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ELECTRICAL CONDUCTIVITY OF MILK AND BACTERIOLOGICAL FINDINGS IN COWS WITH SUBCLINICAL MASTITIS

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Abstract: Intramammary infections change the composition of milk and increase electrical conductivity of milk and decrease milk electrical resistance. Electrical conductivity has been used to detect mastitis during last four decades. The aim of this research was to examine the reliability of the milk electrical conductivity measuring in detection of subclinical mastitis. The experiment was conducted on a dairy farm of Holstein-Friesian breed. A total of 113 quarter milk samples were examined, 55 samples from cows in first stage of lactation and 58 from cows in third stage of lactation. Electrical conductivity (EC) of milk samples was detected by Hand-held EC meter (Draminski mastitis detector). Quarter milk samples for bacteriological analysis were taken aseptically during the morning milking in sterile test tubes. Bacteria growth was not detected in 60 quarter milk samples (53.1%), while in the other 53 samples bacteria was found (46.9%). The most common isolated bacteria in first and third stage of lactation was *Corynebacterium* spp. (38.9%) and coagulase - negative staphylococci (3.54%). High quality and healthy milk with Draminski mastitis detector was observed in 59.29% of the samples (67/113). Cows with mastitis may not always show an increased EC of milk from the infected quarter. Electrical conductivity of milk can give useful informations about udder health status, but hand-held EC meters, such as Draminski mastitis detector, cannot be used alone in diagnosis of subclinical mastitis.

Key words: electrical conductivity, Draminski mastitis detector, subclinical mastitis, cow

Introduction

In dairy industry, intramammary infections (IMI) are among the most important diseases of cows that cause great economic losses (*Boboš et al., 2013*). Mastitis is a response of udder to different internal and external factors (*Varatanović et al., 2010*), and substantially affects on the milk quality and production of dairy cow. Bacterial pathogens are major threat to mammary gland, which cause irritation and pathological changes in mammary tissue. The degree of changes depend on the pathogenicity of bacteria and the inflammatory response (*Sharif and Muhammad, 2008*). Clinical mastitis is easy to detect by clinical signs of the disease (colored and painful udder, oedema, watery appearance of milk, milk with flakes, clots or pus), but subclinical mastitis is difficult to diagnose. Subclinical mastitis is 15 to 40 times more common than the clinical form (*Jasper et al., 1982; Kelly et al., 2011*). The most important major mastitis pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* (*Katić, 2012*). In the last few years, there has been recorded an increase in udder infections with minor mastitis pathogens - *Corynebacterium* spp. and coagulase - negative staphylococci (*Indriss et al., 2013*). Mastitis caused by minor mastitis pathogens is typically a mild, subclinical reaction that is associated with increased milk somatic cell count (*Reyher et al., 2012*).

IMI changes the composition of milk, they can increase somatic cells count and electrical conductivity of the milk (*Pyörälä, 2003*; *Shahid et al., 2011*). Over fifty years, somatic cell count (SCC) is a useful indicator of the health status of mammary gland. SCC in milk from healthy quarters is less than 200 000 cells/mL. Healthy udder quarter contains only 1-11% neutrophils, but, during inflammation, the proportion of neutrophils increases over 90% (*Sharif and Muhammad, 2008*). Beside intramammary infection, the stage of lactation, age of cows, chronic diseases, mechanical and thermal irritations of udder tissue affect the somatic cell count. SCC is high immediately after parturition and increases slightly to the end of the lactation.

Electrical conductivity (EC) has been used to detect mastitis during last four decades (*Linzell and Peaker, 1975; Hamann and Zecconi, 1998*). EC is determined by the concentration of anions and cations in milk. Concentration of sodium and chloride ions increases in milk from infected quarters which leads to increased electrical conductivity of milk (*Kitchen, 1981*). As a results of the damage to the udder tissue, concentration of lactose and potassium decrease, and concentration of sodium and chloride increase. EC of milk can show substantial variation in the absence of mastitis due to factors such as lactation stage, age of the cow, milking interval and oestrus (*Biggadike et al., 2000*). Also, factors such as milk temperature, pH and fat concentration in milk have influence on the measurement of EC. Electrical conductivity of milk has a positive correlation with somatic cell count.

To the best of author’s knowledge, no reports have reported the diagnostic application of measuring milk electrical conductivity in cows with subclinical mastitis in Republic of Serbia. The aim of this research was to examine the reliability of the milk electrical conductivity measuring in detection of subclinical mastitis.

Material and Methods

The experiment was conducted on a dairy farm of Holstein-Friesian breed. General condition and udder status were evaluated by clinical examination of animals. Udders of cows were examined visually and by palpating for the presence of any udder changes (redness, swelling, pain, heat). Also, milk samples from each quarters were examined for the presence of flakes and clots. Animals with visible signs of inflammation were not included in the study. Following the production cycle, milk samples were taken from cows in stage 1 - peak (0-50 d) and from the same cows in stage 3 - late lactation (121-200d) (*Novak et al., 2009*). A total of 113 quarter milk samples were examined, 55 samples from cows in stage 1 and 58 from stage 3.

Electrical resistance of milk samples was detected by Hand-held EC meter (Draminski mastitis detector, Poland). The results of milk electrical resistance measured with the Draminski mastitis detector were interpreted according to the manufacturer's instructions (Table 1). Concentration of sodium and chloride ions increases in milk from infected quarters which leads to increased electrical conductivity of milk and decreased milk electrical resistance.

Table 1. Interpretation of results obtained with Draminski mastitis detector (www.draminski.com)

Readings	Interpretation of results
Above 300 units	The milk sample is of high quality and is healthy. The incidence of subclinical mastitis is very low
Between 250 and 300 units	A progressively increasing incidence of subclinical infection as readings decrease
Below 250 units	This is an indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states. This is typified by somatic cells present rising from less than 1 million up to many millions

Milk samples were collected using aseptic techniques in sterile test tubes. Before sampling, cleaning and disinfection of the udder teats were done using 70% alcohol. The samples were labeled with cow's ID number and the teat from which sample was collected, and submitted to the laboratory for analysis at the temperature of refrigerator. From each sample, 0.1 mL of milk was plated on Columbia blood agar base (Oxoid, Basingstoke, UK, CM0331) with 5%

defibrinated ovine blood, MacConkey agar (Oxoid, CM0007) and Sabouraud dextrose agar (Oxoid, CM0041). Plates were incubated during 72h at 37°C under aerobic conditions, and microbial growth was mentored daily. The isolates were identified by their cultural characteristics, microscopic appearance in Gram stained preparations, catalase reaction, coagulase test with rabbit plasma and CAMP test.

Results and discussion

The study included 113 quarter milk samples from cows without clinical signs of mastitis in first and third stage of lactation for bacteriological examination and determination of electrical conductivity of milk. No bacteria growth was detected in 60 quarter milk samples (53.1%), while in the other 53 samples bacteria was found (46.9%). Results of bacteriological findings are shown in Table 2.

Table 2. Bacteriological findings in milk samples in different stage of lactation

Bacteriological finding	**Stage 1**		**Stage 3**		**Total**	
	N	**%**	**N**	**%**	**N**	**%**
No bacterial growth	30	54.54	30	51.72	60	53.1
Streptococcus agalactiae	1	1.82	-	-	1	0.9
Corynebacterium spp.	22	40	22	37.93	44	38.9
Coagulase - negative staphylococci	1	1.82	3	5.17	4	3.54
Trueperella (Arcanobacterium) pyogenes	1	1.82	1	1.73	2	1.77
Other bacteria	-	-	2	3.45	2	1.77
Total samples	**55**	**100**	**58**	**100**	**113**	**100**

The most common isolated bacteria in first and third stage of lactation was *Corynebacterium* spp. (38.9%) and coagulase - negative staphylococci (3.54%). This indicates an increase of prevalence mammary gland infection with minor mastitis pathogens. These results correspond with the conclusions of *Indriss et al. (2013)* who reported an increase of intramammary infections with minor mastitis pathogenst in 106 out of 390 milk samples (27.18%).

Electrical resistance of milk from cows in different stage of lactation is given in Table 3.

Table 3. Electrical resistance of milk in different stage of lactation

Electrical resistance	**Stage 1**		**Stage 3**		**Total samples**	
	N	**%**	**N**	**%**	**N**	**%**
Above 300 units	52	94.55	15	25.86	67	59.29
Between 300 and 250 units	3	5.45	26	44.83	29	25.67
Below 250 units	-	-	17	29.31	17	15.04
Total samples	55	100	58	100	113	100

Results pointed to increased incidence of subclinical mastitis in late lactation period (74.14%). In third stage of lactation composition of milk is changing along with increasing of somatic cells number. Rapid increase of EC (resistance below 250 units) in milk from cows in first stage of lactation was not detected. High quality and healthy milk with Draminski mastitis detector was observed in 59.29% of the samples.

Electrical resistance of milk and bacteriological findings in first and third stage of lactation are presents in Table 4 and Table 5.

Table 4. Electrical resistance of milk with different bacteriological findings in first stage of lactation

Bacteriological findings	N	Electrical resistance					
		I	**II**	**III**	**Min.**	**Max.**	**Mean value±SD**
No bacterial growth	30	28	2	/	260	700	403±80.14
Streptococcus agalactiae	1	1	/	/	450	450	450
Corynebacterium spp.	22	21	1	/	270	470	404.55±45.33
Coagulase - negative staphylococci	1	1	/	/	360	360	360
Arcanobacterium pyogenes	1	1	/	/	330	330	330

I- Value above 300
II- Value between 300 and 250
III- Value below 250

Value of electrical resistance above 300 unites in first stage of lactation was the most detected value, in samples where bacteria were not isolated (Table 4). Draminski mastitis detector indicated lower milk electrical resistance in two bacteriologically negative samples. Only three samples had value of electrical resistance between 300 and 250 which points to the possibility of appearance subclinical mastitis. *Norberg et al. (2004)* indicates that cows with mastitis may not always show an increased electrical conductivity of milk from the infected quarter, but the variation in EC of milk from infected quarters may be larger than variation in EC of milk from healthy quarters. Bacteria were isolated in 54.54% of milk samples (30/55), while Draminski mastitis detector gave false negative results in 43.64% of samples (24/55).

Table 5. Electrical resistance of milk with different bacteriological findings in third stage of lactation

Bacteriological findings	N	Electrical resistance					
		I	**II**	**III**	**Min.**	**Max.**	**Mean value±SD**
No bacterial growth	30	7	16	7	190	520	277±60.3
Corynebacterium spp.	22	6	6	10	190	400	264.55±56.12
Coagulase - negative staphylococci	3	2	1	/	300	320	310±10
Arcanobacterium pyogenes	1	/	1	/	290	290	290
Other bacteria	2	/	2	/	260	290	275±21.21

I- Value above 300
II- Value between 300 and 250
III- Value below 250

In third stage of lactation, no bacteria growth was noticed in 51.72% of milk samples (30/58), but hand-held meter gave false positive results in 76.67% of these samples (23/30). These findings correspond with results of other authors (*Musser et al., 1998; Ruegg and Reinemann, 2002; Pyörälä, 2003*). False negative results were detected in 28.57% of milk samples (8/28) where minor mastitis pathogens (*Corynebacterium* spp. and coagulase - negative staphylococci) were isolated.

Measuring EC of milk in infected quarters with minor mastitis pathogens sometimes is difficult. *Woolford et al. (1998)* were more readily detected infections of udder with major mastitis pathogens than infections with coagulase - negative staphylococci. The most likely cause of this is less damage and inflammation by minor mastitis pathogens and the possibility that such infections were localized in the teat canal and teat sinus.

Stage of lactation has great influence on the electrical conductivity of milk. Concentration of chloride ions in milk increases physiologically as lactation progresses what affects on EC of milk in all four udder quarters. Higher values of electrical conductivity of milk in infected quarters can be noticed only in that quarter (*Sheldrake et al., 1983*).

Conclusion

Electrical conductivity/resistance of milk can give useful informations about udder health status, but hand-held EC meters, such as Draminski mastitis detector, cannot be used alone in diagnosis of subclinical mastitis. This conclusion correspond with findings of other autors (*Hillerton and Walton*, *1991*). Results of electrical conductivity of milk should be supplemented with bacteriological findings in milk or somatic cell count.

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Električna provodljivost mleka i bakteriološki nalaz kod krava sa subkliničkim mastitisom

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Rezime

Intramamarne infekcije utiču na hemijski sastav mleka dovodeći do povećanja električne provodljivosti, odnosno smanjenja električne otpornosti mleka. Električna provodljivost mleka se koristi u detekciji subkliničkih mastitisa tokom poslednjih četiri decenije. Cilj istraživanja je da se ispita pouzdanost merenja električne provodljivosti mleka u otkrivanju krava sa subkliničkim mastitisom. Istraživanje je sprovedeno na farmi visokomlečnih krava holštajn frizijske rase. Ukupno je pregledano 113 pojedinačnih uzoraka mleka krava, odnosno 55 uzoraka od krava u prvoj fazi laktacije i 58 uzoraka od krava u trećoj fazi laktacije. Električna provodljivost mleka određena je Draminski mastitis detektorom. Pojedinačni uzorci mleka za bakteriološku analizu uzeti su tokom jutarnje muže, aseptičnom tehnikom u sterilne epruvete. Bakterije su izolovane iz 53 uzorka mleka (46,9%), dok je 60 uzoraka mleka (53,1%) bakteriološki bilo negativno. Najčešće izolovane bakterije tokom prve i treće faze laktacije bile su *Corynebacterium* spp. (38,9%) i koagulaza - negativne stafilokoke (3,54%). Prema vrednostima električne provodljivosti dobijenim Draminski mastitis detektorom, 59,29% uzoraka mleka (67/113) pokazalo je higijensku ispravnost visokog kvaliteta. Električna provodljivost mleka ne mora uvek biti povećana u inficiranim četvrtima vimena krava. Merenje električne provodljivosti mleka može da pruži značajne informacije o zdravstvenom statusu vimena, ali Draminski mastitis detektor se ne može koristiti sam u otkrivanju subkliničkih mastitisa.

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