# Influence of some environmental and paratipical factors on the somatic cells count in milk in black-and-white cows

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## Abstract

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The aim of the present research was to study the influence of some environmental and paratypical factors on the somatic cells count (SCC) in milk in black-and-white dairy cows, Holstein-Friesian type. The survey included a total of 484 lactating cows from 8 cattle farms in Plovdiv region, Bulgaria, by using data from the monthly controls of their productive traits – a total of 3473 Test Day records. A statistically significant effect on the SCC in milk for Test Day of the factors herd, parity, calving season, month of Test Day recording and lactation stage was reported. In cows with larger number of parities, a higher SCC was reported compared to the younger ones. The highest was the SCC in cows at third parity (656.4 thousand/ml) and the lowest at first parity (263.9 thousand /ml). With the highest SCC were cows calved in summer (599.5 thousand/ml), and with the lowest – in winter (177.5 thousand/ml). SCC increased considerably in August and September (562.5 and 461.8 thousand/ml) and reached the highest values in October and November (806.4 and 910.9 thousand/ml). The lowest was the SCC during the winter months from December to February (from 104.8 to 151.6 thousand/ml). The highest SCC in milk was recorded in the first 90 days in milk (DIM) (from 800 to 700.0/ml). After this period, SCC was starting to decline, and after 200 DIM, values relative to normal udder health status of less than 200.0 thousand/ml were reached.

Keywords: dairy cows; SCC; parity; calving season; lactation stage; calendar month

## Introduction

Somatic cells are mainly epithelial cells of the mammary gland and white blood cells (leukocytes) occurring in the mammary gland in response to injury or infection (Sharma et al., 2011). They include 75% leukocytes (neutrophils, macrophages, lymphocytes, erythrocytes) and 25% epithelial cells. White blood cells play a protective role against infections and help repair the damaged tissues. During inflammation (mastitis), the large increase in SCC is due to the influx of neutrophils in the milk that fight the infection and represent over 90% of the total SCC (Harmon, 1994). The normal

composition of somatic cells in milk varies depending on the type of secretion or lactation stage. Typically, in the milk from healthy udder SCC is lower than 100.0 thousand/ml, whereas bacterial infection can increase it to over 1 000.0 thousand/ml (Bytyqi et al., 2010). The increase in SCC is widely accepted as an early sign of inflammatory changes in the mammary gland and the presence of SCC between 200 and 299.0 thousand/ml can be considered as a subclinical mastitis (Tahawy & Far, 2010). The somatic cells count is an indicator of cows' resistance to mastitis and is an important component for assessing milk quality, the level of hygiene and mastitis control (Sharma et al., 2011, Banga et al., 2014).

Although the main factor affecting SCC in milk is the infection of the mammary gland (Göncü & Özkütük, 2002; Sederevicius et al., 2006), it is also influenced by a number of other factors such as: parity, age, lactation stage, season, stress level, interval between milkings and environmental and management factors (De Haas, 2003, Skrzypek et al., 2004; Sederevicius et al., 2006, Erdem et al., 2007). The cows breed also influence the SCC (Busato et al., 2000, Koc., 2004).

The specific conditions of the region or country also affect SCC in milk, such as climatic conditions, selection level, selection goals, feeding systems, etc. In this aspect, it is very important to study the effects of these factors on the milk quality in order to improve and control it (according to the standard). Based on the results of these studies, various proper technologies and management can be recommended to reduce SCC in cow's milk and respectively the risk of mastitis and the production of safe food for humans (Bytyqi et al., 2010).

Controlling the SCC allows for their inclusion in the selection indices in dairy cattle farming as a trait associated with both the resistance of cows to mastitis diseases and the possibility of advice and changing management of herds related to milk production with low SCC. The Bulgarian breeding associations carry out Test Day records and collect information about SCC, but the results do not reach the farmers and do not lead to positive management solutions.

The goal of the research was to study the impact of various environmental and paratipical factors such as farm conditions, calving season, calendar month, parity and lactation stage on somatic cells count in milk in black – and – white cows housed in the Central South Bulgaria.

#### **Material and Methods**

The survey was conducted in 8 dairy cattle farms with different housing and milking systems in Plovdiv region, Bulgaria. All cows were black-and-white, Holstein-Friesian type, and they all were under productivity control by same one breeding association. In 5 of the farms the cows were 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 milked in milking parlor. In farms with tie-stall housing in the period from May to October cows were on pasture and in loose-housing farms they were housed indoor all year round.

The study covers the test – day records for 2015, including data on lactating cows in each farm with not less than 7 test – day records – a total of 484 lactating cows. Cows were on different parities, as for the purpose of the study they are presented in classes respectively: on I – 102, on II – 137, on III – 98 and on IV and more parities – 148 cows.

For the purpose of the study, month performance test – day records data were used – a total of 3473 records including data on milk yield and somatic cells count in milk for a test day. To study the effect of some environmental factors, they are presented in classes. Calving season: winter – from December to February; spring – from March to May; summer – from June to August; autumn – from September to November.

The lactation stage is presented in classes by days in milk (DIM) after calving: up to 30th; from 31 to 60th; from 61 to 90th; from 91 to 120th; from 120 to 150th; from 151 to 180th; from 181 to 210th; from 211 to 240th; from 240 to 270th; from 271 to 300 and over 301 DIM.

To determine the degree of influence of the different factors the following model was used:

$$\mathbf{Y}_{ijklmn} = \boldsymbol{\mu} + \mathbf{H}_{i} + \mathbf{S}_{j} + \mathbf{L}_{k} + \mathbf{M}\mathbf{K}\mathbf{l} + \mathbf{P}\mathbf{L}_{m} + \mathbf{e}_{ijklmn},$$

where:

Y<sub>iiklmn</sub> is the depended variable (SCC);

 $\mu$  – population mean;

 $H_i$  – fixed effect of the farm;

 $S_i$  – fixed effect of the calving season;

 $L_{\mu}$  – fixed effect of the parity;

MK1 – fixed effect of the calendar month of Test Day recording;

 $PL_m$  – fixed effect of the lactation period and  $e_{ijklmn}$  is the effect of uncontrolled factors (the error).

By analysis of variances (ANOVA) for each model are obtained by classes of fixed factors least squares of means (LSM), representing the sums of the squares, calculated as a deviation from the average value of the trait derived from the model.

To study the effect of the controlled factors and statistical indicators the relevant modules of STATISTICA 6 was used.

#### **Results and Discussion**

On Table 1 the mean values of the three traits are presented – milk yield for 305 day lactation, milk yield for a Test Day and SCC for a Test Day. The average milk yield for standard lactation (305 days) varies considerably for different farms ranging from 4531.43 kg for farm 3 to 7687.69 kg for farm 8. The difference is 3156.3 kg and is statistically significant. The average milk yield for a Test Day also varies from 16.1 kg to 25.1 kg (farms 1 and 8). The difference is 9 kg and is statistically significant. Between the rest of the farms the differences are small – 1 to 3 kg. In general, for the surveyed farms a good milk yield was reported, as in most of them it corresponding to the average for the breed in the country. The average milk yield of the controlled cows from the Black–and–white breed in Bulgaria is 5300-5600 kg, with 3.6-3.8% fat and 3.2-3.3% protein in the milk (Yordanov et al., 2017).

The simplest and most common method of processing and presenting SCC is as arithmetic mean. Another method widely used especially in Europe is presentation as geometric mean. In this case, 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). For these reasons we present SCC as arithmetic mean values (Table 1).

In the SCC for Test Day, the variation between the herds is considerable. The difference in SCC between farms 6, 7 and 8 and the other five farms is particularly clear. In the first five farms, there are some variations, but all mean values are – up to 200.0/ml, which is the norm for healthy animals without mastitis. In the last three farms, the average values are considerable and well above 400 thousand/ml, which is the value allowed when buying milk.

Koc (2008) also shows a considerable variation in the average SCC for Turkey's cows from 296.483 thousand/ml to 688.811 thousand/ml, and the average milk yield for a Test Day is similar to our results. The author indicates that SCC in milk is higher in cows in Turkey – 389.303 thousand/ml than in some EU countries, such as Poland – 269.0 thousand/ml (Skrzypek et al., 2004), Switzerland – 180.0 thousand/ml (Busato et al., 2000), Sweden – 233.0 thousand/ml (Toledo et al., 2002). In their study of 13 cattle farms in Kosovo, By-tyqi et al. (2010) found a significant variation in mean SCC for total milk in individual herds from 93.310 to 908.860

Table 1. Mean values for milk yield and SCC by farms

thousand/ml. In Brazil, Ribas (2013) established a mean SCC of 486.810 and 553.0 thousand/ml, respectively, in 257 540 and 1 995 034 milk samples taken from dairy tanks in the periods 1999-2001 and 2005-2012.

From the average values for productive traits and SCC for a Test Day, a high dependence between high productivity and SCC is not reported. Cows from farm 8 are with the highest milk yield for Test Day -25.1 kg, but they have the highest values for SCC -982 300 thousand/ml. With relatively high milk yields for Test Day are also cows from farms 4 and 5, 20.5 and 22.0 kg, respectively, but they have a low SCC, within the norm for a healthy udder, respectively 171.1 and 194.4 thousand/ml.

Table 2 presents the analysis of variance for the controlled factors on SCC in milk for Test Day. Included are the conventional factors, usually included in the linear evaluation models: herd, parity, calving season, calendar month of Test Day recording, lactation stage. Since the survey covers Test Day records for one calendar year (2015), the year is not included as a factor in the model. All factors included in the ANOVA model (Table 2) have a statistically significant effect on SCC in milk for a Test Day at P < 0.001.

 Table 2. Analysis of variance for the effect of the controlled factors on the SCC in milk for Test Day

Sources of variation	Degrees	Somatic cells count			
	of freedom	MS	F P		
	(n – 1)				
Total for the model	34	27413033	26.55 ***		
Farm	7	44370508	42.82 ***		
Parity	3	7859021	7.58 ***		
Calving season	3	18420413	17.78 ***		
Calendar month	11	23666830	22.84 ***		
Lactation period	10	16485227	15.91 ***		
Error	3433	1036293			

*Significance at:* \*P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001

Farm, №	Number of cows included in the study	Milk yield for 305 days, kg	For a Test Day		
			Number of records	Milk yield, kg	SCC, thousand/ml
	n	$x \pm SE$	n	$x \pm SE$	$x \pm SE$
1	30	4952.84±114.84	235	16.1±0.29	104.6±12.38
2	44	5908.27±140.80	291	19.6±0.34	135.3±10.24
3	20	4531.43±120.79	155	18.2±0.33	131.6±11.33
4	69	6159.91±142.24	534	20.5±0.25	171.1±8.09
5	76	6784.36±163.19	579	22.0±0.21	194.4±12.60
6	63	5729.25±123.08	474	18.9±0.23	717.5±56.09
7	61	5740.11±123.59	471	19.0±0.23	653.2±44.94
8	121	7687.69±125.65	734	25.1±0.29	982.3±72.59
Average/total	484	6374.67±66.87	3473	20.8±0.11	477.1±19.30

The Koc study (2008) of Holstein cows housed in Turkey showed that the effect of the herd, the parity, the lactation month and the milking time (evening and morning) had statistical significance (P < 0.001) on SCC in milk. The same significant effects of these factors are reported by Bytyqi et al. (2010) in Holstein cows in Kosovo.

On Fig. 1 are presented the LS-mean values of the SCC by parity. A trend for higher SCC values in cows with bigger number of parities is observed. The lowest is SCC for Test Day in cows on first parity -263.9 thousand/ml. The highest is SCC in cows on third parity -656.4 thousand/ml. In the cows on fourth and later parity, also a high SCC is reported, albeit slightly lower than, that in cows on third parity, 502.2 thousand/ml. This group includes cows with different parity numbers, including cows on 7 or 8th parity.



Fig. 1. LS-means for SCC in milk for a Test Day by parity

Gergovska et al. (2012) also found a significant influence of parity on SCC. With the lowest SCC are the cows on first parity (251.54 thousand/ml), with the highest – cows on fourth and more parities (633.22 thousand/ml).

Koc (2008) found that average SCC in milk increased in later parities. Mean SCC for I, II and III parity are 404.296, 416.582 and 555.393 thousand/ml, respectively. De Haas, (2003) also reported a higher SCC in later parities. The author believes this is due to a different protective mechanism against mammary gland infection at a younger and older age. Bytyqi et al. (2010) indicate that cows on first parity and those on fifth and later parities appear to be less exposed to udder infections, so their SCC in milk is about 178.45 thousand/ml and 205.61 thousand/ml, respectively. The highest SCC was recorded in cows on third parity (338.43 thousand/ ml), followed by second (322.61 thousand/ml) and fourth parities (308.66 thousand/ml). Sharma et al. (2011) believe that SCC increases with the increasing of age, and this increase is mainly due to an increased incidence of infections in older cows rather than due to age itself.

Fig. 2 shows the effect of the calving season on SCC values for a Test Day. With the highest value of SCC in milk are cows calved in summer -599.5 thousand/ml, followed by those calved in autumn -404.4 thousand/ml. With the lowest values of SCC in milk are cows calved in winter -177.5 thousand/ml.



Fig. 2. LS-means for SCC depending on calving season

Baul et al. (2011) established that the calving season had a significant impact on SCC. Milk from cows calved in spring had the lowest SCC - 219.0 thousand/ml. The milk from cows calved in autumn was with the highest SCC - 724.0 thousand/ml.

Gergovska et al. (2012) point that the calving season has a statistically significant effect on the SCC in milk, as the highest SCC is reported in cows calved in summer (521.990 thousand/ml) followed by those calved in the winter (413.840 thousand/ml).

On Fig.3 are presented the LS-mean values for SCC depending on calendar months. The highest values of SCC were reported in October and November (806.4 and 910.9 thousand/ml), followed by August and September (562.5 and 461.8 thousand/ml). The lowest values of SCC are reported in the winter months – December, January and February (from 104.8 to 151.6 thousand/ml).



Fig. 3. LS-mean values for SCC in milk depending on calendar month

The reason for the higher SCC in the months from July to November is probably the favorable conditions for the development of pathogenic microorganisms, causing mastitis – warmer and wetter weather, especially during year-round indoor housing.

Bytyqi et al., (2010) found that the effect of the calendar month on SCC in raw milk showed a higher SCC in December (469.0 thousand/ml), followed by November (426.74 thousand/ml) and October (401.14 mg/ml). In August, September, January, February and March, this value is considerably lower (125.06, 147.12, 297.55, 276.64 and 153.65 thousand/ml). In spring and summer the cows are out on pasture and enter the barns only for milking. The milk produced in August containing three times less SCC than the milk produced in December by the same cows, which may be caused by the inability to ensure good management during the indoor housing period (i.e., lack of ventilation and good hygiene, higher stress, etc.), which increases the risk of intramammary infections by environmental pathogens (Wenz et al., 2007).

Atasever & Erdem, (2009) indicate that the increase in SCC is higher in spring or summer. This may be due to the increased presence of flies and other insects that can transmit infectious pathogens from one animal to another.

In general terms, the seasonal variation in the SCC is most often expressed as a peak in the late summer and decrease to the lowest values during the winter months (Gavan et al., 2009). The higher SCC found in the winter compared to the summer months, is most often in cases when the cows are housed in barns during the winter and on pasture in the summer.

The presented varying of SCC by calendar months explains the effects reported in the varying by calving season. With the highest values of SCC in milk are cows calved in the summer. The beginning of their lactation is during the two seasons with the highest SCC values – summer and autumn. In the cows, calved in the winter, the first half of the lactation, which is characterized by the highest risk of mastitis and respectively increased SCC, goes during the winter-spring months, when SCC is usually low.

The presented LS-mean values of SCC in milk for Test Day by lactation stages (Fig. 4), shows that the highest SCC is observed during the first 90 DIM – SCC from 800.0 to 700.0 thousand/ml. After this period, SCC begins to decrease, and after 200 DIM, normal udder health values – below 200.0 thousand/ ml are reached.

The early lactation stage is associated with an increased risk of mastitis and consequently increased SCC in milk, which is often due to inadequate measures during the drying of the cows and inadequate management during the dry period (Steeneveld et al., 2013).

Koç (2008) also found an increased SCC in the first lacta-



Fig. 4. LS-mean values of SCC in milk for Test Day by lactation stages

tion month, and gradually from the second month of lactation to its end, SCC decreased. SCC in the first lactation month is 607.295 thousand/ml and decreases to 387.525 thousand / ml in the second lactation month. De Haas (2003) also reports the same dependence.

As a consequence of the thus presented dependencies between the calving season and the calendar month of Test Day recording, Fig. 5 shows the variation of SCC for a Test Day by lactation stages in cows calved in different seasons.



Fig. 5. Variation of SCC by lactation stage depending on calving season

The figure shows, that the variation of SCC during lactation is not with equal dependency in cows calved in different seasons. In cows, calved during the summer, SCC in milk starts at high levels (1127.0 thousand/ml) and continues to increase, reaching a peak around the 90th DIM (1326.8 thousand/ml). As a season this period coincides with the summer months and in some cows with part of the autumn months. About 120-150 DIM, SCC in milk for a Test Day declines considerably and reaching values much closer to the normal (459.0 thousand/ml), which coincids with the winter and spring months of the year.

In cows calved in autumn, lactation starts with the highest SCC - 1250.0 thousand/ml, then begins to gradually decrease,

as after 150 DIM is about the normal level of 200.0 thousand/ml.

Contrary to these variation curves, is the curve of the cows calved in the spring. They have the lowest SCC at calving, after which it begins to rise and reaches maximum values (700.0 thousand/ml) in the middle of lactation around the 150th day, which coincides with the summer and autumn season. The smoothest is the curve of SCC in milk for Test Day by lactation months in cows calved in winter. There is some variation, but the values remain about 400.0 thousand/ml during the whole lactation.

## Conclusion

There is a clear trend of increasing the somatic cells count in milk depending on the season and in relation to the lactation stage. The highest is the risk of mastitis and high SCC in cows, whose beginning of lactation coincides with favorable seasonal conditions for the development of mastitis infections – summer and autumn. The SCC increases considerably in August and September, reaching the highest values in October and November. The highest SCC in milk is reported in the first 90 DIM. After this period it starts to decrease, as after 200 DIM it riches values associated with normal udder health status – below 200.0 thousand/ml. In cows with bigger number of parities a higher SCC compared to the younger ones was reported.

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