PROCEEDINGS OF V. INTERNATIONAL AGRICULTURAL, BIOLOGICAL, LIFE SCIENCE CONFERENCE AGBIOL 2023

18-20 SEPTEMBER 2023

EDIRNE, TURKEY

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WELCOME NOTES

You are welcome to our V. AGBIOL Conference that is organized by Trakya University. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to get benefit from the interaction with each other.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Due to COVID-19 situation, II. AGBIOL 2020 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Due to COVID-19 situation, AGBIOL 2021 was organized online again. AGBIOL 2022 conference was organized with a worldwide participation from 44 countries over 522 papers contributed by over 1300 authors.

There is a worldwide participation from 33 countries 833 papers contributed by over 2000 authors with 522 oral and 311 poster presentations in AGBIOL 2023.

The AGBIOL 2023 will be normal participation as well as with online participation in Trakya University Balkan Congress Center in Edirne, Turkey on 18-20 September, 2023. The program will include oral talks by invited prominent scientists and oral and e poster presentations by participants in selected topics from the submitted abstracts focusing on Agriculture, Biology and Life Sciences topics.

With care for our nature and environment, we aim the green congress, meaning that as little as possible papers will be used. Abstract book will be published in electronic book and will be distributed to the participants on flash memory stick as well as by e mail for online participants. All the e-posters should be prepared in electronic form and then submit to via the conference e mail and will exhibit in electronical poster boards as well as in online e poster hall in our web page during the conference.

The participants with paid conference fee will be able to access all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided participant ID and codes. The selected ABSTRACTs will be published in the Conference ABSTRACT and Proceedings Book. Participants might send us their full papers, which based on their preferences will be published either in our Conference ABSTRACT and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

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COMPARATIVE METHODS FOR DETECTION OF SUBCLINICAL MASTITIS AT DAIRY COWS IN ORDER TO IMPROVE MILK QUALITY

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ABSTRACT

The control of the health status of the udder is a significant element for obtaining a hygienically and safety milk. The aim of our research was to make a comparative analysis of the methods for determining subclinical mastitis, such as CMT and somatic cell count (SCC/mL) in comparison with electrical conductivity (EC) and lactose as an indirect method for detection of subclinical mastitis. It was determined that by increasing the number of somatic cells in milk (SCC/mL), the percentage of lactose in milk decreases from 4.80% to 4.13%, and the electrical conductivity increases from 4.21 mS/cm to 4.95 mS/cm. The number of somatic cells obtained using the MKC EN ISO 13366-2:2010 method was taken as a standard method for determining the somatic cells in milk, and based on these results, the sensitivity of the other methods was further determined. The results indicate that the California mastitis test (CMT) has 57% sensitivity and 88% specificity, while measuring the electrical conductivity (EC) has a sensitivity of 82% and a specificity of 50%. Whereas the sensitivity of the lactose is 79%, and the specificity is 60%. The sensitivity of the test, the so-called true positive rate, or probability of detection, expresses the percentage of correctly identified infected quarters. According to this with determination of EC and the percentage of lactose, more reliable results are obtained compared to the CMT test. On the other hand the specificity of the test, the ability to detect all negative samples, i.e. healthy cows, better results were obtained with the CMT test.

Keywords: mastitis, California mastitis test (CMT), electrical conductivity (EC), lactose.

INTRODUCTION

Mastitis is still one of the most significant problems in the dairy industry and one of the most expensive diseases affecting dairy cows. The losses that occur are the result of milk reduction, veterinary costs, deterioration of milk quality, and increase in the risk of subsequent mastitis (Lightner, J.K., et al., 1988). These losses are mostly caused by subclinical mastitis, while clinical mastitis can easily be determinate by the farmer (Kaşikçi, G., et al., 2012).

Diagnosing subclinical mastitis can be problematic because the milk still looks normal, but the number of somatic cells is increased (Forsback et al., 2010). These changes can be determined indirectly using several diagnostic methods such as California mastitis test (CMT), pH, chlorides, catalase test, modified White Side test (MWT) (Reddy, B. S. S., et al., 2014) as well as electrical conductivity. These tests are preferred to be used as screening tests for subclinical mastitis and can be easily used and satisfactory and repeatable results can be obtained (Leslie et al., 2002). The diagnosis of mastitis according to the International Dairy Federation (IDF) should be made based on the number of somatic cells (SCC) and the microbiological status of the quarter, i.e. bacteriological cultures of milk samples are the standard method for determining mastitis, which is financially more expensive and therefore not widely used.

For these reasons, the goal was to determine the compliance of several methods with standard protocols for diagnosing subclinical mastitis as somatic cell count. Because, in recent times, the awareness of consumers who expect quality and safety products obtained from healthy animals is increasing more and more. Precisely because of this, it is necessary to control the quality of milk on the farm itself in order to meet the demands of consumers.

MATERIAL AND METHOD

The milk samples (N=69) were taken from a farm in the Pelagonian region, with a tied cow housing system. First, the milk was milked on a black pad in order to determine if there was clinical mastitis or inflammation of the teat canal, then the milk was milked on California mastitis test (CMT) plates in order to determine if there was subclinical mastitis. Two milk samples per quarter were taken, for determination of somatic cell count (SCC/mL) and for determination of conductivity and physicochemical parameters of the milk. The samples taken were transported to the laboratory at a temperature of 5-8°C in a hand-held refrigerator, and the tests were performed within 24 hours.

The obtained results were grouped into four categories depending on the number of somatic cells. At the same time, the first category referred to normal milk, where the number of somatic cells was ≤ 200,000 cells/ml, while the second, third and fourth categories referred to the number of somatic cells from 200,001 to 400,000 cells/ml; 400,001 to 600,000 cells/ml; and ≥601,000 cells/ml, respectively.

California mastitis test (CMT). The test is based on the action of surfactants (alkylaryl sulfonate) on DNA polymer from leukocytes, during which DNA is separated, and the protein part spontaneously turns into a gel. Interpretation of the results was done as previously described by Galfi A., (2016).

The electrical conductivity (EC) was examined using a HANNA HI 98192 EC/TDS/NaCl/Resistivity conductometer, which has a measurement range of 0-400 mS/cm. The samples were analyzed after milking. During the measurement, the temperature of the samples was 20-25 °C. About 50 ml of milk was taken for analysis.

The number of somatic cells was determined using a fluoro-opto-electronic method, BENTLEY SOMACOUNT CC 150, according to standard MKC EN ISO 13366-2:2010: Milk - Somatic cell counting - Part 2: Instructions for use with fluoro-opto- electronic counter ISO 13366-2:2006. Samples intended for determining the number of somatic cells were previously preserved with bronopol and heated to a temperature of 40 °C in a water bath before analysis in the apparatus.

Physicochemical parameters in milk (fat, protein, lactose, dry matter (SNF), density, casein, pH) were analyzed using LactoScope FTIR Advanced.

The examination of the sensitivity and specificity of indirect tests was done as previously described by Sharma et al., (2010).

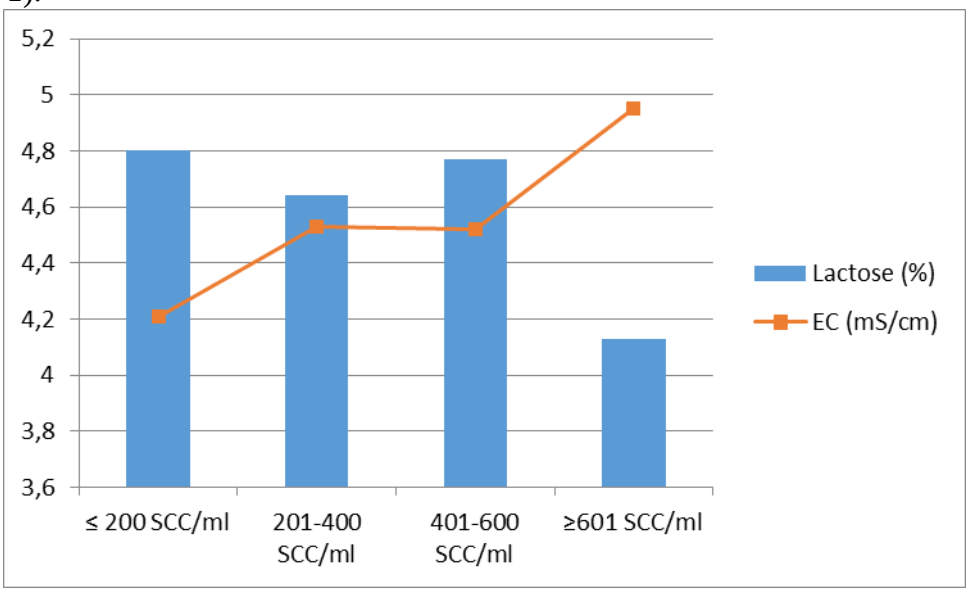
Statistical significance between the studied categories was analyzed at a significance level of 5% ($p<0.05$) and 1% ($p<0.01$) using the Student's t-test. The data are presented in tables and graphs. The results were processed using Microsoft Office Excel and SPSS 20 statistical software.

RESULTS AND DISCUSSION

Monitoring the health status of dairy cows is necessary in order to obtain quality and hygienic milk (Boboš et al., 2012). The somatic cells of the milk are an indicator of the health status of the udder as well as the hygienic quality of the milk. A large number of factors which interact with each other affect the number of somatic cells in milk, such as the lactation period,

the number of lactations, i.e. the age of the animals, milk yield, improper milking, stress, chronic diseases as well as mechanical injury to the udder tissue (Laevens et al., 1997; Pyörälä, 2003; Boboš and Vidić, 2005).

The increased number of somatic cells is usually accompanied by changes in the physicochemical composition in raw milk. The results shown in table 1 refer to the changes that occur in the milk composition, as a result of the increased number of somatic cells. Additionally, electrical conductivity in milk gradually increases with the increase in the number of somatic cells (Graph 1).



Graph 1 Changes in EC and lactose depending on the categories according to the number of somatic cells

Significant differences were determined only between the group where the number of somatic cells was over 600,000 cells/ml compared to the rest of the groups ($p<0.05$) (table 1). In addition, although we have an increase in milk conductivity when the number of somatic cells is above 200,000 cells/ml (4.53 mS/cm (201-400 x 10^3 SCC/ml) and 4.52 mS/cm (401-600 x 10^3 SCC/ml), however, no significant differences were observed, which we believe is due to the small number of samples in these two groups (N=8 and N=9, respectively). Additionally EC can have significant variations even in the absence of mastitis which can be due to a number of factors such as stage of lactation, age of cows, milking intervals as well as cow condition (Biggadike et al. 2000). Factors such as milk temperature, pH, and milk fat percentage can have an effect on EC measurement (Qayyum et al. 2016).

Table 1 Changes in the physicochemical composition of milk by category according to the number of somatic cells (N=69)

Categories according to the number of somatic cell count SCC/ml (N=69)	Milk parameters $\bar{x} \pm SD$							
	SCC/ml x 10^3	**Fat (%)**	**Proteins (%)**	**Lactose (%)**	**SNF (%)**	**pH (%)**	**Casein (%)**	**EC (mS/cm)**
≤200 x 10^3 (N=25)	77,92 ± 56,34	2,06 ± 1,78	3,32 ± 0,24	4,80 ± 0,19 [a]	9,07 ± 0,25	6,74 ± 0,06	2,79 ± 0,20	4,21 ± 0,68 [a]
201-400 x 10^3 (N=8)	322,83 ± 231,00	2,23 ± 0,54	3,48 ± 0,49	4,64 ± 0,31 [a]	9,04 ± 0,52	6,77 ± 0,07	2,92 ± 0,38	4,53 ± 2,42 [a]
401-600 x 10^3 (N=9)	466,03 ± 45,46	2,51 ± 0,69	3,65 ± 0,25	4,77 ± 0,20 [a]	8,87 ± 0,31	6,73 ± 0,10	3,13 ± 0,20	4,52 ± 0,64 [a]
≥601 x 10^3 (N=27)	1.415,65± 726,00	2,21 ± 0,64	3,64 ± 0,34	4,13 ± 0,57 [b]	8,61 ± 0,68	6,87 ± 0,11	3,04 ± 0,25	4,95 ± 1,15 [b]

*** Differences in values with different superscripts in the same column are statistically significant at the level: a:b p<0.05**

The CMT test is accepted as a quick, simple and reliable method for identifying cows with altered secretion and subclinical mastitis. At the same time, based on the results of CMT, the number of somatic cells can be indirectly determined in individual milk samples (Galfi A., 2016). Table 2 shows the results obtained using CMT, where the number of somatic cells is taken as a standard. 4% of the examined samples are false positives, while false negatives are 28%. In comparison with the studies of Galfi A., (2016), that value is 13.33% for false positive in the period before the drying of the cows, where bacteriological tests are taken as a standard. Sharma et al., (2010) states that the false positive reaction of CMT is 23.79%, while the false negative is 25.72%. Additionally, according to Varatanović et al., (2010), CMT test was positive at 11 samples, which were determinate previously as bacteriologically negative, on the other hand CMT give a negative reaction in 10 samples, previously determinate as bacteriologically positive.

The sensitivity of CMT in our research was 57%, and in the research of Galfi A., (2016) in dry cows the sensitivity of the test is 75%, while in the early lactation period the sensitivity is 87.5%. Sharma et al., (2010) found a higher sensitivity of the test (86.07%), while Langer et al., (2014) found a lower sensitivity of 60.1% compared to our research. The specificity of the test is 88% in our research, while in the research of Galfi A., (2016) that specificity of the test in the period before the drying of the cows is 86.67%, and in the period of early lactation it is 87.5%. According to the research of Dingwell et al., (2004) the sensitivity of CMT four days after parturition is 82.4%, and the specificity is 80.6%, which indicates that this method can be applied with success in determining udder secretion disorders and subclinical mastitis during the early lactation period. The validity of the test according to the studies of Langer et al., (2014) is 61.56%, Reddy et al., (2014) 73.33%, while according to the results of Sharma et al., (2010) the validity of the CMT is 75.52%.

Table 2. Results obtained with the California mastitis test (CMT) and using SCC/ml as a standard

Test	CMT	
	N	**%**
TP	25	36
FP	3	4
TN	22	32
FN	19	28
Total number of analyzed samples	69	
Sensitivity (%)	57	
Specificity (%)	88	
Validity (%)	68	
PPV (%)	89	
NPV (%)	54	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

The sensitivity of the test with EC in our research is 82% (table 3). Similar results were obtained by Mansell and Seguya (2003) where they observed a sensitivity of 51%, while Langer et al., (2014) determined significantly low values of sensitivity compared to other authors and it was 12.5%. Galfi A., (2016) states that the sensitivity of the test in the drying period is 74.32%, while during early lactation it was significantly low at 2.86%, and for the specificity of the test, it is 50%. According to research by Mansell and Seguya (2003), the specificity of the test was 71%, while Nielen et al., (1992) observed a high specificity of 94%. In addition, Langer et al., (2014) considers that the possibility of determining the subclinical form of mastitis measured by the Draminski mastitis detector is relatively low 7.6%. While the validity in our research is 55%. According to the obtained results of Galfi A., (2016) when measuring the electrical conductivity with the Draminsky test, it was determined that the validity of the test in the drying period is 52%, while in the early lactation period it is 48.65%. While Langer et al., (2014) determined validity with the Draminski test of 59.05%.

From the results (table 3), it can be noted that the percentage of false positives is high (38%). The validity of manual instruments for measuring electrical conductivity has been investigated by many authors. Musser et al., (1998) indicated that 71% of test positive samples were bacteriologically negative and minor mastitis pathogens were isolated in 11% of negative milk samples. According to Galfi A., (2016) the stage of lactation, type of pathogenic microorganism's plays a significant influence on EC values. Additionally, Seguya and Mansell (2000) observed the lowest electrical conductivity in milk samples infected with major mastitis pathogens. Additionally, during mastitis the electrical conductivity is not always increased (Norberg et al., 2004). Also, Woolford et al., (1998) stated that the difficulties in the interpretation of electrical conductivity measurement results arising from large variations in EC values in uninfected udder quarters between cows, between udder quarters of same cow, as well as between different milking periods in the same udder quarters. Large deviations in the electrical conductivity of milk during the drying period and early lactation are thought to be the result of a physiological increase in chloride concentration in milk (Linzell and Peaker, 1975). Langer et al., (2014) explained that the reduced electrical conductivity of milk in infected udder quarters occurs as a result of increased capillary permeability during intramammary infection

and the transport of sodium, potassium and chlorine ions into the alveolar lumen resulting in to increase their concentration in milk.

IDF experts Hamann J., and Zecconi A., (1998) published a meta-analysis on electrical conductivity (EC) in which they concluded that EC does not provide satisfactory results for the detection of subclinical and clinical mastitis. According to their research the ability of EC to predict clinical mastitis can be considered in two ways. Moreover, if the clinical signs of the animal are taken as a criterion for diagnosis, in that case the sensitivity is 68%, specificity 82%, PPV 58%, NPV 82%. While if the number of somatic cells is taken as a criterion, the sensitivity remains at the same level of 68%, the specificity increases to 88%, the percentage of PPV and NPV is 72% and 85%, respectively. In subclinical mastitis, when intra mammary infection is taken as a criterion, sensitivity is 61%, specificity 66%, PPV 55% and NPV 70%.

Table 3. Results obtained by measuring electrical conductivity (EC) and using SCC/ml as a standard

Test	EC	
	N	**%**
TP	19	33
FP	22	38
TN	13	22
FN	4	7
Total number of analyzed samples	58	
Sensitivity (%)	82	
Specificity (%)	50	
Validity (%)	55	
PPV (%)	46	
NPV (%)	76	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

As a result of tissue damage during the occurrence of mastitis and the reduction of the synthetic ability of the enzyme system of the secretory cells, there is also a reduction in the biosynthesis of lactose (Pyörälä, S. 2003). According to Pyörälä, S. (2003), lactose can be used as an indicator of mastitis, as it decreases during inflammation. According to the results obtained in our research, the sensitivity is 79%, while the specificity is 60% (table 4). The ability of lactose to determine intramammary infection according to the predicted limits of 4.7% whose value applies when the number of somatic cells is up to 100,000 cells/ml is 60.8% for sensitivity, and 80.6% for specificity (Pyörälä, S. 2003).

Table 4 Results obtained by measuring lactose and using SCC/ml as standard

Test	Lactose	
	N	**%**
TP	19	30
FP	16	25
TN	24	37
FN	5	8
Total number of analyzed samples	64	
Sensitivity (%)	79	
Specificity (%)	60	
Validity (%)	67	
PPV (%)	54	
NPV (%)	83	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

CONCLUSIONS

Based on our research there is a positive correlation between somatic cells count and electrical conductivity. The highest values were observed in the fourth defined category according to the number of somatic cells (≥600,000 cells/ml) of 4.95 (mS/cm), compared to the normal milk group (≤ 200,000 cells/ml) 4.21 (mS/ cm). Additionally, with the increase in the number of somatic cells in the milk, there is also a decrease in the percentage of lactose. The lowest values were observed in the fourth defined category according to the number of somatic cells (≥600,000 cells/ml) of 4.13%, compared to the normal milk group (≤ 200,000 cells/ml) 4.80%. The best results in terms of the sensitivity of the test were obtained with EC (82%), then with lactose (79%) and finally with CMT (57%), from the total number of analyzed samples. The best results in terms of specificity were obtained using CMT (88%), lactose (60%) and EC (50%), from the total number of analyzed samples. Sensitivity of the test represents the ability of the test to detect all positive, infected individuals, the application of EC and the percentage of lactose gives more reliable results, compared to the CMT test. In terms of the specificity of the test, where its ability to detect all negative, i.e. healthy cows, better results were obtained with the CMT test, which is just another proof that the person performing the test needs training for correct interpretation of the obtained results

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