EFFICIENT DIAGNOSTICS AND TREATMENT OF BOVINE MASTITIS ACCORDING TO HERD MANAGEMENT PARAMETERS

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Abstract. The objective of the work was to investigate the possibility of early diagnostics of sub-clinical and clinical bovine mastitis according to a set of parameters recorded by a herd management system (production and electrical conductivity of milk and milking time,) taking into account the efficiency of treatment. In the beginning of investigation, three groups of cows were formed (3.5±0.12 and 2.8±0.13 lactation month on average). Cows of the first group (n=20) were diagnosed sub-clinical and cows of the second group (n=20) clinical mastitis; whereas cows of the third group (control) were clinically healthy (n=20). Cows of the first group were treated with udder creams (Karbasept®) and injections of Vitamin E and the preparation containing selenium (Se). Cows of the second group were treated with antibiotics of penicillin group on the basis of data of bacteriological test.

Investigations revealed that in the case of clinical mastitis the milking time ($p<0.05$) before the diagnosis of the disease and after it was longer. Three days before the clinical manifestation of the disease, milk production decreased ($p<0.05$) whereas electrical conductivity of milk increased ($p<0.05$). SCC increased ($p<0.05$) on a day of clinical diagnosis of the disease. The signs for early diagnostics of sub-clinical mastitis might be: decreased production of milk ($p<0.05$), prolonged milking time ($p<0.05$), increased electrical conductivity of milk ($p<0.05$), and increased SCC ($p<0.05$) evidencing on the day of manifestation of clinical symptoms. After the introduction of treatment of sub-clinical mastitis, milk production and SCC recovered in 30 days, electrical conductivity of milk recovered in 1 day, and milking time recovered in 3 days ($p<0.05$). After the treatment of clinical mastitis, the records showed increased milk production ($y = 0.3536x + 21.893$; $R^2 = 0.5845$), decreased electrical conductivity of milk ($y = -0.0426x + 10.974$; $R^2 = 0.3441$), and decreased milking time (after 3 days) ($p <0.05$).

Keywords: mastitis, electrical conductivity of milk, milk production, milking time

Introduction. Rapid and economically justified assessment of cows' health allows for more prompt diagnosing, treatment prescription and alleviation of economic losses resulting from diseases. Major losses are incurred by dairy farms as a result of reduced dairy herd productivity, and low milk quality. Milk production is significantly dependent on reproductive performance and udder health of cows. Typically, the risk of early abortion increases when a cow suffers from mastitis (Santos et al., 2004). The inflammatory process in cow's udder disturbs the homeostatic system of the organism, and negatively affects the sexual cycle regulatory system (Ahmadzadeh et al., 2009). It is widely accepted that detection of mastitis is based on microbiological investigations (Britten, 2012). For the purpose of diagnosis verification the assay of milk enzymatic activities is additionally suggested (Chagunda et al., 2006). Biological techniques used for detecting mastitis warrant accuracy of the diagnosis and allow for selection of the disease-specific treatment tactics. Individualized animal care in major dairy farms is difficult to achieve. Cows are taken care of as a single unit, a herd, by using herd management systems.

Many herd management systems measure cows' productivity and physical parameters of milk. A cow suffering from mastitis yields less milk and the electric conductivity of milk significantly increases in the early stages of disease (Mrode and Swanson, 2003). The milking time of cows suffering from mastitis takes longer as their milk let-down is slower (Clarke et al., 2012). In the cases of various forms of mastitis, milk somatic cell count (SCC) increases (Lindmark-Mansson, 2005). Andrei et al. (2009) argues that increased SCC can be considered a symptom of onset of sub-clinical mastitis development.

An early treatment of mastitis is possible only in case the antibiotics therapy is initiated immediately. The set of the biological parameters measured by herd management software such as reduced milk yield, increased milk conductivity, increased milking time, and increase SCC should be considered as symptoms of mastitis. Advanced and early treatment is believed to enable to recover milk production and composition of a cow more quickly.

The objective of the research was to investigate the possibility of early detection of clinical and subclinical mastitis based on the change of milk yield, milk electrical conductivity, and milking time as well as to evaluate the effectiveness of the immediate treatment.

Materials and methods. Investigation was carried out in the period from 01/06/2012 to 01/08/2012 in the farm with 500 dairy cows of Lithuanian Black and White breed, with the average milk production of 7500 kg per lactation. Cows were kept in a barn loose housing all year round, fed in accordance with the feed rations specifically balanced for each group, milked twice a day – 5:00 a.m. and 5:00 p.m., and showed no seasonality in reproductive performance. The average age of cows was 3.5 lactations. For the purpose of records and management of the cow herd under consideration the herd management software AfiFarm (Israel) was used. This software was used to record milk yield (in kg), electric conductivity of milk (in mS/cm), and milking time (in min.). Milk yield and milking time were recorded during milking, using certified milk counters approved in compliance with valid standards. Computerized herd management software data were collected within 30 days prior to treatment and 30 days after treatment. Milk SCC was found from the general milk sample collected during single morning milking from a cow 30 days prior to diagnosing mastitis, right before the treatment, and 30 days after the treatment by national enterprise "Pieno tyrimai" in accordance with the approved methodologies.

Cows were classified into three groups as follows. The first group (subclinical mastitis) included twenty selected cows with milk SCC over 250 000 cells/ml and decreased day milk yield above 10 %. Clinical mastitis of cows in group 2 (n=20) was detected based on rise in udder local temperature, painfulness, and occurrence of atypical formations in the first jet of milk. The third group (control) included twenty clinically healthy cows with similar stage of lactation to that of cows included in the first and second group of cows.

Milk samples for bacteriological examination of cows with clinical mastitis were collected after morning milking, in sterile tubes. The titre of antibiotics was identified in microbiology research laboratory of national enterprise VĮ "Pieno tyrimai". The maximum disk diffusion zone of penicillin was 22 mm.

Cows with clinical mastitis were treated with intramammary antibiotic suspension Tetra-delta® (10 ml of suspension contains: active substances: 100 mg of novobiocin (sodium salt), 105 mg neomycin (sulphate), 100 mg of benzylpenicillin procaine salt (penicillin G), 100 mg of dihydrostreptomycin (sulphate), 10 mg of prednisolone anhydrous) making injections into the affected quarters of udder through the teat canal at intervals of 24 hours until full disappearance of signs of clinical mastitis (morphological changes of udder and atypical formations in milk). During treatment these cows were marked with specific markers and milked last in the same milking station while diverting their milk into separate container for utilization.

Subclinical mastitis was treated locally using anti-inflammatory and immune system improving preparations. For this purpose CARBASEPT® ointment was used (1 g of ointment contains: active substances: 20 mg of methyl salycilate, 20 mg of camphor) once a day on udder after milking for three consecutive days, together with vitamin E + selenium (1 ml of emulsion contains active substances: 100 mg of α-tocopheryl acetate; 2 mg of anhydrous sodium selenite) subcutaneous injections of 10 ml once a day.

Statistical analysis was conducted using statistics package (SPSS for Windows 15, SPSS Inc., Chicago, IL, USA). The reliability of difference between means (p) was found following a Student's distribution (Juozaitienė, Kerzienė, 2001). The results were considered to be reliable when $p<0.001$; $p<0.01$, and $p<0.05$.

The research was conducted following the provisions of the Law of the Republic of Lithuania No. 8-500 on Protection, Keeping and Use of Animals, dated 06/11/1997 (*Valstybės žinios* (official gazette) No. 108 dated 28/11/1997) and of the by-laws, i.e., orders of State Veterinary Service of the Republic of Lithuania: on Breeding, Care, Transportation of Laboratory Animals (No. 4-361, dated 31/12/1998), and Use of Laboratory Animals for Scientific Tests (No. 4-16, dated 18/01/1999).

Results. According to the data of investigation, 90 % of treated cows with subclinical mastitis, and 95 % of cows with clinical mastitis have recovered.

In the period of research, milk production losses due to subclinical mastitis amounted to 124 kg of milk per cow on the average, whereas it amounted to 272 kg per lactation of non-treated cows in the farm under consideration.

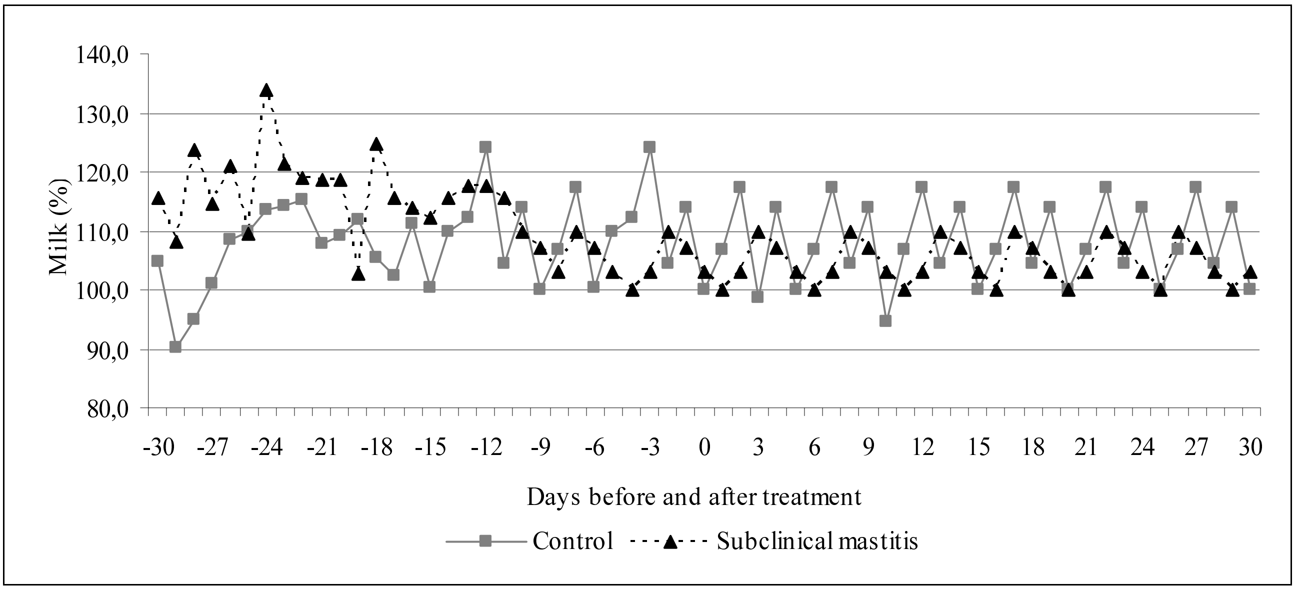

Fig.1.**Variation in milk yield (in percent) before and after the treatment of subclinical mastitis**

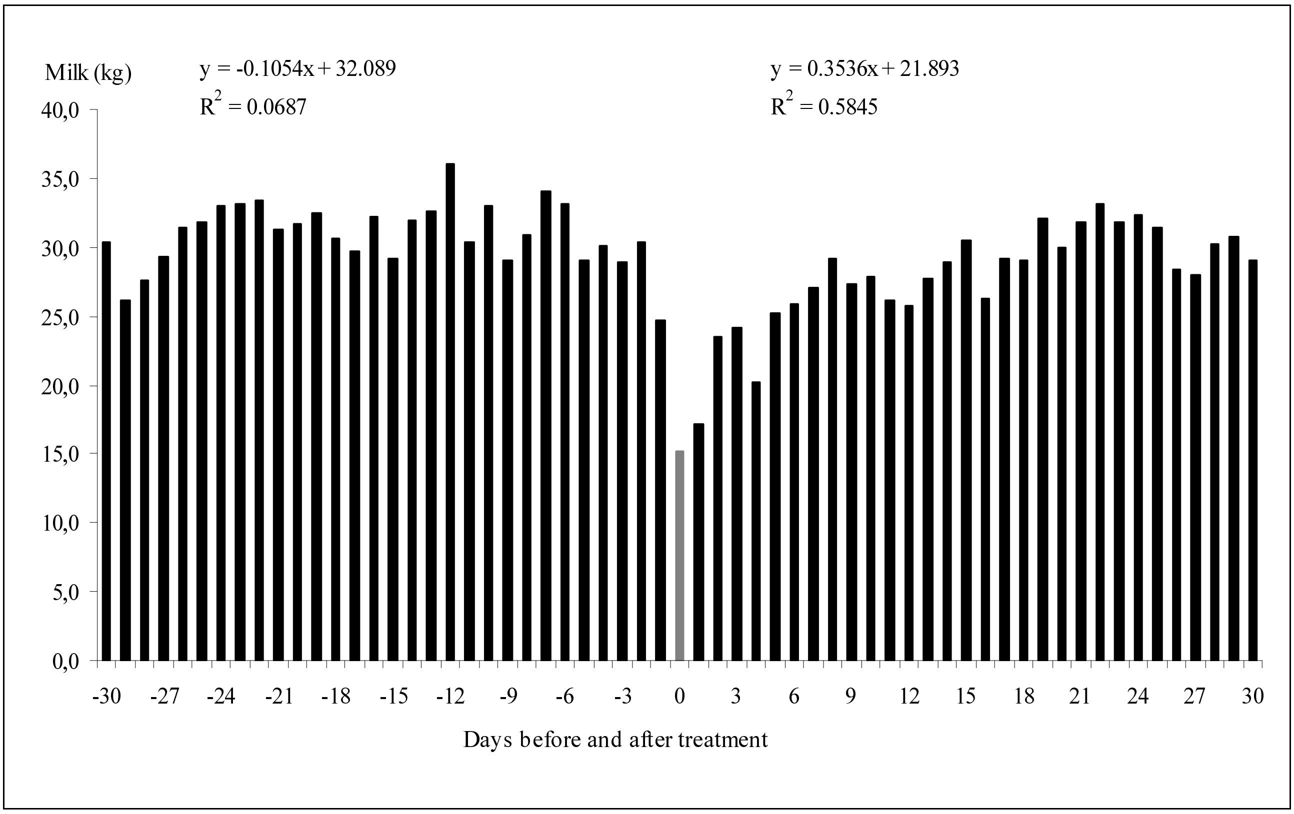

Fig.2. **Variation in milk yield before and after the treatment of clinical mastitis**

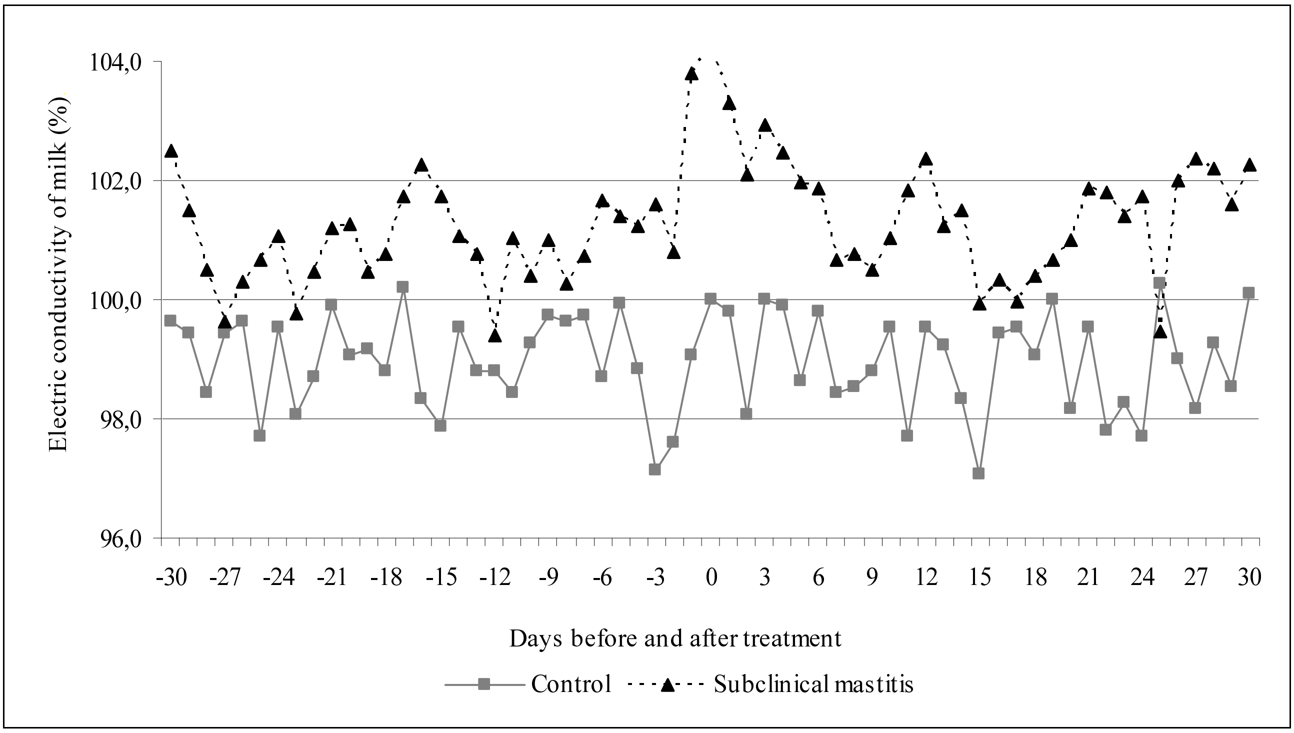

Fig.3. **Variation in electric conductivity of milk before and after the treatment of subclinical mastitis**

Milk yield of cows suffering from subclinical mastitis was gradually decreasing (14%) till the day of treatment, and recovered after 30 days ($p<0.05$) (Fig. 1). In the period of research, milk production losses due to subclinical mastitis amounted to 124 kg of milk per cow, on the average, whereas it amounted to 272 kg per lactation of non-treated cows in the farm under consideration.

In case of clinical mastitis (Fig. 2), milk yield starts decreasing statistically reliably ($p<0.05$) three days prior to development of clinical symptoms of the disease, and increases, on the average, 9 days after the treatment being applied ($p<0.05$). During the development of clinical symptoms of the disease, milk yield decreases, on the average, by 52 % per cow ($y = 0.3536x + 21.893$, $R^2 = 0.5845$). In the period of research, milk production losses due to clinical mastitis amounted to 174 kg of milk per cow, on the average, when compared to the control group ($p<0.01$).

Electric conductivity of milk from cows with subclinical mastitis was found to increase statistically reliably ($p<0.05$) up to 6.5 mS/cm (16%) on the day of treatment. After the treatment, it was found to recover, and no statistically reliable difference when compared to the electric conductivity of milk from cows in the control group was detected (Fig. 3).

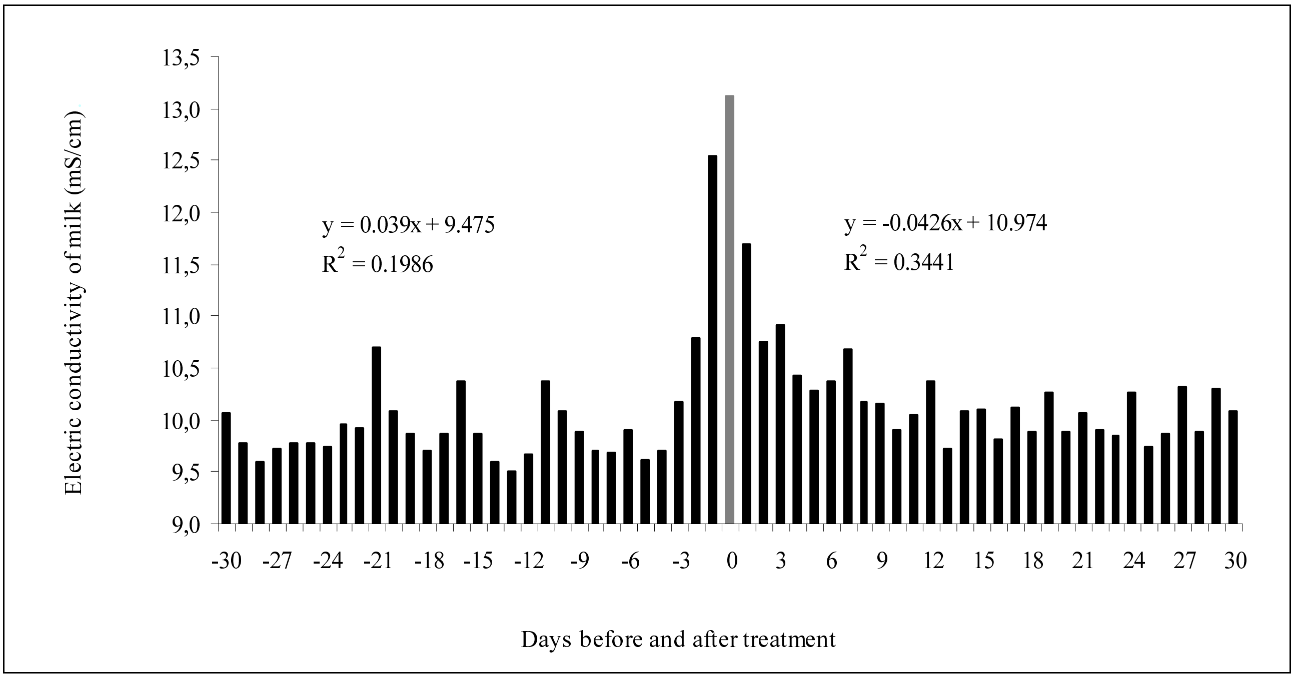

Fig.4. **Variation in electric conductivity of milk before and after the treatment of clinical mastitis**

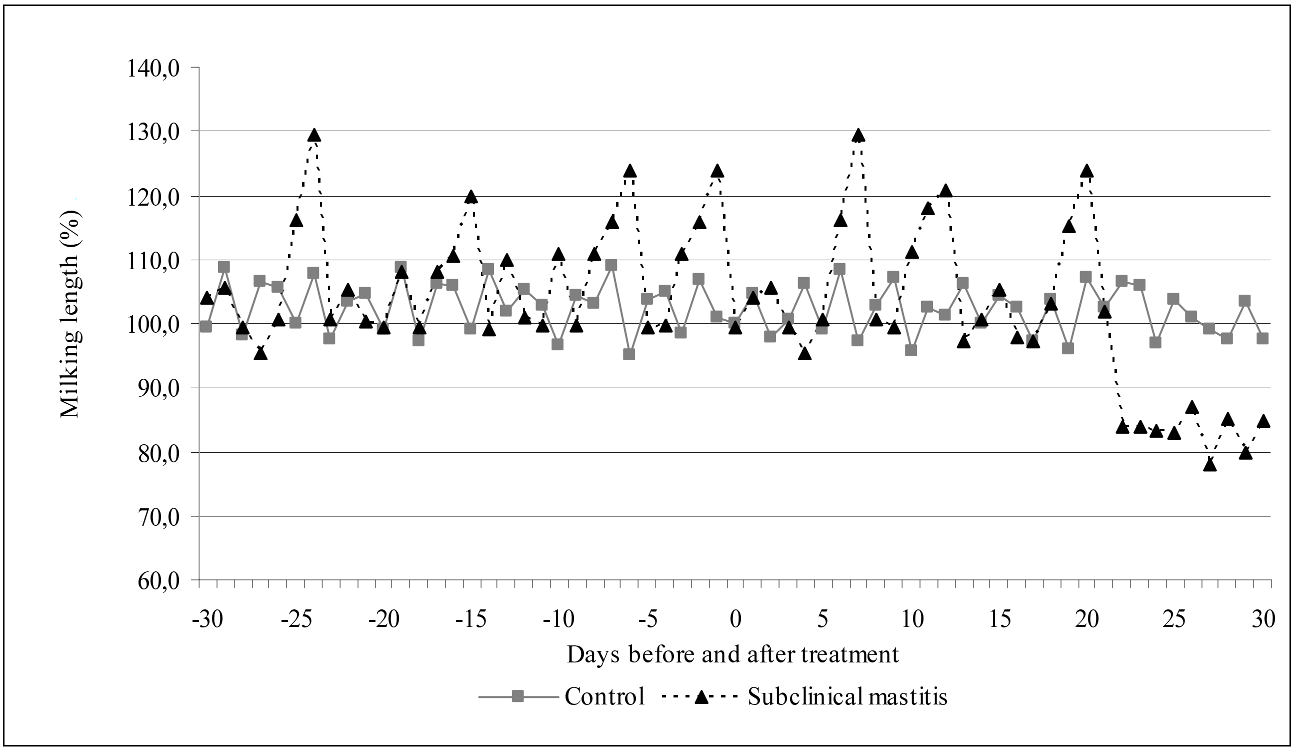

Fig.5. **Variation in milking length before and after the treatment of subclinical mastitis**

Electric conductivity of milk from cows with clinical mastitis (Fig.4) started increasing statistically reliably ($p<0.05$) three days prior to the development of clinical symptoms of the disease, one day prior to that it increased (49%), on the average, up to 8 mS/cm, during the development of clinical symptoms of the disease up to 9 mS/cm (62%), and three days after the onset of the treatment, electric conductivity of milk regained its initial level (on the average, 6.1 mS/cm) ($p<0.05$).

According to the results of regression analysis (Fig.4), the electric conductivity of milk after the treatment of clinical mastitis had a tendency to decrease (on average 0.04 mS/cm per day; $R^2 = 0.3441$).

The milking time was statistically significantly ($p<0.05$) different between groups under consideration over the entire period of investigation. The milking time of cows suffering from clinical mastitis was found to be, on the average, by 5.12 min. longer when compared to that of the control group, and by 4.23 min. longer than that of cows with subclinical mastitis. The average milking time of cows with subclinical mastitis was by 0.89 min. (17%) longer than that of cows in the control group. (Fig.5). After treatment of subclinical mastitis, the milking time of cows tended to decrease ($y = -0.0793x + 10.543$; $R^2 = 0.3257$).

The milking time of cows had a tendency to decrease until clinical mastitis detection ($y = -0.0518x + 12.512$; $R^2 = 0.2719$). After diagnosis of the disease, the milking time of cows increased ($p<0.05$) and remained prolonged for 3 days after the treatment (Fig.6)

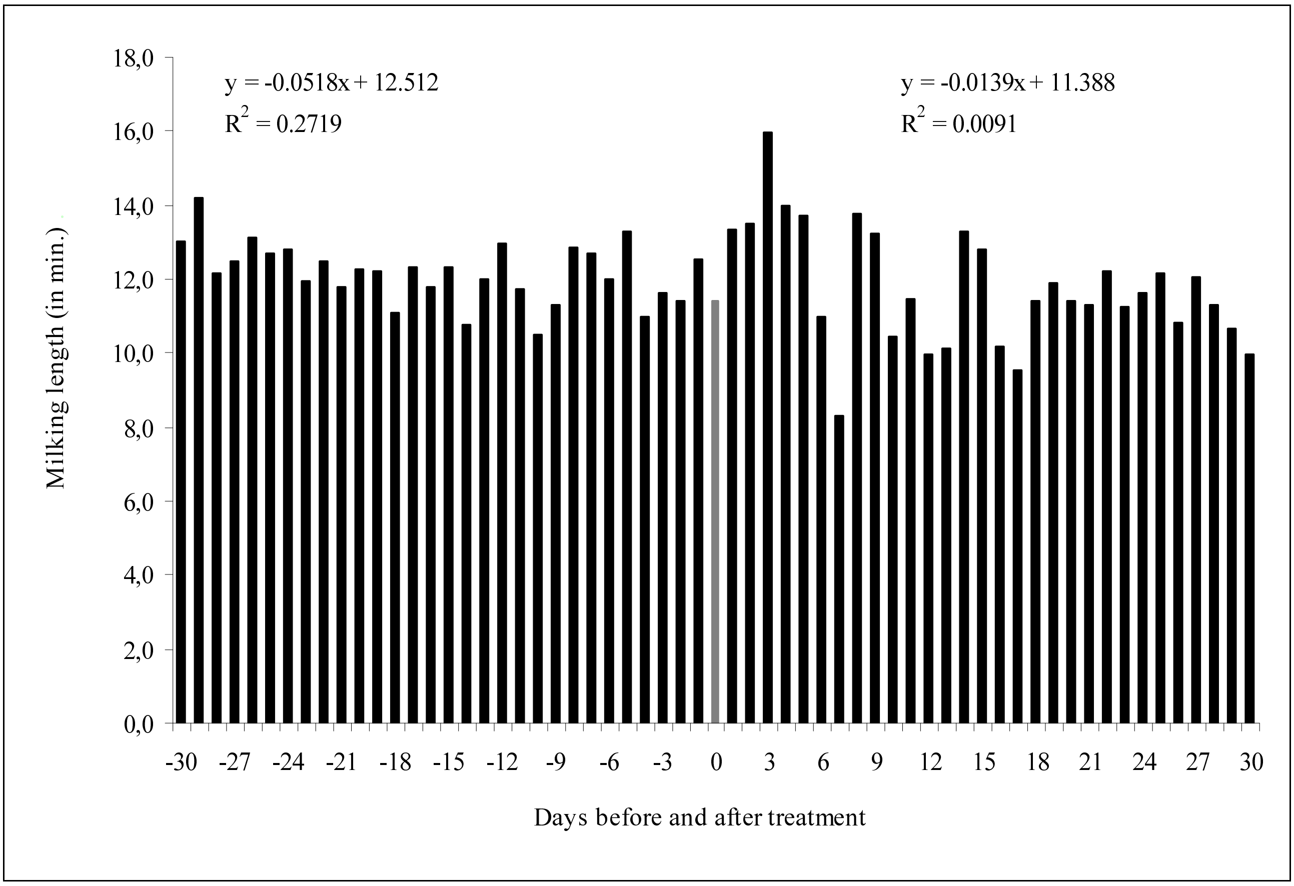

Fig.6. **Variation in milking length before and after the treatment of clinical mastitis**

Response to treatment approved the changes of SCC in milk of cows. In case of clinical mastitis detection, milk SCC, on the average, amounted to 1877 x 10-3ml. One month after the treatment, SCC decreased to 231 thousand/ml. The investigation showed that in case of subclinical mastitis detection, SCC, on the average, amounted to 435.8 x10-3ml., and one month after the treatment, SCC decreased to 55.4 x10-3ml. ($p<0.05$). The variation of milk SCC in the control group of cows was found to be not statistically reliable.

Discussion. Detection of mastitis based on traditional techniques does not meet needs and requirements of modern farming any longer. Consequently, there occurs a need for finding practitioner-friendly, simple, economically justified and reliable ways for detection of mastitis in cows (Polat et al., 2010). Parameters typically recorded by herd management software (milk production, electric conductivity of milk, milking time, SCC) are associated with mastitis, however they do not represent indicative symptoms of mastitis. For example, milk production of cows can be reduced due to metabolic disorders and complicated course of post partum period (Ostergaard et al., 1999). J.M. Lukas et al. (2009) suggest variation in milk production and electric conductivity of milk to be treated as main parameters for cow's health monitoring system. Milk flow rate represents one of the milking parameters, which is mainly inherited (Juozaitienė, Japertienė 2010). According to many authors (De Mol et al., 2001, Hillerton J. E. et al., 1999), detection of mastitis might be performed based on monitoring of variation in milk production, electric conductivity of milk and milking length.

Milk production of cows with clinical mastitis was found to decrease three days prior to development of clinical signs of the disease, and to increase nine days after the confirmation of clinical diagnosis and treatment. Y.T. Gröhn et al. (2004) have reported that milk production typically reduces before detection of clinical mastitis. The reduced milk production prior to development of clinical symptoms should be treated as a signal that veterinary examination of an animal must be arranged immediately. In absence of clinical signs, in case of reduced milk production, the increased SCC was detected. In the case of mastitis, somatic cell count increases with increasing specific milk enzyme activity (Isobe et al,. 2011). Reduced milk production is for many a long day associated with subclinical mastitis (Tsonev et al., 1975). In case of reduced milk production, when increased SCC is detected, it can be considered a symptom of subclinical mastitis. Schukken at al. (2009) found the correlation to exist between the specificity of mastitis agent and decreased milk production. T. Halasa (2009) and others found that the greater is the SCC concentration in milk (above 100^3 cell/ml) the more pronounced decrease in milk production is observed. In practice of mastitis treatment, various penicillin compounds are typically used (Sérieys et al., 2005; Saini et al., 2012). According to laboratory analysis, agents causing clinical mastitis are susceptible for penicillin group of antibiotics; consequently the preparation containing this particular antibiotic was selected for the treatment in our case.

Following the treatment of clinical mastitis, the somatic cell count remained elevated till day 30. An increased SCC concentration evidences a partial recovery of functioning of the mammary gland, and for this reason decreased milk production is observed. According to P.J. Rajala-Schultz (1999), milk production tends to decrease extremely in first two weeks after the detection of mastitis and after the treatment, as well as fails to fully recover in current lactation up to the initial production level that was before mastitis development. This is in perfect agreement with results of our experimentation. Milk yield achieves its initial level existent prior to the disease only two weeks after the treatment; however the average total production over entire period under investigation remains lower. In case of clinical mastitis, the decrease in milk yield is less pronounced. Following the treatment of subclinical mastitis, milk yield failed to achieve its previous levels that were present prior to the disease. Electric conductivity of milk is a reverse measure of the resistance of milk, which is mainly dependent on the strength of blood vessels. It is believed that this parameter can be used for detection of mastitis (Norberg, 2005). Electric conductivity of milk is found by concentration of sodium, potassium, calcium, magnesium, chlorine and other ions (Hamann, Gyodi, 2000; Barth, Worstorff, 2000), which varies in response to the increased electric conductivity. Some researchers (Hillerton, Walton, 1991; Hamann, Gyodi, 2000) claim that electric conductivity of a milk of a healthy cow is in range of 4.0–5.5 mS/cm. If it exceeds 6.0 mS/cm, pathological processes in tissues of udder may be accordingly predicted.

However, S. Pyörälä (2003) contradicts by claiming that assessment of udder health based on the electric conductivity of milk is a rather risky choice. In opinion of many authors, measuring electric conductivity of milk separately, apart from other parameters, is beside the purpose as this parameter is influenced by many different factors: lactation (Barth, Worstorff, 2000), genetic differences between herds (Hamann, Zecconi, 1998; Hamann, Gyodi, 2000), the fat content of milk (Musser et al., 1998), and various diseases (Nielen et al., 1995). Research findings suggest that, instead, a more appropriate way for the detection of mastitis is measurement of variations of electric conductivity of milk, milk production, and milking time (Hillerton J. E. et al., 1999).

Based on the experimental data, the electric conductivity of milk increases irrespectively of the clinical character of mastitis, however in case of clinical mastitis, the increase in this particular parameter is more pronounced when compared to cases of subclinical mastitis. In accordance with V. Špakauskas et al. (2006), the electric conductivity of milk from cows with subclinical mastitis may increase from 6.1 to 8.5 mS/cm. After the treatment, the electric conductivity of milk decreases, however in case of clinical mastitis, it remains at increased level for 10 days. Similar results also have been reported by A. Gáspárdy et al. (2012). The authors point out that electric conductivity of milk remains increased for at least 4 days after the treatment. According to G.T. Fosgate (2012), determination of the etiology of mastitis is more accurate when the test of somatic cell count in milk is used. When measured apart, the increased electric conductivity of milk may only be an evidence of the beginnings of pathology. Changes in electric conductivity of milk when measured in parallel with variations in SCC may serve as a solid argument in detecting the onset of mastitis.

H. Larroque et al. (2005) found a correlation between the milking time and somatic cell count. The research data published by the above-mentioned scholars show that milking rate is "negatively correlated" to the somatic cell count. As research findings suggest, milking time was significantly different between healthy cows and cows with clinical mastitis. This is in good agreement with the findings of H. Larroque et al. (2005) that in severe cases of mastitis, a teat is affected resulting in reduced milk flow rate and causing milking time to be prolonged. The increased milking length was observed in an analogous manner as increased electric conductivity of milk or reduced milk production. It allows determining the general cause behind variations in these parameters – the mastitis.

The tactics for mastitis control must match the criteria for effective and cost-efficient treatment. Starting treatment of mastitis earlier (upon early detection) makes it possible to achieve better treatment results. According to P. Milner et al. (1997), in case of early treatment, to achieve the positive results, the need for antibiotics is reduced up to 50 percent. Undertaking treatment before development of visual signs of mastitis, based on the variation in electric conductivity of milk alone, cows recover much faster (Hillerton, J. E. Semmens, 1999). Many agents causing mastitis are susceptible for penicillin group of antibiotics. For example, as Y. Persson et al. (2011) suggest, most frequently detected agents causing subclinical mastitis are *St.Aureus* and CNS. 4 % of Staphylococcus Aureus and 35 % of conditionally pathogenic staphylococcus strains are resistant to penicillin group of antibiotics. According to A. Lago (2011), the specific treatment based on microbiology assay serves to reduce treatment costs as in result less amount of antibiotics is used. On the contrary, W. Steeneveld et al. (2011) argue that specific treatment is economically not justified. Such a tactics is suitable in cases when not individual cows are affected by mastitis, and additional examinations delay treatment and make it more expensive.

Conclusions

1. Investigation showed statistically significant ($p<0.05$) changes of investigated parameters. Decreased milk production, prolonged milking time, increased electrical conductivity of milk (on a day of manifestation of clinical symptoms), and increased SCC show that such parameters might be the signs of early diagnostics of sub-clinical mastitis. After the treatment of sub-clinical mastitis, milk production and SCC recovered in 30 days, electrical conductivity of milk in 1 day, and milking time in 3 days ($p<0.05$).
2. During the 30 days treatment of clinical mastitis, the records showed constantly increasing milk production ($y = 0.3536x + 21.893$; $R2 = 0.5845$), decreasing electrical conductivity of milk ($y = -0.0426x + 10.974$; $R2 = 0.3441$), and reduced milking time (in 3 days) ($p <0.05$).
3. When decrease in milk production, increase in electric conductivity of milk and prolonged milking time are observed, it is recommended to undertake clinical examination and start treatment immediately which leads to reduced economic losses.

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