

FACTORS OF NATURAL RESISTENCE AT SUBCLINICAL MASTITIS AT THE ACTION OF LIPOSOMAL DRUG

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*The article presents the results of experimental studies on the effect of a new complex liposomal medicine made on the basis of *Hypericum perforatum* L. on the indicators of non-specific resistance of cows suffering from subclinical mastitis.*

Studies have shown that the subclinical form of mastitis leads to an increase in phagocytic activity of blood neutrophils on the background of a decrease in phagocytic number and intensity of phagocytosis. At the same time, the growth of the content of circulating immune complexes and the decrease in the level of bactericidal and lysozyme activity of the blood serum was noted. Intracisternal administration of the drug caused a normalizing effect on the parameters of natural resistance.

Keywords: cows, subclinical mastitis, blood, fagocytosis, neutrophils, bactericidal, lysocytic activity.

Formulation of the problem and analysis of current research. One of the factors that negatively affects the growth of milk productivity of cows and the sanitary quality of milk in farms with different forms of ownership are pathological inflammatory processes in the breast, especially latent.

The subclinical form of mastitis is one of the most common diseases, which according to various authors accounts for 20 to 80% of all inflammatory processes in the breast. Significant economic losses caused to dairy cattle due to this pathology are primarily due to reduced milk productivity of cows, culling of animals (30-35%) and treatment costs [1-3].

In addition to economic losses, the threat is contamination of milk with pathogenic microorganisms, changes in chemical composition, physical and biochemical properties of milk, resulting in loss of nutritional value, which affects its quality and biological safety [4, 5]. Feeding newborn young colostrum from cows with mastitis can lead to gastrointestinal disorders and even their death.

In the treatment of sick cows in most cases, preference is given to the use of antibiotics and sulfonamides medicaments by intracisternal administration. The most negative consequence of the use of antibiotics in the treatment of cows with mastitis is the presence of their residues in the milk, which worsens its technological.

In recent years, there has been a significant expansion of research on the use of liposomal drugs, which do not contain antibiotics, prevent the recurrence of the disease and maximize the restoration of milk productivity. Liposomes are spherical lipid vesicles, which usually include various phospholipids, specific glycopeptides, and cholesterol, which is a steroid component of almost all cell membranes [7-8].

The aim of the article. The aim of the work was to study the effect of a liposomal preparation, made on the basis of *St. John's wort* (*Hypericum perforatum* L.), on the indicators of nonspecific resistance of cows suffering from a subclinical form of mastitis.

Materials and methods. The study was conducted in LLC "Molochni Riky" Brody district of L'viv region on cows, which on the principle of analogues were divided into two groups: control and experimental, 7 animals in each. The material for laboratory tests was the blood of clinically healthy animals and whole milk, in which the number of somatic cells did not exceed 400 thousand/cm³ (control group). The experimental group was formed from animals with subclinical mastitis (SM), the number of somatic cells in the milk of these animals ranged from 500 thousand to 1 million in 1 cm³. In samples of whole milk from cows, the concentration of somatic cells was determined by the viscometry express method on the analyzer "AMB 1-02". To determine the affected quarter of the mammary gland used 2% aqueous solution of mastidine.

Cows of the experimental group in the affected quarters of the udder intracisternal three times with an interval of 24 hours was injected liposomal medicament - the first day 10 cm³, the next two days - 5 cm³. Milk before administration of the medicament was milked by hand, disinfected teat. After

medicament administration, the mammary gland was massaged from the bottom up for its uniform distribution. The cows were transferred to manual milking. Half of the therapeutic dose was prophylactically administered to healthy quarters of the mammary gland.

Liposomal preparation made on the basis of plant raw materials, which is an antibacterial medicament developed in the laboratory of immunology of the Institute of Animal Biology NAAS.

The composition of the medicament includes: novoimanin - extract with St. John's wort (*Hypericum perforatum L.*), vitamins A, D3, E, lecithin, twin. The medicament is active against gram-positive bacteria, including *Streptococcus pyogenes* and *Streptococcus agalactiae*. The anti-inflammatory effect is due to the presence of flavonoids in the medicament. It has the ability to heal the wound surface and stimulates tissue regeneration.

For immunological studies, blood was taken from cows from the jugular vein before morning feeding on the 1st day (before medicament administration) and on the 3-rd and 9-th day after its use.

In heparin-stabilized blood, phagocytosis parameters were determined – phagocytic activity (PA) and the intensity of polynuclear cells to the daily culture of *E. coli* (strain VKM-125; Gostev V.S., 1950). In serum of blood determined bactericidal activity (SBA) to the microbial culture of *E. coli* (strain VKM-125; Markov Y.M., 1968); lysozyme activity (SLA) to the daily culture of *Micrococcus lysodeikticus* (strain VKM-109) - photonephelometric method (Dorofeychuk V.G., 1968), the content of circulating immune complexes (CIC) of average molecular weight (Chernushenko E.F., Kogosova P.S., 1981). The studies were performed according to the methods described in the guide [9].

The obtained digital data were statistically processed using Microsoft Excel software for personal computers, using conventional methods of variation statistics with the determination of mean values (M), their quadratic error (m) and the significance of differences by Student's t-test.

Results and discussion. The body's resistance is provided by both specific, and nonspecific protective factors, and the body's natural (nonspecific) resistance is determined by a complex of cellular and humoral factors. Phagocytosis is the main mechanism of natural resistance, as well as a necessary link in the induction and formation of a specific immune response. One of the main links in the body's natural cellular defense is the phagocytic activity of neutrophilic blood granulocytes.

Studies have shown that the disease of cows in the subclinical form of mastitis leads to changes in the phagocytosis of neutrophils in the blood of animals (table 1). In particular, higher ($47,0 \pm 1,78$ % compared to $41,0 \pm 1,68$ %) ($P < 0,05$) phagocytic activity of neutrophilic granulocytes in the blood of sick cows compared to the control group on the background of reduced phagocytic count (PC) ($4,1 \pm 0,28$ units compared to $4,5 \pm 0,42$ units) and the intensity of phagocytosis (PI) in cows with latent inflammatory process of the breast, which is 16,5% less than in clinically healthy animals.

Table 1. Dynamics of phagocytosis of neutrophils in the blood of cows with subclinical mastitis ($M \pm m$; $n=7$)

Parameters	Control groups animal	Experimental groups animal		
		before treatment	3-rd day of treatment	9-th day from the beginning of treatment
PA, %	$41,0 \pm 1,68$	$47,0 \pm 1,78^*$	$45,2 \pm 1,25$	$42,0 \pm 2,86^\circ$
PI, unit	$10,6 \pm 0,74$	$8,8 \pm 0,56$	$9,5 \pm 0,68$	$10,5 \pm 1,23$
PC, unit	$4,5 \pm 0,42$	$4,1 \pm 0,28$	$4,3 \pm 0,2$	$4,3 \pm 0,3$

Note: In this and table 2: $^\circ$ – $P < 0,05$ – the probability of differences in animals of this group compared with the indicators before the introduction of the medicament (1st day of the experiment); * – $P < 0,05$ - the difference is significant compared to the control group.

The introduction of the study drug to sick cows caused a decrease in blood granulocyte PA. Thus, on the third day of treatment, the activity of phagocytic cells gradually decreased and this trend persisted until the end of treatment, which indicates the complete attenuation of the inflammatory response. Along with this, an increase in phagocytic index and phagocytic number was found, which indicates the normalizing effect of the medicament on the cellular link of the body's natural resistance.

Condition research of the humoral link of nonspecific resistance of cows with latent mastitis showed (table 2) a decrease in the level of intensity of bactericidal activity ($34,9 \pm 1,77\%$ compared to $43,3 \pm 1,2\%$, $P < 0,01$) and lysozyme activity of blood serum ($21,7 \pm 1,29\%$ compared to $28,7 \pm 1,38\%$, $P < 0,01$).

Table 2. Humoral blood protection factors of cows with subclinical mastitis ($M \pm m$; $n=7$)

Parameters	Control groups animal	Experimental groups animal		
		before treatment	3-rd day of treatment	9-th day from the beginning of treatment
SBA, %	$43,3 \pm 1,2$	$34,9 \pm 1,77^{**}$	$38,7 \pm 1,48^*$	$42,3 \pm 1,12^\circ$
SLA, %	$28,7 \pm 1,38$	$21,7 \pm 1,29^{**}$	$26,0 \pm 0,98^*$	$27,3 \pm 1,18^\circ$
CIC, mmol/l	$73,5 \pm 2,5$	$86,5 \pm 2,62^{**}$	$77,0 \pm 1,73$	$74,7 \pm 1,1^\circ$

Note: In this and table 2: $^\circ$ – $P < 0,05$ – the probability of differences in animals of this group compared with the indicators before the introduction of the medicament (1st day of the experiment); * – $P < 0,05$ - the difference is significant compared to the control group.

At the same time, in the serum of cows of the experimental group there was an increase in the content of circulating immune complexes ($86,5 \pm 2,62\%$ compared to $73,5 \pm 2,5\%$, $P < 0,01$), which indicates a significant antigenic load and accumulation of inflammatory metabolites in their body.

Intracisternal administration of the study medicament to sick animals had a normalizing effect on the indicators of the humoral part of nonspecific protection. Thus, on the 9-th day of the experiment, an increase in bactericidal and lysozyme activity of blood serum was registered in animals of the experimental group, respectively ($42,3 \pm 1,12\%$ compared to $34,9 \pm 1,77\%$, $P < 0,01$ and $27,3 \pm 1,18\%$ compared to $21,7 \pm 1,29\%$, $P < 0,05$). At the same time in the specified period of researches the concentration of CIC in blood serum ($74,7 \pm 1,1$ mmol/l against $86,5 \pm 2,62$ mmol/l, $P < 0,01$) significantly decreased that testifies to positive influence of components of medicament on the course of the inflammatory process in sick animals.

Thus, the use of the studied liposomal medicament for the treatment of cows with SM, helps to restore the disturbed conditions of metabolic homeostasis and normalizes the cellular and humoral links of non-specific protection, which has a positive effect on the course of the disease.

Conclusion. The disease of cows with subclinical mastitis leads to a statistically significant increase in phagocytic activity of neutrophils and an increase in the content of circulating immune complexes against the background of reduced bactericidal and lysozyme activity of serum.

Intracisternal administration of the medicament to sick cows had a normalizing effect on the indicators of natural resistance. This is evidenced by an increase in phagocytic index and phagocytic number, bactericidal and lysozyme activity of blood serum, a decrease in the content of CIC and phagocytic activity of blood granulocytes.

In the future, it is planned to conduct a comprehensive functional study of immunocompetent cells under the conditions of using a new complex liposomal preparation based on plant raw materials.

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ФАКТОРИ ПРИРОДНОЇ РЕЗИСТЕНТНОСТІ ПРИ СУБКЛІНІЧНОМУ МАСТИТІ ЗА ДІЇ ЛІПОСОМАЛЬНОГО ПРЕПАРАТУ

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У статті наведені результати експериментальних досліджень щодо впливу нового комплексного ліпосомального препарату, виготовленого на основі звіробою продірявленого (*Huregicum perforatum L.*), на показники неспецифічної резистентності організму корів, хворих на субклінічний мастит.

Дослідження показали, що захворювання корів на субклінічну форму маститу призводить до підвищення фагоцитарної активності нейтрофілів крові на тлі зниження фагоцитарного числа та інтенсивності фагоцитозу. При цьому у хворих корів констатовано зростання вмісту циркулюючих імунних комплексів та зниження рівня бактерицидної та лізоцимної активності сироватки крові. Інтрацистернальне введення хворим коровам досліджуваного препарату спричинило нормалізуючий вплив на показники природної резистентності.

Ключові слова: корови, субклінічний мастит, кров, фагоцитоз, нейтрофіли, бактерицидна, лізоцимна активність.

ФАКТОРЫ ЕСТЕСТВЕННОЙ РЕЗИСТЕНТНОСТИ ПРИ СУБКЛИНИЧЕСКОМ МАСТИТЕ ПОД ДЕЙСТВИЕМ ЛИПОСОМАЛЬНОГО ПРЕПАРАТА

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В статье приведены результаты экспериментальных исследований относительно влияния нового комплексного липосомального препарата, изготовленного на основе зверобоя продырявленного (*Huregicum perforatum L.*), на показатели неспецифической резистентности организма коров, больных на субклинический мастит.

Исследования показали, что заболевание коров на субклиническую форму мастита привело к повышению фагоцитарной активности нейтрофилов крови на фоне снижения фагоцитарного числа и интенсивности фагоцитоза. При этом у больных коров констатировано увеличение содержания циркулирующих иммунных комплексов и снижение уровня бактерицидной и лизоцимной активности сыворотки крови. Интрацистернальное введение больным коровам исследуемого препарата привело к нормализующему влиянию на показатели природной резистентности.

Ключевые слова: коровы, субклинический мастит, кровь, фагоцитоз, нейтрофилы, бактерицидная, лизоцимная активность.