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The electrical conductivity of sheep's milk and the possibility of mastitis detection

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ABSTRACT

Measurement of electrical conductivity (EC) is a method frequently used in dairy cows during milking in milking parlours, but especially in robotic milking as a low-cost mastitis detection method. The aim of this study was to evaluate the relationship between somatic cell count (SCC) and EC of milk in sheep reared in Slovakia as factors for monitoring subclinical mastitis on the basis of a bacteriological examination of udder health. Samples were collected individually from both halves of the udder from 295 sheep of different breeds from eight farms during evening milking. Based on SCC, the samples (590) were divided into classes ($SCC < 2 \times 10^5$, $2 \times 10^5 \leq SCC < 4 \times 10^5$, $4 \times 10^5 \leq SCC < 6 \times 10^5$, and $SCC \geq 6 \times 10^5$ cells.mL^{-1}), ($SCC < 7 \times 10^5$ and $SCC \geq 7 \times 10^5$ cells.mL^{-1}) and ($SCC < 1 \times 10^5$ and $SCC \geq 1 \times 10^5$ cells.mL^{-1}) respectively. Based on the presence of pathogens in the udder halve, they were classified as “major pathogens” (14), “minor pathogens” (161) and “without pathogens” (415). The presence of a pathogen had a significant effect on the increase in EC, SCC and protein content and decrease in content of lactose. We found a significant correlation between EV and SCC at first classification only in cases where all data was analysed jointly ($r = 0.531$), $SCC \geq 6 \times 10^5$ ($r = 0.403$) and $SCC < 2 \times 10^5$ ($r = 0.214$). In the second and third classification, we found significant correlations in both cases, the $SCC < 7 \times 10^5$ ($r = 0.270$) and the $SCC \geq 7 \times 10^5$ ($r = 0.382$) and $SCC < 1 \times 10^5$ ($r = 0.136$) and the $SCC \geq 1 \times 10^5$ ($r = 0.557$). The electrical conductivity showed a stronger correlation with the lactose and protein content than LogSCC. We can argue that measuring the electrical conductivity of sheep milk may be a possible alternative for mastitis detection in sheep. EC can be useful in detecting animals with level of SSC greater than 6×10^5 cells.mL^{-1}.

Keywords: electric conductivity; somatic cell count; sheep milk; mastitis

INTRODUCTION

For sheep farmers it is very important to know the health status of the udder. Increasing SCC leads to a significant reduction in daily milk production, decrease in lactose and a moderate increase in fat and protein **(Caria et al., 2016; Tančin et al., 2017; Baranovič et al., 2018)** however, it significantly aggravates the coagulation properties of milk **(Abdelgawad et al., 2016)**. Measuring the electrical conductivity (EC) of milk during milking has been studied in cattle as a low-cost mastitis detection method that can be easily automated **(Romero et al., 2017)**. Milk normally has an EC of between 4.0 and 6.0 mS.cm^{-1} **(Ferrero, Valledor and Campo, 2014)**, but bacterial infection of the udder results in an increase in Na^+ and Cl^- and decreases in the K^+ levels **(Kitchen, 1981)**, which causes an increase in EC. This is widely used as a method of monitoring mastitis infections. When measured conductivity is in extreme values (6.5 – 13.00 mS.cm^{-1}) at 18 °C, this indicates mastitis **(Ferrero, Valledor and Campo, 2014)**. **Caria et al. (2016)** achieved a sensitivity of 73.08% and a specificity of 75.46% in their study, with an EC threshold of 4.84 mS.cm^{-1} for sheep milk. There are only a few reports that have been published about the effect of mastitis on the conductivity of sheep's milk. This led us to a decision to evaluate the relationship between SCC and EC of milk in sheep reared in Slovakia as factors for monitoring subclinical mastitis on the basis of a bacteriological examination of udder health.

Scientific hypothesis

The presence of pathogens in sheep milk significantly increases the electrical conductivity of milk.

The presence of pathogens in sheep's milk significantly increases SCC in milk.

Increasing the number of somatic cells increases the electrical conductivity in milk.

There is a moderate positive relationship between SCC and EC.

The presence of pathogens in sheep's milk significantly decreases lactose content in milk.

The presence of pathogens in sheep's milk significantly increases protein content in milk.

There is a moderate negative relationship between SCC and EC.

MATERIAL AND METHODOLOGY

Samples from 590 udder halves of 295 machine milking ewes of different breeds from eight farms were collected during evening milking. Milk samples were collected aseptically after cleaning the teats, especially teat-ends with antibacterial wipes (GAMA Healthcare Ltd, UK). Sampling always started with the right udder half, the first two strips were placed separately, next 10 mL were used for EC measurement with a handheld conductometer Milk Checker N-4L (Oriental Instruments Co., Ltd., Japan) with compensation the measured EC on a standard temperature of 25 °C, 1 mL was aseptically gathered into sterile test tube for cytobacteriological analysis and an additional sample of 50 mL was taken for somatic cell count and a basic components analysis. Immediately after removal, the milk sample was stored in a portable refrigerator at 5 – 15 ° C. The samples were transported to the laboratory and refrigerated at 4 °C. Milk samples (inoculum 10 μl) streaked onto selective culture medium PM test (LabMediaServis s.r.o., CZ) were incubated at 37 °C for 24 h. Isolated strains of pathogens were then verified by typing with BBL Crystal® (Becton, Dickinson & Co., New Jersey, USA). Somatic cell count was determined using a Somacount 150 (Bentley Instruments, Inc., Chaska, Minnesota, USA), milk composition was determined by MilkoScan FT120 (Foss, Hillerød, Denmark).

Statistic analysis

The correlation of EC with SCC was analysed (Proc Corr, SAS ver. 9.3; SAS Institute Inc., 2011) according to SCC intervals by **Romero et al. (2017)** ($SCC < 2 \times 10^5$, $2 \times 10^5 \leq SCC < 4 \times 10^5$, $4 \times 10^5 \leq SCC < 6 \times 10^5$, and $SCC \geq 6 \times 10^5$ $cells.mL^{-1}$), by **Caria et al. (2016)** ($SCC < 7 \times 10^5$ and $SCC \geq 7 \times 10^5$ $cells.mL^{-1}$) and **Barth, Burow and Knappstein (2008)** ($SCC < 1 \times 10^5$ and $SCC \geq 1 \times 10^5$ $cells.mL^{-1}$). EC and SCC variables were transformed into base 10 logarithms. The relationship of the EC and SCC variables with fixed effects was analysed by a one-way ANOVA (Proc GLM; SAS/STAT ver. 9.3; SAS Institute Inc., 2011), the mean differences were determined by the Scheffe's test.

RESULTS AND DISCUSSION

After the pathogen analysis, we found that 175 animals were free of the pathogen in the udder and in 120 animals the pathogen was present in at least one half of the udder. 76 animals (25.8%) from the "free of the pathogen" category had $SCC < 1 \times 10^5$ and EC ranging from 0.0 to 0.4. In total, 175 udder halves (29.7%) were infected, from that 55 animals were infected in both halves and

Table 1 Descriptive statistics of EC ($mS.cm^{-1}$) by type of pathogen.

category	N	Mean	SD	Median	Minimum	Maximum
without pathogens	415	4.6335^{B}	0.7579	4.5	3.1	10.3
major pathogens	14	5.8786^{A}	1.6912	5.3	2.9	9.6
minor pathogens	161	5.2919^{A}	1.2376	5.0	3.5	11.5

Note: A, B – means with different letters are significant (p <0.001); SD – standard deviation.

Table 2 Descriptive statistics of LogSCC (log $cells.mL^{-1}$) by type of pathogen.

category	N	Mean	SD	Median	Minimum	Maximum
without pathogens	415	4.8999^{C}	0.5836	4.8325	3.4772	7.0000
major pathogens	14	6.5047^{A}	0.9081	6.6447	4.3424	7.5887
minor pathogens	161	5.8489^{B}	0.7564	6.0418	3.9542	7.4532

Note: A, B – means with different letters are significant (p <0.001); SD – standard deviation.

Table 3 Spearman correlation coefficients and descriptive statistics of EC ($mS.cm^{-1}$) by SCC class (x 10^3 $cells.mL^{-1}$) according to **Romero et al. (2017)**; **Caria et al. (2016)** and **Barth, Burow and Knappstein (2008)**.

SCC class	r	N	Mean	SD	Median	Minimum	Maximum
Romero et al. 2017							
$SCC < 200$	0.214^{***}	392	4.4992^{A}	0.5443	4.5	2.9	7.9
$200 \leq SCC < 400$	0.036^{NS}	34	4.7765^{A}	0.6282	4.8	3.5	6.7
$400 \leq SCC < 600$	-0.138^{NS}	21	4.7810^{A}	0.6022	4.8	3.8	6.2
$SCC \geq 600$	0.403^{***}	143	5.8091^{B}	1.3779	5.5	3.5	11.5
Caria et al. 2016							
$SCC < 700$	0.270^{***}	456	4.5432^{A}	RE0.5624	4.5	2.9	7.9
$SCC \geq 700$	0.382^{***}	134	5.8619^{B}	1.4028	5.5	3.5	11.5
Barth, Burow and Knappstein 2008							
$SCC < 100$	0.136^{*}	301	4.4581^{A}	0.5424	4.5	2.9	7.9
$SCC \geq 100$	0.557^{***}	289	5.2433^{B}	1.1882	5.0	3.1	11.5
all data	0.531^{***}	590					

Note: * p <0.05; *** p <0.001; A, B – means with different letters are significant (p <0.001); SD – standard deviation.

Table 4 Descriptive statistics of lactose (%) and protein (%) by type of pathogen.

category	**N**	**lactose**				**protein**			
		Mean	**SD**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**
without pathogens	415	4.97^{A}	0.48	1.15	6.13	5.66^{B}	0.68	3.94	7.74
major pathogens	14	4.10^{B}	0.80	2.80	5.70	6.55^{A}	1.43	4.82	9.97
minor pathogens	161	4.59^{B}	0.80	1.79	6.06	5.82^{AB}	0.97	3.87	9.95

Note: A, B – means with different letters are significant (p <0.001); SD – standard deviation; Min. - Minimum; Max. – Maximum.

Table 5 Descriptive statistics of lactose (%) and protein (%) by SCC (log x 10^3 mL^{-1}) classes with division according to **Romero et al. (2017)**; **Caria et al. (2016)** and **Barth, Burow and Knappstein (2008)**.

SCC class	**N**	**lactose**				**protein**			
Romero et al. 2017		**Mean**	**SD**	**Min.**	**Max.**	**Mean**	**SD**	**Min.**	**Max.**
SCC < 200	392	5.06^{A}	0.35	3.67	6.13	5.68	0.77	3.94	9.97
200 ≤ SCC < 400	34	4.95^{A}	0.43	3.85	5.85	5.69	0.74	4.76	7.72
400 ≤ SCC < 600	21	4.88^{A}	0.42	3.54	5.47	5.64	0.95	3.87	8.67
SCC ≥ 600	143	4.25^{B}	0.85	1.15	5.89	5.86	0.86	4.41	9.47
Caria et al. 2016									
SCC < 700	456	5.03^{A}	0.37	3.54	6.13	5.67	0.79	3.87	9.97
SCC ≥ 700	134	4.22^{B}	0.85	1.15	5.89	5.85	0.84	4.41	9.47
Barth, Burow and Knappstein 2008									
SCC < 100	301	5.06^{A}	0.36	3.67	6.13	5.74	0.78	3.94	9.97
SCC ≥ 100	289	4.63^{B}	0.75	1.15	5.89	5.71	0.82	3.87	9.47

Note: A, B – means with different letters are significant (p <0.001); SD – standard deviation; Min. - Minimum; Max. – Maximum.

Table 6 Spearman correlation coefficients among milk variables (n = 590).

	LogSCC	lactose	protein	EC
LogSCC	1.000			
lactose	-0.373***	1.000		
protein	-0.022	-0.526***	1.000	
EC	0.531***	-0.393***	-0.152***	1.000

Note: *** p <0.001.

65 animals with only one half. In 14 samples (2.4%) major pathogens were detected (*Staphylococcus aureus* (5 samples), *Streptococcus agalactiae*).

The presence of the pathogen had an significant effect ($F_{(2;587)} = 37.06$; p <0.001) on the increase in electrical conductivity (Table 1), no significant differences were found between the minor and major pathogens. EC of the infected glands (n = 175) without considering the type of pathogen was (Mean ± SD) 5.3389 ± 1.2836 $mS.cm^{-1}$.

Similarly as above, the presence of the pathogens had an significant effect ($F_{(2;587)} = 155.61$; p <0.001) on the increase in LogSCC (Table 2), but the major pathogens increased the LogSCC level significantly higher than minor pathogens. This goes along with the results of other studies **(Linage et al., 2017; Gonzalo, 2018)**.

The correlation between SCC and EC for all animals (Table 3) was higher (a moderate relationship) than that found by **Caria et al. (2016)** (r = 0.306) or **Romero et al. (2017)** (r = 0.33), but corresponds to the data reported by **Peris et al. (1991)**. The strongest correlation was, similarly to **Romero et al. (2017)** (r = 0.25) in SCC ≥ 6 × 10^5 class. This correlation may indicate that EC may be used in this class for mastitis detection. Also, differences in the EC were statistically significant ($F_{(3;586)} = 86.67$; p <0.001) only between the SCC ≥ 6 × 10^5 class and the other classes. When ordering EC according to **Caria et al. (2016)** significant differences between means ($F_{(1;588)} = 261.08$; p <0.001) were found. Lower value of EC in class SCC < 7 × 10^5 as in classes 2 × 10^5 ≤ SCC < 4 × 10^5 or 4 × 10^5 ≤ SCC < 6 × 10^5 in classification above (Table 3) was caused by counting a greater number of cases from the SCC < 2 × 10^5 class to this class.

The lactose and protein content was significantly affected by the presence of pathogens (Table 4) but without significant differences between minor and major pathogens groups. In the SCC class classification (Table 5), we found significant differences in lactose content only between the SCC ≥ 6 × 10^5 class and the other classes. In the classifications according to **Caria et al. (2016)** and **Barth, Burow and Knappstein (2008)**, the differences between the classes were statistically significant. However, there are no differences between classes in protein content.

The negative correlation between LogSCC and lactose content (Table 6) corresponds to findings from other authors **(Scharch, Süß, and Fahr, 2000; Olechnowicz et al., 2009; Caria et al., 2016)**, but our values are lower than those reported by **Olechnowicz et al. (2009)** and **Scharch, Süß, and Fahr (2000)**. The electrical conductivity showed a stronger correlation with the LogSCC than lactose and protein content reported, although it is still only a weak relationship.

CONCLUSION

We can argue that measuring the electrical conductivity of sheep milk may be a possible alternative for mastitis detection in sheep. EC can be useful in detecting animals with level of SSC greater than 6×10^5 cells.mL^{-1}. But we can not estimate a threshold for healthy animals. Perhaps, if we obtain more data from animals in the $2 \times 10^5 \leq$ SCC $< 4 \times 10^5$ and $4 \times 10^5 \leq$ SCC $<6 \times 10^5$ (cells.mL^{-1}) categories, it will be possible to specify the threshold in the future. However, since electrical conductivity is influenced by several factors **(Romero et al., 2017)**, it would be more appropriate to think about multiple individual assessments in milking parlours rather than using a portable device for mastitis detection.

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