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## Clinical And Morphological Characteristics At Combined Pathology Of The Mammary Gland, Endometrium At Cows And Its Influence On The Protein Component Of Milk.

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### ABSTRACT

Researches on simultaneous studying of hematological, biochemical and immunological structure of blood of cows, a protein component of milk and histological structure of a mammary gland and an endometrium at cows both with subclinical mastitis, and with the combined course of subclinical mastitis and postpartum endometritis are carried out. It was revealed that the blood parameters had deviations from the physiological norm, and the immune responses of the body were reduced, while there was an increase in the percentage of rod neutrophils, leukocytosis, as well as lymphocytopenia, an increase in the percentage of eosinophils, accompanied by a decrease in albumins,  $\beta$ -globulins, an increase in  $\alpha$  - and  $\gamma$  - globulin fractions, the activity of redox processes decreased, the level of resistance of the body with a violation of homeostasis decreased, indicating the presence of acute inflammatory process in animals body. At the same time, the inflammatory process at cows with combined pathology was more expressed. The histological examination of the mammary gland found that the alveoli were predominantly polymorphic, of different sizes and contained serous fluid with an abundance of fat and milk inclusions. In some places, the alveolar septum expanded due to loose connective tissue and blood capillaries. Lactocytes were sometimes vacuolated and rejected significantly from the basal membrane of the alveoli. At the same time, with the increase in the number of somatic cells in milk, the content of whey proteins increased, but the number of caseins decreased. Because of the imbalance of essential amino acids and the presence of a limiting amino acid tryptophan protein component of the milk had a low biological value, and with an increase in the concentration of somatic cells in milk it is expressed to a greater extent.

**Keywords.** Subclinical mastitis, endometritis, mammary gland, protein component.

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## INTRODUCTION

According to many authors, inflammation of the uterine mucosa in cattle in the postpartum period is one of the most common pathologies in most livestock farms [1,3], which occurs both on the basis of detention of the placenta or during childbirth, and on the basis of infectious diseases, accompanied by a significant decrease in milk yield and, as a rule, inflammation of the mammary gland [2,6,7]. At mastitis the composition and properties of milk change and milk protein is subject to large changes [8].

The purpose of this research was to study the state of homeostasis, the protein component of milk, the histological structure of the diseased animals with combined pathology in this disease at cows in a comparative perspective.

## MATERIAL AND METHODS

The material was samples of udder and uterus of 7, and samples of blood and of milk from 24 cows (healthy cows, suffering from subclinical mastitis and at the same time subclinical mastitis and acute postnatal endometritis). Material for light microscopy was fixed in 10.0% neutral formalin solution and Carnoy fluid, dehydrated in alcohols, chloroform, embedded in paraffin, preparing sections on the microtome MPS-2 with a thickness of 5-7  $\mu\text{m}$ , were had deparaffinization and stained with hematoxylin-eosin by Van Gieson, Azur-2 in combination with basic fuchsin and toluidine blue. Endometritis at cows was established using clinical and laboratory research methods. Mastitis was diagnosed starting 4 days after calving at cows using clinical research methods and KerbaTest. The reaction was taken into account by the degree of formation of a jelly-like clot, which is the main criterion for assessing the reaction, as well as by an additional sign – a change in the color of the mixture. Blood was examined for 1-3 and 10-14 days after calving at cows suffering from postpartum endometritis and subclinical mastitis. Studies of the protein component of milk were carried out on the 14th day after cows calving. Mass fraction of protein was determined by Kjeldahl formalinum method, casein – by Maltipoo method, whey protein – by calculation method [8]. Amino acid composition was identified by liquid chromatography using Shimadzu LC-20 Prominence chromatograph (Japan). At calculating the biological value of the protein, the recommended (FAO, 2011) method for determining the amino acid score was used, taking into account the biological availability of individual essential amino acids and the refined formula of the reference protein [9,10]. Hematological studies were carried out by conventional methods, and biochemical – in accordance with the “Guidelines for the use of standardized biochemical methods of blood, urine and milk study in veterinary laboratories” [4].

The digital material obtained in the course of research was processed by the method of variation statistics on algorithms using the computer program Microsoft Office “Excel”.

## RESULTS AND THEIR DISCUSSION

3 groups of 8 full-aged cows were formed: 1 – clinically healthy animals, 2 – animals with subclinical mastitis, 3 – animals with a combined course of subclinical mastitis and postpartum endometritis. In the course of studies, it was found that the clinical condition of cows with subclinical mastitis was within normal limits, while a decrease in milk productivity was observed, and some cows had mild oppression. Mammary gland was not changed externally, but sometimes had slightly firm consistency by touch. The nipples were in the normal range, and excretions from nipple channels are not marked. The lymph node of the mammary gland under the skin was slightly enlarged and had a soft consistency. Their sizes varied within 7,53×2,95, against 3,80×1.50 cm at clinically healthy cows. The surface of the mammary gland incision was juicy and sometimes serous liquid with a yellowish tinge drained slightly.

Hematological, biochemical and immunological studies at cows with both subclinical mastitis and the combined course of subclinical mastitis and postpartum endometritis revealed that blood parameters had deviations from the physiological norm, and the immune responses of the body were reduced.

Analyzing the obtained data (table 1), it can be noted that in the blood of sick animals with combined pathology an increased content of leukocytes in the animals in the range of  $8,90 \pm 0,56$  was observed, 1,8 times more in comparison with clinically healthy animals.

**Table 1: Blood parameters of cows with subclinical mastitis and combined course of subclinical mastitis and postpartum endometritis**

Parameter	Group		
	first	second	third
Erythrocytes, 10 <sup>12</sup> /l	6,01±1,81	6,48 ± 0,58	6,25 ± 0,41
Hemoglobin, g/l	101,23±0,27	109,24 ± 4,7	107,40 ± 5,06
Leukocytes, 10 <sup>9</sup> /l	5,06±2,03	6,84 ± 0,32	8,90 ± 0,56
Neutrophils, % :	0	0	0
- young			
- rod, %	3,0±1,40	7,466 ± 0,20	5,466 ± 1,20
- segmented, %	18,0±2,80	20,066 ± 3, 50	21,066 ± 3,15
Eosinophils, %	3,5±0,61	4,20 ± 0,78	5,40 ± 2,68
Monocytes, %	2,5±2,020	3,20 ± 1,17	3,166 ± 1,07
Lymphocytes, %	63,1±1,20	42,90 ± 1,80	41,90 ± 3,90
Total protein, g/l	79,4±4,21	76,27 ± 2,40	78,77 ± 4,64
Protein fractions:			
Albumins, %	42,3±1,33	35,19 ± 3,15	31,19 ± 2,585
α- globulins, %	13,02±0,20	14,10 ± 0,17	15,70 ± 3,05
β- globulins, %	12,8±2,11	13,28 ± 1,04	17,18 ± 1,03
γ- globulins, %	33,15±0,50	31,25 ± 1,08	29,985 ± 1,08

The increased content of the number of rod leukocytes in comparison with clinically healthy animals while reducing the number of lymphocytes and increasing the number of eosinophils and monocytes, respectively, 2,5 times and 60,0% in the second and third groups, testified to a higher intoxication of animals and simultaneously the beginning of the process of neutralization and removal of harmful metabolites.

In the study of indicators of protein metabolism it was found that the total protein content in the serum of cows suffering from subclinical mastitis, decreased by 2,0%, and in the serum of cows with a combined course of subclinical mastitis and postpartum endometritis, in the protein fractions the number of albumins decreased by 11,0%, at the same time the number of α - and β-globulins increased. The increase in the levels of the latter parameters indicated an acute inflammatory process complicated by infection.

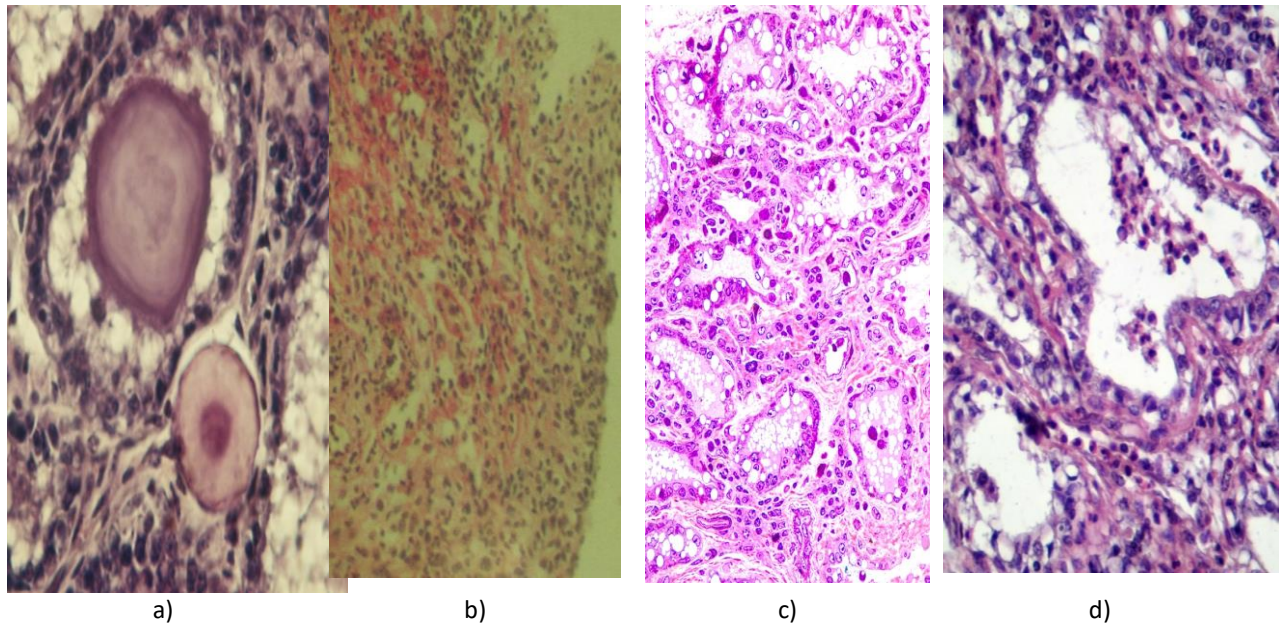
In the study of the biochemical composition of the blood of experienced cows the following differences were revealed. The increase of urea content in the blood in 2 and 3 experimental groups, creatinine by 1,5 and 1,4 times in comparison with the first group of animals, and the glucose content decreased, which indicated a decrease in the level of carbohydrate metabolism and, accordingly, bioenergetic processes, as glucose is the main source of energy for many cells of the body. Glucose accounted for more than 90,0% of all low molecular weight carbohydrates (table 2).

**Table 2: Biochemical parameters of blood serum of cows with subclinical mastitis and combined course of subclinical mastitis and postpartum endometritis**

Parameters	Group		
	first	second	third
Urea (mM/l)	2,9±0,15	3,25 ± 0,23	3,507 ± 0,62
Creatinine (μm/l)	60,2±2,56	93,24 ± 6,72	86,916 ± 8,56
Cholesterol mM/l)	2,36±0,87	4,25 ± 0,81	3,153 ± 0,50
AlAT (U/L)	25±2,50	28,06 ± 2,66	31,76 ± 3,66
AsAT (U/L)	35±2,36	75,55 ± 6,54	78,87 ± 8,48
Glucose (mM/l)	2,3±1,61	1,53 ± 0,14	1,83 ± 0,14
Phosphorus (mM/l)	1,7±1,02	2,02 ± 0, 31	0,92 ± 0,09
Calcium (mM/l)	3,0±0,98	2,99± 0,43	4,2 ± 0,14

In addition, attention was drawn to the decrease in the body of cows with a combined course of subclinical mastitis and postpartum endometritis of phosphorus and an increase in the blood calcium in 1,5 times, which was a consequence of their low content in feed for a long time, this in turn led to the resorption of calcium from bone tissue and inhibition of sexual function, without affecting significantly the functioning of other systems of the body.

Histological examination revealed that mammary gland alveoli in subclinical mastitis at cows were predominantly polymorphic, of various sizes and contained serous fluid with an abundance of fat and milk inclusions. Here single rounded balls with pinkish and homogeneous parenchyma, filling 2/3 of the alveoli were observed (Figure 1a).



**Fig. 1. a) Structural changes in the mammary gland at cows with serous-catarrhal subclinical mastitis of cows. Huge rounded homogeneous formations in the lumen of the alveoli. Sur. by heme. – eosin. Exp. r. 7, about 40; b) Expressed inflammatory reaction on the uterine mucosa at cows with purulent-catarrhal endometritis. Sur. by heme.-eosin. Exp. r. 8, ab. 40; c) Development of serous-catarrhal inflammation in the mammary gland in cows with subclinical mastitis. Sur. by Azur-2 with the main fuchsin. Exp. 400; d) The transition of catarrhal inflammation to purulent-catarrhal in the mammary gland in cows with subclinical mastitis. Exp. r. 8, ab. 40;**

At layering to the subclinical mastitis of acute postpartum endometritis for 10-12 days after calving in cows, the clinical condition slightly worsened, although their body temperature was within normal limits (37,7-38,9°C). The number of respiratory movements was 14-17 per minute, heart rate – 65-72 per minute, and scar contractions – 2-3 per minute.

At sick cows, there was a release from the genitals of purulent-mucous exudate of semi-liquid consistency with a yellowish or brownish tint in an amount of 75,0-95,0 to 240,0-275,0 ml. Especially a large amount of exudate was found in the morning on the floor, after a night rest of animals. Exudate was also noted on the root of the tail and in the lower corner of the vulva in the form of dried crusts. Often sick cows arched their backs and stood in a pose for urination.

In cows on 10-12 days after calving in rectal examination, the uterus was palpated in large sizes, was in the abdominal cavity, reacted poorly to massage, after which the genitals were isolated from the liquid or semi-liquid lohi of dark brown color with an admixture of a small amount of mucus.

In general, endometritis at cows was characterized by catarrhal discharge from the uterus during defecation, urination and, as a rule, after a night rest of cows. Catarrhal purulent endometritis occurred in cows in the presence of a pronounced inflammatory reaction on the uterine mucosa (figure 1b) [5]. At the same time, in those cows that had acute inflammatory processes in the uterus in the form of catarrhal-

purulent endometritis, inflammatory processes were detected in the structure of the mammary gland up to catarrhal – catarrhal-purulent mastitis (figure 1 c, d).

The study of changes in the protein component of cow milk was carried out on day 14 after calving cows. The summary results of the studies are presented in table 3.

**Table 3: Characteristics of the protein component of cow milk in different forms of mastitis**

Parameter	Group		
	first	second	third
Total protein, %	3,64±0,011	3,81±0,017*	3,64±0,032
among them casein, %	3,0±0,061	2,91±0,021	2,87±0,042
whey protein, %	0,67±0,012	0,91±0,011*	0,78±0,013*
∑ essential amino acids	41,1±0,31	41,3±0,51	41,7±0,28
∑ nonessential amino acids	58,7±0,41	58,8±0,27	58,2±0,23
Scor essential amino acid tryptophan, %	91,4	86,4	85,8

In our studies it was revealed that the amount of total protein in the second group relative to the first is higher by 0,17 % ( $p < 0,05$ ), and when compared with the third they are equal. The concentration of casein fraction of proteins in the first group relative to the second is higher by 0,09 % ( $P < 0,05$ ), and the third – by 0,13 ( $P < 0,01$ ). The serum protein content, on the contrary, is higher in the second and third groups by 0,28 % ( $P < 0,01$ ) and 0,15 % ( $P < 0,05$ ), respectively.

This is because the inflammation of the mammary gland of the cow to eliminate pathogenic microflora in its alveoli and ducts from the blood sharply increases the flow of leukocytes and whey proteins. This reduces the synthesis of casein, but increases the amount of total protein by increasing the level of whey proteins and form elements. In addition, the biological value of the protein component is reduced. Because of the imbalance of essential amino acids and the presence of limiting amino acid tryptophan milk protein had a low biological value and it is expressed more in the second and third group, that is, in the milk of cows with different forms of mastitis.

### CONCLUSIONS

Thus, hematological and biochemical parameters of blood in cows with combined treatment of subclinical mastitis and acute postpartum endometritis were characterized by a violation of carbohydrate, mineral and protein metabolism. At the same time, the activity of redox processes and the level of resistance of the body with a violation of homeostasis decreased. The mammary gland in cows with subclinical mastitis on the background of postpartum endometritis had a dense consistency, and its subcutaneous lymph node increased, its size varied within 7,53×2,95 cm. Histologically, alveoli were predominantly polymorphic, of various sizes and contained serous fluid with an abundance of fat and milk inclusions. In some places, the alveolar septum expanded due to loose connective tissue and blood capillaries. Lactocytes were vacuolated in some places and significantly detached from the basal membrane of the alveoli.

At the same time, with the increase in the number of somatic cells in milk, the content of whey proteins increased, but the number of caseins decreased. Because of the imbalance of essential amino acids and the presence of a limiting amino acid tryptophan protein component of the milk had a low biological value, and with an increase in the concentration of somatic cells in milk it is expressed to a greater extent.

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