Original Paper

Relationships Between and Variation of Cows' Somatic Cell Score and Milk Traits

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The objective was to analyse (1) relationships between somatic cell score (SCS) i.e. logarithmic transformation of somatic cell count (SCC) and daily milk yield and milk components (fat, protein and lactose contents), and (2) factors affecting variation of these traits in Hostein cows. Test day records (10,892) from 2015 to 2020 were investigated. Relationships were characterized using linear correlations. Effects of fixed factors: SCC class, parity, month in milk, sampling year and season, and random factors of cow and residual error were evaluated using mixed model. The factor SCC class was omitted in the analysis of SCS. There were found negative correlation coefficients between SCS and milk yield (-0.25) and lactose content (-0.36), and positive correlation coefficients between SCS and fat content (+0.12), and between SCS and protein content (+0.15). The considered fixed factors showed the significant influence on investigated traits. With increasing SCC, milk yield and lactose content decreased and fat and protein contents increased (this pattern agreed with pattern of correlation coefficients). Milk yield, fat and protein contents and SCS were lower in primiparas than in multiparas; lactose content showed the opposite pattern. The lowest milk yield and lactose content were found in autumn; the lowest fat and protein contents were found in summer. The highest SCS found in winter did not confirm a hypothesis that higher temperatures and humidity have impact on cows' susceptibility to infections and the number of pathogens to those cows are exposed. Further research is needed.

Keywords: Holstein, milk traits, SCS, correlations, mixed model

1 Introduction

Milk and its composition are key factors influencing profitability of dairy farms (Cinar et al., 2015). Somatic cell count (SCC) in milk, in addition to other milk components, is crucial for monitoring milk quality and health security.

Mastitis is an inflammatory response of mammary gland to infection; economic loss due to this disease is considerable (El-Tahawy and El-Far, 2010). Both clinical and subclinical (with no visible symptoms shown) mastitis are characterized by an increase of SCC. This trait is thus recognized as an important indicator of udder health (Bobbo et al., 2017). Due to increased SCC, significant economic losses result from reduced milk yield and milk quality (unfavourable changes in fat and casein contents) as indicated by El-Tahawy and El-Far (2010) and Hand et al. (2012). The detrimental effect of high SCC negatively affects cheese-making process mainly in terms of slower milk coagulation (Bobbo et al., 2017). Cows with increased SCC can be found in each herd. Somatic cells of a healthy cow's consist of 75% to 85% leucocytes and 15% to 25% epithelial cells. A level of 200 ths.$\cdot$ml^{-1} is indicative of determining threshold between udder health and disease (Barret, 2002; Cinar et al., 2015).

Bulk tank SCC is routinely used to define national and international regulatory standards that govern hygienic milk (Ruegg and Pantoja, 2013; Vieira et al., 2021; Hisira et al., 2023). The thresholds vary among countries and the limits are as follows: <400 ths.$\cdot$ml^{-1} (Australia, Canada and Switzerland, European Union including Slovakia), <500 ths.$\cdot$ml^{-1} (Brazil and India), <750 ths.$\cdot$ml^{-1} (USA).

Somatic cell count, similarly to milk and its basic components (fat, protein and lactose contents), are influenced by various genetic and non-genetic factors (Cinar et al., 2015; Kul et al., 2019). These are: parity

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(Atasever and Stádnik, 2015), stage of lactation (Cerón-Munoz et al., 2002), sample year and season (Bertocchi et al., 2014; Kul et al., 2019). Rajčevič et al. (2003) reported that also breed, farm, housing technology and feed are eligible to investigate. With milk yield and its components, their variation in dependence on SCC was confirmed (Bernabucci et al., 2002; El-Tahawi and El-Far, 2010). Some studies on these topics were done in Slovakia recently (Bujko et al., 2018, 2022; Tančin et al., 2020; Čobirka et al., 2022; Oravcová et al., 2022). The present study is thus intended to provide a complex view.

The objective was to analyse (1) relationships between somatic cell score and milk traits (milk yield, fat, protein and lactose contents) and (2) factors affecting their variation in Holstein cattle. Special attention was given to factor of sampling season (season in which measurements were done).

2 Material and methods

Data of dairy Holstein cows (10,892 test-day records of 736 heads) from a farm located in south-western Slovakia during the period from 2015 to 2020 were considered. Milk was recorded once per month and samples were analysed monthly as well. Milk components (fat, protein and lactose contents) were determined using MilkoScan FT120 (Foss, Hillerød, Denmark) and somatic cell counts (SCC) were determined using Fossomatic 90 (Foss Electric, Hillerød, Denmark) after heating of milk to 40 °C and incubated in a water bath for 15 min. Six classes of SCC (<100 ths., 100 ths. <200 ths., 200 ths. <400 ths., 400 ths. <600 ths., 600 ths. <1 mil., ≥1 mil.) were considered. According to sampling season, measurements were assigned to spring (March to May), summer (June to September), autumn (July to September) and winter (December to February). A total, 437 first, 339 second, 209 third, 109 fourth and 66 fifth (including ≥5) parities were available. Number of records within individual months in milk fell between 807 and 1130. Somatic cells are not normally distributed; therefore, SCC were transformed to somatic cell score (SCS) using the Dabdoutb and Shook (1984) equation: $SCS = \log_2(SCC/100{,}000) + 3$.

Statistical analyses were done using statistical programme SAS Studio 3.8 (2022). Correlation analysis (CORR procedure) was applied to reveal relationships between SCS and milk traits: milk yield, and fat, protein and lactose content, respectively. The mixed model methodology (MIXED procedure) was applied to study the influence of factors affecting variation of studied traits. Two models were considered:

$$y_{ijklmn} = \mu + L_i + M_j + Y_k + S_l + u_m + e_{ijklmn} \qquad (1)$$

where: y_{ijklmn} – individual SCS; μ – overall mean; L_i – fixed factor of parity (1..., 5+), $\sum_i L = 0$; M_j – fixed factor of month in milk (1..., 10), $\sum_j M = 0$; Y_k – fixed factor of sampling year (2015..., 2020), $\sum_k Y = 0$; S_l – fixed factor of sampling season (spring, summer, autumn, winter), $\sum_l S = 0$; u_m – random factor of cow (1..., 736), $u_m \sim N(0, \sigma^2_m)$; e_{ijklmn} – random residual error, $e_{ijklmn} \sim N(0, \sigma^2_e)$

$$y_{ijklmno} = \mu + L_i + M_j + C_k + Y_l + S_m + u_n + e_{ijklmno} \qquad (2)$$

where: $y_{ijklmno}$ – individual observations of milk yield, fat, protein and lactose contents; μ – overall mean; L_i – fixed factor of parity (1..., 5+), $\sum_i L = 0$; M_j – fixed factor of month in milk (1..., 10), $\sum_j M = 0$; C_k – fixed factor of SCC class (<100 ths., 100 ths. <200 ths., 200 ths. <400 ths., 400 ths. <600 ths., 600 ths. <1 mil., ≥1 mil.), $\sum_k C = 0$; Y_l – fixed factor of sampling year (2015..., 2020), $\sum_l Y = 0$; S_m – fixed factor of sampling season (spring, summer, autumn, winter), $\sum_m S = 0$; u_n – random factor of cow (1..., 736), $u_n \sim N(0, \sigma^2_n$; $e_{ijklmno}$ – random residual error, $e_{ijklmno} \sim N(0, \sigma^2_e$

Fixed factors included in the models (1) and (2) were estimated using the Least Squares Means (LSM method). Statistical significances of fixed factors were tested by Fischer's *F*-test; individual differences between estimated levels of fixed factors were tested by Scheffé's multiple-range tests. Differences were considered statistically significant when $P < 0.01$ or $P < 0.05$. Random cow and residual error variances were estimated using the Restricted Maximum Likelihood (REML method). These estimate repeatability of studied traits that may be interpreted as the proportion of total variance attributable to within-individual variance.

3 Results and discussion

Linear correlation coefficients are given in Table 1. Correlation coefficients between SCS and milk yield and between SCS and lactose content were moderate and negative (-0.25 and -0.36, $P < 0.01$), whereas correlation coefficients between SCS and fat content and between SCS and protein content were lower and positive (+0.12 and +0.15, $P < 0.01$). Atasever and Stádník (2015) reported higher correlation coefficients between SCS and milk yield (-0.52) and between SCS and protein content (+0.168) in Holstein primiparous cows; Antanaitis et al. (2021) reported higher negative correlation coefficient between SCS and lactose content (-0.471) in Holstein cows identified as having subclinical mastitis. These authors suggested that milk lactose may be used as a biomarker of suspected udder inflammation. Silva da et al. (2018)

reported correlation coefficients between SCS and milk yield by 50% lower (-0.118) and between fat content and SCS by 75% lower (+0.036). In contrast, these authors reported higher correlation coefficient between SCS and protein content (+0.282). Cunha et al. (2008), who carried out a similar study also with Holstein cows reported the same correlation coefficient between SCS and protein content (+0.1505), whereas, correlation coefficients between SCS and milk yield and SCS and fat content were lower (-0.1837 and +0.0719, respectively). In contrast, Rajčevič et al. (2003) reported higher correlation coefficients between SCS and lactose content (-0.423) and between SCS and protein content (+0.240); correlation coefficients between SCS and milk yield and between SCS and fat content were similar to those found in presented study (-0.286 and +0.130, respectively).

Analyses of variances showed the significant influence of fixed factors included in the models (1) and (2) on analysed traits (*P* <0.01). This was in accordance with studies of Koc (2006), Ceyhan et al. (2011), Mariani et al. (2022) who also reported a significant influence of considered factors. Cunha et al. (2008), Mijic et al. (2012), Marinov et al. (2021) found the significant influence of parity, stage of lactation, sampling season, calving season and farm; Also, Harmon (1994) opined that these factors are of the great influence if the mammary gland is infected.

The influence of month in milk is not reported in detail; the same pattern as mentioned by Cerón-Munoz et al. (2002), Koc, (2006), Saravan et al. (2015) was found in this study: daily milk yield and lactose content decreased, whereas SCS increased (after a shorth decrease in the beginning of lactation). According to Koc (2006), this is a result of cow physiology which, among others, also reflects infection and damage of mammary gland. Cerón-Munoz et al. (2002) similarly assumed that an increase of SCS could be attributed to lesions caused by regular milking or the progress of bacterial infections throughout the lactation or due to dilution effect.

Table 2 shows the dependence of milk yield and its components on SCC class. The classes were formed from the lowest (<100 ths.·ml^{-1}) to the highest (≥1 mil.·ml^{-1}) SCC. The number of observations gradually decreased between classes. The exception was the class with the highest SCC which showed higher frequency than the previous two classes. Analyses of SCC indicated that about 73% of records were of SCC less than 200 ths.·ml^{-1}, 10% were records with SCC between 200 and 400 ths.·ml^{-1}, 4% were proportions of records in each of further two classes and 9% was proportion of records with SCC higher than 1 mil.·ml^{-1}. In accordance with findings of Koc (2006), an increase of records with the highest SCC indicates that mastitis was spread to some extent and a need of some measures to be taken (improvement of bedding, dry environment, more hygienic milking). The pattern of dependence of milk traits on SCC followed the pattern of correlations. With increasing SCC, the estimated means of daily milk yield decreased (from 35.3 ±0.22 to 30.5 ±0.33 kg); of lactose content also decreased (from 4.84 ±0.01 to 4.61 ±0.01%). In contrast, fat and protein contents increased (from 3.87 ±0.02 to 4.07 ±0.02% and from 3.13 ±0.01 to 3.29 ±0.02%). The differences between respective means were significant (*P* <0.01 or *P* <0.05) with few exceptions as summarized in Table 3. The same patterns were reported by Cinar et al. (2015) and Silva da et al. (2018). Kul et al. (2019) reported the opposite pattern: fat and protein contents decreased

Table 1 Pearson's correlation coefficients

	Milk (kg.day^{-1})	Fat (%)	Protein (%)	Lactose (%)
Somatic cell score	-0.25**	+0.12**	+0.15**	-0.36**

** *P* <0.01

Table 2 Least squares means and standard errors by SCC class

SCC class	N	Milk (kg.day^{-1})	Fat (%)	Protein (%)	Lactose (%)
		$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$
<100 ths.ml^{-1}	6,599	35.3 ±0.22^{A}	3.87 ±0.02^{A}	3.13 ±0.01^{A}	4.84 ±0.01^{A}
100 <200 ths.ml^{-1}	1,395	33.5 ±0.25^{BbD}	3.99 ±0.03^{BCDEF}	3.18 ±0.01^{BCD}	4.80 ±0.01^{BC}
200 <400 ths.ml^{-1}	1,023	32.7 ±0.27^{CcDE}	4.03 ±0.03^{CDEF}	3.23 ±0.02^{CDEF}	4.78 ±0.01^{CD}
400 <600 ths.ml^{-1}	487	32.6 ±0.33^{DE}	4.03 ±0.03^{DEF}	3.25 ±0.02^{DEF}	4.75 ±0.01^{DE}
600 ths. <1 mil.ml^{-1}	427	32.2 ±0.34^{E}	4.07 ±0.03^{EF}	3.29 ±0.02^{EF}	4.73 ±0.01^{E}
≥1 mil.ml^{-1}	943	30.5 ±0.33^{F}	4.07 ±0.02^{F}	3.29 ±0.02^{F}	4.61 ±0.01^{F}

N – number of observations; SCC – somatic cells count; ABCDEF – means with different superscripts are significantly different at *P* <0.01; abc – means with different superscripts are significantly different at *P* <0.05

with increasing SCC. In comparison to findings of this study, Silva da et al. (2018) reported higher differences in traits (decrease of milk yield from 40 kg to 20 kg and of lactose content from 4.65% to 4.2%). Kul et al. (2019) reported lower differences (decrease of milk yield from 33.92 kg to 31.56 kg and of lactose content from 4.93 to 4.84%). Cinar et al. (2015) did not find any influence of SCC class on fat content.

Table 3 shows the dependence of investigated traits on parity (1 to 5+). With increasing parity, SCS increased; milk yield increased up to the third parity and fell down in higher parities probably due to more frequent health issues in older cows. Young primiparous cows had significantly lower milk yield and SCS than multiparous cows as agreed with study of Alhussien and Dang (2018). According to Sebastino et al. (2020), parity and age are correlated variables; primiparous cows are still growing and developing their mammary system; consequently, they have lower milk yield and SCC. The increase of SCS in dependence on parity was confirmed by Mijič et al. (2012). Lactose content showed opposite pattern to SCS i.e. decreasing trend, fat and protein contents were of unclear trends. According to Antanaitis at al. (2021), the increase of SCC and the decrease of lactose content are directly related to presence of subclinical mastitis. Rajčevič et al. (2003) supposed that the biosynthesis of lactose in milk is diminished due to infection of mammary gland. Similarly, Costa et al. (2020) hypothesized that a decrease of lactose content is the result of accumulated effects of intramammary infections on epithelium of mammary gland across cow's productive life.

Table 4 shows the dependence of investigated traits on sampling year. Annual trends were mostly of fluctuating pattern (fat and lactose contents) or tended to increase (milk yield, protein content) or decrease (SCS), mainly in last two years (milk yield, SCS). Fluctuating annual changes of fat and protein contents were reported by Bertocchi et al. (2014). These authors assumed that variations of sampling year and season demonstrate associations of milk components and logarithmic SCC with climatic conditions.

Table 5 shows the dependence of investigated traits on sampling season. Daily milk yield was the highest in spring (34.4 ±0.24 kg) and lower in the remaining seasons. Fat and protein contents were the lowest in summer. The highest milk yields in spring agreed with findings of Marinov et al. (2021), Ferreira and De Vries (2015), Čobanovic et al. (2022) and Luo et al. (2023); disagreed with findings of Rajčevič et al. (2003) who reported the highest milk yields in summer. The highest SCS found in winter agreed with findings of Rajčevič et al. (2003) and Kul et al. (2019) and disagreed with findings

Table 3 Least squares means and standard errors by parity

Parity	N	Milk (kg.day^{-1})	Fat (%)	Protein (%)	Lactose (%)	SCS
		$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$
1	4,103	30.1 ±0.23^{A}	3.97 ±0.02^{ACDE}	3.18 ±0.01^{ADE}	4.81 ±0.01^{A}	2.43 ±0.06^{AB}
2	3,131	33.2 ±0.23^{BDE}	4.05 ±0.03^{BCDE}	3.28 ±0.01^{BCD}	4.77 ±0.01^{B}	2.51 ±0.06^{B}
3	1,910	34.7 ±0.26^{C}	4.01 ±0.02^{CDE}	3.25 ±0.01^{CDE}	4.75 ±0.01^{C}	3.36 ±0.07^{C}
4	970	33.4 ±0.32^{DE}	4.02 ±0.03^{DE}	3.24 ±0.02^{DE}	4.72 ±0.01^{Dd}	3.87 ±0.09^{D}
5+	778	32.4 ±0.42^{E}	4.00 ±0.04^{E}	3.19 ±0.02^{E}	4.70 ±0.01^{Ee}	4.68 ±0.12^{E}

N – number of observations; SCS – somatic cells score; ABCDEF – means with different superscripts are significantly different at *P* <0.01; de – means with different superscripts are significantly different at *P* <0.05

Table 4 Least squares means and standard errors by sampling year

Year	N	Milk (kg.day^{-1})	Fat (%)	Protein (%)	Lactose (%)	SCS
		$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$	$\mu \pm s_\mu$
2015	1,166	33.7 ±0.37^{AaEF}	4.00 ±0.04^{ABCDEF}	3.15 ±0.02^{A}	4.75 ±0.01^{ABDF}	3.65 ±0.10^{ADE}
2016	2,368	32.2 ±0.30^{BCDE}	4.02 ±0.03^{BDEF}	3.20 ±0.01^{B}	4.76 ±0.01^{BbD}	3.23 ±0.08^{BbCF}
2017	1,325	32.5 ±0.29^{CcDE}	3.92 ±0.03^{CcF}	3.16 ±0.02^{C}	4.79 ±0.01^{C}	3.26 ±0.08^{CcD}
2018	1,168	31.9 ±0.32^{DE}	4.04 ±0.03^{DdEF}	3.22 ±0.02^{D}	4.75 ±0.01^{DF}	3.53 ±0.08^{DdE}
2019	2,732	32.6 ±0.22^{E}	4.09 ±0.02^{E}	3.29 ±0.01^{E}	4.71 ±0.01^{EF}	3.59 ±0.06^{E}
2020	2,133	34.0 ±0.25^{F}	4.00 ±0.02^{F}	3.34 ±0.01^{F}	4.74 ±0.01^{Ff}	2.95 ±0.07^{Ff}

N – number of observations; SCS – somatic cells score; ABCDEF – means with different superscripts are significantly different at *P* <0.01; abcdf – means with different superscripts are significantly different at *P* <0.05

Table 5 Least squares means and standard errors by sampling season

Season	N	Milk (kg.day^{-1})	Fat (%)	Protein (%)	Lactose (%)	SCS
		$\mu \pm s_{\mu}$	$\mu \pm s_{\mu}$	$\mu \pm s_{\mu}$	$\mu \pm s_{\mu}$	$\mu \pm s_{\mu}$
Spring	2,813	34.4 ±0.24^{A}	3.92 ±0.02^{A}	3.21 ±0.01^{A}	4.79 ±0.01^{A}	3.32 ±0.07^{ABC}
Summer	2,642	32.9 ±0.24^{BD}	3.80 ±0.02^{B}	3.07 ±0.01^{B}	4.73 ±0.01^{BC}	3.37 ±0.07^{BbC}
Autumn	2,612	31.3 ±0.23^{C}	4.17 ±0.02^{CD}	3.34 ±0.01^{C}	4.73 ±0.01^{C}	3.36 ±0.07^{C}
Winter	2,825	32.5 ±0.25^{D}	4.15 ±0.02^{D}	3.28 ±0.01^{D}	4.75 ±0.01^{D}	3.53 ±0.07^{Dd}

N – number of observations; SCS – somatic cells score; ABCDEF – means with different superscripts are significantly different at $P < 0.01$; bd – means with different superscripts are significantly different at $P < 0.05$

of Bertocchi et al. (2014) who reported the highest SCS in summer. Rajčevič et al. (2003) assumed that milk yield is affected by photoperiod and increases with increasing day length, and, consequently, a dilution effect on fat and protein contents appears. The decrease of fat and protein contents in summer is related to the negative influence of hot temperatures above 30 °C on the synthesis of these components (Bertocchi et al., 2014) and/or reduced food intake (Rajčevič et al., 2003). Heinrichs et al. (2016) and Čobanovic et al. (2022) also reported that low milk components in summer are indicative of feed patterns. The highest SCS in winter disagreed with findings of Green et al. (2006), Hogan and Smith (2012), Sebastino et al. (2020) and Stocco et al. (2023) who found the highest SCS in summer. This is in line with the hypothesis that hot season is suitable condition for reproduction of pathogens. The decrease of fat and protein contents in spring and summer as temperatures increase (found also in this study) was reported by Bernabucci et al. (2002, 2015) and Renna et al. (2010); however, the mechanism responsible for this phenomenon is not well understood according to Bertocchi et al. (2014). In contrast to results found in this study, El-Tahawy and El-Far (2010), Kul et al. (2019) and Tančin et al. (2020) reported the lowest fat and protein contents in autumn and winter, in spring and in spring and winter, respectively. The latter authors found the highest milk yields in winter and spring and the highest logarithmic SCC in autumn; they reported higher differences between estimated means of milk yield (10 vs. 2 kg). Based on analyses of the influence of sampling season, it can be assumed that an increase of SCS is not simply related to reduced milk yield (as agreed with Green et al., 2006).

4 Conclusions

Investigations of relationships between SCC and milk traits confirmed a negative effect of SCS on milk yield and lactose content; this enables to conclude that high SCC causes losses due to reduced milk yield. Variations of studied traits with regard to sampling year showed desirable pattern: the increase of milk yield and the decrease of SCS indicating that mastitis on herd level was, to some extent, reasonably managed. In contrast, findings related to sampling season were less clear and further research is needed.

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