

Impact of various milking equipment on incidence of mastitis in dairy herd

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It was found that the lowest percentage of cows with mastitis was recorded in winter: 10.3% ($P < 0.001$) when milking with 'DeLaval' equipment and 17.7% ($P < 0.001$) when milking with the ADM-8A unit. The highest incidence of mastitis in cows was observed in summer: 21.0% ($P < 0.001$) and 27.1% ($P < 0.001$), respectively, when milking cows at milking parlors 'DeLaval' and ADM-8A. It was found that, when using milking equipment ADM-8A, adult cows are 2.1-1.7 times more prone to mastitis compared to cows of the 1st and 2nd calving. When milking cows with the 'DeLaval' unit, the incidence of mastitis in adult cows is 4.3-1.1 times higher than in cows of the 1st and 2nd calving. Gentle milking mode on the 'DeLaval' equipment allowed to increase the number of completely healthy animals to 74%, which is 9.6% more than when using the milking unit ADM-8A (64.4%), and to reduce the incidence of clinical mastitis in cows by 3.4 times. It has been established that in cows with disorders in the udder the content of somatic cells in the secretion of the udder significantly changes (with a high degree of correlation) in all periods of the functional state of the body. Thus, during subclinical mastitis $r = 0.72$ ($P < 0.001$) and udder irritation $r = 0.58$ ($P < 0.05$). At the beginning of lactation, subclinical mastitis is accompanied with significant changes in the activity of enzymes: muramidase $r = 0.84$ ($P < 0.001$), lactoperoxidase $r = 0.65$ ($P < 0.01$) and lactoferrin $r = 0.66$ ($P < 0.01$).

Keywords: cow; milking; mastitis; udder secretion; milk quality; milking equipment

Introduction

Production and sale of milk and dairy products in modern conditions are most closely related to biosafety issues. The main goal when working in this field is to comprehensively protect people and their environment from environmentally unfavorable factors. Raw milk is the main raw material in the production of all dairy products without exception. The quality and safety of raw milk is crucial in the production of dairy products (Paliy, 2016; Shkromada et al., 2019).

The bacteriological status of milk significantly deteriorates in udder disease in cows, or mastitis. The number of bacteria detected in the milk of cows depends on the form of mastitis and its stage, as well as the type of pathogens and milking equipment (Bobbo et al., 2017; Kromker & Leimbach, 2017; Paliy et al., 2018).

Data analysis (Silva et al., 2016) shows that milk quality is often reduced due to the high content of microflora. According to the authors' research for the recent 15 years, pathogenic streptococci and staphylococci have been most often detected from raw milk and udder secretions of patients with mastitis in cows. A special place, as noted (Hadzevych et al., 2019; Wente et al., 2019), in studies of mastitis have enterobacteria from the group of *Escherichia coli*, such as *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia*, as well as corynebacteria and pseudomonads.

The increase in the content of somatic cells in milk due to the growth in the number of leukocytes and the entry of microorganisms into the mammary alveoli signals that a cow has mastitis. It is very important that the number of somatic cells in milk can be easily and quickly determined, so the primary diagnosis of mastitis is carried out by monitoring somatic cells, which in themselves do not affect the safety and quality of milk. It is the somatic cells that make up the tissues of the mammary ducts and alveoli, which are involved in the secretion of milk (Paliy, 2019). In the udder of cows there is a constant renewal of epithelial tissue cells. This is due to the fact that the old cells die and come off. Those are accompanied by the cells that perform protective functions in the body (leukocytes). For this reason, somatic cells are constantly part of milk (Deshapriya et al., 2019). According to many scientists, the physiological norm of safe content of somatic cells in milk of cows makes 500 thous./cm³. In healthy cows, the somatic cell content within the physiological norm depends on the breed, age and term of lactation. Scientists have found that the content of somatic cells in the milk of first-calving cows is lower than in that of cows of subsequent lactations

(Zhelavskiy & Dmytriv, 2018). During the lactation period of 3-4 months after calving, the maximum number of somatic cells is observed in animals (Palii et al., 2020e). When the milk contains about 500 thous./cm³ of somatic cells, the admixture of mastitis milk in the bulk milk does not exceed 6%, which is acceptable and safe for dairy products. However, when the content in the milk collection of somatic cells increases and reaches 1000 thous./cm³, the admixture of mastitis milk in the bulk milk reaches 30% or more. Such milk is dangerous for human consumption and becomes a source of pathogenic microorganisms in dairy products (Palii et al., 2020b).

On farms that do not pay enough attention to the health of the cows' udder, the incidence of mastitis is 25-30% with the involvement of the pathological process from 9 to 12%; atrophy of parts of the udder is registered in 7-10% of cows in the herd (1.7-2.3% of parts) (Gokceoglu et al., 2020). The number of mastitis cases in cows increased dramatically after the introduction of machine milking. Farms suffer more from mastitis than from other bovine diseases taken together. With mastitis, milk yields are reduced by 10-15% or more, milk becomes unsuitable for processing into cheese or butter, because its composition and properties change (Freitas et al., 2017; Palii et al., 2020f). Inflammation of the mammary gland is the most common disease of farm animals, especially cows, which causes significant damage to livestock: milk loss during the period when a cow is ill, as well as during the current and subsequent lactation, drug consumption, early culling, sickness rate and death of young animals. Along with this, in modern conditions, the requirements for the quality of milk for the content of somatic cells and microorganisms are increasing (Bortolami et al., 2015; Palii et al., 2019; Wolfenson et al., 2015). In this regard, breeders are interested in preventing mastitis in the udder of cows and improving the quality of milk.

Good condition and operation of the milking equipment are crucial factors in terms of maintaining the health of the cow's udder. It is important to remember the role of the operator, as he is responsible for the condition of the milking equipment and the accuracy of milking. Studies of domestic and foreign scientists have shown that, in dairy herds, mastitis and udder atrophy in 16-45% of cases are caused by malfunction of milking machines and violation of the rules of machine milking (Palii et al., 2020c). The dangerous effect of the milking machine on the condition of the cow's udder is based primarily on two points: it can spread bacteria that cause mastitis, or damage the skin of the teats. Bacteria are spread from cow to cow through teat cups (Paliy et al., 2020). The pressure fluctuations of the milking parlor can result in reverse milk jets, which also carry bacteria from one part of the cow's udder into another. Failure of the milking machines or violations of the rules of milking cause damage to the teat tips, which increases the number of bacteria that cause mastitis on the skin of the teat, which increases the risk of new mastitis infections.

Thus, a comprehensive study of technological factors that significantly affect animals during milking is one of the pressing issues of modern animal husbandry.

Materials and Methods

The research was carried out on the state experimental farm Gontarivka, IT NAAS of Kharkiv region. Until 2020, clinical mastitis was diagnosed by a general examination and visual examination of the udder. Besides, the number of cows with clinical mastitis when using the ADM-8A milking equipment was conclusively 5 times higher than the number of cows with clinical mastitis when using the 'DeLaval' milking parlor. In the period from December 2019 to September 2020, a study was conducted to detect subclinical mastitis in the herd of cows using the 'Alpha Test' from 'De Laval' and a milk control plate (Paliy, 2018). The diagnostic procedure used included, at the initial stage, milking the first streams of milk into a separate container. A small amount of milk was milked from each teat of the udder into the pool on the milk control plate, observing that the rings at the bottom of the pool were slightly above its layer. Then to this milk, using a special dispenser, a portion of the reagent (2 ml), mixed in light circular motions, was added. A few seconds later, the determination was performed. Two groups of cows were tested for mastitis for 10 months. A total of 969 milk samples were examined.

Statistical processing was performed by the method of variation statistics on the reliability of the differences of the compared indicators. The values of arithmetic mean (M), standard deviation (σ), mean error (m) were calculated using the computer program Microsoft Office Excel 2007. The degree of reliability of the differences in the mean values in cases of normal distribution was determined by Student's t test.

Results and Discussion

As a result of the research conducted it was revealed that when using the milking equipment ADM-8A 4 out of 59 heads of cows had clinical mastitis that made 6.8% (Table 1). When using the 'DeLaval' milking equipment only 1 cow out of 50 heads was registered to have clinical mastitis, which was 2.0%. Subclinical mastitis in cows during milking with the ADM-8A equipment was 8.5%, and when milking with the 'DeLaval' parlor, it was 12.0%.

Table 1. Incidence of mastitis in cows when applying various milking equipment

Indicator	ADM-8A		'DeLaval'	
	cows	%	cows	%
Conditionally healthy animals	12	22.3	6	12.0
Subclinical mastitis	5	8.5	6	12.0
Clinical mastitis	4	6.8	1	2.0
Healthy animals	38	64.4	37	74.0
Total	59	100.0	50	100.0

When using domestic and imported equipment, 20.3% and 12.0% of conditionally healthy animals, respectively were found. In total, when using the ADM-8A unit, 64.4% of healthy animals were detected, and with the 'DeLaval' parlor, this amount was 74.0%, i.e. 9.6% more. It was found that the number of cows with mastitis during the year was different (Table 2).

Table 2. Analysis of the incidence of mastitis in cows by seasons, %

Form of mastitis	XII	I	II	III	IV	V	VI	VII	VIII	IX
'DeLaval'										
Number of cows, heads	43	46	46	46	47	46	42	43	48	48
Conditionally healthy animals	7.0±0.6	6.5±0.6	8.7±0.6	6.5±0.6	10.6±0.5	10.9±0.5	11.9±0.7	11.6±0.7	10.4±0.6	8.3±0.6
Subclinical	2.3±0.4	4.3±0.4	2.2±0.4	2.2±0.4	2.1±0.4	6.5±0.5	9.5±0.5	9.3±0.5	10.4±0.6	10.4±0.6
Clinical	-	-	-	-	-	-	-	-	-	2.1±0.3
Healthy	90.7±0.7	89.2±0.7	89.1±0.7	91.3±0.7	87.3±0.7	82.6±0.7	78.6±0.7	79.1±0.7	79.2±0.7	79.2±0.7
ADM-8A										
Number of cows, heads	51	54	54	54	53	46	45	52	50	51
Conditionally healthy animals	11.8±0.6	13.0±0.6	11.1±0.6	13.0±0.6	9.4±0.5	17.4±0.7	22.2±0.9	19.2±0.7	18.0±0.7	13.7±0.6
Subclinical	2.0±0.2	1.9±0.2	7.4±0.4	7.4±0.4	7.5±0.4	4.3±0.3	4.4±0.3	3.8±0.3	2.0±0.2	2.0±0.2
Clinical	2.0±0.2	1.9±0.2	1.9±0.2	3.7±0.3	3.8±0.3	4.3±0.3	4.4±0.3	3.8±0.3	4.0±0.3	5.9±0.5
Healthy	84.2±0.7	83.2±0.7	79.6±0.7	75.9±0.7	79.3±0.7	74.0±0.7	69.0±0.7	73.2±0.7	76.0±0.7	78.4±0.7

The lowest percentage of cows with mastitis was registered when using imported equipment in March 2020 and December 2019: 8.7% and 9.3%, respectively. When milking with ADM-8A, the least amount of mastitis was observed in December 2019 which was 15.8%.

Thus, when using the 'DeLaval' milking equipment in December, less by 6.5% ($P < 0.001$) cases of mastitis were registered than when using ADM-8A. Data on the incidence of mastitis in cows on average by season of the year are shown in Table 3.

It was found that the lowest percentage of cows with mastitis was recorded in winter: 10.3% ($P < 0.001$) when milking with the 'DeLaval' and 17.7% ($P < 0.001$) when milking with the ADM-8A unit.

The highest incidence of mastitis in cows was observed in summer: 21.0% ($P < 0.001$) and 27.1% ($P < 0.001$), respectively, when milking cows at the 'DeLaval' milking parlors and ADM-8A. When calculating the average indicators, conditionally healthy cows were not considered healthy. Thus, the highest percentage of healthy cows was observed in the winter during milking with the 'DeLaval' and ADM-8A milking equipment: 89.7% ($P < 0.001$) and 82.3% ($P < 0.001$), respectively. At the same time, when the imported equipment was used, there were by 7.4% more healthy animals ($P < 0.001$). Often cows with signs of the disease come from the maternity ward, where milking is carried out with the domestic plant ADM-8A. It is important to note that only one such a cow was found when milking with the imported equipment, and 4 cows or 19% of all sick cows when using ADM-8A.

Table 3. Incidence of mastitis in cows by seasons, %

Form of mastitis	Seasons			
	Winter	Spring	Summer	Autumn
'DeLaval'				
Number of cows, heads	135	139	133	48
Conditionally healthy animals	7.4±0.2	9.3±0.2	11.3±0.3	8.3±0.5
Subclinical	2.9±0.1	3.6±0.1	9.7±0.2	10.4±0.6
Clinical	-	-	-	2.1±0.3
Healthy	89.7±0.1***	87.1±0.1***	79.0±0.3***	79.2±0.8
ADM-8A				
Number of cows, heads	159	153	147	51
Conditionally healthy animals	12.0±0.2	13.3±0.2	19.8±0.3	13.7±0.7
Subclinical	3.8±0.1	6.4±0.1	3.4±0.1	2.0±0.3
Clinical	1.9±0.1	3.9±0.1	3.9±0.1	5.9±0.5
Healthy	82.3±0.1	76.4±0.1	72.9±0.3	78.4±0.8

*** - $P < 0.001$

The disease later turns into more severe forms. That is, if the cow comes from the maternity ward conditionally healthy, but later, as a rule, subclinical mastitis develops. It was found that, when using ADM-8A milking equipment, adult cows are 2.1-1.7 times more prone to the incidence of mastitis compared to cows of the 1st and 2nd calving (Table 4). When milking the cows with the 'DeLaval', incidence of mastitis of adult cows was 4.3-1.1 times higher, compared to cows of 1st and 2nd calving. The results of laboratory tests of the secretion of the udder of cows with functional disorders in the mamma of different genesis are presented in Table 5. The obtained data prove that, in cows with disorders in the mammary gland, the content of somatic cells in the secretion of the udder in all periods of the functional state of the body significantly changes (with a high degree of correlation). Thus, during subclinical mastitis $r = 0.72$ ($P < 0.001$) and udder irritation $r = 0.58$ ($P < 0.05$).

At the beginning of lactation subclinical mastitis is accompanied by significant changes in the activity of enzymes: muramidase $r = 0.84$ ($P < 0.001$), lactoperoxidase $r = 0.65$ ($P < 0.01$) and lactoferrin $r = 0.66$ ($P < 0.01$).

The analysis of the obtained data showed that an increase in the number of somatic cells, lactoferrin and decreased activity of the enzyme muramidase form the general pattern of changes occurring in the secretion of the affected udder lobes in comparison with healthy ones with different functional state of the udder.

Table 4. Incidence of mastitis in cows of various age, %

Indicator	Age of a cow when calving		
	1	2	older than 3
	ADM-8A		
Number of cows, heads	10	8	41
Conditionally healthy animals	20.0	12.5	22.0
Subclinical	-	-	12.2
Clinical	-	12.5	7.3
Conditionally healthy cows and cows with mastitis	20.0	25.0	41.5
Healthy animals	80.0	75.0	58.5
	'DeLaval'		
Number of cows, heads	13	13	24
Conditionally healthy animals	7.7	7.7	16.7
Subclinical	-	15.4	16.7
Clinical	-	7.7	-
Conditionally healthy cows and cows with mastitis	7.7	30.8	33.4
Healthy animals	92.3	69.2	66.6

Table 5. Indicators of mammary gland secretion in cows with functional disorders in the mamma, (n=35)

Indexes	Udder irritation	Subclinical mastitis
Somatic cells, thous./ml	570±0.13	6763.3±217*
Total protein, %	3.19±0.13	3.23±0.18
Muramidase, NU	0.59±0.02	0.40±0.09*
Lactoperoxidase, NU	650.7±42.1	987.2±72.6**
Lactoferrin, µg/ml	139.4±3.56	300.0±56.7**
Catalase activity, p	35.5±42.7	6.87±0.42

* - $P < 0.05$; ** - $P < 0.01$ compared to udder irritation

A significant entry of somatic cells from the bloodstream into the udder is subdued to the fact that the udder needs a sufficient number of phagocytes. As the phagocytic activity of the blood cells entering the affected organ is significantly reduced compared to healthy ones, the cellular defense begins to work by an extensive type.

Neutrophils and lactocytes, being a source of lactoferrin in the udder secretion, release it from special granules due to degranulation of the former during phagocytosis and destruction of these granules, which causes its high concentration in functional disorders in the breast regardless of lactation. A low activity of muramidase in the secretion of the affected lobes indicates a decrease in the antistaphylococcal properties of the enzyme and local resistance of the organ. That is the content of lactoperoxidase, the activity of which in inflammation increases at the beginning and middle of lactation which is a feature of changes in the secretion of the affected lobes of the udder.

The formation of the udder function and stabilization of lactogenesis necessitate regular emptying of the udder and the influx of fresh neutrophils involved in the phagocytosis of microorganisms and intensive secretion of the enzyme, as evidenced by its increased activity at the beginning and middle of lactation. Moreover, the additional entry of lactoperoxidase into the secretion occurs during the destruction of lactocytes. Therefore, in dairy cows, functional disorders in the udder are manifested by activation of cellular protection and a factor of nonspecific local resistance of the lactoferrin enzyme. The nature of the functional state of the udder determines the features of lactoperoxidase activity of the secretion.

Some researchers (Penry et al., 2017; Krawczel et al., 2017; Guarín & Ruegg, 2016) have shown that teat canal is suppressed and closed between milkings. Peristalsis of the smooth muscles lining the canal promotes the excretion of bacteria. The canal expands during milking (8.6 mm long, 1.2 mm in diameter), after which, remaining in the same state, has no peristalsis for 2 to 4 hours. Temporary saturation of the tissues of the teat tips with fluid (blood and intercellular fluid) occurs after milking. It takes the fluid time to leave the teat. Through a short wide canal, milk is milked faster, but such a cow is more susceptible to mastitis. The length and diameter of the teat canal change with age, which plays an important role in susceptibility to mastitis (Wieland et al., 2019).

A healthy cow's udder is one of the factors in producing high quality milk. Udder diseases are widespread and are a major problem in ensuring the hygiene of milk production worldwide (Klaas & Zadoks, 2017; Palii et al., 2020d). Primary contamination of raw milk with microorganisms starts in the teat canals and the surface of the udder, especially in the case of inflammatory

process of the udder, i.e. mastitis. Scientists have found that in subclinical forms of mastitis in the milk of cows can get up to 105 CFU/cm³ of pathogens, and in clinical mastitis - up to 108 CFU/cm³. The causative agents of mastitis in cows are various representatives of the microbial environment: bacteria, mycoplasmas, yeast and algae. Researchers have identified 137 species of microorganisms that may be the cause of its occurrence, but only 20 of them are well investigated. The causative agents of mastitis live and develop inside the infected parts of the udder. They provoke the spread of subclinical mastitis in the udder lobes, which usually manifests itself in the form of an increase in the number of somatic cells (leukocytes and epithelial cells of the mammary gland) in the milk expressed from the affected lobe. Pathogens are rapidly transmitted from cow to cow or from one lobe of the udder to another, and are spread during milking through reusable towels for wiping the hands of operators and milking machines (Vlieghe et al., 2012). The problem of sanitary and hygienic quality of cow's milk and a set of factors affecting the content of microorganisms in cow's milk are reflected in the works (Asma et al., 2019; Degen et al., 2015; Paliy, 2019).

Bezman et al. (2015) and Kock et al. (2018) made significant contribution to the study of the sanitary and hygienic condition of cow's milk and the level of its contamination by various microorganisms. Domestic science and practical experience have developed effective veterinary and sanitary measures to control the problem of mastitis in cows. Methods of early diagnosis, prevention and treatment of udder disease through the use of various antimicrobial drugs, physiotherapy and antiseptic treatment of teats have been created and implemented (Leimbach & Krömker, 2018). Despite the progress made, the problem of udder disease in cattle continues to be one of the most relevant for veterinary science and practice in all countries of the world with intensive dairy farming, which is a socio-economic problem. Nowadays, the heads of agro-industrial enterprises face the problem of veterinary and sanitary quality of milk, the solution of which is a difficult task, as it depends on many factors, both objective and subjective (Paliy & Paliy, 2019). There are many studies that confirm that mastitis is not only a serious disease, but also one of the cost items of dairy farming (Doehring & Sundrum, 2019; Nolan, 2017; Paliy et al., 2020a; Samson et al., 2016; Soest et al., 2016). The development of diagnostic measures which aim to identify cows with mastitis that should consist of a history, general clinical examination, thorough examination of the cow's udder and bacteriological examination of the secretion of the udder is a promising area.

Conclusion

Gentle mode of milking on the 'DeLaval' parlor allowed to increase the number of completely healthy animals up to 74%, which is 9.6% more than when using the milking equipment ADM-8A (64.4%); and to reduce the incidence of clinical mastitis in cows by 3.4 times.

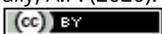
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