Mastitis detection based on electric conductivity of milk

M. Janzekovic [a,*], M. Brus [a], B. Mursec [a], P. Vinis [a], D. Stajnko [a], F. Cus [b]

[a] Faculty of Agriculture and Life Sciences, University of Maribor,
Pivola 10, 2311 Hoce - Slivnica, Slovenia

[b] Faculty of Mechnical Engineering,University of Maribor,
Smetanova 17, 2000 Maribor, Slovenia

* Corresponding author: E-mail address: marjan.janzekovic@uni-mb.si

Properties

ABSTRACT

Purpose: If measurements of the increased total count of somatic cells in the individual udder quarter or in the whole udder could be determined with electric conductivity by means of the microprocessor-controlled device Mastitron LF 3000 enough precisely to predict the presence of subclinical mastitis.

Design/methodology/approach: The occurrence of increased count of somatic cells in milk was found out group by group by the method of measuring the electric conductivity of milk. For the milk conductivity the average measurement from all four udder quarters was taken into account. The population of 102 lactating cows (Black and white, Simmental and Brown Swiss breed) on seven farms fore three summer months was observed.

Findings: It was established that higher average electric conductivity than 6.5 mS/cm confirmed in 80% also the increased count of somatic cells in milk. When evaluating the differences between the quarters exceeding 1 mS/cm also a higher total count of somatic cells was confirmed in 73.7%. Moreover, statistically significant relation (P<0.01) between the CMT test and the ECM was found out so it can be claimed that the two methods do not exclude themselves mutually.

Research limitations/implications: It was found that the reliability of the ECM in our case was 80% depending to a large extent on the milk composition which changed depending on the stage of lactation, type of feed and general health condition of animals. For a more reliable, accurate and faster implementation of the ECM method further researches will be necessary.

Practical implications: Though the ECM method of determination of the subclinical mastitis of the milch-cows is well established in the world, it is not yet known well enough in the Slovene practice as a method of diagnosing the subclinical mastitis.

Originality/value: It was found that the results of our research in production circumstances were comparable with the indications of the maker and foreign researchers.

Keywords: Electric properties; Milk, Somatic cells; Mastitis; Electric conductivity

1. Introduction

Healthy udder of milking cows is one of the most important factors of successful breeding from the health as well as economic point of view. According to [5], the cattle breeder has 43% higher costs due to occurrence of mastitis or increased count of somatic cells in the milk, since less milk is produced. Out of that, 16% of costs can be attributed to unusable milk, 23% to additional care of cows and 18% to veterinary services. The affected animals produce less milk in lactation and in the entire life. Due to permanent dry-out of one or several quarters of the udder the market value of the animal is reduced or the animal may be isolated from production. Measurements of electric conductivity of milk (ECM) were aimed at establishing, whether the presence of hidden mastitis and, consequently increased TCSC (total count of somatic cells) in the individual udder quarter or in the entire udder could be determined by means of the microprocessor-controlled device Mastitron LF 3000 precisely enough.

Mastitis features

The occurrence of mastitis depends on the balance between the virulence of pathogenic organisms and udder resistance and also on the period of the exposure of the lactic gland to infection (Veber, 2001). If the udder resistance is high, clinical form of mastitis does not appear. The clinically ill quarter is treated with antibiotics. In most cases bacteria return into the ill quarter, only rarely they are eliminated.

It is estimated that about 50% of milch-cows have pathogenic bacteria on the average in two quarters; 1 to 3% of cows always show symptoms of mastitis. For the control and/or prevention, diagnosing and treating of mastitis the microbic causers as well as the manner of their transmission must be known. On the basis of certain features and similarities they are grouped into individual groups. Mastitis is caused by at least 20 different bacteria, mainly of streptococcus and staphylococcus varieties.

Varieties of mastitis

The illness can progress with noticeable changes or clinical symptoms. In case of clinical mastitis the structure of milk (change of colour, flakes) and udder (swellings, tissue hardenings, higher temperature) changes and also the general condition of animals may deteriorate.

Very often the animal may fall ill without visible health changes-subclinically. Such type of inflammation can be detected only by cytological and/or bacteriological examination of milk. Subclinical mastitis without visible health changes may pass into clinical mastitis and inversely [1].

Three major forms of mastitis, differing in clinical symptoms and resulting consequences, are known:

- Serious illness is accompanied by pain, red and warm swelling, easy to be noticed. This is an acute illness with serious infection of one udder quarter only or the whole udder.
- Festering mastitis appears, when the whole animal is infected. In such cases difficult problems, resulting even in death, can arise.
- In case of chronic mastitis the outside symptoms are not visible and it can reside in the udder for a long period. It may spread and calm down again.

Bacterial causers of mastitis and manners of infection Hlebec - Logar (2000) state two manners of infection with bacteria:

a) Bacteria living in an environment, where unfavourable conditions (barn, ground, litter, manure, feed, water etc.) ensure their formation and the udder infection and cause infection.

b) Bacteria associated with udder for their survival do not survive for a long time in the environment, are contagious and are transmitted from the infected udder of one cow to the healthy udder of other cow.

According to [7,8] in case of spontaneous appearance of mastitis the microbic causers may enter into the lactic gland in the following ways:

1. Through teat mouth and teat canal into the teat and milk cistern and outgoing canals - galactogenic infection;
2. Through injured spots on the teat and udder skin - wound infection;
3. With blood from another inflamed focus in the organism - haematogenic infection.

Most often the microorganisms enter into udder through the teat opening into the udder tissue. The gland tissue with outgoing canals is arranged into 4 quarters; both udder halves are divided with the supporting middle ligament. The front and rear quarters are separated, too, though no anatomical barrier is visible. So, direct transmission of inflammations from one to the other quarter is prevented [11].

Frequently, the milk samples sent to laboratory for bacteriological examination because of suspicion about mastitis are found sterile. In such cases non-specific or aseptic inflammations are in question. In such cases an increased count of somatic cells are present in the milk, while the causer is not present.

According to [11,] the existing illnesses are treated by the antibiotic therapy. To that end the dry - out period is most efficient. In that case no problems concerning antibiotic remainders in the milk arise. The effective programme of the mastitis control comprises prevention of new infections and elimination of the existing ones (about one half of the milch-cow herd remains infected). By successful practical strategies of mastitis suppression it is possible to reach only 80 to 85% of healthy cows in the herd. New infections are prevented by isolating infected cows, hygienic milking and disinfection of teats after milking.

It is well known that the first milk jets contain more bacteria than the last ones, whereas the residual milk is supposed to be even sterile. A healthy udder always secretes faultless milk which is infected only subsequently (outside conditions), while according to the contrary theory always the most important mastitis causers are present in the cows' udder. However, often the pathogenic bacteria are not detected in the udder in spite of increased TCSC. In addition to the presence of bacteria the appearance of mastitis is influenced also by other factors, such as unfavorable outside conditions, innate or acquired anomalies of teats and udder, incorrect milking and increased sensitivity of animals [7,8].

The increase of the TCSC in the milk can be affected by a number of factors [6, 17]:

- stalls - if too narrow or too short, they can cause teat and udder damages;
- humid and dirty litter contributes to procreation of bacteria, does not insulate cold floor;
- barn - excessive occupation with animals and bad air in it;
- season - in spring the TCSC in the milk is lower than in autumn;
- daily fluctuations - the milk of morning milking contains less SC than the milk of evening milking;
- grazing - the udder is exposed to fast temperature changes between the day and night; heat; during the autumn months the cows lie on cold and wet ground;
- dirty exits, watering-places and trodden paths contaminate the udder, which contributes to the development of infections;
- milking techniques - ingress of causers is possible by direct transmission of germs from cow to cow, from one quarter to another, by reduced resistance to infection causers and due to mechanical action of milking-machine on teat canals;
- the vacuum fluctuations and blind milking cause strong irritation of udder, therefore the udder reacts with increased formation of somatic cells in the udder. In that case the presence of bacteria and other microorganisms can not be proved;
- incorrect milking out;
- milking hygiene - dirty udder and wet cleaning are most favorable to appearance of diseases;
- feeding and/or change of feeding - feed rations poor in E vitamin and selenium supposedly have particular influence on occurrence of mastitis with microorganisms transmitted from animal to animal. Change of feed and its irregularity cause metabolic trouble (many carbohydrates, little proteins, and too little voluminous feed). The way of feeding has particular influence on the count of microorganisms. That implies fast changing of rations, offering mouldy, rotten, muddy and frozen feed in winter and in summer too much young succulent or limp grass causing diarrhea and having effect on the count of microorganisms in milk. On the other hand, it was not found out that the feed had an express effect on the count of somatic cells;
- stresses occurring due to fast weather changes, ill treatment of animal, transport, increased temperature.

Individual properties of animals

The CSC also depends on the individual properties of animals [2, 14]:

- cow's age - older cows are more susceptible due to reduced immune and physical resistance of the organism. With the increase of the number of lactations also the CSC can increase. The count of cells in the milk of cows after first calving is lower. Older cows and cows with higher milk flow (higher milk production) are more susceptible to diseases;
- animal's health condition - negative effects of diseases, such as puerperal paresis, delaying of placenta and long postnatal discharge, disease during previous lactations increase the hazard of occurrence of mastitis;
- chronic diseases;
- hoof trouble;
- infection with the causers of udder inflammation, infections and/or inflammations - the raiser must pay highest attention to cows with chronic mastitis, subject often to consecutive increases of the CSC;
- treatment with antibiotics;
- dry - out period - the highest hazard of occurrence of mastitis occurs during the dry - out period, during the first week and during the last two weeks of dry - out, when the udder is subject to changes and is, therefore, very much exposed to infections. When the daily production drops below 4 kg, the CSC is increased. Table 5 shows the frequency of occurrence of new infections during the individual period;
- lactation period - at the beginning the CSC is slightly increased, afterwards it drops and reaches the minimum at the peak of lactation (Table 2);
- lactation time - according to the results of researches of the extent and time of duration of increased formation of SC the cows can be grouped into three principal groups with respect to the curve during lactation;
  1. CSC in cows with optimally healthy udder (50000 to 120000)
  2. CSC in cows with sudden appearance of inflammation
  3. CSC in cows with chronic udder inflammations (great fluctuations, hidden and acute mastitis interchange)
- heat period;
- calving - 8 to 14 days after calving the CSC in milk is naturally increased. The blood vessels in the udder are then permeable for transmission of the defense substances from cow to calf and also for leucocytes. In this way, the transmission of the defense substances from cow to the new-born calf is ensured;
- shapes of teats and udder - incorrect shapes of teats, teat canals, state of teat openings (too short canal with damaged epithelium, weak sphincter) and udder (large and hanging) allow easier transmission, penetration and procreation of bacteria;
- genetic properties - heritability for susceptibility of cows to mastitis is low (1 to 3%), while for the SC it is considerably higher, i.e., 10 - 15% according to recent studies. In the future, the selection program will include also the breeding value "CSC" which will provide precious information for selection of the bull;
- raising of animals with increased resistance to mastitis (Dutch geneticists). This will be ensured by immediate accompanying of appearance of SC and raising the animals having high and well attached udder. All this is combined in the so-called M-index and/or index of the udder health. The higher the index, the greater the resistance to mastitis.

According to [6, 16] the highest risk of occurrence of the lactic gland inflammation occurs during the first and second period after dry - out. During the entire dry - out period mastitis can appear on one half of cows, i.e., 42%. Most new infections occur during the first month of lactation, when the animal's organism is most burdened. During the first week after calving the cows are more susceptible to infection, too. During the entire lactation, only slight fluctuation of the CSC occurs on cows with healthy udder. The acute variety of mastitis is accompanied by a

very high CSC (over 1.000.000 SC/ml) which, however, drops to the normal level again after recovery. Greatest problems appear on animals with chronic and/or subclinical mastitis, where a very high fluctuation during the entire lactation is characteristic of the CSC. The CSC drops below 400.000/ml very rarely, while it can increase for several millions.

Studies of the influence of some factors on the basic milk ingredients which are considered to be the indicators of subclinical mastitis; they have come to the following conclusions [9]. By constant monitoring they performed the milk profile test including a number of parameters with which the metabolic and health state of milking cows can be monitored. Monthly, they took the milk samples (n = 804) of milking cows (n = 105) from five farms and determined the basic milk ingredients (% of fats, % of proteins and % of lactose) and the CSC in milk. With respect to the CSC the milking cows were divided into five groups: 1st group up to 50.000/ml, 2nd group from 50.000 to 100.000/ml, 3rd group from 100.000 to 200.000/ml, 4th group from 200.000 to 400.000/ml and 5th group over 400.000/ml. They established that linearly with the increase of the CSC the protein content increased statistically significantly, whereas the lactose in the milk dropped. By the multiple analyses of variance they estimated the influence of other factors (farm, breed variety, physiological period, lactation, and annual season) and showed the share of explained variance. By the statistical model they explained 44.18% of variability for the TCSC in milk. The consecutive lactation, physiological period and the TCSC had a statistically significant high influence ($P<0.05$) on the milk ingredients.

Studied on the correlation between the CSC and some milk ingredients (fat, lactose, proteins and dry matter) during different lactation periods. They took samples of 90 Holstein-Frisian cows. The cows were divided into three groups with respect to the lactation period. A standard lactation period of 305 days was taken into account. The milk samples were taken during the morning and evening milking. After completion of analyses they came to the conclusion that the correlation between the CSC and the milk fat dropped with the increase of quantity of fat. It was highest, i.e., 0.82 during the stage I of lactation, 0.50 during the stage II and 0.41 during the stage III. The correlation between the CSC and the lactose quantity was higher during later periods and increased from 0.38 over 0.91 to 0.91 during the lactation stage III. The correlation between the CSC and the protein quantity had similar tendencies on the fat; stage I: 0.72, stage II: 0.70 and stage III: 0.27. The correlation between the quantity of dry matter and the CSC was 0.79 during the stage I, 0.53 during the stage II and 0.41 during the last stage.

Detection of mastitis by fast methods in barn

When finding out the udder diseases also the fast barn methods are available in addition to laboratory researches, where the bacteriological examinations (with respect to microbic causers) and the cytological examinations of milk (count of cells) are performed. Those methods include:

- clinical examinations of udder and secretion;
- test for milk taste;
- finding out the milk secretion trouble;
- establishing the milk electric conductivity.

Clinical examination according to [7] requires first the information about the animal's general condition which is the result of feeling of the animal, feeding, possible trouble or diseases, but also age and milk productivity of the individual cow. The next step is the clinical examination of the udder itself by paying attention to:

- size and shape of individual quarters and entire udder;
- shapes and particularities of teats;
- possible damages and medical changes of skin;
- self secretion of milk.

Test for milk taste is an additional possibility of mastitis detection, but it is little used. Looking outside, hardly any changes are noticed in the initial stages. However, the increased count of somatic cells as the principal symptom of mastitis causes the change of the milk composition. It can be detected by testing because the milk has a slightly salty taste. The protein quality and the ratio between minerals change (More sodium and chlorine, less potassium), there are more enzymes, the quantity of lactose and fat is reduced. The casein and fat content drops, while the pH of milk grows simultaneously with the increased chloride quantity. The milk is of slightly salty taste [10, 15]. With the increase of the CSC in the milk the production drops, all changes affect the milk technological properties, which have an effect on the defects in the manufacture of milk products.

California test or CMT It is used for finding out the milk secretion trouble based on proving the presence of SC in the milk. Routine checking implies milking out the first jets onto a dark check base on which the color and consistence of milk can be observed [11].

The California (Schalm's) test with mastitis reagent (California Mastitis Test - CMT) detecting the occurrence of mastitis on a number of white bodies in milk is most frequently used. In addition to the dye the mastitis reagent contains still some chemical substances with sulphur, which are bound to the cells in milk.

Establishing the milk electric conductivity on the basis of the operating instructions for the device Mastitron LF 3000 for measuring of the milk electric conductivity state that this method ensures:

- measuring of milk conductivity
- checking of the udder health condition directly in the barn
- early detection of mastitis
- functioning of the device LF 3000.

Fig. 1. Milk conductivity device MASTITRON LF 3000

Mastitron LF 3000 is a microprocessor - controlled device ensuring timely and simple detection of changes (mastitis) in the udder and measuring the milk electric conductivity in milisiemensov per cm (mS/cm). The membranes of mechanically damaged or infected lactic gland cells allow the input of salt from blood into milk, which results in increased conductivity of milk. Conductivity of alveolar milk even of ill animals is within the range of normal physiological conductivity. LF 3000 assures early detection of the lactic gland disease before the milk visibly changes and allows early and safe action (Figure 1).

The milk conductivity was measured by MASTITRON LF 3000 on the basis of electrical conductivity measure the ability of a solution to conduct an electric current between two electrodes, and it is measure in milisiemensov (mS). The concentration of anions and cations, with Na^+, K^+, and Cl^- as the most important, determines the EC of milk. In the mammary gland, the sodium pumps located on the baso-lateral membrane of the secretory cells are pumping Na^+ into the extracellular fluid and K^+ into the cells, while Na^+ and K^+ are transported passively between the secretory cells and the milk, across the apical membrane. In addition, a paracellular pathway is present across the epithelium (tight junctions), where Na^+ and Cl^- are moving into the milk and K+ and lactose are moving into the extracellular fluid. When a cow is exposed to an intramammary infection, EC of the milk increases due to an increased concentration of Na^+ and Cl^- in the milk. This increase is caused by destruction of tight junctions and the active ion-pumping system. As a result of the cell damage, Na^+ and Cl^- leak into the lumen of the alveolus, and K^+ and lactose move together out of the milk. However, factors other than mastitis, like breed parity, lactation stage, milking interval and milk composition may affect EC of milk [3, 13].

Further development of milk conductivity measuring devices were presented in the new conductivity meter LF 4000 immediately calculates the difference of electric conductivity between the individual quarters. If the difference exceeds 15%, it warns that the milk from the measured quarter is susceptible to inflammation. It warns by acoustic signal and red print on the display.

At the EIMA 2000 the award for the innovation went to the milking unit sensor measuring the milk productivity separately for each teat during milking. As in case of diseases the milk conductivity changes considerably, it can be established in that way, whether something is wrong with the udder and appropriate action can be taken.

2. Description of the approach, work methodology, materials for research

For the purpose of the research all cows in lactation were monitored on seven farms during a period of three summer months - July, August and September. The population of 102 animals of the Black and white, Simmental and Brown Swiss breed was observed. By the use of a more recent method of milk conductivity measuring the occurrence of subclinical mastitis on animals was detected. In total 11 examinations were performed on seven herds in the morning and in the evening. The selected farms had common characteristics in animal breeding. Barns with tied animals (Grabner's system) with short stalls were predominant.

Littering down was effected with saw-dust, as necessary. For milking they were equipped with the milk duct system, only with small herds the milking vessel was still used.

The principal problem on the farms concerns the barns which are too small for extension of the basic herd. Excessive occupation of the barn results in bad air, particularly during the hot summer months, which, however, can also be a result of improper ventilation. Too narrow and too short stalls, causing many a damage to udder and feats, are also a major drawback.

The feed rations in summer are based on all-day grazing with addition of hay and energy-rich concentrate (dried beet chips, wheat meal) and a complete concentrate for the milking cows. A considerable surplus of proteins due to high-quality young pasture occurred in all the herds concerned during that period.

Table 1.
Milk conductivity in healthy and ill quarters (operating instructions)

	Difference between quarter with highest and lowest conductivity (mS/cm)	Conductivity in individual quarter (mS/cm)
Healthy udder	<0.6	<5.5
Doubtful quarter	0.6 - 0.9	5.5 - 6.4
Subclinical disease	>1.0	>6.5
Milk must not be delivered	>1.0	>8.0

Operating instructions used in comparisons (Table 1).

Collection of data

On each farm first all the necessary data on the cow herd were collected. For each animal in lactation a register was made, where the following information was recorded:
- breed (black and white, brownswiss, Simmental, cross-breeds)
- age (from 2.5 to 13 years)
- consecutive lactation (1 to 11)
- date of latest calving
- evaluation of udder (attachment, shape, health changes)
- daily milk production (data from A inspection, farmer's estimate)
- examination of milk for the CSC

The process of milk inspection was as follows:

Once a week in the morning or in the evening we visited each farm and measured the milk conductivity on each cow in the herd, which was in lactation. On the average, the interval between the morning and evening milking was 12 - 13 hours. Before sampling the udder was not cleaned because of the likelihood of stimulation causing the entry of the alveolar milk into the udder cistern. For accuracy of the results of measurements, however only the milk from the udder cistern must be taken.

The first jets were milked out into the measuring cup of the device. The value of electric conductivity was read on the display. The procedure was repeated for all quarters and the results were entered into the tables prepared.

The conductivity measurements were performed during three months and 11 examinations on each herd were made. On the individual animals with increased measured electric conductivity of milk the CMT was simultaneously performed.

3. Description of achieved results of own researches

The results of the milk conductivity measurements during the three months' period of inspection, collected in tables for cows in lactation on each farm, were presented in Excel. Statistical processing of data was effected by statistical package SPSS 10.0.

First, the obtained results of the milk conductivity measurements were compared mutually with respect to the individual cows on the farm. For each cow the measured milk conductivity was compared for all four quarters (I - IV) and for comparison between the front and rear udder half (I - IV, II - III). Then, the milk conductivity was compared between all the cows during one inspection and, afterwards, for all inspections on the individual farm. The basic statistical parameters - average value, minimum, maximum values, standard deviation and variability coefficient (%) were presented with respect to the milk conductivity measurements performed on each farm. The data were analyzed by LSD method. Statistically significant differences were designated $P \leq 0.05$ (*), $P \leq 0.01$ (**).

To study the influence on the property examined the following statistical model was used:

$$Y_{ijklmno} = \gamma + M_i + S_j + P_k + L_l + K_m + KM_n + CSC_o + e_{ijklmno}$$

γ = constant; mean value of model
M_i = influence of i - th milk quantity (i = 5,6,...,29)
S_j = influence of j - th group (j = 1,2)
P_k = influence of k - th breed (k = 1,2,3,4)
L_l = influence of l -th lactation (l = 1,...,11)
K_m = influence of m - th cow (m = 1,...,102)
KM_n = influence of n - th farm (n = 1,...,7)
CSC_o = influence of o - th count of somatic cells (o = 7000,...,3421000)
$e_{ijklmno}$ = influence of random variable

By Pearson's correlation coefficient the relation between the ECM in quarters and the CSC was established.

Table 2.
Basic statistical parameters ($\bar{x} \pm SD$, CV) of measurements of the ECM in mS/cm on the examined animals on all the selected farms

Farms	$\bar{x} \pm SD$	CV %
A	6.1 ± 0.8	13.4
B	6.5 ± 0.7	10.4
C	5.8 ± 0.7	12.7
D	6.5 ± 0.5	8.1
E	5.6 ± 0.6	10.2
F	6.6 ± 1.1	16.8
G	6.7 ± 0.8	11.3

Legend: $\bar{x} \pm SD$ - mean value with standard deviation, CV = variability coefficient

In the Table 2 with basic statistical data the differences occurring between the mean, highest and lowest values, the standard deviation and the variability coefficient can be seen.

Particularly conspicuous is the farm F, where the difference between the measured minimum and maximum conductivity is 7.25 mS/cm. The standard deviation exceeds 1, in comparison with the arithmetic mean the CV exceed 16%.

The lowest measured values of electric conductivity of milk are on farms C and F. They reflect the fairly stable health state of animals with respect to udder diseases. The average mean value of all inspections on farms is 6.245, the mean standard deviation is 0.7309 and the average CV is 11.829.

Table 3.
Average values ($\bar{x}$) of electric conductivity of milk with standard deviation (SD) and coefficient of variability (CV) from breed to breed

Property	n	$\bar{x} \pm SD$	CV %
Black and white breed	45	6.4 ± 0.90	14.1
Simmental breed	19	6.1 ± 0.74	12.1
Brown Swiss breed	33	5.9 ± 0.74	12.6

On the average the basic statistical parameters applicable within the breeds (Table 3), shows the highest milk conductivity measured on the Black and white breed was 6.4 mS/cm with greatest standard deviation 0.90 and 14.1% CV. On the herds observed there are no significant differences between the Simmental and Brown Swiss breed (Table 3).

Table 4.
Average values ($\bar{x}$) of electric conductivity of milk with standard deviation (SD) and CV between consecutive lactations

Lactation	n	$\bar{x} \pm SD$	CV %
first and second	37	6.0 ± 0.8	13.9
third and fourth	43	6.4 ± 0.8	12.1
over five	22	6.3 ± 0.9	15.2

There are no differences between the first 4 consecutive lactations (Table 4). On animals older than 7 years having completed 5 or more lactations the difference is already more than 14% in the comparison of the SD with the arithmetic mean. In that case also the CV which was between 12.1 and 13.9 until the 5th lactation changes to 15.2 which demonstrate that in case of higher lactations the variability of the measured values is greater. Hlebec-Logar (2000) state that with the increase of the number of lactations the CSC increases as confirmed by our results.

Table 5.
Average ($\bar{x}$) values of electrical conductivity of milk with standard deviation (SD) and CV with respect to lactation period or days after bringing forth

Property	n	$\bar{x} \pm SD$	CV %
up to 150 days after bringing forth	66	6.4 ± 0.8	13.019
over 150 days after bringing forth	36	6.2 ± 0.9	14.874

In the Table 5 it can be seen that the milk conductivity does not change with the lactation period.

The first conductivity measurement on the cows after calving was performed only after 7 days. At that time, the milk is already suitable for sale to the dairy. Increased conductivity was not to be traced. According to Hlebec-Logar also the CSC is slightly

increased in the beginning of lactation, then it drops and reaches the minimum at the peak of lactation.

The next table shows the results of the performed measurements of electric conductivity with simultaneous cytological examinations. A direct comparison between the conductivity test and the CSC was made and on the basis of results of the milk inspection the cows were divided into healthy animals and animals ill with subclinical mastitis. For each cow the average calculated ECM for the entire udder was considered.

Table 6.
Comparison of the ECM test and CSC with 6.5 mS/cm limit value (average conductivity for all quarters)

CSC in 1000	n	Limit milk conductivity in mS/cm	n1	% of animals	Conductivity	Milk
< 400	55	<6.5	44	80	5.7	A
		>6.5	11	20	6.8	
>400	19	<6.5	6	32	6.3	I
		>6.5	13	68	7.0	

The Table 6 shows that in 80% of cases, i.e., on 44 cows, having average calculated ECM of 5.7 mS/cm and /or below the limit value 6.5 mS/cm, the milk contained less than 400000 SC/ml as established by the cytological examination. The udder health was confirmed by both tests. The test reliability was 80%.

In 11 cases the milk contained less than 400000 SC/ml according to the cytological examination in spite of increased ECM which amounted to 6.8 mS/cm. On those 20% of cows deviations occurred between the results of the ECM test and the CSC. Such results were reached, when the ECM was uniformly higher in alll quarters (6.1 to 7.1 mS/cm) or if the ECM was normal in three quarters (below 6.5) and increased in one quarter (from 6.7 to 8.4 mS/cm).

On 19 cows with increased CSC above the limit value 400000 SC/ml also the ECM was higher in 68%, since it reached the average value of 7.0 mS/cm. The test results were confirmed by the cytological examination.

However, on 6 cows, i.e., in 32% the milk contained more than 400000 SC/ml in spite of lower ECM which was equal to 6.3 mS/cm. This might be caused by high differences between the individual quarters (from 1.6 to 3.4 mS/cm) and/or by the type of bacterial infection.

According to the maker's leaflets and pamphlets the udder can be considered to be healthy, if the measured value of the ECM in the individual quarter is smaller than 5.5 mS/cm and/or if the difference in conductivity between the quarters is less than 0.6 mS/cm. If the ECM is higher than 6.5 mS/cm and/or if the difference between the quarters is higher than 1 mS/cm, suspicion about disease is justified. If the conductivity is higher than 8 mS/cm, the milk must be delivered no more. In our case similar results were reached as stated by the equipment maker. In most cases, with increases ECM the milk acceptable.

CSC in milk is as the key parameter indicating the actual health state of the milking cows' udder in the barn. In August also the cytological examination was performed in addition to the ECM test.

Table 7.
Average CSC in August on the farms and/or on individual herds and fluctuations of the CSC on individual cows

Farms	Average CSC/ml per cow	Minimum CSC/ml per cow	Maximum CSC/ml per cow
A	154.000	7.000	537.000
B	393.000	38.000	2.319.000
C	300.000	7.000	1.436.000
D	189.000	54.000	921.000
E	296.000	7.000	1.910.000
F	299.000	9.000	3.421.000

The average CSC on all farms was 272.000 (Table 7). An increased CSC was traced on the farm B. Out of the herd observed 4 animals showed subclinical chronic mastitis which periodically passed into chronic form. On three animals, representing 17.6% of the entire herd, the CSC exceeded 400.000/ml.

On the farm F an extraordinarily high deviation between the lowest and highest CSC/ml (3.412.000) on the individual cows occurred. In spite of that the total sample with 299.000 CSC/ml was favorable. A satisfactory total sample was reached due to thorough milking, where the animals with increased CSC are milked for calves. Once a month, the farm delivers the milk samples to the dairy for cytological examination. The obtained results are a basis for all the further action for milking itself and for treatment of animals.

The strongly increased CSC (over 1.000.000) was caused by the individual cows that did not show any symptoms or only weakly expressed symptoms of clinical mastitis (extended quarters, tissue thickenings). As it can be seen, an increased CSC, resulting from hidden udder disease on the individual cows in the herd, occurs periodically on all farms. As stated by M. Hlebec-Logar (2000) in their researches in this case the cows can be divided into three main groups with respect to occurrence of mastitis. On cows with the optimally healthy udder the CSC varies among 50.000 to 120.000. The CSC (over 400.000) is increased on the cows with acute inflammation, but most often in case of chronic udder inflammation, when clinical symptoms of the disease can not be noticed.

Table 8.
Pearson's correlation coefficients between individual quarter and the CSC

Property	Q1	Q2	Q3	Q4	CSC
Q1	1.00	0.556[*]	0.414[**]	0.518[**]	0.272[**]
Q2		1.00	0.535[**]	0.457[*]	0.397[**]
Q3			1.00	0.457[*]	0.461[**]
Q4				1.00	0.434[**]
CSC					1.00

Legend: *P≤0.05; statistical significance with 5% risk; **P≤0.01; statistical significance with 1% risk; Q1 - quarter I; Q2 - quarter II; Q3 - quarter III; Q4 - quarter IV

The Table 8 shows that statistically significant correlations occurred in measuring of the ECM and the CSC in all four

quarters. A statistically significant relation (P≤0.05) exists between the ECM of the first quarter and the ECM of the second quarter. A statistically significant positive relation (P≤0.01) exists between the ECM of the first quarter and the ECM of the third and fourth quarter. A statistically significant positive relation (P≤0.01) exists between the ECM of the second and the ECM of the third quarter. A statistically significant positive relation (P<-0.05) exists between the ECM of the second and the ECM of the fourth quarter and between the ECM of the third quarter and the ECM of the fourth quarter. Those statistically significant relations confirm the thesis that, if the udder is healthy, there are no differences between the measurements of the ECM as confirmed also by other authors. Musser et al. (1998) state that the difference in the ECM between the affected quarters is statistically significantly higher than on cows without subclinical mastitis.

Statistically significant positive relations (P≤0.01) exist between the ECM of all four quarters and the CSC. The statistically significant relation between the two mentioned properties tells that the increased CSC in the milk affects the ECM of the individual quarter on the entire udder.

4. Conclusions

On the basis of the statistical analysis of data obtained by measurements of the ECM on 102 cows in lactation the following conclusions have been reached:

- the average mean value of the electric conductivity of milk during all inspections was 6.3, the lowest being 4.4 and the highest measured value 12 mS/cm. The mean standard deviation was 0.739 and the average CV (%) was 11.829.
- the higher ECM above the limit value 6.5 mS/cm was not necessarily confirmed by increased CSC above the limit value 400.000 SC/ml.
- if the ECM is lower than 6.5 mS/cm, it does not imply that the udder does not pose any risk and that the milk contains less than 400.000 SC/ml.
- if the ECM measurements between the quarters vary strongly (differences exceeding 1 mS/cm), the milk is most often inacceptable with more than 400.000 SC/ml.
- the CSC shows a statistically positive relation to the ECM (P ≤ 0.01).
- It has been established that the average electric conductivity higher than 6.5 mS/cm confirms also an increased CSC in 80%. When evaluating the differences between quarters, exceeding 1 mS/cm, also a higher CSC has been confirmed in 73.7%.
- statistically significant relation (P ≤ 0.01) between the CMT test and the ECM has been confirmed. This proves that the two methods do not exclude each other and that they complement themselves in doubtful cases.
- in Slovenia the discussed method is not yet enough known.

It can be concluded that much additional detailed information about the use of the ECM as a method of diagnosing the subclinical mastitis will be required.

References

[1] A. Mijovic, Somatic cells in milk, European regulation – not European conditions for producers, Black and Whiter Cattle 10 (1998) 11-14 (in Slovene).

[2] A. Arkink, Breeding healthier cows, High lights, September, 2000, 9.

[3] E. Norberg, Electrical conductivity of milk as a phenotypic and genetic indicator of bovine mastitis: A review, Livestock Production Science 96 (2005) 129-139.

[4] J. Musser, K. Anderson, M. Caballero, D. Amaya, J. Maroto – Puga, Evaluation of a hand – held electric conductivity meter for detection of subclinical mastitis in cattle, American Journal of Veterinary Research 59 (1998) 1087-1091.

[5] L. Diklić, More about somatic cells and cows nutrition, Advices for farmers in Celje in Koroska region, Celje (1999) 17-18 (in Slovene).

[6] M. Hlebec-Logar, Mastitis has more images, Farmer (2000) 16-17 (in Slovene).

[7] M. Janžekovic, M Brus, L. Mužerlin, Quality and delivery of milk in Slovenia, Dairying 57 (2007) 275-288.

[8] M. Janzekovic, M. Brus, B. Mursec, F. Cus. Accuracy of calculation of body mass on the basis of measurements. Journal of Achievements in Materials and Manufacturing Engineering 23/1 (2007) 47-50.

[9] M. Klinkon, T. Zadnik, M. Nemec, Influence of some factors on the bases of milk components, Milk and milk products, Proceedings of the 4th Slovene Congress with International Participation, Portorož, 1999, 27 (in Slovene).

[10] M. Klopcic, Variability of milk components by cows in AP control and estimation of fix factors, Milk and milk products, Proceedings of the 2nd Slovene Congress with International Participation, Portorož, 1997, 21 (in Slovene).

[11] P. Caput, Cattle breeding, 1996 (in Croatian).

[12] User`s manual Mastitrona LF 3000, Scala d.o.o., Maribor, 5 (in Slovene)

[13] M.N. Sloth, and P. Løvendahl, Electrical Conductivity of Milk: Ability to Predict Mastitis Status, Journal of Dairy Science 87 (2004) 1099-1107.

[14] P. Vindis, B. Mursec, M. Janzekovic, F. Cus, Processing of soybean meal into concentrates and testing for Genetically Modified Organism (GMO), Journal of Achievements in Materials and Manufacturing Engineering 20 (2007) 507-510.

[15] B. Mursec, M. Janzekovic, F. Cus, U. Zuperl, Comparison of rollers after sowing of buckwheat, Journal of Achievements in Materials and Manufacturing Engineering 17 (2006) 269-272.

[16] B. Mursec, F. Cus, Integral model of selection of optimal cutting conditions from different databases of tool makers, Journal of Materials Processing Technology 133 (2003) 158-165.

[17] B. Mursec, F. Cus, J. Balic, Organization of tool supply and determination of cutting conditions, Journal of Materials Processing Technology 100 (2000) 241-249.