a model cheese-making procedure developed by Cipolat-Gotet et al. (2013), which mimics all phases of cheese production. However, there is little information available regarding the relationships between technological traits of milk and SCC.

Even if bulk tank milk is used for cheese production, information at the cow level might be useful in order to include milk technological traits as breeding goals in dairy cows. Moreover, the individual variation, which is higher compared with that of bulk samples, helps to clarify the relationships between milk SCC and cheese-making traits. Therefore, the aim of this study was to elucidate the relationship between SCC and milk quality and technological traits at the individual cow level. In particular, we performed a detailed investigation of the effects of a range of SCC (from very low to very high) on (1) the milk yield and composition (i.e., fat, protein, casein, casein number, lactose, urea, and pH); (2) the coagulation properties (traditional MCP and the new CF model parameters); and (3) the cheese yield and recovery of milk nutrients in the curd and loss in the whey.

## MATERIALS AND METHODS

### *Milk Sample Collection*

This study is part of the Cowability-Cowplus Projects, which were described in detail by Cipolat-Gotet et al. (2013) and Cecchinato et al. (2013). Briefly, individual milk samples of 1,271 Brown Swiss cows were collected once from 85 herds (a maximum of 15 cows/herd, 1 or 2 herds per week, 13 mo in total) located in Trento Province, northern Italy. The relevant environmental conditions were described in detail by Sturaro et al. (2013). The milk samples (one per cow) were collected during the evening milking. After collection, each sample was divided into 2 subsamples, which were refrigerated (4°C, without preservative). One subsample (50 mL) was transferred to the Milk Quality Laboratory of the Breeders Federation of Trento Province (Trento, Italy) for milk composition analysis. The other (2,000

mL) was transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) at the University of Padova (Legnaro, Padova, Italy) for MCP analysis and model cheese making. Data on herds and cows were provided by the Breeders Federation of Trento Province and the Superbrown Consortium (Italy).

### Analysis of Quality Traits, MCP, CF Modeling, Cheese Yield, and Whey Losses

**Milk Composition Traits.** In the Milk Quality Laboratory in Trento (Italy), each milk subsample was analyzed within 20 h of milking for fat, protein, casein, lactose (%), and urea (mg/100 g) using a Milkoscan FT6000 (Foss Electric A/S), calibrated according to the following reference methods: fat (ISO, 2010b; ISO 1211|IDF 1; gravimetric method, Rose-Gottlieb); protein (ISO, 2014; ISO 8968–1|IDF 20-1; titrimetric method, Kjeldahl); casein (ISO, 2004a; ISO 17997–1|IDF 29; titrimetric method, Kjeldahl); lactose (ISO, 2002; ISO 5765-1|IDF 79-1; enzymatic method); urea (ISO, 2004b; ISO 14637|IDF 195; differential pH method); TS (ISO, 2010a; ISO 6731|IDF 21; determination of TS content). Ten reference milk samples per month (Italian Breeders Association, Rome, Italy) were used for adjusting calibrations, and 1 repeated sample every 50 analyzed samples was used to control repeatability of analyses.

Milk pH, adjusted for sample temperature, was measured using a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). Each SCC was obtained with a Fossomatic FC counter (Foss Electric A/S) and log-transformed to an SCS (Ali and Shook, 1980). Bacterial count was not measured on individual milk samples.

**Traditional MCP.** In the cheese-making laboratory of the University of Padova (Italy), within 20 h of milking, the time from rennet addition to milk gelation (RCT, min), curd-firming rate (min to a curd firmness of 20 mm;  $k_{20}$ ) and curd firmness after 30 min from rennet addition ( $a_{30}$ , in mm) were determined using a mechanical lactodynamograph (Formagraph; Foss Electric A/S). The duration of the test was extended to 90 min, so that almost all (99.7%) of the milk samples coagulated and yielded  $k_{20}$  values. The details of the experimental conditions (e.g., temperature of the milk samples, concentration and type of rennet) were as reported in Cipolat-Gotet et al. (2012).

**New Curd Firming and Syneresis Traits.** For each milk sample, 360 curd firmness values were recorded (1 every 15 s for 90 min). The new parameters of curd firmness modeled on time  $t$  ( $CF_t$ ) were estimated

using the 4-parameter model proposed by Bittante et al. (2013):

$$CF_t = CF_P \times \left(1 - e^{-k_{CF} \times (t - RCT_{eq})}\right) \times e^{-k_{SR} \times (t - RCT_{eq})},$$

where  $CF_t$  (mm) is the curd firmness at time  $t$ ;  $CF_P$  (mm) is the maximum asymptotic curd firmness;  $k_{CF}$  ( $\% \times \text{min}^{-1}$ ) is the curd-firming instant rate constant;  $k_{SR}$  ( $\% \times \text{min}^{-1}$ ) is the curd syneresis instant rate constant; and  $RCT_{eq}$  (min) is the rennet coagulation time. Moreover, 2 additional traits related to maximum curd firmness (MCF) were calculated: the maximum  $CF_t$  value ( $CF_{max}$ , mm) and time at  $CF_{max}$  ( $t_{max}$ , min).

**Individual Cheese Yield and Curd Nutrient Recovery.** These phenotypes were obtained through a model cheese-making procedure performed on a milk subsample (1,500 mL) from each individual cow. Cheese yield was assessed using 7 components that formed 2 groups of traits: (1) 3 %CY traits that expressed the weights of the fresh curd (%CY<sub>CURD</sub>), the curd DM (%CY<sub>SOLIDS</sub>), and the water retained in the curd (%CY<sub>WATER</sub>) as percentages of the weight of the processed milk; and (2) 4 REC traits representing the proportions of milk nutrients and energy retained in the curd (REC<sub>SOLIDS</sub>, REC<sub>FAT</sub>, REC<sub>PROTEIN</sub>, and REC<sub>ENERGY</sub>, calculated as the percentage ratios between a given component in the curd versus the processed milk). The energy within the curd was calculated as the difference between the energy in the milk and in the whey (NRC, 2001). A detailed description of the individual model cheese-making procedure used to obtain the phenotypes analyzed in this study, as well as the relevant sources of phenotypic variation, can be found in Cipolat-Gotet et al. (2013). Finally, all analyses (milk composition, coagulation, and cheese-making traits) were performed within 20 h from milk collection.

### Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the following linear model:

$$y_{ijklm} = \mu + \text{DIM}_i + \text{Parity}_j + \text{SCS}_k \\ + \text{Herd-date}_l + e_{ijklm},$$

where  $y_{ijklm}$  is the observed trait;  $\mu$  is the overall mean;  $\text{DIM}_i$  is the fixed effect of the  $i$ th class of days in milk [ $i = 6$  classes: class 1  $\leq 60$  ( $n = 178$ ); 60 < class 2  $\leq 120$  ( $n = 265$ ); 120 < class 3  $\leq 180$  ( $n = 226$ ); 180 < class 4  $\leq 240$  ( $n = 167$ ); 240 < class 5  $\leq 300$  ( $n = 176$ ); class

6 > 300 (n = 188)]; Parity<sub>j</sub> is the fixed effect of the jth parity [j = 1 (n = 368); 2 (n = 362); 3 (n = 201); 4 (n = 144); ≥ 5 (n = 186)]; SCS<sub>k</sub> is the fixed effect of the kth class of SCS [k = 1 to 7; class 1 ≤ 0.66 (n = 131); 0.66 < class 2 ≤ 1.59 (n = 193); 1.59 < class 3 ≤ 2.52 (n = 215); 2.52 < class 4 ≤ 3.45 (n = 224); 3.45 < class 5 ≤ 4.38 (n = 210); 4.38 < class 6 ≤ 5.31 (n = 137); class 7 > 5.31 (n = 147)]; Herd-date<sub>l</sub> is the random effect of the lth herd-date (l = 1 to 85); and e<sub>ijklm</sub> is the random residual. For the studied coagulation properties, cheese yield traits, and nutrient recoveries in curd, the pendulum/vat<sub>m</sub> effect was added to the above-described model as the fixed effect of the mth pendulum (m = 1 to 10) for coagulation properties or the mth number of the vat or waterbath (m = 1 to 15) for cheese yield and nutrient recoveries. Herd-date and residuals were assumed to be normally distributed with a mean of zero and variances of σ<sub>h</sub><sup>2</sup> and σ<sub>e</sub><sup>2</sup>, respectively. The proportion of variance explained by herd-test date was calculated by dividing the corresponding variance component by the total variance.

Polynomial contrasts (P < 0.05) were estimated to look at the response curve of the data for the SCS effect; the first-order comparisons measured linear relationships, whereas the second- and third-order comparisons measured quadratic and cubic relationships, respectively.

## RESULTS

### Descriptive Statistics

As all the investigated traits were characterized by normal distributions (Cipolat-Gotet et al., 2012, 2013), only the 1st and 99th percentiles are provided in Table 1. The milk yield of the Brown Swiss cows reared in the different dairy farming systems averaged 24.2 kg/d and showed a large variability (CV = 31.7%). Among the milk composition traits, casein number and pH showed the lowest variabilities, with CV of 1.65 and 1.19%, respectively. The other milk composition traits confirmed the good quality that characterizes milk of Brown Swiss cows and presented intermediate variabilities (from 3.7% for lactose to 31.4% for milk urea).

The SCS ranged from -0.47 (1st percentile) to 7.77 (99th percentile), corresponding to SCC of 9,000 and 2,722,000 cells/mL, respectively. Both SCS and SCC exhibited very high variabilities.

The mean values for RCT and k<sub>20</sub> were 19.9 and 5.6 min, respectively, whereas a<sub>30</sub> averaged 29.6 mm. In the present work, the CF<sub>t</sub> parameters had mean values as follows: 20.9 min for the coagulation time calculated for each milk sample on the basis of all 360 data points (RCT<sub>eq</sub>); 54.6 mm for the asymptotic potential curd

firmness theoretically achievable at infinite time in absence of curd syneresis (CF<sub>p</sub>); 12.6% × min<sup>-1</sup> for the instant rate constant of curd firming (k<sub>CF</sub>), leading the CF<sub>t</sub> curve toward a value of CF<sub>p</sub> shortly after RCT; and 1.39% × min<sup>-1</sup> for the instant rate constant of syneresis, leading CF<sub>t</sub> toward zero over an extended duration (k<sub>SR</sub>). On average, the maximum CF value (CF<sub>max</sub>) was 36.5 mm, and it was achieved (t<sub>max</sub>) 41.5 min after rennet addition.

The mean %CY<sub>CURD</sub>, which corresponded to the sum of %CY<sub>SOLIDS</sub> and %CY<sub>WATER</sub>, was 15%. The curd nutrient recovery was, on average, close to the mean casein number for protein, almost 90% for fat, slightly more than half for total solids, and about two-thirds for milk energy (Table 1).

### Sources of Variation Among Quality Traits

The results of our ANOVA for the milk yield and composition traits are summarized in Table 2. The proportion of variance explained by herd-test date was more than 70% for urea; approximately 50% for milk production, casein number, and pH; 20% for fat, protein, and casein contents and fat:protein ratio; and slightly more than 10% for lactose and SCS. As expected, DIM and parity effects played important roles in explaining the variation of almost all considered traits, with the exceptions of fat, fat:protein, and urea traits, for which parity had a negligible effect. Detailed information on the effect of DIM and parity on the investigated traits was reported by Cipolat-Gotet et al. (2012, 2013) and Bittante et al. (2015). The influence of DIM and parity was tested also on milk SCS (Table 2) and the results showed an increase in SCS with advancing age and stage of lactation (data not shown).

In the present study, we focused on the effect of SCS, which had strong influences on casein number and lactose, and also affected pH and urea. The results of the polynomial contrasts are reported in Table 2. The least squares means (**LSM**) and the corresponding standard errors of the milk yield and composition traits for which SCS had significant effects are presented in Figure 1, together with the corresponding curve of the data across the different classes of SCS (according to the obtained significant linear or quadratic contrasts). Even though the ANOVA did not reveal a significant effect of SCS on milk yield (Table 2), the contrasts showed a negative linear relationship (P < 0.05) between these 2 traits (Figure 1a). Milk fat, protein, and casein contents were not affected by milk SCS. Urea exhibited an erratic trend across the SCS classes and was characterized by a high standard error of the means (Figure 1d). The casein number and lactose showed clear quadratic patterns: their LSM remained relatively constant across

**Table 1.** Descriptive statistics of single test-day milk yield, composition, traditional milk coagulation properties (MCP), curd firming, cheese yield (%CY), and curd nutrient recovery (REC)<sup>1</sup>

Trait <sup>2</sup>	N <sup>3</sup>	Mean	SD	P1	P99
Milk yield, kg/d	1,233	24.2	7.7	8.5	44.8
Milk composition					
Fat, %	1,242	4.20	0.65	2.71	5.86
Protein, %	1,250	3.70	0.42	2.86	4.70
Fat:protein	1,239	1.14	0.18	0.74	1.62
Casein, %	1,250	2.89	0.32	2.26	3.65
Casein number, %	1,246	77.1	1.3	73.9	79.9
Lactose, %	1,246	4.86	0.18	4.36	5.22
Urea, mg/100 g	1,252	25.9	8.1	9.0	45.2
pH	1,248	6.64	0.08	6.45	6.83
SCC, 10 <sup>3</sup> /mL	1,257	252	615	9	2,722
SCS, units	1,257	2.98	1.86	-0.47	7.77
Traditional MCP					
RCT, min	1,253	19.9	5.7	10.3	38.3
k <sub>20</sub> , min	1,241	5.62	3.59	2.00	19.30
a <sub>30</sub> , mm	1,192	29.6	11.0	0.7	50.8
Curd firming					
RCT <sub>eq</sub> , min	1,250	20.9	6.4	11.1	41.1
CF <sub>p</sub> , mm	1,141	54.6	13.9	26.1	97.9
k <sub>CF</sub> , % × min <sup>-1</sup>	1,155	12.6	5.7	2.4	28.4
k <sub>SR</sub> , % × min <sup>-1</sup>	1,153	1.39	0.56	0.15	2.95
CF <sub>max</sub> , mm	1,248	36.5	7.3	18.5	53.4
t <sub>max</sub> , min	1,226	41.5	12.0	22.4	81.4
Cheese yield (%CY)					
%CY <sub>CURD</sub>	1,247	15.0	1.9	11.0	19.6
%CY <sub>SOLIDS</sub>	1,238	7.22	0.94	5.37	9.93
%CY <sub>WATER</sub>	1,241	7.80	1.28	5.04	11.23
Nutrient recovery (REC)					
REC <sub>PROTEIN</sub> , %	1,242	78.1	2.4	72.4	83.3
REC <sub>FAT</sub> , %	1,231	89.9	3.6	78.7	95.9
REC <sub>SOLIDS</sub> , %	1,244	52.1	3.6	44.1	60.9
REC <sub>ENERGY</sub> , %	1,231	67.3	3.3	59.2	75.1

<sup>1</sup>P1 = 1st percentile; P99 = 99th percentile.

<sup>2</sup>SCS =  $\log_2(\text{SCC}/100,000) + 3$ ; RCT = rennet coagulation time; k<sub>20</sub> = curd firming rate as minutes to a curd firmness of 20 mm; a<sub>30</sub> = curd firmness after 30 min from rennet addition; RCT<sub>eq</sub> = rennet coagulation time estimated using the equation; CF<sub>p</sub> = potential asymptotic curd firmness at infinite time; k<sub>CF</sub> = curd firming instant rate constant; k<sub>SR</sub> = syneresis instant rate constant; CF<sub>max</sub> = maximum curd firmness achieved within 45 min; t<sub>max</sub> = time at achievement of CF<sub>max</sub>; %CY<sub>CURD</sub> = fresh cheese yield; %CY<sub>SOLIDS</sub> = total solids cheese yield; %CY<sub>WATER</sub> = water entrapped in the curd; REC<sub>PROTEIN</sub>, % = protein retention; REC<sub>FAT</sub>, % = fat retention; REC<sub>SOLIDS</sub>, % = total solids retention; REC<sub>ENERGY</sub>, % = energy retention.

<sup>3</sup>Number of samples.

the first 3 classes of SCS, and then decreased almost linearly as the SCS increased beyond 2 (Figures 1b and 1c). Finally, although the LSM differences for milk pH were modest, decreases in lactose and the casein number were accompanied by decreases in milk acidity (Figure 1e).

### Sources of Variation Among MCP and CF Modeling

The results from our statistical analysis of the various coagulation properties are summarized in Table 3. Notably, the proportion of variance explained by herd-test date was lower for the coagulation properties than for the quality traits and was much lower than for milk yield; the values for coagulation properties ranged between ~4 and 16%, with the exception of t<sub>max</sub> (22%). As expected, DIM had strong influences on both the

traditional MCP and the new CF<sub>t</sub> model parameters. Parity was significant ( $P < 0.05$ ) in explaining the variation of only RCT among the traditional traits, but it was significant for all of the CF<sub>t</sub> traits (with the exception of CF<sub>p</sub>).

The effect of pendulum, which was the only effect related to instrument repeatability, was confirmed to play important roles in explaining the variation of all traits except for RCT and RCT<sub>eq</sub>. Somatic cell score influenced almost all of the tested coagulation traits, both traditional and modeled, with the only exceptions being k<sub>20</sub>, CF<sub>p</sub>, and k<sub>SR</sub>.

The LSM of the coagulation traits affected by SCS are reported in Figure 2. The most rapid gelation was exhibited by milk samples with an SCS in the class centered on a value of 2, both when expressed as single point trait (RCT) or as a CF<sub>t</sub> equation parameter

( $RCT_{eq}$ ). Gelation was delayed when SCS decreased (slightly) or increased (strongly) with respect to the class centered on an SCS of 2, as confirmed by the quadratic patterns observed for both RCT and  $RCT_{eq}$ . Considering the curd firming process, within the traditional MCP, SCS did not show any significant effect on  $k_{20}$ , whereas the effect on  $a_{30}$  seemed to be opposite to that seen for RCT (Figure 2b). In the case of the  $CF_t$  parameters,  $CF_P$  and  $k_{SR}$  were not affected, but  $k_{CF}$  decreased linearly as SCS increased (Figure 2d). This yielded a linear decrease of  $CF_{max}$  (Figure 2e) and a quadratic pattern for  $t_{max}$ , which exhibited a relevant delay only when SCS exceeded 3 (Figure 2f).

### Sources of Variation Among Cheese Yield and Whey Losses

Table 4 shows the roles of the considered effects in explaining variations of the cheese yield traits and milk nutrient recovery in the curd. For these traits, the proportion of variance explained by herd-test date (20 to 40%) was much greater than that seen for the coagulation and curd-firming traits. As expected, DIM was the most important source of variation affecting all of the studied traits. Parity had a strong influence on  $\%CY_{WATER}$  and (consequently)  $\%CY_{CURD}$ , and affected  $REC_{PROTEIN}$ . Unlike the coagulation and curd firming traits, the effects of the instruments used (waterbaths and individual vats) were negligible in explaining the variations of the cheese yield and curd recovery traits. Similar to parity, SCS attained significance exclusively for  $\%CY_{CURD}$ ,  $\%CY_{WATER}$ , and  $REC_{PROTEIN}$ .

Figure 3 depicts the LSM results for cheesemaking-related traits across the different classes of SCS. All

of the affected traits showed nonlinear effects for SCS. As SCS increased beyond 3, there was a progressive decrease in water retained in the curd (decreasing  $\%CY_{WATER}$ ; Figure 3b), which caused a parallel decrease in  $\%CY_{CURD}$  (Figure 3a). For  $REC_{PROTEIN}$ , the negative effect of SCS was almost linear (Figure 3c). In contrast,  $REC_{FAT}$  (Figure 3d) and (consequently)  $REC_{ENERGY}$  (Figure 3e) showed more evident quadratic trends, with the most favorable values being observed for milk with an intermediate SCS content, whereas lower values were seen for milk samples with smaller and greater SCS.

## DISCUSSION

### SCS Affects the Variation of Milk Yield and Composition

An infection in the udder triggers an inflammatory response characterized by recruitment of white cells from the bloodstream and altered secretion of various molecules (Wellnitz and Bruckmaier, 2012). Because cell numbers in milk are associated with inflammation, SCC is recognized as the international standard indicator of udder health and milk quality (Sharif and Muhammad, 2008). Bovine mastitis increases the milk SCC, decreasing milk production and changing the composition of the milk (Kitchen, 1981; Le Maréchal et al., 2011). Inflammation damages the mammary gland tissue, inhibiting the biosynthesis of fat, protein, and lactose, and thereby decreasing milk yield (Harmon, 1994; Schallibaum, 2001). In the present study, we observed a negative linear relationship between milk production and the classes of SCS (Figure 1a), although

**Table 2.** Results from ANOVA (*F*-value and significance) for single test-day milk yield and composition traits

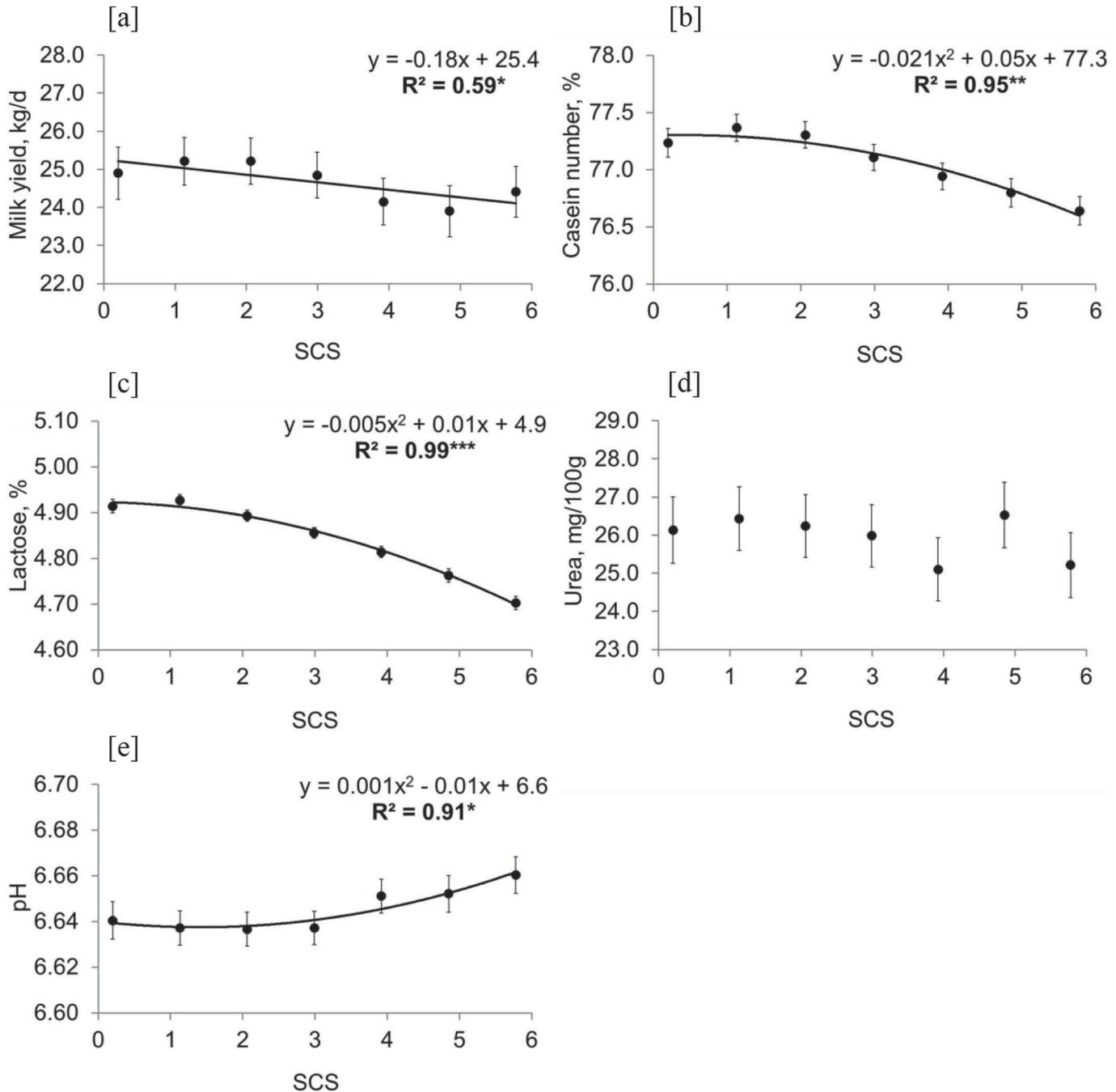
Trait	DIM	Parity	SCS <sup>1</sup>	SCS Contrast			Herd-date, <sup>2</sup> %	RMSE <sup>3</sup>
				Linear	Quadratic	Cubic		
Milk yield, kg/d	118.4***	35.7***	1.5	4.2*	0.1	3.7	48.9	4.67
Milk composition								
Fat, %	14.4***	0.3	1.0	0.7	0.4	0.1	19.8	0.57
Protein, %	175.1***	8.2***	0.4	0.9	0.2	0.2	21.3	0.27
Fat:protein	12.3***	2.3	0.6	0.5	0.0	0.0	17.0	0.16
Casein, %	167.4***	12.0***	0.6	0.5	1.1	0.1	22.1	0.21
Casein number, %	4.5***	14.0***	13.5***	60.5***	8.1**	4.3*	57.1	0.79
Lactose, %	11.4***	21.2***	38.4***	198.6***	17.1***	1.2	10.5	0.14
Urea, mg/100 g	4.9***	0.5	2.5*	2.6	0.1	0.0	72.9	4.26
pH	5.5***	6.4***	3.6**	12.4***	4.4*	0.7	50.8	0.06
SCS, units	26.8***	16.7***	—	—	—	—	11.3	1.62

<sup>1</sup>SCS =  $\log_2$  (SCC/100,000) + 3.

<sup>2</sup>Herd-date effect expressed as proportion of variance explained by herd/test date calculated by dividing the corresponding variance component by the total variance.

<sup>3</sup>RMSE = root mean square error.

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



**Figure 1.** Least squares means of milk composition traits across SCS. Results of the polynomial contrasts have been reported: the response curve of the data across classes of SCS (linear or quadratic), the coefficient of determination ( $R^2$ ) of the regression and the  $P$ -value of the polynomial contrasts.  $^*P < 0.05$ ;  $^{**}P < 0.01$ ;  $^{***}P < 0.001$ . Error bars correspond to SE of LSM.

the numeric pattern seemed slightly curvilinear. This trend agrees with the results obtained by Koldewej et al. (1999), who found a linear relationship between  $\log_{10}(\text{SCC})$  and test-day milk yield. The same authors evaluated quadratic and cubic effects, but found that they contributed little to the overall fit of the models.

The linear relationship predicts that milk production will decrease by 2.04 kg for each unit increase in  $\log_{10}(\text{SCC})$ , leading to a 10-fold increase in SCC. This corresponds to a decrease of 0.6 kg/d of milk per unit of increase in  $\log_2(\text{SCC})$ , which corresponds to a 2-fold increase in SCC. Our estimate ( $-0.18$  kg/d per

unit) is much smaller, even in the interval between SCS values of 2 and 5 ( $-0.5$  kg/d). However, it should be noted that we excluded any cow that showed signs of clinical mastitis. Previously, the relationships between repeated records of milk yield and the SCS of 33,453 first-lactation Norwegian Red cows were described by de los Campos et al. (2006) using equations that considered possible recursive or simultaneous effects between traits. Those authors found evidence that SCS has a negative effect on milk yield (the infection effect, for which SCS is a standard indirect indicator), with an increase of 1 unit of SCS decreasing milk yield by about 1.1 kg/d; however, they did not find any reciprocal effect of milk production on SCS (dilution effect). Similar results were obtained by Wu et al. (2007), who used a Bayesian approach, compared with the maximum likelihood method used by de los Campos et al. (2006).

The lactose level in milk decreases during mastitis not only because of lower biosynthesis, but also because membrane permeability increases, allowing lactose to leak from the milk into the blood (Shuster et al., 1991); consequently, the milk contents of some minerals increase. As shown in Figure 1c, we observed that an increase in SCC was accompanied by a nonlinear reduction in lactose content, with lactose values remaining relatively constant in milk samples with SCS <2 but decreasing almost linearly above this value. The association between a high SCC and a decreased lactose concentration is well documented (Kitchen, 1981; Auld et al., 1995; Wickström et al., 2009), and it

has been proposed that lactose could be used to monitor udder health (Pyörälä, 2003). However, most of the existing reports have used a 2-level evaluation (low vs. high) to examine the effect of SCC on milk yield and composition; far fewer studies have examined response curves across different classes of SCC. Bruckmaier et al. (2004) observed lower lactose concentrations (43.8 vs. 48.1 g/L) in infected quarters ( $\log_{10}\text{SCC/mL} >6$ ) compared with healthy contralateral quarters ( $\log_{10}\text{SCC/mL} <5.2$ ), which was consistent with the previous findings of other authors (Fox et al., 1985; Harmon, 1994). As lactose content decreases, the concentrations of certain minerals (sodium and chloride) increase to maintain the osmotic equilibrium (Batavani et al., 2007). Moreover, lower values of casein might be observed in high SCC milk because of plasmin- and somatic-cell-protease-mediated activity against caseins, especially  $\alpha_{S1}$ - and  $\beta$ -casein (Urech et al., 1999). The changes in the ionic environment and the degradation of casein due to a higher enzymatic activity (Verdi et al., 1987; Franceschi et al., 2003) are responsible for the increase in milk pH observed during mastitis. This increase seems to follow a nonlinear quadratic trend (Figure 1e), with constant LSM values found for the first 4 classes of SCS, followed by moderate increases in classes 5, 6, and 7. Batavani et al. (2007) compared milk samples collected from healthy quarters and quarters with sub-clinical mastitis (defined as a leukocyte count >500,000 cells/mL) and found that the pH of mastitic milk was significantly higher than that of healthy milk (6.69 vs.

**Table 3.** Results from ANOVA (*F*-value and significance) for traditional milk coagulation properties (MCP) and curd firming traits

Trait <sup>1</sup>	DIM	Parity	Pendulum <sup>2</sup>	SCS <sup>3</sup>	SCS Contrast			Herd-date, <sup>4</sup> %	RMSE <sup>5</sup>
					Linear	Quadratic	Cubic		
Traditional MCP									
RCT, min	25.7***	2.9*	1.0	6.7***	15.5***	12.1***	1.3	14.6	4.95
k <sub>20</sub> , min	4.6***	0.4	2.3**	1.0	1.0	1.6	0.0	4.3	3.51
a <sub>30</sub> , mm	9.0***	2.0	2.4**	2.6*	5.1*	4.6*	0.3	6.4	10.28
Curd firming									
RCT <sub>eq</sub> , min	20.6***	2.9*	0.7	5.2***	11.7***	10.1**	0.1	11.1	5.68
CF <sub>p</sub> , mm	18.8***	1.7	8.3***	0.7	0.0	0.0	1.8	11.9	12.13
k <sub>CF</sub> , % × min <sup>-1</sup>	15.9***	3.3*	2.9***	2.3*	4.0*	1.2	0.1	13.8	5.00
k <sub>SR</sub> , % × min <sup>-1</sup>	2.4*	5.0***	18.4***	2.0	3.1	2.6	1.8	15.6	0.47
CF <sub>max</sub> , mm	20.1***	4.7***	5.1***	2.7*	9.7**	2.6	0.8	21.9	6.02
t <sub>max</sub> , min	14.7***	5.2***	4.3***	3.0**	7.3**	5.7*	0.3	15.0	10.49

<sup>1</sup>RCT = rennet coagulation time; k<sub>20</sub> = curd firming rate as time (min) to a curd firmness of 20 mm; a<sub>30</sub> = curd firmness after 30 min from rennet addition; RCT<sub>eq</sub> = rennet coagulation time estimated using the equation; CF<sub>p</sub> = potential asymptotic curd firmness at infinite time; k<sub>CF</sub> = curd firming instant rate constant; k<sub>SR</sub> = syneresis instant rate constant; CF<sub>max</sub> = maximum curd firmness achieved within 45 min; t<sub>max</sub> = time at achievement of CF<sub>max</sub>.

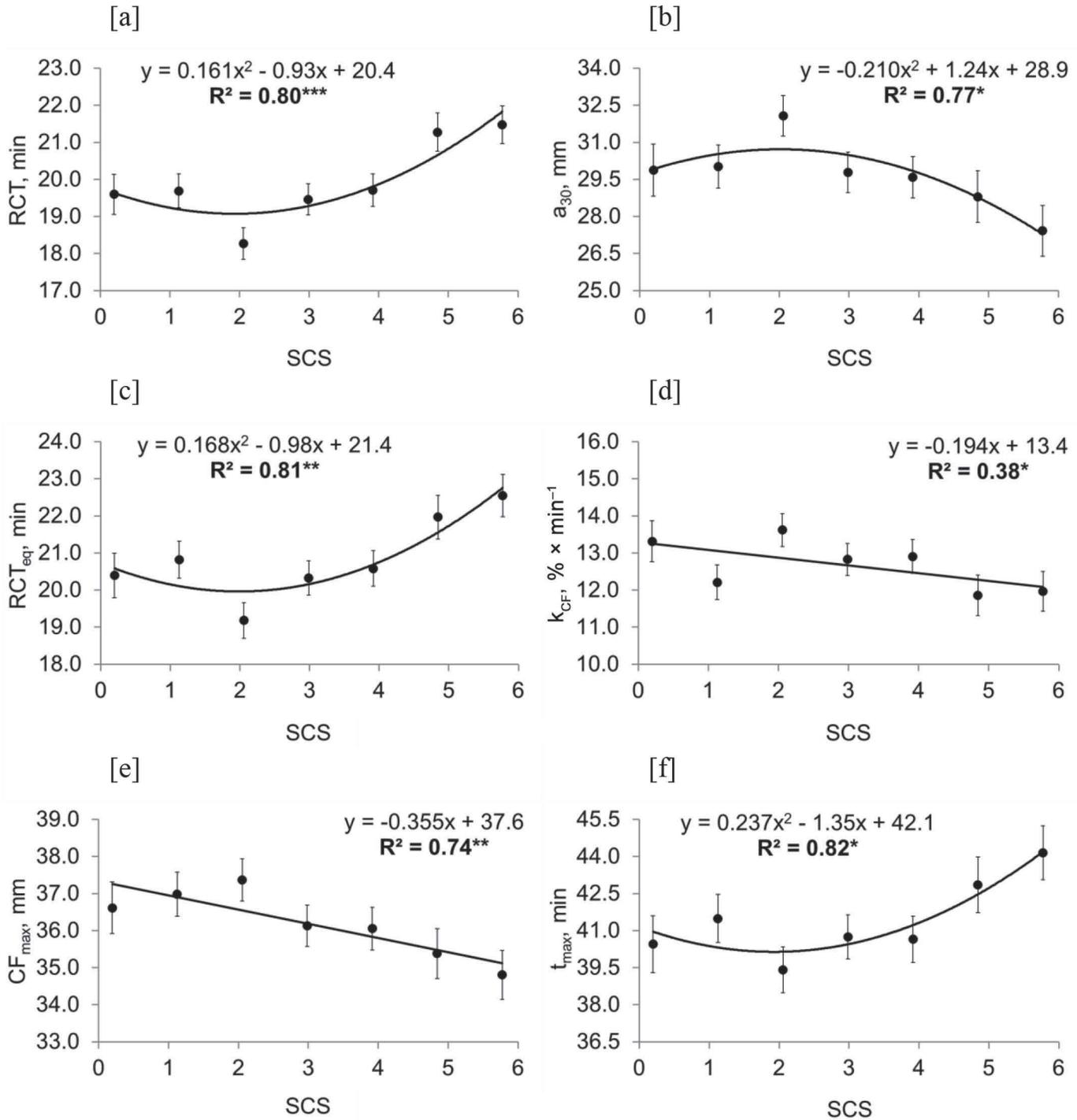
<sup>2</sup>Pendulum = measuring unit of the coagulation meter.

<sup>3</sup>SCS =  $\log_2(\text{SCC}/100,000) + 3$ .

<sup>4</sup>Herd-date effect expressed as proportion of variance explained by herd-test date calculated by dividing the corresponding variance component by the total variance.

<sup>5</sup>RMSE = root mean square error.

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



**Figure 2.** Least squares means of traditional milk coagulation properties (MCP) and curd firming traits across SCS. Results of the polynomial contrasts are reported: the response curve of the data across classes of SCS (linear or quadratic), the coefficient of determination ( $R^2$ ) of the regression and the  $P$ -value of the polynomial contrasts. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . RCT = rennet coagulation time;  $a_{30}$  = curd firmness after 30 min from rennet addition; RCT<sub>eq</sub> = rennet coagulation time estimated using the equation;  $k_{CF}$  = curd firming instant rate constant; CF<sub>max</sub> = maximum curd firmness achieved within 45 min;  $t_{max}$  = time of achievement of CF<sub>max</sub>. Error bars correspond to SE of LSM.

6.59, respectively;  $P < 0.01$ ). Vianna et al. (2008) investigated the effect of low (<200,000 cells/mL) and high (>700,000 cells/mL) SCC on raw milk composition and observed higher pH (6.85 vs. 6.76) and lower lactose values (4.53 vs. 4.69%) in high SCC milk. The effect of mastitis on the total protein content is controversial (Le Maréchal et al., 2011), but drastic changes clearly occur in the protein profile (Urech et al., 1999). The proteolysis of casein and the decreased synthesis of the major whey proteins,  $\beta$ -LG and  $\alpha$ -LA, are balanced by increases in the contents of BSA and immunoglobulin via leakage through the blood–milk barrier (Haenlein et al., 1973). Thus, the total protein content in milk may not be significantly influenced by high SCC (Munro et al., 1984). In the present study, the casein and protein contents were not affected by the SCS class, but the casein:protein ratio was affected (Table 2). In fact, the casein number was influenced by high SCC, and showed a nonlinear quadratic pattern similar to that observed for lactose content (Figure 1b). Geary et al. (2013) performed a meta-analysis on the data available in the literature and estimated the relationships between SCC and milk composition traits. A total of 32 published articles, mostly based on 2- or 3-class (low, intermediate, and high SCC) evaluations, were included in the analysis. Significant ( $P < 0.01$ ) negative linear relationships were found between SCS and lactose content, and between SCS and casein number. Quadratic and cubic effects were also tested in the random regression models but they were not significant. Coulon et al. (1998) reported that the decrease in casein number became significant when SCC >200,000 cells/mL; this value corresponds to our SCS class 4, which is the class

in which we observed the casein number begin to decrease as SCS increased (Figure 1b). Notably, an SCC >200,000 cells/mL is considered the threshold value for detecting subclinical mastitis at the individual cow level (Guidry, 1985).

### SCS Affects Variation in Milk Coagulation and Curd Firming Pattern

Although cheese production is a major use of milk, relatively few studies have examined the effect of SCC on the cheese-making process or the properties of the produced cheese (Le Maréchal et al., 2011). Nonetheless, a high milk SCC is generally recognized to affect not only milk composition, but also the technological traits related to clotting ability and cheese processing (Barbano et al., 1991; Auld and Hubble, 1998). As variation in milk pH could strongly affect both traditional MCP and the  $CF_t$  parameters (Stocco et al., 2015), the main effect of SCS on these traits could reflect increases in pH caused by subclinical mastitis. Higher pH, in association with elevated SCC, negatively affects the cheese-making ability of milk by decreasing the activities of clotting-related enzymes (Swaisgood, 1982). Moreover, alterations in casein may also affect milk coagulation, as proteose-peptones released during the degradation of casein seem to have negative effects on clotting time, curd firmness, and curd formation (Le Maréchal et al., 2011). In the present study, the increased alkalinity and lower casein numbers of milk samples characterized by SCS >3 were responsible for a prolonged coagulation time ( $RCT$ , Figure 2a;  $RCT_{eq}$ , Figure 2c) and a weaker coagulum ( $a_{30}$ , Figure 2b) with

**Table 4.** Results from ANOVA ( $F$ -value and significance) for cheese yield (%CY) and milk nutrient recovery in curd (REC)

Trait <sup>1</sup>	DIM	Parity	Wat/WB <sup>2</sup>	SCS <sup>3</sup>	SCS contrast			Herd-date, <sup>4</sup> %	RMSE <sup>5</sup>
					Linear	Quadratic	Cubic		
Cheese yield (%CY)									
%CY <sub>CURD</sub>	53.2***	4.1**	1.2	3.0**	9.1**	6.4*	0.0	29.8	1.41
%CY <sub>SOLIDS</sub>	39.8***	1.2	1.6	1.1	0.2	3.5	0.1	19.7	0.76
%CY <sub>WATER</sub>	37.0***	5.6***	1.3	4.2***	16.5***	5.0*	0.6	41.5	0.90
Nutrient recovery (REC)									
REC <sub>PROTEIN</sub> , %	8.0***	11.1***	1.2	22.1***	121.5***	2.8	0.0	32.2	1.86
REC <sub>FAT</sub> , %	15.4***	0.2	1.3	1.9	0.5	6.4*	0.3	31.1	2.92
REC <sub>SOLIDS</sub> , %	33.8***	0.5	1.7	1.3	1.7	2.2	0.8	20.1	2.92
REC <sub>ENERGY</sub> , %	9.5***	0.2	1.7	1.5	0.9	5.5*	0.8	20.2	2.89

<sup>1</sup>%CY<sub>CURD</sub> = fresh cheese yield; %CY<sub>SOLIDS</sub> = total solids cheese yield; %CY<sub>WATER</sub> = water entrapped in the curd; REC<sub>PROTEIN</sub>, % = protein retention; REC<sub>FAT</sub>, % = fat retention; REC<sub>SOLIDS</sub>, % = total solids retention; REC<sub>ENERGY</sub>, % = energy retention.

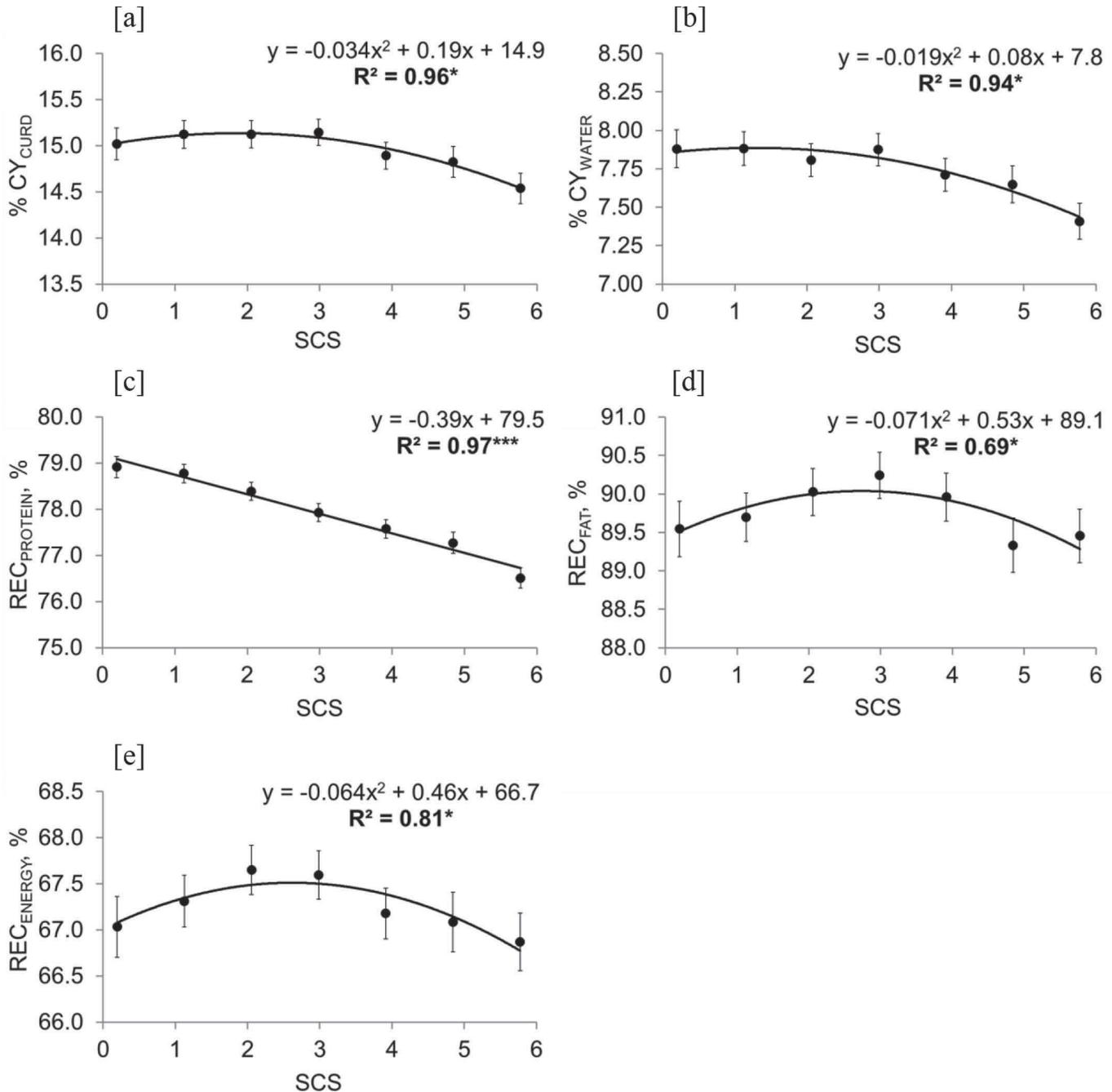
<sup>2</sup>WB = water bath.

<sup>3</sup>SCS =  $\log_2$  (SCC/100,000) + 3.

<sup>4</sup>Herd-date effect expressed as proportion of variance explained by herd/test date calculated by dividing the corresponding variance component by the total variance.

<sup>5</sup>RMSE = root mean square error.

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



**Figure 3.** Least squares means of cheese yield traits (%CY) and milk nutrient recovery in curd (REC) across SCS. Results of the polynomial contrasts have been reported: the response curve of the data across classes of SCS (linear or quadratic), the coefficient of determination ( $R^2$ ) of the regression and the  $P$ -value of the polynomial contrasts. \* $P < 0.05$ ; \*\*\* $P < 0.001$ . Error bars correspond to SE of LSM.

reduced syneresis; moreover, a nonlinear quadratic relationship was observed between MCP and SCS. Associations between elevated SCC and reduced MCP (e.g., longer rennet clotting time and lower curd firmness) have been described in the literature (Munro et al., 1984; Rogers and Mitchell, 1994; Klei et al., 1998). Ng-Kwai-Hang et al. (1989) reported that gelation was

delayed (+5 min) as milk SCC increased from 100,000 to 600,000 cells/mL. Pellegrini et al. (1994) found that the coagulation time of ewe milk increased ~5 min between class 1 (SCC <100,000 cells/mL) and class 4 (SCC >500,000 cells/mL). In a recent paper, Vázquez et al. (2014) reported a positive (unfavorable) linear relationship between SCC and RCT, with the latter

increasing by 0.9 min (54 s) for each increase in SCC of 100,000 cells/mL. It should be noted that the effect of clinical or subclinical mastitis is not fully represented in the traditional MCP because these tests are often limited to 30 min. Certain relevant factors (e.g., an increased SCC) can delay gelation time, thereby increasing the incidence of samples that fail to coagulate within the test duration [called noncoagulating (NC) milk, as reviewed by Bittante et al. (2012)].

The newer strategy of prolonging observation time and modeling all available information allows the estimation of more informative parameters. In the present study, the test was prolonged to 90 min, which allowed almost all of the samples to coagulate, as previously described (Cipolat-Gotet et al., 2012). Moreover, the modeling of all 360 data points available for each milk sample increased the repeatability of the trait measures. The traditional (RCT; Figure 2a) and modeled (RCT<sub>eq</sub>; Figure 2c) gelation times both showed clear nonlinear patterns, with the best results yielded by milk samples of the SCS class centered on the value of 2 (corresponding to a SCC of 50,000 cells/mL). The high-SCS-related delay in gelation time was consistent with previous reports (Barbano et al., 1991; Auldust and Hubble, 1998). However, we also observed that gelation was delayed in milk samples with very low SCS (<2).

It is difficult to directly compare the present work with prior studies that used linear covariates, assessed only 2 or 3 classes of SCC, or (when more classes were examined) tested distributions from milk with higher SCC than examined in the present study. For example, Politis and Ng-Kwai-Hang (1988) reported that RCT values remained relatively constant for classes with 100,000 to 500,000 somatic cells/mL, and thereafter increased as the SCC increased to over 1,000,000 cells/mL. However, the authors did not report any SCC class below 100,000 cells/mL. Regarding the variation of traditional RCT with increasing SCS, our results are consistent with those of Toffanin et al. (2012), although they used bulk milk samples and did not test the data for nonlinearity.

The published reports regarding the effect of SCC on the other traditional MCP are more variable. This is also due to the biases induced on the  $a_{30}$  trait by NC samples, which has often been excluded from data analyses or assumed to have a 0 value, or on the  $k_{20}$  trait, which presents a much higher (compared with noncoagulating samples) frequency of samples not reaching 20 mm of curd firmness within the usual 30-min time limit (Bittante et al., 2012). Because of this problem, many studies do not consider  $k_{20}$ . Here, prolonging the test duration allowed us to record  $a_{30}$  and  $k_{20}$  values for almost all of the analyzed samples. Our results showed that SCS did not have a significant effect

on  $k_{20}$ , whereas its effect on  $a_{30}$  was characterized by a pattern (Figure 2b) opposite than that observed for RCT (Figure 2a). This confirms the strong correlation of these 2 traits and emphasizes that little information is gained from the latter when the former is known.

Considering the new model-based curd firming traits, SCS did not affect potential curd firmness (CF<sub>P</sub>) or the syneresis rate constant of curd ( $k_{SR}$ ; the velocity at which whey is expelled from the curd). It did, however, exert an almost linear negative effect on the curd firming instant rate constant ( $k_{CF}$ , Figure 2d), which was reflected by the similar negative effect on maximum curd firmness (CF<sub>max</sub>, Figure 2e) and the curvilinear effect on the time at which CF<sub>max</sub> was achieved ( $t_{max}$ , Figure 2f).

### **SCS Affects Variation of Cheese Yield and Milk Nutrient Recovery in Curd or Loss in Whey**

Milk coagulation properties are important for 2 main reasons: (1) they are technologically valuable for optimizing the cheese-making process and predicting possible abnormalities during the process and in the final product; and (2) they may be used to indirectly predict cheese yield, especially with respect to fines losses in whey and the moisture retained in the curd. This second aspect is important because the MCP are relatively easy to measure in multiple samples at the laboratory level, whereas direct measurements of the %CY and REC traits are expensive and time-consuming.

The experimental results about the possibility of indirect prediction of cheese yield are controversial. Some authors failed to find significant differences in cheese yield between milk with good versus poor clotting abilities (Ikonen et al., 1999; Bonfatti et al., 2014), whereas others found that better MCP were associated with higher cheese yield and increased recovery of milk protein and fat in the curd (Aleandri et al., 1989; Malacarne et al., 2006). Recent work performed by Cecchinato and Bittante (2016) on a large data set revealed weak relationships between traditional MCP and the %CY and REC traits but stronger correlations with the parameters depicting the latter portion of the CF<sub>t</sub> curve (especially CF<sub>P</sub> and  $k_{SR}$ ).

In studies involving a small number of experimental cheese-making sessions, some authors reported that the cheese yield decreased as the SCC increased (Ali et al., 1980; Munro et al., 1984; Barbano et al., 1991). In particular, high SCC was shown to reduce the yields of cottage (Vianna et al., 2008), Parmigiano Reggiano (Summer et al., 2015), and Cheddar (Grandison and Ford, 1986; Auldust et al., 1996; Marino et al., 2005) cheeses, whereas it seemed not to affect Prato cheese (Mazal et al., 2007; Vianna et al., 2008) or mozzarella

(Andreatta et al., 2007). However, most of these studies used bulk milk, enrolled a limited number of samples to compare low versus high SCC, and did not consider the patterns across different classes of SCS.

In the present study, we observed a longer coagulation time and weaker curd firmness at the time of cutting; this likely reflected the impaired acidification of mastitic milk, which leads to a greater loss of nutrients in the whey (Figure 3c-e). Moreover, the mean value of  $REC_{PROTEIN}$  (78.1%, Table 1) was, as expected, close to the mean value of the casein number (77.1%, Table 1), and the 2 traits showed similar, almost linear, patterns with increasing SCS. The total variation induced by SCS on  $REC_{PROTEIN}$  (Figure 3c) was 4-fold its effect on casein number (Figure 1c), indicating that the negative effect of somatic cells in milk is not limited to modification of the casein:total protein ratio but also involves a loss of casein in the whey. In fact, although the nitrogen content remains constant, less casein is incorporated in the reticulum of the curd during cheese-making (Ali et al., 1980), decreasing the curd tension at the time of the cutting (Yun et al., 1982), reducing cheese yield, and triggering a greater nutrient loss (Bynum and Olson, 1982). Positive relationships between SCC and protein losses into the whey were also reported by Politis and Ng-Kwai-Hang (1988) and Barbano et al. (1991).

Unlike the recovery of protein, the recoveries of milk fat ( $REC_{FAT}$ , Figure 3d) and (consequently) milk energy ( $REC_{ENERGY}$ , Figure 3e) showed evident nonlinear quadratic trends, with the highest recoveries yielded by the milk samples with intermediate SCS and slightly lower values for milk samples with both smaller and greater SCS. Summer et al. (2015) reported that high SCC has a negative effect on fat recovery in cheese due to increased lipolysis. In contrast, no change in the loss of fat into the whey was reported by Politis and Ng-Kwai-Hang (1988), Mazal et al. (2007), or Vianna et al. (2008).

Increasing SCS was associated with a decrease in the water retained in the curd ( $\%CY_{WATER}$ , Figure 3b), especially for  $SCS < 3$ , yielding a parallel decrease of  $\%CY_{CURD}$  (Figure 3a). Interestingly, both high and low SCC appeared to affect some of the technological traits of milk. For example, milk belonging to SCS classes 1 and 2 (corresponding to  $SCC < 38,000$  cells/mL) was characterized by slower coagulation (Figure 2a), lower curd firmness (Figure 2b), and lower recoveries of fat (Figure 3d) and energy (Figure 3e) in the curd compared with milk of the intermediate classes of SCS. When we represented the equations using the LSM of the new curd firming traits (Figure 4), we detected differences in curd firmness between SCS classes 1, 3, and

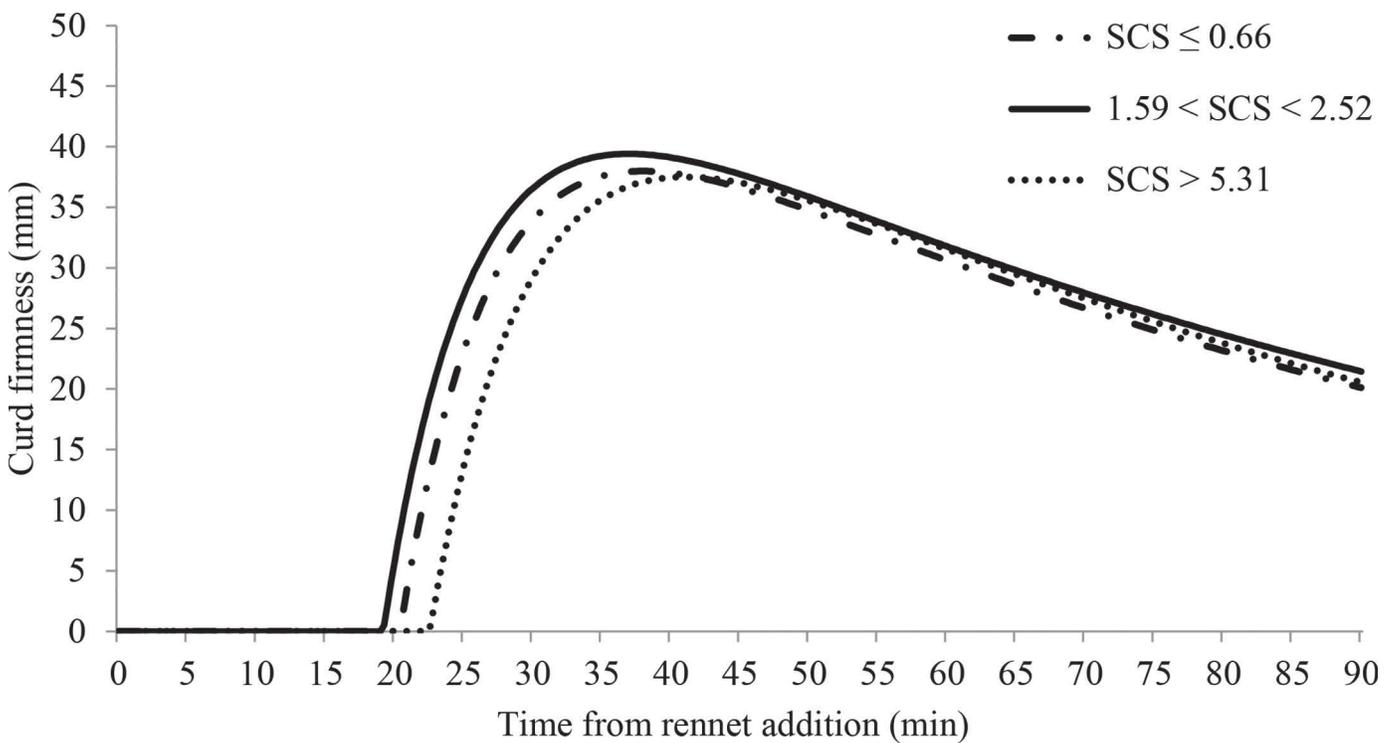


Figure 4. Curd firmness modeling for classes 1 ( $\leq 0.66$ ), 3 (1.59–2.52), and 7 ( $> 5.31$ ) of SCS.

7, with the best results obtained from milk belonging to the third class. Low SCC (<200,000 cells/mL) was previously reported as a possible risk factor for clinical mastitis (Suriyasathaporn et al., 2000) and might be associated with a reduced immune response to infections. The milk of healthy cows contains a resident leukocyte population that plays a key role in innate defense, and cows with very low milk SCC were reported to exhibit a less-efficient response to IMI (Wellnitz et al., 2010). Thus, we speculate that some of the cows belonging to SCS class 1 had deficits in their immune responses and were affected by an undetectable mastitic event that had slight effects on milk yield (Figure 1a), casein number (Figure 1b), and lactose content (Figure 1c). This could explain why these values were lower for class 1 than for classes 2 and 3. Moreover, the pH was slightly higher in class 1 milk compared with the intermediate classes, worsening the technological traits of this low-SCC milk.

## CONCLUSIONS

The results of the present study confirmed the negative effect of high SCC (indicator of mammary gland inflammation) on milk yield, milk composition, milk coagulation properties, and cheese-related traits. This study offers new insights into the relationships between the aforementioned variables, and explores the response curve of data obtained from individual milk samples across different classes of SCS. Our results show nonlinear trends for some milk composition and technological traits with respect to SCS. This is the first report to test the relationships between SCS and new technological traits [i.e., curd firming traits, syneresis traits, individual cheese yield (%CY) and curd nutrient recoveries (REC)]. As SCS increased, we observed a linear loss of milk production and several changes in milk composition. These variations decreased the quality and clotting ability of the milk, which showed slower coagulation and weaker curd firmness. This consequently decreased the cheese processing and cheese-making properties (as shown by reductions in the cheese yield and recoveries of nutrients in the curd). Further studies will be required to clarify the relationships between results obtained analyzing individual and bulk samples, and the nonlinear relationships between SCS and milk technological traits observed in this study should be considered in genetic analysis. New studies are needed to explore the negative effect of low SCC on milk technological traits, as milk of SCS classes 1 and 2 showed slower coagulation, lower curd firmness, and lower nutrient recoveries in the curd. Moreover, as mastitis may be caused by different pathogens that raise different immune responses, such variations should be consid-

ered in future investigations of how mastitis affects milk quality and technological traits.

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