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Somatic cells count in milk and its relation with productive traits in dairy cows

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Abstract. *The aim of the study was to determine the dependence between Test Day (TD) milk productivity traits and somatic cells count (SCC) in milk and also the influence of the factors: farm, parity and TD recording season of Holstein cows. The survey included a total of 484 lactating cows from 8 cattle farms in Bulgaria. A total of 3473 TD records including data on TD milk yield, fat %, protein % and SCC in milk were used. A statistically significant effect of the farm, recording season, and SCC on TD milk yield, fat % and protein % was reported. The highest TD milk yield was reported in spring (22.42 kg), followed by winter (21.95 kg). In winter and autumn were reported higher mean values for TD fat (3.77 and 3.84%) and TD protein (3.38 and 3.40%) content in milk. At the highest SCC - above 999 000 cells/ml, the highest average daily milk yield - 24.1 kg and the lowest fat (3.48%) was reported. With the highest and positive statistically significant value was the phenotypic correlation between TD milk yield and SCC (0.21). The correlation with TD fat % was statistically significant, with negative value (- 0.07).*

Keywords: dairy cattle, fat, milk yield, phenotypic correlation, protein, season, Test Day

Introduction

Somatic cells are mostly the immune system cells (80% in not infected quarters, 99% in mastitis quarters). They are part of the natural defense mechanism and include lymphocytes, macrophages, polymorphonuclear cells and some epithelial cells (Pavel and Gavan, 2011). Therefore, somatic cells are a reflection of the inflammatory response to intramammary infection. When udder is healthy, the SCC in milk is between 50 000 and 100 000 cells/ml (Skrzypek et al., 2004). If the SCC is greater than 200 000 cells/ml, it is considered to be a threshold for distinguishing healthy udder from ill (Harmon, 2001; Skrzypek et al., 2004). Milk with increased SCC is associated with changes in milk protein, milk fat, lactose and minerals, which degrades the quality of milk and milk products (Rajčevič et al., 2003; Fernandes et al., 2004; Lindmark et al., 2006). Subclinical mastitis is also associated with a decrease in milk production, changes in milk consistency, a decrease in the possibility of adequate processing, and a high risk of the hygiene of milk, which may even contain pathogens (Stojanovski et al., 2021). Milk with low content of SCC means better milk products with a longer shelf life (Alhussien and Dang, 2018).

A number of authors have studied the dependency between SCC, milk yield and milk composition in different dairy cow populations. The results presented are contradictory. A number of studies have found a moderate but negative genetic correlation between milk yield and SCC (Carlen et al., 2004; Koivula et al., 2005; Juozaitiene et al., 2006; Samoré et al., 2008, 2010; Jia-Zhong et al., 2010). But others have reported moderate but positive correlations between SCC and milk yield, such as Carlen et al. (2004) and Dadpasand et al. (2013). The same applies to the fat and protein content of milk.

According to Sharma et al. (2011) the simplest and most common method of processing and presenting SCC is as arithmetic mean. Another method widely used especially in Europe is the so-called geometric mean. When using it, the mean value is usually lower than the arithmetic mean value for the same data. This is due to the difference in processing procedure between the two methods. In the arithmetic mean, the presence of one high value has a stronger influence on the mean than in the geometric mean. However, the differences are not so substantial as to influence a particular solution (Ingalls, 2001).

The specific conditions of the region or country affect the milk yield level, milk composition, as well as SCC in milk, such as climatic characteristics, level of selection, breeding goals, feeding systems, etc. In this respect, it is very important to study the interconnections between these traits under specific conditions, which may be relevant for both breeding models and herd management strategies.

The goal of the study was to determine the dependence of Test Day (TD) milk productivity traits (milk yield, fat and protein percentage) and somatic cells count (SCC) in milk of Holstein cows in 8 dairy cattle farms in Bulgaria.

Material and methods

The study included 484 Holstein cows from 8 cattle farms in Plovdiv region. All farms were under productivity control by the same breeding association. In 5 of the farms the cows were

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housed under the conditions of tie-stall housing system, in three of them the milking was with a bucket milking machine, and in two - with a milk pipeline. The other three farms were with loose housing system and milking in milking parlor.

The average milk yield of a cow for 305 days in milk (DIM) ranged from 4883 to 8643 kg.

The study covered the 2015 Test Day (TD) records and included data for lactating cows with no less than 7 TD records in each farm. Cows were on different parities, as for the purpose of the study they are presented in classes, respectively: I-st – 102, II-nd – 137, III-rd - 98 and IV-th and more parities - 148 cows. In the study the monthly performance TD records data were used - a total of 3473 records including data on TD milk yield, fat (%), protein (%) and SCC in milk. To study the relationship between TD productive traits and SCC, they are presented in classes as follows: up to 200 000 cells/ml, 201 000 to 500 000 cells/ml, from 501 000 to 999 000 cells/ml (by Jia-Zhong et al., 2010 and Cinar et al., 2015).

The statistical processing of data for TD SCC is to arithmetic mean.

TD records for productive traits were grouped according to the season of recording, respectively: TD records from December to February (including) - winter season, March to May - spring, June to August - summer and September to November - autumn.

A linear model was used to study the variation and relationship between factors and traits, the solutions were obtained by using the Least Squares Generalized Method (LSM), and the variance components were estimated using the ANOVA algorithm.

To determine the degree of influence of SCC on milk productivity traits the following model was used:

$$Y_{ijkln} = \mu + H_i + S_j + L_k + SCS_l + e_{ijkln},$$

Where: $\mathbf{Y_{ijkln}}$ is the depended variable (TD milk yield, fat, % and protein, % in milk); $\boldsymbol{\mu}$ is the population mean; $\mathbf{H_i}$ is the fixed effect of the farm; $\mathbf{S_j}$ is the fixed effect of the TD recording season; $\mathbf{L_k}$ is the fixed effect of the parity, $\mathbf{SCS_l}$ is the fixed effect of the SCC and $\mathbf{e_{ijkln}}$ is the effect of the non-included random factors.

By analysis of variances (ANOVA) for each model by classes of fixed factors the least squares of means (LSM) were obtained. To study the effect of the controlled factors and statistical indicators and correlations the relevant modules of STATISTICA 6 were used.

Results and discussion

Table 1 shows the average values of the three productive traits – TD milk yield, fat and protein content (%) and SCC in milk by farms. The average TD milk yield varied considerably in different herds. With the lowest milk yield were cows from farm 1 - 16.1 kg and with the highest from farm 8 - 25.1 kg. The difference was 9 kg and statistically significant (p<0.001). Between the rest of the farms the differences were smaller - 1 to 3 kg, but also are statistically significant (p<0.001). The TD fat (%) indicated differences between herds, smaller and insignificant between 1, 2, 3 and 4 farms, and farm 8 (p>0.05), and bigger and significant and between 5, 6 and 7 farms, and farm 8 (p<0.001).

The lowest fat content was reported for cows on farm 7 - 3.44% and the highest for cows on farm 2 - 4.01%, with a difference of 0.5%. Much less was the variation of the TD protein percentage by farms. It was the highest in cows from farm 5 - 3.5% and the lowest in farm 3 - 3.19%, the difference was only 0.3%.

Overall, at the farms surveyed a good milk yield was reported; in most of them, the average percentage of fat and protein corresponded to the average values for the cows of the breed reared in Bulgaria. The average milk yield of controlled cows of the Black-and-white breed in Bulgaria is 5300-5600 kg, with 3.6-3.8% fat and 3.2-3.3% protein content in milk (Yordanov et al., 2017).

For the trait TD SCC variation between the farms was considerable. Especially clear was the difference in SCC between farms 6, 7 and 8 and the remaining five farms. The differences in values of that parameter between the observed farms were statistically proven (p<0.05-0.001), except for those between farms 1 and 2, 1 and 3, 2 and 3, 4 and 5, 3 and 7 and 6 and 7 (p>0.05) (Table 1). In the first five farms (1-5), there was some variation, but all average values were up to 200 000 cells/ml, which is normal for healthy animals free of mastitis. In the last three farms, the average values were considerable and quite over 400 000 cells/ml, which is the upper threshold value allowed when buying milk. These farms were with loose housing production systems and cows were milked in milking parlor, while at the others the milking was with a bucket milking machine and with a milk pipeline.

Santman-Berends et al. (2012) found that in herds where milking is performed with automated milking systems, the incidence of subclinical mastitis is on average 6.9% higher than the other milking systems. The authors indicate that subclinical mastitis is widespread in all dairy herds, with large variations in density. Bytyqi et al. (2010) also found large differences in SCC in raw milk from the different Kosovo farms included in their study - from 93 310 to 908 860 cells/ml on average per herd. These differences between farms, they believe, are due to managerial differences in many aspects, which in most cases are not only related to expensive infrastructure (milking parlors and equipment, etc.), but come directly from negligence and the implementation of inappropriate housing and milking practices.

Kos (2008) also points a considerable variation in the average SCC for Holstein cows in Turkey, from 296 483 cells/ml to 688 811 cells/ml, respectively, with average TD milk yield similar to our results. The author points out that SCC in milk is higher in cows in Turkey - 389 303 cells/ml than in some EU countries, such as Poland - 269 000 cells/ml (Skrzypek et al., 2004), Switzerland - 180 000 cells/ml (Busato et al., 2000), Sweden - 233 000 cells/ml (Toledo et al., 2002). In Brazil, Ribas (2013) established a mean SCC of 486 810 and 553 000 cells/ml, respectively, in 257 540 and 1 995 034 milk samples taken from dairy tanks in the periods 1999-2001 and 2005-2012.

Table 1. Average values for TD productive traits and SCC by farms

Farm	Number of TD records	TD Milk yield, kg x ± SE	TD fat, % x ± SE	TD protein, % x ± SE	TD SCC, cells(x10^3)/ml x ± SE
1	235	16.1±0.292	3.96±0.055	3.22±0.010	104.6±12.38
2	291	19.6±0.340	4.01±0.054	3.37±0.018	135.3±10.24
3	155	18.2±0.328	3.99±0.078	3.19±0.014	131.6±11.33
4	534	20.5±0.254	3.88±0.039	3.42±0.014	171.1±8.09
5	579	22.0±0.212	3.78±0.026	3.50±0.014	194.4±12.60
6	474	18.9±0.232	3.59±0.035	3.41±0.007	717.5±56.09
7	471	19.0±0.229	3.44±0.034	3.38±0.008	653.2±44.94
8	734	25.1±0.291	3.95±0.032	3.47±0.005	982.3±72.59
Average	3473	20.8±0.109	3.80±0.014	3.41±0.004	477.1±19.30

Farm	1	2	3	4	5	6	7	8
Statistical differences between farms for TD Milk yield, kg								
1	-	1-2***	1-3***	1-4***	1-5***	1-6***	1-7***	1-8***
2			2-3**	2-4*	2-5***	2-6^{ns}	2-7^{ns}	2-8***
3				3-4***	3-5***	3-6^{ns}	3-7^{ns}	3-8***
4					4-5***	4-6***	4-7***	4-8***
5						5-6***	5-7***	5-8***
6							6-7^{ns}	6-8***
7								7-8***
Statistical differences between farms for TD fat, %								
1	-	1-2^{ns}	1-3^{ns}	1-4^{ns}	1-5***	1-6***	1-7***	1-8^{ns}
2			2-3^{ns}	2-4^{ns}	2-5***	2-6***	2-7***	2-8^{ns}
3				3-4^{ns}	3-5**	3-6***	3-7***	3-8^{ns}
4					4-5*	4-6***	4-7***	4-8^{ns}
5						5-6***	5-7***	5-8***
6							6-7**	6-8***
7								7-8***
Statistical differences between farms for TD protein, %								
1	-	1-2***	1-3*	1-4***	1-5***	1-6***	1-7***	1-8***
2			2-3***	2-4*	2-5***	2-6*	2-7 ^{ns}	2-8***
3				3-4***	3-5***	3-6***	3-7***	3-8***
4					4-5***	4-6^{ns}	4-7**	4-8***
5						5-6***	5-7***	5-8*
6							6-7**	6-8***
7								7-8***
Statistical differences between farms for TD SCC, cells(x10^3)/ml								
1	-	1-2^{ns}	1-3^{ns}	1-4***	1-5***	1-6***	1-7*	1-8***
2			2-3^{ns}	2-4**	2-5**	2-6***	2-7*	2-8***
3				3-4*	3-5*	3-6***	3-7^{ns}	3-8**
4					4-5^{ns}	4-6***	4-7**	4-8***
5						5-6***	5-7***	5-8***
6							6-7^{ns}	6-8**
7								7-8**

Significant at: *- $p<0.05$; **- $p<0.01$; ***- $p<0.001$; ^{ns}- Non Significant difference

From the presented average values for TD productive traits and SCC, a high dependence between high productivity and SCC was not observed. Cows from farm 8 are with the highest TD milk yield - 25.1 kg and slightly above the average for the breed in Bulgaria values of fat and protein (%) in milk, but they have the highest values for SCC - 982 300 cells/ml. With comparatively high TD milk yields are also cows from farms 4 and 5, 20.5 and 22.0 kg, respectively, with good fat and protein content in milk, but they have low SCC, within the norm for a healthy udder, 171 100 and 194 400 cells/ml, respectively.

Table 2 presents the results of the analysis of variance for effect of controlled factors on productive traits - TD milk yield, fat and protein % in milk. The analysis shows a statistically significant effect of farm, TD recording season, and SCC (presented in classes) on the three productive traits, at p<0.001. The parity had a significant effect only on the trait TD milk yield, at p<0.05. In traits TD fat and protein (%), no significant effect of parity was reported (p>0.05). A slightly lower milk yield in cows on first parity compared to older cows was reported. The lack of significant differences by parity is more likely due to the fact that the cows on different parities in the analysis are grouped from different farms, and they have different levels of productivity.

Table 2. Analysis of variance for effect of controlled factors and SCC on TD productive traits

Sources of variation	Degrees of freedom (n-1)	TD Milk yield, kg		TD fat, %		TD protein, %	
		MS	F P	MS	F P	MS	F P
Total for the model	16	2033.0	63.21***	10.32	15.82***	2.53	49.12***
Farm	7	2943.8	91.20***	14.02	21.49***	3.10	57.5***
Parity	3	90.7	2.81*	0.10	0.15^{ns}	0.02	0.32^{ns}
TD recording season	3	824.3	25.54***	5.44	8.33 ***	1.32	24.4 ***
SCC, in classes	3	1944.1	60.23***	6.56	10.05***	0.95	17.6 ***
Error	3451	32.2		0.65		0.05	

Significant at: *- p<0.05; **- p<0.01; ***- p<0.001; ^{ns}- Non Significant difference

Season, lactation stage, parity and feed are considered to be among the main factors affecting the milk composition by a number of authors (Noro et al., 2006; Heck et al., 2009; Lambertz et al., 2014). The reported significant effect of the farm on the three productive traits is in accordance with the reported differences by farms in Table 1. The animals on different farms, in addition to having a difference in heredity, are placed under different feeding and management conditions.

The significant effect of the TD recording season is also confirmed by the LS-mean values by seasons, Table 3. The highest TD milk yield was reported in spring (22.42 kg), followed by winter with a slight difference (21.95 kg). During the summer and autumn a lower with 1.5-2 kg milk yield was reported. This effect is due, on the one hand, to higher temperatures in the summer and even in early autumn in this region of southern Bulgaria. On the other hand, it is due to the difference in feed used during these seasons. In almost half of the farms surveyed, those with less capacity, cows were on pasture in the summer. In large farms cows were housed year-round indoors.

Table 3. LS-mean values for the studied TD productive traits by TD recording season

TD traits	Season			
	Winter x ± SE	Spring x ± SE	Summer x ± SE	Autumn x ± SE
Number of TD records	740	936	924	873
Milk yield, kg	21.95 ± 0.21	22.42 ± 0.27	20.40 ± 0.24	20.38 ± 0.22
Fat, %	3.77 ± 0.031	3.65 ± 0.039	3.69 ± 0.034	3.84 ± 0.031
Protein, %	3.38 ± 0.009	3.30 ± 0.011	3.37 ± 0.009	3.40 ± 0.008

In winter and autumn, higher mean values were also reported for fat (3.77 and 3.84%) and protein (3.38 and 3.40%) content in TD milk yield. During the spring, the lowest mean values for milk composition, 3.65% fat and 3.30% protein, were reported.

Rajčevič et al. (2003) indicate that, of the environmental factors, the season of the year and the cow's nutrition had a considerable influence on the main milk components. The season of the year is often associated with different feeds. The authors found that the fat and protein content of milk was the highest in winter and the lowest in summer. The protein drop in milk during the summer is influenced by high temperatures (above 30°C), at which food intake is reduced.

Figure 1 shows the variation in TD milk yield depending on SCC. There was a tendency for a higher daily milk yield at higher values of SCC.

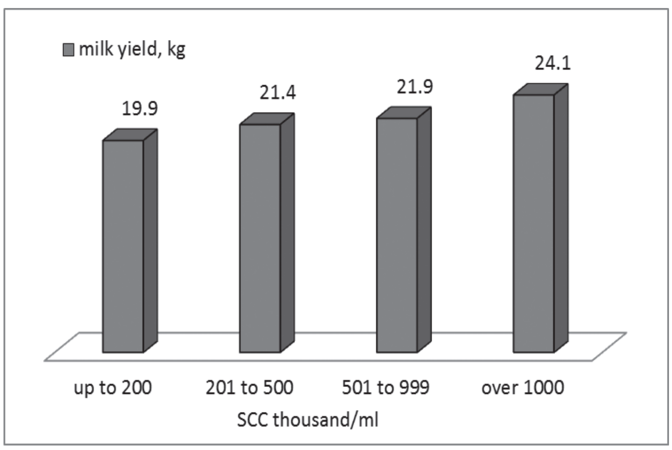

Figure 1. LS-mean values for TD milk yield (kg) depending on SCC

At SCC up to 200 000 cells/ml (an indicator of a healthy udder), the mean TD milk yield value was 19.9 kg. At the highest mean daily milk yield - 24.1 kg, the highest SCC - above 999 000 cells/ml is reported. This effect may be due to the fact that, for example, the farm with the highest milk yield was also with the highest SCC, and also to the combining of TD records from the beginning of lactation, when milk yield is at its highest, but it is also a risky period for mastitis infections.

The variation in TD fat percentage depending on SCC presented on Figure 2 indicates that when reporting the high SCC (over 1 000 000 cells/ml), which is associated with a particular infection of the mammary gland, the lowest fat percentage (average 3.48%) was also reported.

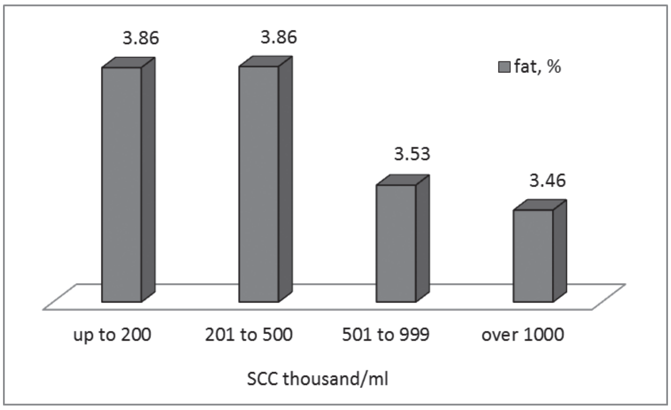

Figure 2. LS-mean values for TD fat (%) depending on SCC in milk

Azzara and Dimmick (1985) indicated that higher SCC leads to a decrease in fat synthesis due to damage of the secretory epithelium and lipolytic activity or proteolytic activity of leukocyte enzymes. Garcia et al. (2015) found that the milk fat content decreases with increasing the SCC, with the correlation value of -0.29 at p<0.01. Fenerova et al. (2008) found in the cows of the Brown breed in our country that the traits milk yield, milk fat yield and milk protein yield decrease when the somatic cell level increases above 1 000 000 cells/ml, as the most considerable decrease is for milk fat yield - 9.34%, for milk yield - 5.40% and for milk protein yield - 4.54%.

Figure 3 presents the LS-means for the TD protein % in milk, depending on SCC. At this productive trait a clear dependence on SCC was not reported as in milk yield and fat %, and the differences between the different SCC classes were very low (below 0.1%). A trend for a lower TD protein % in milk in cows with SCC up to 200 cells/ml - 3.34% was reported, which suggests healthy animals. The highest was protein (%) in milk with a reported SCC of 201 to 500 cells/ml - 3.42%. These SCC values were indicative of infection, subclinical mastitis. The increase in SCC above 500 cells/ml showed a slight decrease in the TD protein (%) in milk, the lowest being at the highest SCC - above 1000 cells/ml - 3.33%.

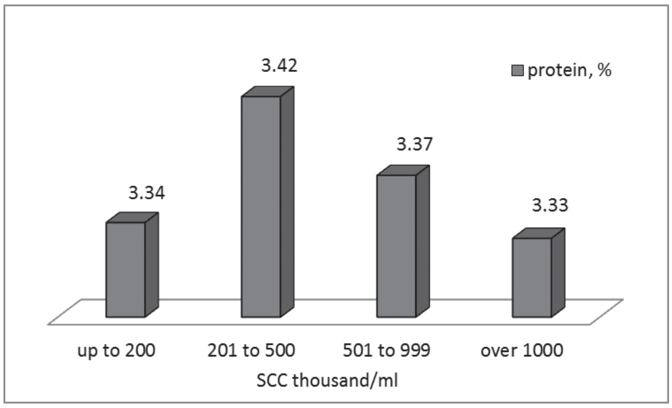

Figure 3. LS-means for TD milk protein (%) depending on SCC

Other authors have found increased milk protein of cows with increased SCC. The milk protein of healthy cows consists of approximately 80% casein and 20% whey protein. The studies of Le Maréchal et al. (2011) showed that there is a change in the protein profile of cows with mastitis, and they have an increase in whey protein levels. However, they may show a decrease in α- and β-caseins. Damage to the epithelium of the mammary gland causes the inflow of blood proteins in milk. Consequently, protein content may increase or remain unchanged, but with a considerable change in composition (Urech et al., 1999; Batavani et al., 2007).

Garcia et al. (2015) found a reverse effect of SCC on milk protein content compared to fat, i.e. an increase in protein (%) with an increase in SCC, with a correlation value of 0.46 at p<0.01. Due to the increased vascular permeability caused by inflammation, whey protein content may be higher in milk with high SCC, this phenomenon overcomes the deficiency of casein synthesis (Alonso-Fauste et al., 2012). Some serum proteins, such as cellular proteins and lactotransferrin, are only detected in milk samples from animals with mastitis.

The phenotypic correlations between productive traits and SCC were with low values, Table 4. With the highest and positive value was the phenotypic correlation between TD milk yield and SCC r_p=0.21. This shows a trend towards higher SCC in cows with higher daily milk yield. This higher daily milk yield may be higher in general or to be associated with beginning of lactation when and cases of mastitis are prevalent and consequently SCC is higher.

Table 4. Phenotypic correlations between TD productive traits and SCC

Indicators	Milk yield, kg	Fat, %	Protein, %
SCC	0.21*	-0.07*	0.02
Fat, %	-0.17*	-	-
Protein, %	0.08*	0.08*	-

*Correlations are significant at p<0.05

Dadpasand et al. (2013) believe that positive genetic correlations between SCC and milk yield that they found, indicate that selection for higher milk yields increases SCC in milk to some extent, which ultimately increases the sensitivity of cows to clinical mastitis. Positive genetic correlation between milk yield and SCC was also found in Finnish Ayrshire (Poso and Mantysaari, 1996), Holstein in France (Rupp and Boichard, 1999) and Swedish Holstein cows (Carlen et al., 2004). Other studies have found a moderate but negative genetic correlation between milk yield and SCC (Carlen et al., 2004; Koivula et al., 2005; Samoré et al., 2008, 2010; Bondan et al., 2018).

The percentage of fat and protein in milk had very low phenotypic correlations with SCC, about 0. For the fat (%), the correlation, although low, was statistically significant and negative - r_p = -0.07. This value was also related to the reported effect of SCC on milk fat, % - a lower fat, % was reported in milk with high SCC. The phenotypic correlation between SCC and protein (%) in milk had the lowest value (r_p=0.02) and no statistical significance.

Dadpasand et al. (2013) indicated that the results of their study show that cows with high genetic potential for milk, fat and protein tend to have higher SCC. They also report a negative, low-value genetic correlation between fat (%) and SCC in cow's milk. The authors conclude that the interacting effects of different infections with the SCC may change the sign or intensity of the correlation coefficients between milk and protein and fat content with SCC.

The reported dependencies between milk productivity traits and SCC may also be due to the relationship between milk productivity levels and milk composition at different lactation stages and the associated with them differences in susceptibility to mastitis. Samoré et al. (2008) found in the Italian Holstein that the genetic correlation of milk protein with SCC was high and positive at the beginning of the first stages of the first lactation, but approaching zero or negative values in the later stages of lactation. Positive genetic correlations between protein content and SCC were reported in other populations also by Carlen et al. (2004), Miglior et al. (2007) and Muir et al. (2007).

Samoré et al. (2008) reported genetic correlations close to zero (0.02 to 0.08) between SCC and the percentage of protein in milk in Italian Holstein cows. Similar values (0.07) were also obtained for Italian Brown cattle from Samoré et al. (2010) and Bondan et al. (2018) between SCC and fat, % (0.076) and protein, % (0.174).

Silva et al. (2018) obtained correlations between SCC and milk yield with negative and low value (-0.118) and positive with fat, % (0.036) and protein, % (0.282) for Holstein cows in Brazil. The authors indicated that, despite the observed increase in fat, protein and dry mater in milk at higher SCC, this fact is not favorable for milk quality, as this may be due to a decrease in milk production when SCC increased. Cinar et al. (2015) conclude that high SCC affects not only the quantity but also the composition and quality of milk. Therefore, they recommend the monthly control of SCC on farms as one of the most effective method of monitoring and taking timely and adequate measures in relation to animal health and the production of good quality milk with better composition. According to Alhussien and Dang (2018) for consumers, the lower value of SCC in milk means extended shelf life and improved taste, and for farmers, low SCC in milk means less treatment, lower costs and higher milk yield per cow. Furthermore, the use of SCC in milk as a management tool, on a routine basis, will help to increase immunity and improve the quality and quantity of milk as a comfort and well-being of cows.

Conclusion

Somatic cells count (SCC) had a statistically significant effect ($p<0.001$) on the productive traits (milk yield, fat and protein content in the milk) of Holstein cows. In cows with higher SCC (over 500 cells/ml), a higher average milk yield and a lower percentage of Test Day (TD) fat and protein were reported. With a highest and positive value was the phenotypic correlation between SCC and TD milk yield (0.20). The correlation with fat, % was statistically significant, but with low and negative value (-0.07). Between SCC and the percentage of protein in milk, the phenotypic correlation was with low positive value (0.02), but not statistically significant.

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