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## Indicators of phagocytosis of granulocytes neutrophils in cows blood sick on subclinical form of mastitis and under the influence of preparation «Antymast»

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*The article contains the results of the experimental researches of the influence of preparation «Antymast», is made from natural bee raw material, on the indicators of neutrophils granulocytes phagocytosis of cows blood, which have subclinical form of mastitis.*

*The research was conducted during the spring period on two groups of cows of Black–Spotted Dairy breeds (2–3 lactation) 5 – 7 animals in each group. The cows before starting the investigations were diagnosed with the help of viscose metric express method for subclinical forms of mastitis: control group – clinically healthy animals, research group – with signs of subclinical mastitis. Cows from the research group were administered intracisternally with one injection of syringe tube (13 ml) three times at intervals of 24 hours of preparation «Antymast» in the affected udder quarters, half of the therapeutic dose was prophylactically injected into the healthy quarter of breast.*

*It was established that in cows, sick on subclinical form of mastitis, was observed the activation of phagocytic activity of blood granulocytes neutrophils against decrease in phagocytic index ( $p < 0,05$ ) and the number and increase ( $p < 0,05$ ) spontaneous NBT test.*

*Intracisternal introduction of preparation «Antymast» to sick cows contributed to the normalization of phagocytic activity of blood neutrophils and increase their absorption capacity and the reduction of NBT test ( $p < 0,05$ ).*

**Key words:** *cattle, subclinical mastitis, somatic cell, phagocytosis, phagocytic activity, phagocytic index, phagocytic number, neutrophil granulocytes, NBT–test of blood.*

## Показники фагоцитозу нейтрофільних гранулоцитів крові корів, хворих на субклінічну форму маститу, та за дії препарату «Антимаст»

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*У статті наведені результати експериментальних досліджень впливу препарату «Антимаст», виготовленого на основі натуральної бджолоїної сировини, на показники фагоцитозу нейтрофільних гранулоцитів крові корів, хворих на субклінічну форму маститу.*

*Дослідження проведено у весняний період на двох групах корів чорно–рябої молочної породи (2 – 3 лактації) по 5 – 7 тварин у кожній. Корови перед початком досліджень були продіагностовані віскозиметричним експрес–методом на субклінічні форми маститу: контрольна група — клінічно здорові тварини, дослідна група — з ознаками субклінічного маститу. Коровам дослідної групи інтрацистернально в уражені чверті вимені тричі з інтервалом 24 години вводили по одному шприцу–тубі (13 мл) препарату «Антимаст», у здорові чверті молочної залози профілактично вводили половину лікувальної дози.*

*Встановлено, що у корів, хворих на субклінічну форму маститу, спостерігається активація фагоцитарної активності нейтрофільних гранулоцитів крові на тлі зниження фагоцитарного індексу ( $p < 0,05$ ) та числа і підвищення ( $p < 0,05$ )*

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спонтанного НСТ-тесту. Інтрацистернальне введення хворим коровам препарату «Антимаст» сприяло нормалізації фагоцитарної активності нейтрофілів крові й підвищенню їх поглинальної здатності та зниженню НСТ-тесту ( $p < 0,05$ ).

**Ключові слова:** велика рогата худоба, субклінічний мастит, соматичні клітини, фагоцитоз, фагоцитарна активність, фагоцитарний індекс, фагоцитарне число, нейтрофільні гранулоцити, НСТ-тест, кров.

## Показатели фагоцитоза нейтрофильных гранулоцитов крови коров, больных субклинической формой мастита, при действии препарата «Антимаст»

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В статті приведені результати експериментальних досліджень впливу изготовленого на основі пчелиного сир'я препарату «Антимаст» на показателі фагоцитоза нейтрофільних гранулоцитів крові коров, хворих субклінічної формою мастита.

Дослідження проведено в весняний період на двох групах коров чорно-рябій молочної породи (2–3 лактації) по 5–7 тварин у кожній групі. Корови перед початком досліджень були продіагностовані вискозиметричним експрес-методом на виявлення субклінічної форми мастита: контрольна група – клінічно здорові тварини, опытная група – з ознаками субклінічного мастита. Коровам опытной групи інтрацистернально в поразенні четверті вымени трижды з інтервалом в 24 години вводили по одному шприцу-тубе (13 мл) препарату «Антимаст», в здорові четверті молочної залози профілактично вводили половину лікувальної дози.

Встановлено, що у коров, хворих субклінічною формою мастита, спостерігається активація фагоцитарної активності нейтрофілів крові на фоні зниження фагоцитарного індексу ( $p < 0,05$ ) і числа підвищення ( $p < 0,05$ ) спонтанного НСТ-тесту. Інтрацистернальне введення хворим коровам препарату «Антимаст» сприяло нормалізації фагоцитарної активності нейтрофільних гранулоцитів крові і підвищенню їх поглинаючої здатності, зниження НСТ-тесту ( $p < 0,05$ ).

**Ключові слова:** велика рогата худоба, субклінічний мастит, соматичні клітини, фагоцитоз, фагоцитарна активність, фагоцитарний індекс, фагоцитарне число, нейтрофільні гранулоцити, НСТ-тест, кров.

### Introduction

The important condition of milk and dairy products of domestic production in the world market is their biological safety and high quality according to the standards of the European Union. Research was aimed at solving these important issues highlighted in a number of scientific papers (Yakubchak and Homenko, 2002; Kasyanchuk et al., 2006; Kuhtyn, 2008; Rudenko et al., 2009).

The pathological inflammatory processes in the mammary gland reduces milk production of cows and sanitary quality of milk, leading to premature culling of highly productive animals. Especially big economic losses to dairy cattle has widespread hidden (subclinical) form of mastitis (Plakhotnjuk and Ordin, 2013; Murska, 2014).

*Actuality of theme.* Violation of the homeostasis of the organism of cows sick on subclinical form of mastitis, which occurs as a result of failure of the process (rules of animals launch, conditions of keeping and feeding, exploitation), is accompanied by significant changes in immunological reactivity of the organism (Shpileva, 2003). One of the main criteria for evaluating of immune homeostasis is system of phagocytosis, in which polymorphonuclear leukocytes perform the main role. It is established, that neutrophils play one from the main roles in the development of disease (Drannyk, 2003; Broda, 2009; Broda, 2013).

The organism resistance against infection and effective elimination of disease agents from the blood of dif-

ferent nature, largely depends on the functional activity and efficiency of phagocytic cells of the system. Spontaneous test with nitroblue tetrazolium to evaluate the state of oxygen dependent mechanisms of phagocytes bactericidal action (granulocytes) of blood *in vitro*. It characterizes the state and the degree of activation of intracellular NADPH-oxidase antimicrobial systems (Masljanko, 1999; Bilovol et al., 2011).

In the treatment of sick cows in most cases preference is given to use of antibiotics and sulfanilamide preparations, which almost completely replaced all other therapeutic agents. However, widespread use of these drugs has certain negative consequences: reducing their effectiveness due to acquisition of resistance of mastitis pathogens to many of them; reducing the resistance of animal organism and breast tissue; occurrence of atrophy and indurations and thus the development of hypo- and agalactia, causing significant losses to farms.

*The aim* of our research was to investigate the influence of the preparation «Antymast», made from natural bee raw materials, on indices of phagocytosis of the cows blood neutrophils, sick on subclinical form of mastitis.

### Material and methods

The research was conducted in SE HH «Obroshyno» Pustomyty district, Lviv region during the spring period on two groups of cows (lactation 2–3) 5–7 animals in each. Cows before the investigations were diagnosed with

the help of viscose metric express method on subclinical forms of mastitis: control group – a clinically healthy animals, research group – with signs of subclinical mastitis. To determine the affected breast quarter was used 2% aqueous solution of mast dine. Cows of the experimental group were administered intracisternally one injection of syringe tube (13 mL) of the drug «Antymast» (RP number AB-03772-01-12) into the affected udder quarter three times at intervals of 24 hours, into healthy quarter of the breast were prophylactically administered half of the therapeutic dose. Before using the drug milk was milked from all quarters of the udder and was disinfected the nipple. After injection of the drug massages of the breast were performed for its uniform distribution.

The preparation «Antymast» includes: bee propolis, extract of bee mahr, beeswax, castor oil, vegetable oil.

Blood for immunological tests were taken from the jugular vein in cows before injection of the drug and on the 3-rd – and 9-th day after injection.

The functional activity of neutrophils was evaluated by the indexes of phagocytosis, defining the phagocytic activity (TA) – relative content of neutrophils, participating in the process of phagocytosis, phagocytic index (FI) – absorption capacity of active neutrophil and and phagocytic number (PN) – the number of microbial cells, attributable per one calculated phagocytosis.

State of functional and metabolic activity of leukocytes was determined on the basis of the research of no substrate restoration of nitro blue tetrazolium reaction (NBT spontaneous test) (Vlizlo, 2012).

During the research period it was carried out the observation on clinical condition of cows, control of breast gland – examination, palpation, trial milking. To control the quality of milk restoration it was used the analyzer «AMV 1-02» intended for measuring the relative viscosity of the whole milk and the concentration calculation of somatic cells in it (ISO 7357: 2013).

The obtained digital data had been processed statistically by using Microsoft Excel software package for personal computers, using standard methods of variation statistics

with the definition of average values (M), their quadratic error (m) and reliability of differences, which are set for the Student t-criterion.

## Results and discussion

Phagocytosis is one of the most important factors of structural and immune homeostasis, which is aimed at the preservation constancy of internal environment. This process combines various cellular reactions in the direction of phagocytosis object recognition, its neutralization and removal from the body. Studying the phagocytic activity of neutrophils granulocytes of blood – one of the main parts of the natural cellular immunity of organism, it was found that the disease of cows on subclinical form of mastitis leads to indexes changes of phagocytosis of reactive blood cells.

In particular, the FA granulocytes neutrophils in the blood of sick cows was 4.4% higher than in the animals in the control group, but the difference was not reliable. Nevertheless, the results of these researches indicate a strengthening of inflammation in the body (Table.). It also indicates decrease in the intensity of phagocytosis (FI) in cows with subclinical inflammation of the mammary gland. So, FI index, which characterizes the intensity of phagocytosis of sick cows was 16.8% lower ( $p < 0,05$ ), than in clinically healthy animals. In this case the blood of cows of from the experimental group is fixed a tendency to reduce the number of microbial cells, attributable for one calculated phagocytosis (AX). At the same time it was observed changes in such important functional parameters of phagocytic cells, as the spontaneous NBT-test, which reflects the activity of intra leukocyte bactericidal system – an important link of nonspecific resistance of the organism. In particular, in sick cows this indicator was 1.8 times ( $p < 0,05$ ) higher than in clinically healthy. These data indicate the presence of cows body, sick on subclinical mastitis, toxic products of the pathological process, therefore neutrophils are not capable of complete phagocytosis.

Table.

**Indicator changes of blood phagocytosis in cows, sick on subclinical mastitis in the dynamics of treatment (M ± m; n = 5)**

Indexes	Gr.	The period of researches		
		before injection of the preparation	3rd day after introduction	9th day after introduction
FA, %	C	42,00 ± 1,70		
	E	46,40 ± 1,81	44,00 ± 2,07	43,00 ± 1,70
FI, un.	C	10,78 ± 0,43		
	E	8,97 ± 0,60*	9,40 ± 0,55	9,82 ± 0,31
SF, un.	C	4,53 ± 0,28		
	E	4,14 ± 0,25	4,18 ± 0,42	4,24 ± 0,30
NST-test (spontaneous)%	C	7,40 ± 1,03		
	E	13,20 ± 1,46*	11,6 ± 0,93*	8,20 ± 0,86°

Note. In this table is statistically reliably difference between investigated indicators in experimental animal group, compared to the control: \* – with  $< 0.05$  cows of the experimental group concerning the indicator before treatment: ° –  $p < 0.05$ .

But, in the dynamics of treatment there was some correction of phagocytic defense. So, on the third day of curation the reactivity of phagocytic cells is gradually

decreased and this tendency continued until the end of the treatment, which indicated a complete attenuation of the inflammatory reaction. Along with this, during the treat-

ment phagocytic cells were active in their attractive absorption capacity, which is manifested by increased phagocytic index and phagocytic number.

This is consistent with the dynamic changes of spontaneous NBT test – between the intensity and the ability of cells to complete phagocytosis – exists an inverse dependence. In the experimental group of cows on the third day after the injection "Antymast" this figure was 4,2% ( $p < 0,05$ ) higher than in the control group of cows, but to the ninth day of the experiment it was reduced to the level of normative values, and the difference compared to treatment were probable.

So, the results of using of the applied schemes of cows treatment, sick on subclinical mastitis, showed that intracisternally injection into the affected udder quarters at intervals of 24 hours per one syringe–tube of preparation «Antymast», and in healthy breast quarters prophylactic half of the therapeutic dose has a positive effect on functional activity of neutrophils granulocytes of peripheral cows blood, proving the advisability of its use in the treatment of subclinical mastitis in veterinary practice.

### Conclusions

1. In cows suffering from subclinical form of mastitis, is observed the activation of phagocytic activity of neutrophils granulocytes of blood against decrease in phagocytic index ( $p < 0,05$ ) and the number and increase ( $p < 0,05$ ) of spontaneous NBT–test.

2. The treatment of cows suffering from subclinical form of mastitis, with preparation "Antymast" positively affected on the recovery of phagocytosis parameters of cows. In particular, ascertained decrease of phagocytic activity of blood neutrophils, increase of their absorption capacity and reduction of spontaneous NBT test.

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