

THE ELECTRICAL CONDUCTIVITY OF CATTLE MILK AND THE POSSIBILITY OF MASTITIS DIAGNOSIS IN ROMANIA

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Summary

In this study, there have been investigated several traits of EC, in order to associate them with the health status of the udder. Fifty dairy cows were classified as: healthy/clinically infected or subclinically infected, and EC was measured repeatedly from samples collected when milking each quarter. Four traits of EC have been defined: the ratio between the highest and the lowest values of EC for each quarter (IQR), the maximum value of EC for each animal; the ratio between the highest and the lowest value of EC variation between the quarters, and the maximum value of EC variation for each cow. The values of these traits were calculated for each milking in the testing period. All these traits increased significantly when subclinical or clinical infections were present. In order to validate this method, a limit value test and a discriminatory function analysis were used. The traits that reflect a certain level of infection and less the EC variance, especially the IQR value, are the most used in order to classify the cattle from the health status point of view. By using this trait, 81.4 % of the clinical cases and 44.0 % of the subclinical cases were correctly classified. Among the cattle classified as healthy, 73.5 % were correctly categorized. However, additional information was obtained considering the health status of the udder when using the correlation between the EC traits.

Key words: electrical conductivity, mastitis, udder, milk

Milk electrical conductivity has been used as a main indicator of mastitis since the last decade (3, 8). The electrical conductivity is determined by the anions' and cations' concentration, and if the cow is affected by mastitis, the sodium and chloride concentrations would increase, determining an increment of EC value too, especially from the infected udder quarter.

Electrical conductivity (EC) was repeatedly expressed as a maximum value for each quarter or each milking when studied in the last decades (1, 7, 9). The detection models were based on the maximum values and analysis of the periodic series, by using archived information, and these showed good results when diagnosing mastitis (4, 5). Nevertheless, there still exist problems concerning the infected cattle which are not identified and the clinically healthy cattle, which are classified as ill, these errors coming from a superficial description of each trait. By selecting the high EC values and using them in these studies, precious information could be obtained (2, 6). A dairy cow with mastitis will not reveal constantly high values of milk EC, but the values of variation between milking times from an infected quarter can be higher than the EC variation of milk obtained from healthy

quarters. The cause for a situation like this can be correlated with the physical transformations suffered by the milk during mastitis, with consequences on the milk flow. A combination of the measuring level and EC variation can determine an improvement when describing a trait like this.

The objectives of this study are:

(1) To quantify the variation' association of different EC traits, in this case the level and variation between the milking times with the health status of the udder.

(2) To determine whether a combination of the traits could improve the classification ability of cattle when taking into account the health status of the udder.

Materials and methods

The experimental data were obtained for a 2 month period, at the end of 2009, and the cattle included in the investigation are classified as dairy cows, from Romanian Black Spotted breed, and hybrids of Holstein Friesian and Jersey. A number of 50 dairy cows were investigated during this study, these being raised and exploited in tie stalls systems, being fed on balanced meals, considering the physiological status.

The EC measurements were realized by using EcoMilk Ultrasonic Analyzer. This is a device for multi-parameter analysis of milk, with the ability to determine precisely the fat percentage, the protein content, the solids-non-fat, lactose, density, freezing point value, added water percentage, pH, temperature and electrical conductivity for cattle, sheep, goat and buffalo milk.

The sampling was realized weekly, and the EC analysis was conducted for each quarter individually.

Among the data, the ones with EC values of < 3 and > 12 mS were considered extreme, being excluded from the study. Statistically, there have been obtained the average value for EC highest values from a single milking (X_{20}) and the variation of all valid EC measurement from a single milking (σ^2_{EC}), for each quarter. The next EC measurements were calculated for each milking : Max X_{20} (the highest value among the quarter data for each animal and each milking time), Max σ^2_{EC} (the highest value for σ^2_{EC} for each animal and milking time), IQR X_{20} (the ratio between the quarters considering the highest and the lowest value of X_{20} for each animal and each milking time) and IQR σ^2_{EC} (the ratio between the quarters for the highest and the lowest value of σ^2_{EC} for each animal and milking time individually).

In this investigation, there has been applied a surveillance plan considering the udder health status. The samples for each quarter were collected once a week. These samples were subjected to analysis with EcoMilk Ultrasonic Analyzer, in order to obtain the physico-chemical parameters, among them EC. In this study, there have been included only morning milking times.

At the cattle exploitation unit, a series of bacteriological examinations of the milk were realized, in order to identify the etiological agents of mastitis.

The dairy cows investigated in this study were defined as: healthy, clinically infected and subclinically infected. Those dairy cows with bacteriologic negative results were classified as healthy. The subclinically infected cows were the ones which received drug treatment after presenting mastitis symptoms. All the drug treatments were realized by a veterinarian, using the same protocol for each infected cow. Considering the clinically infected cows, the microbiological examination presented positive results. The infected cows (clinically and subclinically) with no anterior registered information were excluded from this study, as well as the ones with all four quarters infected. So, after applying this selection, there have been obtained samples from 47 cows.

From the statistical point of view, there has been used a variance models' analysis method, (PROC GLM – “general linear method”) and the significant difference testing for EC traits, between the healthy udders and the infected ones, and also between the animals. The quantified traits for each animal (Max X_{20} , Max σ^2_{EC} , IQR X_{20} , IQR σ^2_{EC}) were tested in order to associate them with the health status of the udder. For each week testing, a certain health status was diagnosed, taking into account the results obtained for EC. There have also been defined a series of limit values for each category, being investigated the ability of the results to distinguish between the infected and healthy cattle. The values used in this stage were selected as to cover the cases in which the cattle were diagnosed presumptively as well as the ones in which they were considered healthy.

The ability of this parameter to reflect the health status of cattle was expressed as sensibility and specificity. The cases in which EC was correlated with the clinical signs of mastitis were considered true positive (TP), and the ones in which EC was not the indicator for mastitis, but the clinical signs were present were classified as false negative (FN). The cases in which mastitis was not diagnosed through EC and the clinical signs were not present were considered true negative (TN) and the cases in which healthy cows were considered infected were called false positive (FP). The sensitivity represents the percentage of infected cows which were classified as such ($TP / (TP + FN) \times 100$) and the specificity represents the percentage of clinically healthy cows, correctly classified ($TN / (FP + TN) \times 100$).

In order to determine if a combination of traits permitted the increment of the ability to classify the cattle correctly considering the health status, it has been used a discrimination analysis, a separation method for groups of two or more animals, for data obtained by measurement and for variable data. Several EC traits, obtained by measurement observations can be used as input variable distributed equally, in order to transform logarithmically the traits obtained in the ratios (IQR X_{20} and IQR σ^2_{EC}), before analysis. The four parameters of EC were separately tested and in different combinations. The separated analysis were realized on subcategories, which contained only healthy and clinically infected cows (1) or healthy and subclinically infected cows (2), in order to compare the results through a simple detection model and by discrimination function analysis. So, the clinically and subclinically infected cows, were defined as infected (3) and

in the end, the analyses were applied on all the data comprised in a set. The discriminatory analysis function performance was transposed also in sensitivity and specificity, in order to classify the cows in the existent categories.

Results and discussions

The level and variation for EC data

The results obtained for EC at quarter level are included in table 1.

Table 1

The EC value distribution (main values \pm standard error) for healthy, subclinically and clinically infected quarters.

	Healthy	Subclinically infected	Clinically infected
Number of quarters	21	115	52
X_{20}^1	$4.53^a \pm 0.01$	$5.42^b \pm 0.02$	$6.31^c \pm 1.53$
σ_{EC}^2	$0.112^a \pm 0.004$	$0.205^b \pm 0.008$	$0.747^c \pm 0.013$

In comparison with the healthy quarters, X_{20} increased significantly ($P < 0.01$) for both categories of infected quarters (subclinically and clinically). The difference in this case between the clinically infected quarters and the subclinically infected ones was also significant. In addition, the σ_{EC}^2 parameter increased for subclinically infected quarters as well as for the clinically infected ones, and also for the higher difference between the healthy quarters, in comparison with the subclinically infected ones. At the organism level, all the EC traits presented higher values for the infected cows, in comparison with the healthy ones (table 2). All the differences observed for the EC values obtained for healthy cattle, subclinically and clinically infected ones presented a high significance ($P < 0.01$). Considering the variance, the difference was even higher for the clinically infected quarters, in comparison with the healthy cows, that the difference between the healthy quarters and the subclinically infected ones.

Table 2

The distribution of EC traits (main values \pm standard error) for healthy, subclinically and clinically infected ones

	Healthy	Subclinically infected	Clinically infected
Number	22	112	54
Max X_{20}^1	$6.72^c \pm 0.06$	$5.26^a \pm 0.03$	$5.67^b \pm 0.04$
Max σ_{EC}^2	$0.807^c \pm 0.030$	$0.239^a \pm 0.015$	$0.318^b \pm 0.020$
IQR X_{20}^3	$1.342^c \pm 0.009$	$1.121^a \pm 0.004$	$1.176^b \pm 0.006$
IQR σ_{EC}^2	$16.87^c \pm 1.03$	$6.72^a \pm 0.52$	$7.54^b \pm 0.68$

¹ = maximum value of X_{20} for quarters (the average of the highest 20 values) for each dairy cow and each milking;

² = maximum value of σ_{EC}^2 for quarters (the variance registered for each quarter) for each dairy cow and each milking;

³ = the ratio between the quarters, between the maximum and the minimum value for X_{20} , for each dairy cow and each milking;

⁴ = the ratio between the quarters, between the maximum and the minimum value for σ_{EC}^2 , for each dairy cow and each milking;

^{a,b,c} = the average values for each row, with significant differences concerning the categories.

The classification of cattle considering the health status

The sensitivity and the specificity of each EC parameter for differentiation between the healthy cows and the infected ones are included in figure 1. The sensitivity was increased when separating the clinically infected cows from the healthy ones, in comparison with the differentiation of healthy cows from the subclinically infected ones. The IQR and X_{20} parameters manifested the highest sensitivity and specificity for classifying the clinically and subclinically infected cows. By using these parameters, 81.4 % of the clinical cases and 44.0 % of the subclinical cases were correctly classified. Among the cattle classified as healthy, 73.5 % were correctly categorized.

The results for the discriminatory function are included in table 3. The correct classification of healthy cows (specificity) was higher than the one comprising the rest of the limit values. In order to compare the clinically infected cows with the healthy ones (category 1), specificity had limits of 94.1 % and 96.9 % for individual parameters. In the situation of including all the parameters in this analysis, the specificity was 94.8 %. Between 17.5 and 48.1% of the clinically infected cows were classified correctly, when the parameters were calculated individually, in analyzing the discriminatory function. When combined, 29.5 % of the infected cows were classified correctly. In these analyses, IQR σ^2_{EC} was better defined than Max σ^2_{CE} .

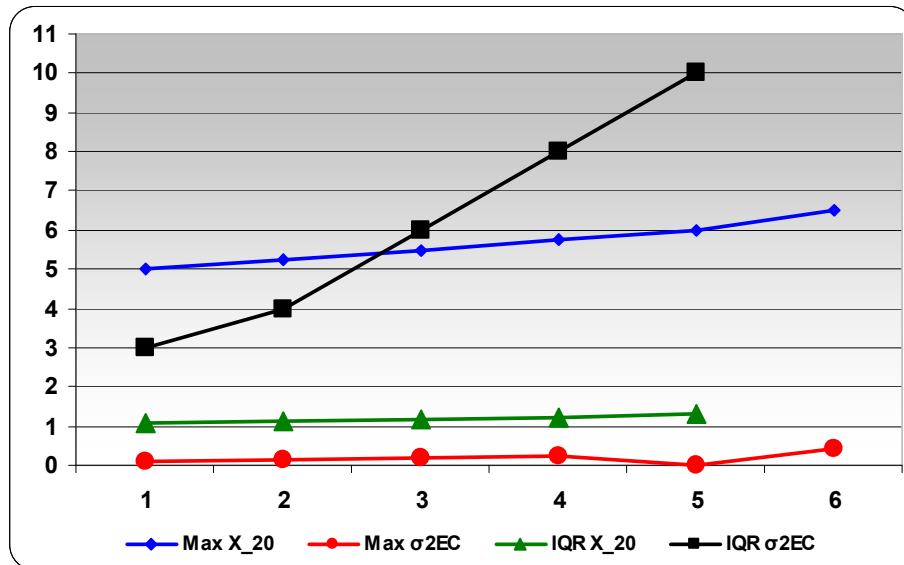


Fig.1. The sensitivity and specificity of differentiating the healthy cows from the clinically and subclinically infected ones, by using different statistical indicators calculated for milk electrical conductivity

Specificity for category 2 (only healthy cows and subclinically infected ones) was approximately similar to category 1. The two parameters of EC were similar when classifying the subclinically infected cows, so 16.9 % of them were detected when using IQR X_{20} and 17.8 % when using $MaxX_{20}$. For EC variation parameters, the discriminatory function analysis was not able to differentiate the clinically infected cows from the subclinically infected ones. When introducing all the parameters in calculus, almost 12.5 % of the subclinically infected cows were classified correctly.

For category 3 (healthy cows compared to cows infected with mastitis in general), the specificity was lower than the one from the other categories. The sensitivity was 42.7 % for IQR X_{20} , 43.2 % for $MaxX_{20}$, 23.1 % for $Max \sigma_{CE}^2$ and 32.8 % for IQR σ_{CE}^2 .

When discriminatory function analysis was performed for all the data available, the cows were classified in the three main groups: healthy, subclinically infected and clinically infected. The specificity had limits of 96.4 and 95.8 %. The measurement of EC through IQR X_{20} presented the highest sensitivity, and 44.8 % of the clinical cases were classified correctly. Only 10.5 % of the subclinically infected cows were classified correctly. By using the IQR σ_{CE}^2 and $Max \sigma_{CE}^2$ parameters, 17.1 and 16.8 % of the clinically infected cows were classified correctly. When using a combination of all the parameters, 95.2 of the healthy cows were classified correctly, in the meantime clinically infected cows were classified correctly only by 41.8 % and the subclinical infection cases were classified in 5.4 % correctly. The infected cows which were not correctly classified were introduced in healthy cow group.

Table 3

Sensitivity and specificity values used for differentiating healthy cows from infected ones by using CE statistical indicators for different categories

Statistical EC indicator	Category 1		Category 2		Category 3		Category 4		
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity clinically infected cows	Sensitivity subclinically infected cows	Specificity
$Max X_{20}^1$	17.5	94.1	17.8	92.1	43.2	78.7	30.6	10.5	93.6
$Max \sigma_{EC}^2$	16.8	98.0	8.1	97.7	23.1	90.4	16.8	1.1	95.8
$IQR X_{20}^3$	48.1	96.3	16.9	91.7	42.7	86.2	44.8	7.6	97.3
$IQR \sigma_{EC}^4$	35.6	96.9	7.2	96.5	32.8	78.5	17.1	2.5	94.2
Combination	29.5	96.3	12.5	94.5	35.4	83.4	27.3	8.9	95.2

For the variation parameter among the several milking times when samples were collected, there have been observed significant differences between the

healthy quarters, the subclinically and the clinically infected ones. The differences between the milk which comes from the healthy quarters and the one from clinically infected quarters can be explained in part through the physical transformation of the mastitis milk. Clinical mastitis determines the clotting of milk, thus decreasing the flow from the teat level, and this type of milk usually sticks on the measurement device, slowing or even making impossible the performance of milk analysis.

The values of σ_{EC}^2 were higher for the healthy cows than for the infected ones.

In some clinical mastitis cases, EC measurements from infected quarters can vary widely, without extreme values in the initial and final period of investigation. In this study, X_{20} did not present significant values for the infected quarters, in comparison to the healthy ones, but the EC value for the milk obtained from the infected quarters had a higher variation in comparison with the healthy quarters.

Among the parameters which reflect EC level, IQR X_{20} showed the highest degree of association between the clinical and subclinical cases of mastitis, no matter the type of discriminatory function analysis or limit values test used to classify the cows in health status groups. By using IQR, there are included also the factors which affect EC value equally from all the quarters. These are not correlated with the udder health status also but refer more to the breed, parity, milking periods, the milking moment of the day, the milk fractions, the milk composition, estrous phase and illness apparition.

In addition, Max σ_{EC}^2 and IQR σ_{EC}^2 were calculated in order to express the variation of EC between the milking times and variation between the quarters. It was evident that these parameters will contribute faultlessly with information considering the health status of the udder, and also the level of EC values obtained in this investigation. The sensibility and specificity were lower when evaluating these parameters individually in comparison to others. Max σ_{EC}^2 had a higher accuracy than IQR σ_{EC}^2 . Covering distributions and high variation for variance parameters can be taken into account when several difficulties appeared in differentiating the proposed categories, just when using Max σ_{EC}^2 and IQR σ_{EC}^2 .

By using discriminatory function analysis and taking into account the health status of the udder, a combination of all the statistical indicators for EC raised the correctitude of classification when taking into account the clinically and subclinically infected cows. The highest specificity was obtained by using the discriminatory function in comparison to the data obtained directly by measuring EC, but the ability of classification was low.

Conclusions

Milk electrical conductivity is influenced by the health status of the udder. The parameters which reflect the level of electrical conductivity describe the health status of the udder in a more precise way than the ones which reflect the EC

variation between the milking times. The ratios between the values obtained for each quarter are parameters for a more precise behavior than the absolute level of EC, in what concerns the classification of infected cows (either clinically or subclinically). Nevertheless, additional data must be obtained considering the health status of the udder, in order to realize a combination of EC results. The ability of EC parameters to separate the cases with subclinical infection for the healthy ones was not precise enough. Milk electrical conductivity can represent a possible indicator used for breeding programs, but there are several additional investigations needed to estimate the genetic parameters which concern the correlations of mastitis presence.

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