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ELECTRICAL CONDUCTIVITY OF MILK IN DIFFERENT MILKING PHASES AND RELATIONSHIP WITH SUBCLINICAL MASTITIS AND MASTITIS PATHOGENS OF COWS

V. Juozaitienė[1*], L. Anskienė[1], E. Čereškienė[1], A. Juozaitis[1], J. Žymantienė[2], V. Žilaitis[3] and R. Bobinienė[4]

[1]Veterinary Academy, [2] Department of Anatomy and Physiology, [3] Department of Non-Infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės 18, Kaunas, Lithuania; [4]Institute for Scientific Research, Lithuanian University of Educational Sciences, Studentų St. 39, Vilnius, Lithuania
[*]Corresponding author's e-mail: Vida.Juozaitiene@lsmuni.it

ABSTRACT

The aim of the study was to investigate the relationships between the electrical conductivity of milk in different milking phases with subclinical mastitis and productivity of cows. Three hundred fourteen Lithuanian Black and White cows in 2-4 lactation months were evaluated with Lactocorder®. We found on average 6.28 mS/cm and 7.17 mS/cm, for respectively electrical conductivity at highest milk flow (ELHMF) and electrical conductivity during the initial time (ELAP). The average was 6.66 mS/cm for the maximum electrical conductivity after reaching the highest milking speed (ELMAX) and 0.79 mS/cm for (beginning peak difference of the electrical conductivity (ELAD). The somatic cell count (r=0.196) and milk yield (r=-0.222) showed the strongest unfavorable correlation ($P<0.01$) with ELHMF. Higher ELHMF was related to a greater frequency of mastitis. Pathogens of intramammary gland were found by 39 % of cows in group of ELHMF<6 mS/cm and 52% - in group of ELHMF≥6 mS/cm ($P=0.002$). The milk from cows infected subclinically with *Staphylococcus aureus* had 0.55 - 0.68 mS/cm higher ELHMF, than with subclinically *Streptococcus agalactiae, Streptococcus uberis*, *Streptococcus dysgalaetia* ($P<0.001$).

Key words: electrical conductivity of milk, milking phases, subclinical mastitis.

INTRODUCTION

Mastitis is a persistent, inflammatory reaction of the udder tissue, which entails a decline in potassium, and is responsible for a higher somatic cell count and electrical conductivity of milk (Gáspárdy *et al*., 2012).

The somatic cells present in the milk of a healthy cow belong mainly to the macrophages (66–88%); in addition there are neutrophils, and epithelial and mononuclear cells The proportion of neutrophils is only 1–11% in a healthy quarter but increases up to 90% and more in a quarter with intramammary infections. The proportion of neutrophils as the percentage of the somatic cell count has been proposed as a mastitis indicator (Sandholm, 1995; Hamann and Zecconi, 1998).

Investigation of Yarabbi *et al.* (2014) showed that the somatic cells count in raw milk has a significant effect on the electrical conductivity. In increasing the number of somatic cells, electrical conductivity of milk will increase ($P<0.05$). differentiate (2005) and Ogola *et al.* (2007) found that increasing somatic cells count changes in the amount of raw milk mineral. These changes will affect in type and amount of milk mineral in pH, acidity and electrical conductivity of milk. So that by increasing anions and cations such as sodium and chloride and by reducing potassium and calcium, the electrical conductivity will increase. The reason for this is that due to increasing permeability of blood vessels the amount of sodium and chloride has increased, whereas the amount of potassium phosphorus, zinc and magnesium has decreased and by reducing the absorption of calcium from blood to milk, the amount of calcium has reduced. On the other hand, because of the important role of calcium in the casein micelle structure, calcium level of milk is reduced with defects in synthesis of casein. So when cattle exposed into mammary glands, milk electrical conductivity increases (Yarabbi *et al.,* 2014).

Electrical conductivity of milk can be used as a phenotypic and genetic indicator of bovine mastitis. According to results Norberg *et al.* (2004b), Norberg (2005) genetic correlations between electrical conductivity of milk and mastitis have been estimated to be in the range from 0.65 to 0.8, hence, obtaining genetic response for mastitis should be possible by using information of electrical conductivity of milk in genetic evaluation. However, collecting and implementing electrical conductivity of milk information in a breeding program may be a challenge.

Measuring electrical conductivity of milk to detect mastitis is based on the ionic changes, which occur during inflammation, since the sodium and chloride concentrations increase in milk. Bansal *et al.* (2005) estimated that the average electrical conductivity of milk increases from infected udder quarters, as they increase in Na^+ and Cl^- ion concentration due to damaged mammary epithelium and there appears milk barrier.

Nielen *et al.* (1992) reported a decline of 0.88 kg/d in milk production with a rise of 1 mS of the mean electrical conductivity. The authors also suggested that the electrical conductivity and somatic cell count have an additive effect on the loss in daily milk production.

In accordance with Špakauskas *et al.* (2006), the electrical conductivity of milk from cows with subclinical mastitis may increase from 6.1 to 8.5 mS/cm. Norberg *et al*. (2004a) found that the average electrical conductivity of healthy cows was 4.87 mS/cm, when identified with subclinical mastitis – 5.37 mS/cm, clinical mastitis – 6.44 mS/cm. Hamann and Gyodi (2000) reported that electrical conductivity of milk of healthy cows is 4.0-5.5 mS/cm.

Lee and Choudhary (2006) found the averages of ELHMF (electrical conductivity at highest milk flow) was 6.13 mS/cm, ELAP (beginning peak level of the electrical conductivity) - 6.47 mS/cm and ELMAX (maximum electrical conductivity) - 6.45 mS/cm

Cho *et al.* (2009) reported that while the cows with higher electrical conductivity at the beginning of milking had less somatic cell counts, cows with higher electrical conductivity after the peak of milk yield had more somatic cell counts in milk.

For mastitis diagnosis, it is important to investigate the relationships between electrical conductivity of milk in different milking phases with milk somatic cell count, productivity and mastitis and to evaluate an influence of various factors on milk electrical conductivity changes.

The goal of this study was to determine subclinical mastitis by electrical conductivity of milk in different stages of milking.

MATERIALS AND METHODS

The research was carried out at herd of the Lithuanian Black-and-White cattle improvement association, at the State Laboratory for Milk Control "Pieno tyrimai", at the Centre of State Rural Business Development and Information and at the Laboratory of Establishment of Animal Breeding Value and Selection of Lithuanian University of Health Sciences.

The 314 Lithuanian Black and White cow's with lactation period of 2-4 months, on average of 2.8±0.06 lactation were evaluated with electronic milk flow meter Lactocorder® (Lactocorder, WMB, Switzerland) three times evening milking. Lactocorder® as a measurement device it has been recognized by ICAR (Internacional Committee for Animal Recording).

Table 1 reports a general description of investigated traits in this research. Bimodality of milk flow was detected when a curve had a flow pattern with 2 increments separated by a clear drop in milk flow for more than 200 g/min within 1 min after the start of milking (Dzidic *et al*., 2004).

Table 1. Description of evaluated traits with Lactocorder®.

Parameter	Commentary
MGG	Total milk yield (from the beginning to the end of the measurement), kg
ELHMF	Electrical conductivity at highest milk flow, mS/cm
ELAP	Electrical conductivity during the initial time, mS/cm of milking (beginning peak level of the electrical conductivity)
ELAD	Beginning peak difference of the electrical conductivity, mS/cm
ELMAX	Maximum electrical conductivity after reaching the highest milking speed, mS/cm
ELST	Step in the electrical conductivity, mS/cm
ELND	Level difference of the electrical conductivity, mS/cm
ELMNG	Maximum electrical conductivity after main milking, mS/cm
BIMO	The bimodality (sudden drop in milk flow at start of milking)

The results of our measurements with LactoCorder® (http://www.lactocorder.ch) were processed by the software program pack "LactoPro 5.2.0" (Biomelktechnik Swiss).

The research on the somatic cell count in milk and microbiological testing of milk samples from cows for diagnose of mastitis was performed at the State enterprise „Pieno Tyrimai". Mastitis status of milk samples was determined by diagnostic procedures recommended by National Mastitis Council (NMC, 1987). Somatic cell count in milk was determined using measuring device "Somascope" (CA-3A4, 2004; Delta Instruments, the Netherlands), which operates on the principle of flow cytometry technology. State enterprise "Pieno tyrimai" operates under quality management system conforming to the requirements of International Standard ISO/IEC 17025:2005 to ensure the accuracy of milk composition and quality tests.

The % of Holstein genes in cows genotype has been estimated according to records of cows with complete (3 ancestor's generations) pedigree information from database of State Agricultural Information and Rural Business Centre in the Laboratory of the Establishment of Animal Breeding Value and Selection of Lithuanian University of Health Science.

Statistical characteristics in the sample (n) – arithmetic mean (M), standard error (SE), P – value (P) – were calculated using R 2.1.0" package (http://www.r-project.org/). Data analysis was performed by using Student-t and Chi-Square statistical significance tests. The results were considered statistically significant when $P \leq 0.05$.

We investigated the influence of fixed factors and their interaction on cow's milk yield (MGG) and somatic cells count (using a $\log_{10}$ transformation normalized to SCC $\log_{10}$). ELHMF effect was used in the model, as this indicator from all investigated traits of electrical conductivity showed the strongest correlation with the amount of milk and the somatic cell count. The bimodality of milking curves was associated with an increase of electrical conductivity of milk, which also was included in the model.

Data of cows were analyzed by using a linear model:

$$Y_{ijklm}=\mu + E_i + L_j+B_k + G_l+ EL_{ij} + EB_{ik} + EG_{il} + LB_{jk} + LG_{jl} + BG_{kl} + ELB_{ijk} + ELG_{ijl}+ EBG_{ikl} + LBG_{jkl} + ELBG_{ijkl} + e_{ijklm}$$

Where: Y_{ijklm}=dependent variables (MGG and SCC log10); μ=general mean, E_i - ELHMF group (group 1 <6 mS/cm, group 2 ≥6 mS), L_j - lactation (class 1 – lactation 1 and class 2 – 2 and more), B_k - evaluation of the bimodality of milk flow curves (class 1 – bimodal curve, class 2 – normal curve), G_l – group of cows according to the level of genes of Holstein breed in genotype of cows: HF1 - less than 75% and HF2 - 75% and more, EL_{ij} – effect of ELHMF and lactation interaction, EB_{ik}- effect of ELHMF and bimodality of milk flow curves interaction, EG_{il} – effect of ELHMF and bimodality and genotype interaction, B_{jk} – effect of lactation and bimodality interaction, LG_{jl}- effect of lactation and genotype interaction, BG_{kl}– effect of bimodality and genotype interaction, ELB_{ijk}– effect of interaction, ELG_{ijl} - effect of interactions ELHMF x lactation x genotype, EBG_{ikl}– effect of interactions ELHMF x bimodality x genotype, LBG_{jkl} – effect of interactions lactation x bimodality x genotype, $ELBG_{ijkl}$– effect of interactions ELHMF x lactation x bimodality x genotype, e_{ijklm} - residual error.

RESULTS

We estimated that the average milk yield (MGG) of cows was 13.32±0.225 kg (12.22±0.342 kg in lactation 1 and 14.28±0.279 kg in lactation 2 and older; P<0.001), the average somatic cell count - 355.74±43.334 thousand/ml (188.72±11.115 thousand/ml in lactation 1 and 559.10 ±56.033 thousand/ml in lactation 2 and older (P<0.01).

The averages of electrical conductivity are demonstrated in a Table 2. The indicators of electrical conductivity of milk in all phases of the milking has been greater for older lactation cows, but the greatest statistically significant differences were determined for ELHMF and ELAP (4.8-5.3%, P<0.001).

Table 2. Descriptive statistics of electrical conductivity of milk in the different phases of the milking.

Statistic	ELHMF	ELAP	ELAD	ELMAX	ELST	ELND	ELMNG
			All cows (n=314)				
M	6.29	7.17	0.79	6.66	0.24	0.82	6.15
SE	0.036	0.048	0.048	0.041	0.020	0.033	0.061
			Lactation 1 (n=167)				
M	6.13	7.00	0.70	6.54	0.18	0.78	6.07
SE	0.041	0.058	0.060	0.055	0.023	0.048	0.049
			Lactation 2 and more (n=147)				
M	6.47^{***}	7.35^{***}	0.89^{*}	6.79^{**}	0.29^{**}	0.87	6.25
SE	0.059	0.075	0.077	0.058	0.032	0.045	0.118

Differences of means between lactations is significant at the level: *P<0.05, **P<0.001, ***P<0.001.

The bimodal milk flow curves where determined in 29.62% of cows. The bimodality of milk flow curves was associated with the increase of ELAP, ELAD, ELMAX, ELST and ELHMF (0.02 – 0.36 mS/cm) and the decrease of ELND and ELMNG (-0.11 – 0.3 mS/cm).

The biggest influence of bimodality (Table 3) was determined on ELAD (it was 13.2% higher of cows with bimodal milk flow curve, compared with the normal curve), ELND (12.9% less of cows with bimodal milk flow curve) and ELST (8.7% higher of cows with bimodal milk flow curve); bimodality on ELMAX almost had no influence (the difference 0.6%).

We investigated, that ELHMF<6 mS/cm of bimodal milk flow curves was 21.01%, then ELHMF≥6 mS/cm - 34.87%.

Cows of group HF1 accounted 66.8% of all investigated cows. Their milk yield (12.59±0.273 kg) was 14.7% lower and milk somatic cell count (372.93±60.102 thousand/ml) was 15% higher (P<0.001) than the other group of cows (HF2). Statistically significantly differed indicators of ELAD, ELMAX, ELND and ELMNG (from 6.3% ELMAX to 63.9% ELST) between classes of genotype of cows (Table 4).

Assessing the relationships between indicators of electrical conductivity of milk, the strongest correlation coefficients were estimated between ELAP and ELAD, ELMAX and ELND (r=0.682-0.786). The data are presented in the Table 5.

Table 3. Influence of bimodality of milk flow curves on electrical conductivity of milk.

Bimodality of milk flow curves	Statistic	ELHMF	ELAP	ELAD	ELMAX	ELST	ELND	ELMNG
Normal curve	M	6.18	7.07	0.76	6.64	0.23	0.85	6.24
(n=221)	SE	0.034	0.048	0.054	0.051	0.022	0.042	0.048
Bimodal curve (n=93)	M	6.5394	7.40**	0.86**	6.68	0.25	0.74	5.94*
	SE	0.088	0.111	0.100	0.064	0.041	0.054	0.170

Differences of means between groups is significant at the level: *P<0.05, **P<0.001, ***P<0.001.

Table 4. Influence of genotype on electrical conductivity of milk.

Genotype	Statistic	ELHMF	ELAP	ELAD	ELMAX	ELST	ELND	ELMNG
HF1	M	6.26	7.20	0.85	6.50	0.13	0.75	5.98
(n=203)	SE	0.052	0.067	0.064	0.0432	0.024	0.039	0.084
HF2	M	6.40	7.10	0.61*	6.94**	0.36**	0.90*	6.48**
(n=111)	SE	0.053	0.061	0.065	0.076	0.032	0.065	0.066

Differences of means between groups is significant at the level: *P<0.05, **P<0.001, ***P<0.001.

Table 5. Correlation coefficients between investigated traits.

Correlation	ELAP	ELAD	ELMAX	ELST	ELND	ELMNG	ELHMF	SCC_log10
MGG	-0.078	-0.058	-0.066	-0.032	0.113*	0.005	-0.251**	-0.222**
SCC log10	0.125*	-0.023	0.180**	0.188**	0.083	0.018	0.196**	1
ELAP	1	0.786**	0.241**	0.028	0.007	-0.222**	0.340**	0.125*
ELAD	0.786**	1	-0.075	-0.119*	-0.107	-0.281**	-0.067	-0.023
ELMAX	0.241**	-0.075	1	0.355**	0.682**	0.381**	0.494**	0.180**
ELST	0.028	-0.119*	0.355**	1	0.357**	0.247**	0.156**	0.188**
ELND	0.007	-0.107	0.682**	0.357**	1	0.450**	0.148**	0.083
ELMNG	-0.222**	-0.281**	0.381**	0.247**	0.450**	1	-0.207**	0.018
ELHMF	0.340**	-0.067	0.494**	0.156**	0.148**	-0.207**	1	0.196**

Correlation is significant at the level: *P<0.05, **P<0.001.

Milk yield and the somatic cell count (from all indicators of electrical conductivity) showed the strongest correlation with ELHMF (P<0.01). It was estimated negative low correlations between milk yield and somatic cell count (P<0.01). ELHMF statistically significantly (P<0.01) was associated with almost all (except ELAD) investigated indicators of electrical conductivity in different milking phases, but mostly - with ELMAX and ELAP. Negative correlation of ELHMF was estimated just between ELAD and ELMNG (P<0.01).

We estimated that of all fixed effects the biggest influence on the production of cows were by ELHMF and lactation (Table 6), on the somatic cell count – ELHMF and bimodality (P<0.0001). We determined that a production of cows mostly was associated with the interaction between these factors: genotype with lactation and with bimodality (P=0.002-0.005), somatic cell count statistically significantly dependent of lactation x bimodality x genotype (P<0.001).

ELHMF <6 mS/cm was established in 119 (37.9%) cows of group 1, ELHMF ≥6 mS/cm - 195 (62.1%) cows of group 2. Higher electrical conductivity of milk was related to a greater (1.33 times) frequency of mastitis. Pathogens of intramammary gland were found by 39% of cows from group 1 and 52% - group 2 (Fig.1). The bacteriological status of udder was significantly differ (P=0.002) among groups of cows.

The most prevalent pathogens of mastitis were *Staphylococcus aureus* and *Streptococcus agalalactiae* (isolated in 25-37% samples). The prevalence of *Streptococcus uberis* (7-8%) and *Streptococcus dysgalaetia* (4-5%) was similar in both groups of cows.

The prevalence of mastitis pathogens by infected cows between groups (P=0.993) is presented in a figure 2. The most prevalent pathogen of mastitis was *Staphylococcus aureus* (39-42%). Frequency of *Streptococcus agalactiae (1.5-1.6 times), Streptococcus u*beris (2.1-2.6 times) and *Streptococcus* dysgalaetia (3.5-3.7 times) was lower.

In general, the average of ELHMF from milk samples of infected cows (6.32±0.006 mS/cm) were significantly 0.35 mS/cm higher than from uninfected animals (P<0.001). We indicated that the milk from cows infected subclinically with *Staphylococcus aureus* (6.88±0.007mS/cm) had a statistically significant higher electrical conductivity (P<0.001) than with subclinically *Streptococcus agalactiae* (6.20±0.032 mS/cm), *Streptococcus uberis* (6.28±0.048), *Streptococcus* dysgalaetia (6.33±0.123 mS/cm).

Table 6. Investigation of milk yield and somatic cell count affecting factors influence.

Source	MGG	SCC $\log_{10}$
E_i	0.000	0.000
L_j	0.000	0.000
B_k	0.000	0.000
G_l	0.000	0.000
EL_{ij}	0.546	0.061
EB_{ik}	0.024	0.976
EG_{il}	0.477	0.809
LB_{jk}	0.563	0.051
LG_{jl}	0.019	0.004
BG_{kl}	0.002	0.452
ELB_{ijk}	0.396	0.043
ELG_{ijl}	0.005	0.052
EBG_{ikl}	0.213	0.014
LBG_{jkl}	0.430	0.000
$ELBG_{ijkl}$	0.257	0.004
Model	0.000 R^2= 0.949 (Adjusted R^2= 0.944)	0.000 R^2= 0.984 (Adjusted R^2= 0.983)

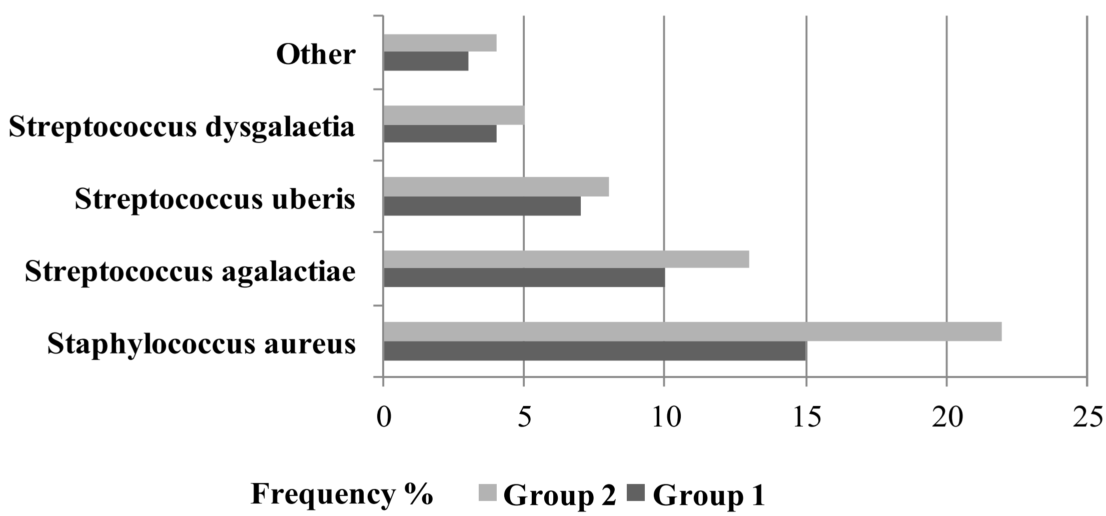

Fig.1. Bacteriological status of udder

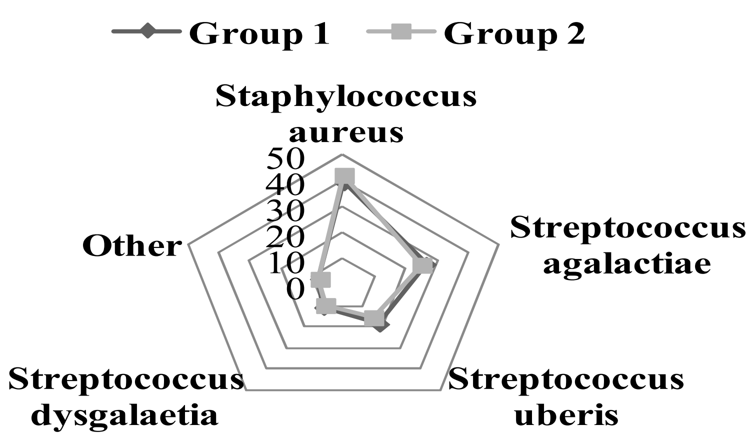

Fig. 2. Frequency of mastitis (%) by EMHF group

DISCUSSION

Mastitis, as one of the most costly disease in the dairy industry, is the result of the interactions between a combination of microbiological factors, host responses in the udder, and management practices.

Different methods have been suggested for detection of subclinical mastitis. Electrical conductivity, which increases during the infection of dairy cows, is also one of the diagnostic methods. Electrical conductivity of milk as a component of an early warning system for udder health monitoring is only suitable when all influencing factors are taken into consideration and the measured values are corrected accordingly.

Ilie *et al.* (2010) reported that the mean electrical conductivity of milk was 4.53 mS/cm for the healthy and 6.31 mS/cm for clinically infected cows.

Cho *et al.* (2009) estimated the averages of ELHMF and ELAP were 6.81 mS/cm and 7.58 mS/cm, respectively. The average of ELMAX was 7.48 mS/cm and that of ELAD was 0.61 mS/cm, which are quite similar to our results.

The values of electrical conductivity obtained in our study (6.15–7.17 mS/cm) were higher than those observed by others (e.g. 5.36–5.44 mS/cm; Cavero *et al.,* (2007).

Lee and Choudhary (2006) found the averages of ELHMF and ELAP were 6.13 mS/cm and 6.47 mS/cm, respectively. The average of ELMAX was 6.45 mS/cm and MGG had low positive correlation with electrical conductivity traits ELHMF, ELAP and ELMAX (r=0.05 to r=0.13), which are quite different that our research findings.

We estimated that of all fixed effects the biggest influence on the production of cows were by ELHMF and lactation, on the somatic cell count – ELHMF and bimodality (P<0.0001).

Bimodality is associated with premilking delay time and teat preparation (Dzidic *et al.,* 2004; Sandrucci *et al.,* 2007) and has a negative effect on milking efficiency, causing increased machine time and modified milk flow parameters (Sandrucci *et al.,* 2007).The mean incidence of bimodality in the present study (29.62 %) was lower than reported elsewhere (33.8% in Samoré *et al.,* 2011; 35.1% in Sandrucci *et al.,* 2007; 50 to 56 % depending on parity in Strapák *et al.*, 2009).

It was shown by Hillerton and Semmens (1999) that electrical conductivity can be used for prediction of clinical mastitis in experimental *Streptococcus uberis* mastitis model.

Kaşikçi *et al.* (2012) found that electrical conductivity showed similarity with California mastitis test and somatic cell count in the detection of subclinical mastitis; furthermore, its reliability would further increase when used together with the other diagnostic methods.

Usually, the majority of the infections is caused by the contagious pathogens *Staphylococcus aureus, Streptococcus agalactiae*, and by the environmental pathogens *Streptococcus uberis, disgalactiae, Coliforms.Staphylococcus aureus* is the most problematic pathogen in many countries (Persson *et al*., 2011; Keane *et al.,* 2013; Verbeke *et al.,* 2014) and can negatively influence somatic cell count and milk yield throughout lactation (Paradis *et al.,* 2010).

We also found that the most prevalent pathogens of mastitis were *Staphylococcus aureus* and *Streptococcus agalalactiae* (isolated in 25-37 % samples). The higher electrical conductivity of milk was related to a greater frequency of mastitis (P=0.002). Results obtained from the study indicate that the milk from cows infected subclinically with *Staphylococcus aureus* had from 0.55 to 0.68 mS/cm higher ELHMF, than with subclinically *Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalaetia.*

We determined that somatic cell count (r=0.196) and milk yield (r=-0.222) showed the strongest unfavorable correlation (P<0.01) with ELHMF, which statistically significantly related with almost all investigated indicators of electrical conductivity. We estimated that of all fixed effects the biggest influence on the production of cows were by ELHMF and lactation, on the somatic cell count – ELHMF and bimodality (P<0.0001). The values of ELHMF from milk samples of infected cows were significantly higher than from uninfected samples (P=0.002). In addition, the milk from cows infected subclinically with *Staphylococcus aureus* had a higher electrical conductivity than with subclinical *Streptococcus agalactiae, Streptococcus uberis* and *Streptococcus dysgalaetia* (P<0.001).

For diagnosis of mastitis it is important ELHMF of milking, because of it's strong correlation with others traits of mastitis. The electrical conductivity detection in ELHMF can give further information of the udder health and predict kind of germs of mastitis. Results obtained from the study showed that ELHMF was related with contagious mastitis form.

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