



## Biochemical Indicators of Blood and Milk in the Cows Affected by Latent Mastitis

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### ABSTRACT

Latent mastitis harms the health of animals, their milk production, and the quality of milk. In cows affected by this disease sharp changes in hematological parameters, and biochemical parameters of blood and milk, which is the focus of our research. This work studies the morphological and biochemical parameters of blood and the biochemical parameters of milk in dairy cows affected by latent mastitis. The research was performed in several farms and farm households of Kotayk and Ararat regions, as well as in the ANAU Research Center of Veterinary Medicine and Veterinary Sanitary laboratories. The research outcomes prove that latent mastitis significantly impacts the composition of blood and milk components. Data obtained during the research enables a better understanding of the causes of mastitis as well as the necessity of using informative diagnostic methods in farm veterinarians' practices.

### Introduction

Subclinical mastitis is a common disease insidiously developing in cow udders which causes considerable damage to the dairy industry. Mastitis entails an increase in infertility rate, raises the rate of culling and medical treatment costs of livestock, and causes a 10 to 15 percent decrease in milk yield in cows so that the underproduction of milk throughout the lactation period amounts to 500-700 liters of milk per dairy cow, on average (Radionov et al., 2011; Velikanov et al., 2021).

Mastitis milk has altered biochemical composition as well as deteriorated technological properties; it has transformed the concentration of somatic cells in the milk, changed the rate of proteins and lactose, and decreased the content of free fatty acids (Andreev, 2019; Sidorova et al., 2020). Therefore, the presence of mastitis milk in feedstock milk is undesirable (Yefimova et al., 2019). Veterinary experts need to understand the processes running on in the malfunctioning lacteous glands since modern treatment regimens are impossible without such understanding (Momsikova et al., 2017; Cherenok, 2020).

## Materials and methods

Both clinically healthy cows and cows with latent mastitis were studied and compared for their hematological and biochemical parameters of blood and milk. The research was performed regarding lactating pluripara cows of the Brown Caucasian and Jersey breeds, aged 5 to 6 years, that were not impaired by post-parturient complications. The animals were kept in stalls and pastures, and their diet consisted of concentrated roughage feed and root crops. The research was carried out in certain farms and farm households of the Kotayk and Ararat regions. In addition, it was carried out in the Laboratory of Veterinary Medicine and Veterinary Sanitary Expertise.

Tested materials included whole raw cow's milk and blood taken from healthy animals and animals affected by latent mastitis. 60 cows were tested during the experiment, and 14 animals were affected by latent mastitis (23.3 %). Based on the analogy, 11 animals were selected from healthy cattle and a reference group. 11 cows were affected by latent mastitis to form an experimental group. All animals were kept under similar conditions.

By hand milking, milk samples were collected from the experimental and reference groups, in the amount of 50 ml from each quarter of the udder. They were promptly delivered to the laboratory for biochemical testing. The fore milk was disposed of, while the rest was tested. To detect latent mastitis, the California Mastitis Test was used, which allows a rapid count of somatic cells in milk.

The high sensitivity of this test is given by tracking two factors at once - the viscosity and color intensity of the samples (Polyantsev, 2015). To increase the reliability of subclinical mastitis detection, milk sedimentation tests were performed in the following way. Whenever a California Mastitis Test result was positive, 10-15 ml of

milk was poured into test tubes from each udder quarter. After milking, the milk was placed in the refrigerator for 16-18 hours, and the response was checked one day later.

In the case of milk taken from cows affected by mastitis, sediment appeared at the bottom of the test tube. The sediment height was measured. The cow was considered to have mastitis if the sediment height exceeded 0.1 cm. Biometrical processing was performed on a computer using Microsoft Office Excel software. The values of obtained data were presented in  $M \pm m$  format, where  $M$  is for a mean value, and  $m$  is for a standard error of the mean (Grachev, 2012).

For morphological and biochemical analyses, blood samples were taken from cows' jugular vein early in the morning. The morphological composition of blood was examined by use of a MicroCC-20 plus analyzer, total protein was determined by refractometry method, the total calcium content in blood serum – by the method of Karakashov and Vichev, and immunoglobulin content – by the method of enzyme immunoassay.

## Results and Discussions

As Table 1 shows, 46 animals (76.6 %) from among 60 tested ones had a negative response to the mastitis test, 6 animals (10 %) gave a positive response, and 8 animals (13.3 %) gave an explicit positive response.

According to Table 2, blood protein composition changes in cases of latent inflammation and mastitis in cow udders. The total protein content (total amount of all circulating proteins) in the experimental group had decreased in comparison with the reference group from  $85.7 \pm 0.3$  g/l to  $62.3 \pm 0.7$  g/l, i.e., by 23.4 g/l on average and by 5.7 g/l compared to the standard values.

**Table 1.** Response to mastitis test in the examined animals\*

| Test Response     | Number of Animals, unit | Body Cell Count, thous/cm <sup>3</sup> | Ratio, % |
|-------------------|-------------------------|----------------------------------------|----------|
| Negative          | 46                      | 100 - 300                              | 76.6     |
| Positive          | 6                       | 300 - 500                              | 10       |
| Explicit Positive | 8                       | over 500                               | 13.3     |

\*Composed by the authors.

**Table 2.** Hematological and biochemical parameters of blood in clinically healthy cows and the cows affected by latent mastitis\*

| Parameter       | Unit of measurement | Standard Values | Reference Group (n=11) | Experimental Group (n=11) |
|-----------------|---------------------|-----------------|------------------------|---------------------------|
| Erythrocytes    | 10 <sup>12</sup> /l | 5 – 7.5         | 5.63 ± 0.3             | 5.76 ± 0.2                |
| Hemoglobin      | %                   | 90 – 120        | 98.6 ± 0.4             | 97.3 ± 0.6                |
| Leukocytes      | g/l                 | 4.5 – 12        | 8.1 ± 0.6              | 3.2 ± 0.5                 |
| Total protein   | g/l                 | 68 – 87         | 85.7 ± 0.3             | 62.3 ± 0.7                |
| Immunoglobulins | g/l                 | 29 – 49         | 28.6 ± 0.8             | 15.1 ± 0.2                |
| Total calcium   | mmol/l              | 2.1 – 2.8       | 2.54 ± 0.03            | 1.5 ± 0.02                |

**Table 3.** Biochemical parameters of milk in the healthy cows and the cows affected by latent mastitis\*

| Indicator                | Unit                  | Standard      | Reference group (n=7) | Experimental group (n=7) |
|--------------------------|-----------------------|---------------|-----------------------|--------------------------|
| Mass Fraction of Fat     | %                     | 3.8           | 3.4                   | 3.0                      |
| Mass Fraction of Protein | %                     | 3.3           | 3.2                   | 2.6                      |
| Density                  | kg/m <sup>3</sup>     | 27 - 33       | 28.93 ± 0.04          | 27.84 ± 0.09             |
| Acidity                  | °T                    | 16 - 18       | 16.71 ± 0.06          | 20.32 ± 0.04             |
| Somatic cells            | thous/cm <sup>3</sup> | up to 200.000 | 237.22 ± 1.24         | 436.23 ± 1.22            |

\*Composed by the authors.

**Picture.** Alteration of texture and color in the mastitis milk.

Besides, the immunoglobulin content of blood had decreased in the affected animals from 28.6±0.8 to 15.1±0.2 g/l, because of being consumed for immune response and combat with the pathogenic microbes causing

inflammation in the affected udder. The average difference in this parameter between the reference and experimental groups was 12.9 g/l. The average difference between the experimental group and the standard value was 13.9 g/l.

A decrease in the number of leukocytes in the reference group was detected compared to the experimental group, from 8.1±0.6 to 3.2±0.5 g/l, reflecting the transfer of leukocytes into the affected mammary gland for phagocytosis. The average difference in this parameter between the reference and experimental groups was 4.9 g/l. In contrast, the average difference between the experimental group and the standard value was 1.3 g/l.

Moreover, the blood of animals in the experimental group had a lower rate of total calcium 1.5±0.02 mmol/l compared to 2.54±0.03 mmol/l in animals in the reference group. The difference in this parameter between the reference and experimental groups was 1.4 mmol/l, while

the average difference was 0.6 mmol/l by 0.11 mmol/g. About 10 g of calcium circulates in the cow blood, and there is a large reserve of this microelement (6000 g) in the cow bones as well. After calving, calcium is eliminated from the cow's body with every milking. On the other hand, the animal cannot extract calcium from its bone reserves or food; which leads to decreased calcium rate in the blood (Zimnikov et al., 2020). In addition, there was an alteration in some biochemical parameters when testing the milk (Table 3).

The milk tests revealed that in the cows affected by subclinical mastitis (experimental group), certain biochemical parameters of milk are altered in comparison with the clinically healthy animals (reference group) because the ability of secretory cells to synthesize the main components of milk influences the inflammation of mammary glands. As follows from Table 3, the density of standard milk (ratio of milk mass at 20 °C to the mass of the same quantity of water at 4 °C) ranges from 27 to 33 kg/m<sup>3</sup>, however, in the experimental group of animals, it decreased by 1.09 kg/m<sup>3</sup> compared to the reference group. The acidity of milk in the experimental group animals had increased by 6.61°T compared to the reference group.

This is caused by the acidic compounds in milk, such as citric, lactic, carbonic, ascorbic, and fatty acids.

The standard mass fraction of fat is 3.8%, but it decreased in the experimental group compared to the reference group, from 3.6% to 3.0%. At the same time, the mass fraction of protein decreased as well, from 3.6% to 3.0%.

Healthy milk contains somatic cells, at a rate of up to 200 thous/cm<sup>3</sup>. However, the tests revealed that, in the case of latent mastitis, the somatic cell content of milk in the animals of the experimental group had reached up to 436.23±1.22 thous/cm<sup>3</sup>, which is an indication of subclinical mastitis and the need for timely treatment of the affected animals (Stepanova et al. 2019).

## Conclusion

Research proves that latent mastitis affects the hematological and biochemical parameters of blood (a decrease in the number of leukocytes and a decrease in the content of total protein, immunoglobulins, and total calcium).

Latent mastitis changes milk biochemical parameters, it increases milk acidity and decreases the ratio of protein

to fat, reducing its nutritional value and suitability for further processing. The altered component ratio in milk negatively affects its organoleptic properties. When milk is contaminated with mastitis, it becomes salty and bitter to the taste. It creates unfavorable conditions for dairy milk processing into fermented milk products. Regular analysis of the hematological and biochemical parameters of blood and the biochemical parameters of milk can be a valuable diagnostic aid for monitoring animal health, so it shall be widely used by farm veterinarians wherever possible.

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**Declarations of interest**

*The authors declare no conflict of interest concerning the research, authorship, and/or publication of this article.*

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