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Efficiency of the data generated by the robotic milking system for comprehensive diagnosis of mastitis in cows

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SUMMARY

Early mastitis diagnosis and treatment play a significant role in reducing the disease incidence in a dairy herd. Examination of the animals ($n = 61$) milked with VMS™ V300 automated voluntary milking system (DeLaval, Sweden) showed that mean milk yield was 15.03 kg (*min* – 4.50 kg, *max* – 24.52 kg); mean milking time in the group was 8 min 14 sec (*min* – 5 min 24 sec, *max* – 12 min 29 sec) during the observation period equal to 10,300 milkings. Milking time for the majority of the cows (67.2%) complied with the standards and equaled to 4–7 min, mean milking time for 32.7% of the animals was 8 minutes. Mean interval between milkings in the test animal group was 11 hours 30 minutes (*min* – 6 h 04 min, *max* – 18 h 54 min). Mean electrical conductivity of the milk was 4.14 1/Om×cm^3 for the whole group of animals. Determined mean mastitis detection index (MDi) was 1.6 and varied in the range of 1.03 to 1.41. Minimal and maximal MDi was 1.0 and 11.1, respectively. Diagnostically representative increase in MDi within 1.8–2.2 was observed in 24.6% of animals. Significant MDi increase to more than 2.2 was found in 21.3% of high-yielding cows. All animals with MDi higher than 1.8 (28 animals) were examined for mastitis. Inflammatory reactions in udder were detected in 28.6% of the animals, clinical and latent inflammations were detected in 7.1 and 21.4% of the cows, respectively. Tests of mammary gland secretion showed that average somatic cell count was up to 200 and 201–300 ths cells/mL in 45.9 and 37.7% of the animals, respectively. Udder secretions of 4.9% of cows contained 301–400 ths somatic cells/mL. In 9.8% of tested animals average somatic count was 401–700 ths somatic cells/mL, and in 1.6% of the animals – more than 701 ths somatic cells/mL. Microbiological and PCR tests of mammary gland secretion samples taken from the animals with mastitis detected the following contagious and coliform mastitis agents: *Staphylococcus* spp. (*St. epidermidis*, *St. saprophyticus*, *St. haemolyticus*), *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium*. Various diagnostic techniques are found to be used for detection of mastitis in the herd and the data generated by robotic voluntary milking station such as mastitis detection index (MDi) can be used for earlier detection of changes in cow's mammary gland.

Keywords: high-yielding cows, mastitis, diagnosis, robotic voluntary milking systems, milk yields, milking time, interval between milkings, electrical conductivity, mastitis detection index (MDi), somatic cells, mastitis agents, contagious mastitis, coliform mastitis

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Эффективность использования данных, полученных с электронной системы роботизированного доения, при комплексной диагностике мастита у коров

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РЕЗЮМЕ

Важную роль в снижении заболеваемости молочного стада маститом играют ранняя диагностика и своевременные лечебные мероприятия. В результате исследования животных ($n = 61$), доение которых осуществлялось при помощи автоматизированной системы добровольного доения VMS™ V300 (DeLaval, Швеция), установлено, что за период наблюдения, равный 10 300 актам доения, средний надой составил 15,03 кг (*min* – 4,50 кг, *max* – 24,52 кг); средняя продолжительность доения по группе – 8 мин 14 сек (*min* – 5 мин 24 сек, *max* – 12 мин 29 сек). Период времени, за которое

происходил цикл доения большинства коров (67,2%), соответствовал нормативным показателям и составил 4–7 мин, у 32,7% животных средняя продолжительность доения была более 8 мин. Средний интервал между доениями в исследуемой группе животных равнялся 11 ч 30 мин (*min* – 6 ч 04 мин, *max* – 18 ч 54 мин). Средняя электропроводность молока по всей группе животных составила 4,14 1/Ом×см^3. Определили, что средний показатель MDi (индекс выявления мастита) был равен 1,16 с диапазоном от 1,03 до 1,41. Минимальное и максимальное значение MDi находилось на уровне 1,0 и 11,1 соответственно. Диагностическое увеличение индекса MDi в пределах 1,8–2,2 наблюдали у 24,6% животных. Достоверное повышение индекса более 2,2 установлено у 21,3% высокопродуктивных коров. Все животные с уровнем MDi более 1,8 (28 гол.) были обследованы на мастит, воспалительные реакции в вымени обнаружили у 28,6% особей, клиническое и скрытое воспаление имели 7,1 и 21,4% коров соответственно. При исследовании секрета молочной железы установили, что у 45,9 и 37,7% животных среднее содержание соматических клеток находилось в диапазоне до 200 и 201–300 тыс/мл соответственно. В секрете вымени 4,9% коров содержалось 301–400 тыс/мл соматических клеток, у 9,8% исследуемых животных показатель был на уровне 401–700 тыс/мл, у 1,6% – свыше 701 тыс/мл. Микробиологические и ПЦР-исследования проб секрета молочной железы от животных с маститом показали, что спектр возбудителей контагиозного и колиформного маститов представлен: *Staphylococcus* spp. (*St. epidermidis*, *St. saprophyticus*, *St. haemolyticus*), *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium*. Установлено, что для выявления мастита в стаде должны быть использованы различные инструменты диагностики, а полученные данные с автоматизированных систем добровольного доения, такие как индекс выявления мастита (MDi), могут применяться для более раннего выявления изменений, происходящих в молочной железе коров.

Ключевые слова: высокопродуктивные коровы, мастит, диагностика, автоматизированные системы добровольного доения, надои, продолжительность доения, интервал между доениями, электропроводность, индекс выявления мастита (MDi), соматические клетки, возбудители мастита, контагиозный мастит, колиформный мастит

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INTRODUCTION

Milk production scaling-up focusing on raw milk quality improvement is of great importance in the modern dairy industry [1–3]. Inflammatory reactions of cow's mammary gland are one of the factors affecting the milk quality at the stage of primary milk production [3–5]. Mastitis in high-yielding cows is a significant financial problem especially in dairy industry. Inflammatory cow's mammary gland diseases are one of the obstacles to extra premium and premium milk production and marketing to processing establishments due to high somatic cell levels in the milk especially in animals with latent mastitis, increased milk contamination with pathogenic and opportunistic microflora and changes in milk fat-to-protein ratio [6]. Therewith, early diagnosis and treatment play an important role in reducing the disease incidence in dairy herd. Detection of somatic cells and analysis of their levels in cow's milk enable subclinical mastitis diagnosis at an earlier stage, when no clinical manifestations are observed. Microbiological and PCR tests of cow's mammary gland secretions allow for detection of a range of agents responsible for inflammatory processes in cow's udder and for identification of the disease etiology. In our country, there has been recently a steady trend for construction of large dairy holdings for keeping large lactating cow populations that hampers early diagnosis of the pathological process in the mammary gland. Automated milking systems capable of registering different parameters during milking can be used for solving this problem. Analysis of the said parameters allows for detection of changes in cow's mammary gland [7].

Automatic milking systems (AMS) were first introduced on dairy farms in 1990s [8]. For several decades, the dairy farming industry of our country has been transitioning to labor automation [9–14]. AMSs are increasingly being introduced into practice owing to their undoubted advantages, such as milk quality improvement and labor cost reduction [12–16]. This voluntary milking technology for dairy cattle provides for full automation of the process, that is based on computer control and significant average increase in milking frequency. AMSs are economically, technically and socially relevant for agriculture industry as well as animal physiology, health and welfare [17–21]. The cow's udder is not examined at every milking when the above said automatic milking systems are used. Therefore, analysis of online measurements is of great importance [22–24]. Parameters registered by robotic milking systems can vary depending on the robotic milking sustem model and equipment configuration. Standard parameters to be controlled are as follows: milk yields, milking time, interval between milkings, milk electrical conductivity, blood in milk [12, 24, 25]. Mastitis detection index (MDi) is less known value registered by robotic milking system. It is calculated based on three parameters: milk electrical

conductivity, interval between milkings and presence of blood in each udder quarter [12]. Currently, basic information on this index can only be found in the user manual for DeLaval VMS™ milking system (Sweden). MDi can be within the range of 0.8 to 4.0. When it is lower than 1.8 it means that this animal has no problems with its mammary glands. When MDi is higher than 1.8 this indicates that the particular cow should be examined for mastitis, MDi higher than 2.2 is indicative of inflammation in the cow's udder. However, there is no sufficient obvious scientific evidence of relationship between MDi and mastitis in high yielding cows.

The study was aimed at complex mastitis diagnosis in cows, analysis of parameters registered by automated voluntary milking system as well as assessment of MDi effectiveness for mastitis diagnosis in cows.

MATERIALS AND METHODS

The study was performed within the governmental programme of the Ministry of Science and Higher Education of the Russian Federation: research area No. 160 – Federal Research Programmes of the Governmental Academies of Sciences, research topic No. 0532-2021-0009 "Development of biological technologies for animal health management and lifetime animal and poultry product quality management" at the Reproductive Technologies Department of the Federal State Budgetary Scientific Institution "Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences" (FSBSI UrFASRC, UrB of RAS) in 2020–2021.

The experiments were performed in high-yielding cows (milk production – more than 8,000 kg) kept in the breeding holding located in the Kamyshlovsky Raion, Sverdlovsk Oblast. VMS™ V300 automated voluntary milking system (DeLaval Company) was put into operation for group of 61 cows in September 2020. A total of 10,300 milkings were examined during the tested period (mean period – 4.9 months; *min* – 1 month, *max* – 7 months), the following parameters were examined: milking time, interval between milkings, milk electrical conductivity, MDi. The animals with MDi higher than 1.8 were additionally examined for clinical and subclinical mastitis. The animals were also examined for mastitis clinical signs by test milkings including examination for symmetry and size of udder quarters, changes in mammary gland skin and temperature. Special attention was paid to supramammary lymph nodes: they were examined for indurations. Changes in teat sphincters and drawn udder secretions were registered.

Tests for subclinical mastitis were performed using Keno™test diagnostic rapid test-kit (CID LINES, Belgium). Somatic cell counts in the mammary gland secretions were measured with viscometric milk analyzer "Somatos Mini" (Sibagropribor Ltd., Russia) and DeLaval DCC counter (Sweden). The method for somatic cell count quantification complies to the Russian Federation standards, GOST 23453-2014[1].

Samples of mammary gland secretions ($n = 8$) were collected from animals with mastitis during the observation period for further microbiological and PCR tests aimed at identification of the disease etiology. Tests were carried out with real-time polymerase chain reaction (PCR) using set of reagents: Vetscreen.STREPTOROL-V, Vetscreen.STAFIPOL, Vetscreen.KOLIPOL, Vetscreen.STREPTOROL (OOO IDS, Russia) and Rotor-Gene 3000 system (Corbett Research, Australia). Cow udder secretion samples were inoculated in liquid and solid nutrient media: meat peptone broth (MPB), meat peptone agar (MPA), Endo's medium, Sabouraud medium, mannitol-salt agar, enterococcus agar, Gissa's colour media, for bacteriological and mycological testing. The recovered isolates were identified in according to Bergey's Manual of Determinative Bacteriology and Manual for determination of pathogenic and opportunistic fungi.

[1] GOST 23453-2014 Milk. Methods for determination of somatic cells. Available at: https://docs.cntd.ru/document/1200115756. (in Russ.)

RESULTS AND DISCUSSION

Average milk yield in the group was 15.03 kg (*min* – 4.50 kg, *max* – 24.52 kg) during the observation period. The mean milking time in the group was 8 min 14 sec, minimal milking time was 5 min 24 sec, maximum milking time was 12 min 29 sec. Mean milking time was 5–7 min, and was consistent to physiological parameters of milk ejection reflex and complied with the limits required for machine cow milking. It was found that 67.2% of cows were milked out in 4–7 minutes and average milking time in 32.7% of cows was more than 8 minutes (Fig. 1).

Mean interval between milkings was 11 h 30 min in the tested group, ranging from minimal interval of 6 h 04 min up to maximum interval of 18 h 54 min. Therewith, the maximum interval of 20 h 11 min – 24 h 00 min was registered once or more times in 31.2% of cows during the observation period (Fig. 2).

Our previous tests showed that milk electrical conductivity in healthy cows was 3.5–4.5 1/Om×cm^3, milk electrical conductivity in cows with subclinical and clinical mastitis was 4.5–6.0 and 6.1–7.0 1/Om×cm^3, respectively [26].

Milking of cows with VMS™ V300 automatic voluntary milking system enables generation of the data on the electrical conductivity of the milk from each mammary gland quarter. Analysis of the data for the whole observation period showed that mean electrical conductivity in the test group was 4.14 1/Om×cm^3; therewith, in 16.4% of cows the milk electrical conductivity was 4.5–6.0 1/Om×cm^3. At the level of individual animal, electrical conductivity of the milk from left front quarter of the udder was 4.50–5.23 1/Om×cm^3 in 23.0% of the cows and more than 7.11 1/Om×cm^3 in 4.92% of cows; electrical conductivity of the milk from right front quarter of the udder was 4.52–5.05 1/Om×cm^3 in 13.1% of the cows and 6.24–9.39 1/Om×cm^3 in 8.2% of cows during the whole observation period. Similar analysis showed that the electrical conductivity of the milk from left hind quarter of the mammary gland was in the range of 4.54–5.20 and 6.06–9.14 1/Om×cm^3 in the same number of animals (13.1%). Increase in electrical conductivity of the milk from the right hind quarter of the udder from 4.51 to 5.73 1/Om×cm^3 and from 6.22 to 7.93 1/Om×cm^3 was registered in 21.31% and 4.92% of the animals, respectively.

Mean MDi was 1.16 (range: 1.03–1.41) during the 7-month test period and 10,300 milkings. Minimum and maximum MDi was 1.0 and 11.1, respectively (Fig. 3). Therewith, MDi was 1.8 up to 2.2 in 50.8% of high yielding cows, single index increase was registered in 26.2%

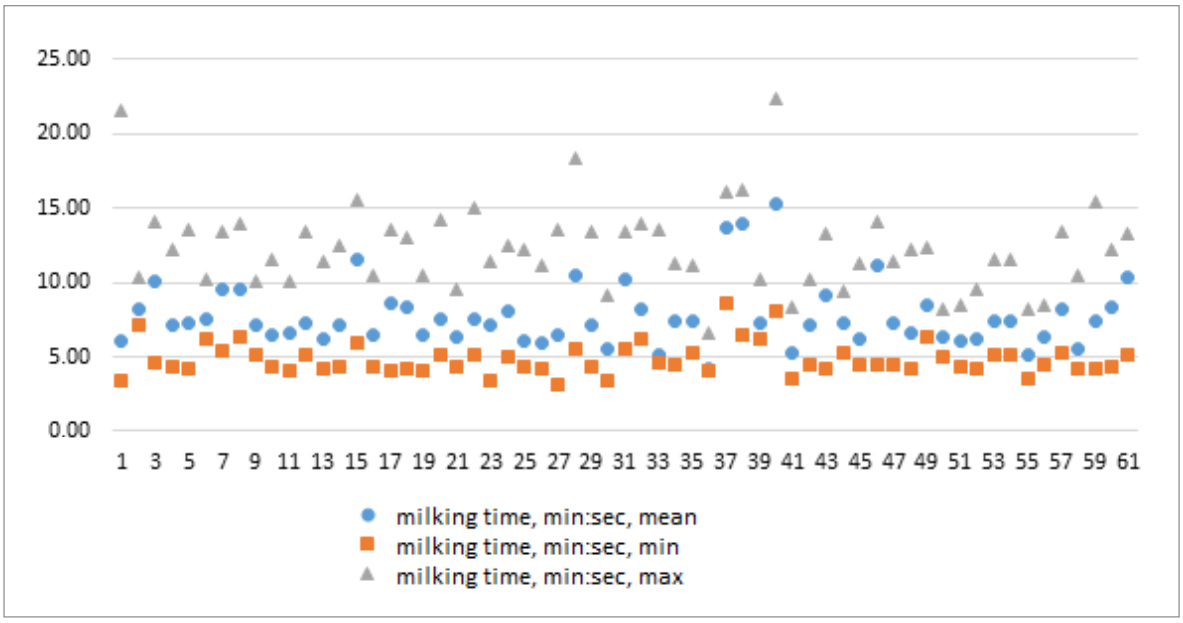

Fig. 1. Distribution of the time of cow milking with robotic milking system

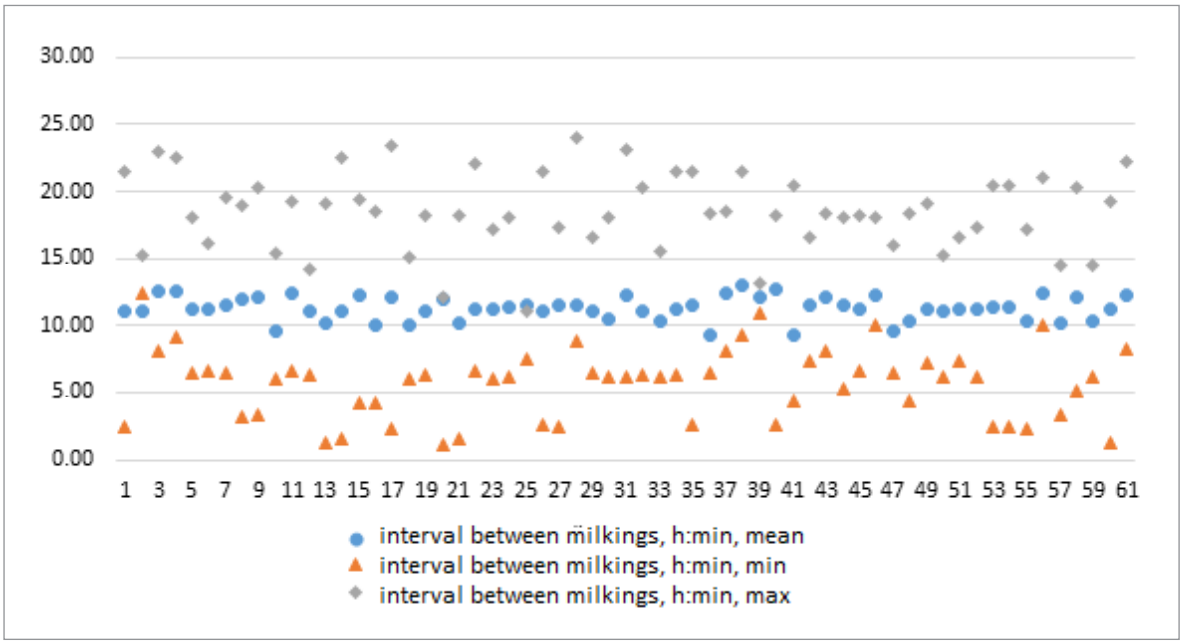

Fig. 2. Distribution of the interval between cow milkings with robotic milking system

of animals. Thereat, diagnostically significant MDi increase within the range of 1.8–2.2 was registered in 24.6% of cows. MDi higher than 2.2 was registered in 36.1% of animals; therewith increased MDi was observed in 14.8% of cows during one up to four milkings but subsequently found not be associated with mastitis in these animals. As a result, MDi higher than 2.2 in 21.3% of high-yielding cows was considered indicative of mastitis. Increased MDi, 1.8–2.2 and higher than 2.2, was registered during maximum 38 and 19 milkings, respectively.

All animals with MDi of 1.8–2.2 and higher than 2.2 (15 and 13 cows, respectively) registered during more than four milkings were examined for subclinical and clinical mastitis. Mastitis was diagnosed in 28.6% of 28 examined cows. Clinical and latent inflammation was detected in 7.1 and 21.4% of cows, respectively. Clinical mastitis was detected in the cows with MDi higher than 2.2. registered during 13–18 milkings. Subclinical mastitis was detected in cows with MDi of 1.8–2.2 registered during 17–28 milkings. No mastitis was detected in the cows with MDi less than 1.8 during the whole test period. Examinations for association between MDi and diagnosed mastitis showed positive correlation (correlation coefficient: $r = 0.78$).

Tests of mammary gland secretions showed that mean somatic cell count was 200 and 201–300 ths cell/mL in 45.9% and 37.7% of animals, respectively, that was indicative of absence of pathological processes in mammary glands. Therewith, MDi for these animals was less than 1.8. There were 301–400 ths/mL of somatic cells in the udder secretions from 4.9% of cows with MDi of 1.8–2.0. Clini-

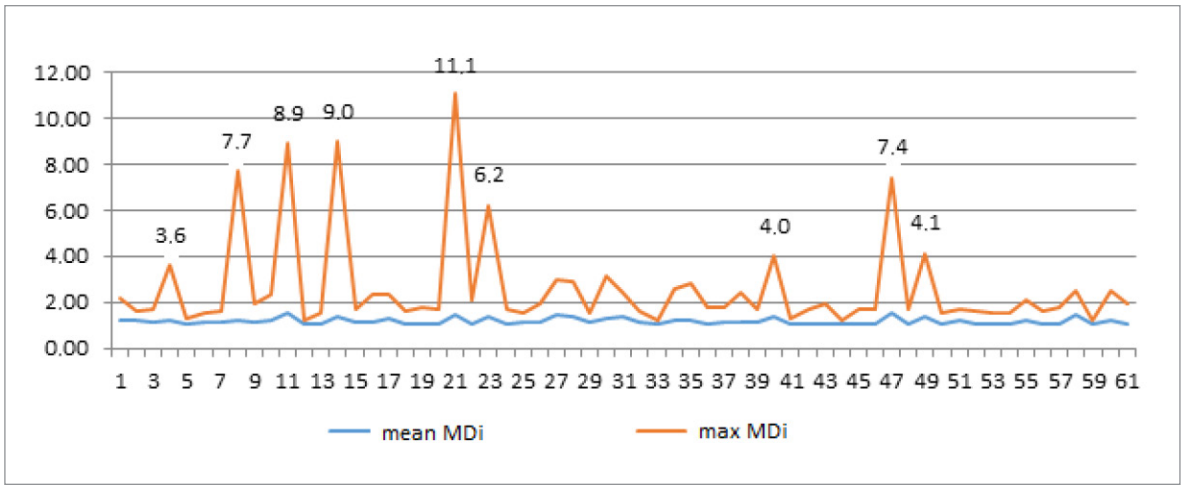

Fig. 3. Mastitis detection index (MDi)

cal examination of these cows revealed subclinical mastitis in 3.6% of them. Somatic cell count was 401–700 ths cell/mL in 9.8% of tested cows and their MDi was in the range of 2.0–2.2. All these animals had inflammation in the mammary gland and subclinical mastitis was detected in 7.1 and 14.3% of cows, respectively. More than 701 ths somatic cells/mL were detected in the milk from one cow (1.6% of tested animals) with MDi higher than 2.2. Clinical mastitis (3.6%) was detected during the clinical examination of the cow. Examination results are given in the Table below.

Fourteen bacterial isolates were recovered from mammary gland secretion samples collected from the cows with mastitis and tested with PCR. The following etiological agents of contagious mastitis were detected in the samples: *Staphylococcus* spp. (*St. epidermidis*, *St. saprophyticus*, *St. haemolyticus*) were detected 100% of samples; *Streptococcus agalactiae* and *Staphylococcus aureus* were detected in 25.0% and 12.5% of samples, respectively. *Escherichia coli* inducing coliform mastitis in cows was recovered from 37.5% of samples.

Microbiological tests of mammary gland secretion samples from the cows with diagnosed mastitis detected *Enterococcus faecium,* environmental microorganism, in 100% of samples as well as *Escherichia coli* and *Staphylococcus epidermidis* in 62.5% and 37.5% of samples, respectively, and *Staphylococcus aureus* in 12.5% of samples.

Thus, microbiological and PCR tests are complementary methods for mastitis etiology identification enabling detection of wide range of pathogens and selection of effective treatment.

CONCLUSION

All available diagnostic techniques (clinical examination, rapid tests, somatic cell counting, analysis of milk electrical conductivity and microbiological and PCR tests) should be used identification of mastitis of a particular type and for prescribing treatment and prediction of the disease course. Tests results showed that mean mastitis detection index (MDi) varied from 1.03 to 1.41, therewith, minimal and maximum MDi was 1,0 and 11.1, respectively. Diagnostically representative MDi increase within 1.8–2.2 was registered in 24.6% of animals. Significant MDi increase to more than 2.2 was found

Table
Somatic cell counts in mammary gland secretion collected from tested cows (*n*=61)

Somatic cell count, ths cells/mL	Number of animals	
	n	%
less than 200	28	45.9
201–300	23	37.7
301–400	3	4.9
401–700	6	9.8
more 701	1	1.6

in 21.3% of high yielding cows. Mastitis was detected in 28.6% of animals with MDi more than 1.8, therewith clinical and subclinical inflammations were detected in 7.1% and 21.4% of cows, respectively. Tests of cow udder secretions showed that mean somatic cell count was not more than 200 ths cells/mL in 45.9% of animals; somatic cell count was 201–300 ths cells/mL in 37.7% of cows, and MDi for these animals was less than 1.8. There were 301–400 ths somatic cells/mL in mammary gland secretions collected from 4.9% of cows with MDi within the range of 1.8–2.0. Somatic cell counts in 9.8% and 1.6% of tested cows with MDi within the range of 2.0–2.2 were 401–700 and more than 701 ths cells/mL, respectively. Microbiological and PCR tests of mammary gland secretion samples from the animals with mastitis detected the following pathogens responsible for contagious and coliform mastitis: *Staphylococcus* spp. (*St. epidermidis*, *St. saprophyticus*, *St. haemolyticus*), *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium*. Estimated coefficient of correlation between MDi and diagnosed mastitis was +0.78. Taking into account recommendations given in VMS™ V300 Milking System Manual, where mastitis detection indices are roughly divided into the following ranges: less than 1.8 – "normal udder", 1.8–2.2 – "udder requires attention", more than 2.2 – "mastitis", performed experiments have confirmed that MDi can be used as an additional tool for mastitis diagnosis that allows early prompt measures to be taken.

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