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Natural Sorbent Zeolite in a Complex for Streptococcal Septicemia of Lambs.

[1]Dunyamalyeva K.I., [2]Suleymanova R.A, [3]Kulieva K.A, [4]Sardarly V.V., [5]Alverdiev G.R, [6]Meifalieva A.D, [7]Mamedrzaev G.S.

[1]Senior Lecturer, Department of Epizootiology, Microbiology and Parasitology, Azerbaijan State Agricultural University, Azerbaijan

[2] Senior Lecturer at the Department of Anatomy, Pathological Anatomy and Pathophysiology, Azerbaijan State Agricultural University, Azerbaijan

[3]Senior Lecturer at the Department of Anatomy, Pathological Anatomy and Pathophysiology, Azerbaijan State Agricultural University, Azerbaijan

[4]Doctor of Philosophy in Biology, Acting Associate Professor of the Department of Epizootiology, Microbiology and Parasitology, Azerbaijan State Agricultural University, Azerbaijan

[5]Candidate of Veterinary Sciences, Acting Associate Professor of the Department of Anatomy, Pathanatomy and Pathophysiology, Azerbaijan State Agricultural University, Azerbaijan

[6]Assistant at the Department of Anatomy, Pathanatomy and Pathophysiology, Azerbaijan State Agricultural University, Azerbaijan

[7]Assistant at the Department of Anatomy, Pathanatomy and Pathophysiology, Azerbaijan State Agricultural University, Azerbaijan

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KEYWORDS
Amoxicillin, zinc oxide, streptocide, natural sorbent, zeolite, streptomycin, immunobiological parameters of blood serum.

ABSTRACT:

The article presents data on the use of amoxicillin and a mixture of zeolite, zinc oxide and streptocide for the treatment of patients with streptococcal septicemia in lambs. Amoxicillin was administered intramuscularly at a dose of 1 ml/10 kg of weight, and after cleaning, a mixture of zinc oxide, streptocide and natural zeolite sorbent, which had the ability to absorb and firmly retain various contaminants, was applied to the umbilical cord area after cleaning. Lambs of the second group were treated according to a scheme in which intramuscular Amoxicillin alone was administered once a day, at a dose of 1 ml/10 kg of body weight, intramuscularly; lambs of the third group were treated with streptomycin at a dose of 20,000 units/kg three times a day intramuscularly.

The result revealed that the difference between the treatment effects of the first, second and third groups was significant. During the treatment period, significant differences in the recovery of animals were revealed. In animals of the first group, immunobiological indicators were higher than in other groups of animals; already on the third day of therapy, the lambs felt normal, there was no pain in the navel area, there was no purulent exudate, in animals of the second and third groups the condition remained satisfactory, pain was felt, There was a slight discharge from the navel.

Introduction.

Despite significant advances in the study of infectious diseases of small ruminants, some issues related to diagnosis, prevention and treatment remain unsolved and require further improvement.(4)

Infectious diseases that are often found in livestock farms among young small cattle include streptococcal infections, in particular streptococcal septicemia.(1,2)

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Based on the epizootological analysis carried out in livestock farms of our Republic, data were collected on their problems with streptococcal infections.

Streptococcal septicemia, an acute infectious disease with a septic form of the course and damage to the umbilical cord. The causative agent of the disease is predominantly beta-hemolytic streptococci of serogroup C - Str.zooepidemicus. Premature and weak animals are susceptible to the disease. Predisposing factors for the disease are unbalanced and inadequate feeding of the queens, non-compliance with zoohygienic conditions for keeping animals and the sanitary condition of childbirth. When the umbilical cord cult comes into contact with contaminated and microorganism-contaminated care items, bedding and the hands of attendants after childbirth.(3)

The disease is mainly accompanied by pain in the umbilical cord area and the discharge of purulent exudate from the navel. Untimely therapeutic intervention is accompanied by septicemia and metastases in various organs.(5)

Currently, for purulent-inflammatory processes, along with antibacterial drugs, drugs with sorption properties are used. These drugs increase the vitality of tissues due to the adsorption of waste products of microorganisms and toxic products of tissue decay. (6) Such natural sorbents also include zeolites, which are volcanic tuff, the reserves of which are huge in Azerbaijan (Aydag deposits).

Zeolites are minerals with molecular-sized pores that, like a sponge, can absorb and firmly hold a wide variety of contaminants (7).

The purpose of this work was to conduct monitoring in livestock farms, identify sick lambs with signs of septicemia, collect samples for laboratory testing and carry out therapeutic measures according to various schemes.

Materials and methods. As a result of monitoring, we identified sick lambs that showed anxiety when pressing on the umbilical cord area, and swelling was observed in this area. Exudate with a fetid odor flowed from the opening of the umbilical ring. In order to clarify the diagnosis, blood and material from the umbilical ring area were taken from sick lambs and sent to the laboratory for bacterial indication of the causative agent of the disease.

Blood was taken in the morning, before feeding the animals. To obtain serum, part of the blood was kept for 20-30 minutes in a thermostat at a temperature of 37 - 380 C, as soon as a blood clot formed, serum samples were transferred to the refrigerator and stored at 0 - 15 0 C.

In order to indicate and identify the causative agent of the disease, a number of studies were carried out in the laboratory, which included; preparation of fingerprint smears, Gram and Romanovsky Giemsa staining, inoculation on universal and enriched nutrient media, study of the hemolytic properties of isolated strains, bioassay on white mice and identification of isolated strains using a precipitation reaction in order to clarify the serogroup. Immunobiological studies were also carried out: determination of the bactericidal activity of blood serum, phagocytic activity of neutrophils (phagocytic activity, phagocytic index, phagocytic number), cellular reactions (plasmocyte reaction).

The pathogenicity of the isolated strains was determined on four white mice weighing 14-16 g. Streptococcal cultures grown in glucose-whey broth were used for infection. The culture was administered in a volume of 0.5 ml intraperitoneally. The mice were observed for several days; after the death of the mice, smears were prepared from the organs of the dead mice, stained with Gram and microscoped. Inoculations were also carried out on glucose-blood agar and glucose-whey broth.

In order to determine the serogroup of epizootic strains, the test culture was first grown in Hottingham broth with 1% glucose at a temperature of 37°C for 20 hours. Then the culture medium was centrifuged for 25 min at 4000 rpm. The sedimentary liquid was sucked off with a pipette, and a suspension was prepared from the sediment; for this, 0.3 ml of 0.2 N hydrochloric acid was added to the sediment, and the resulting mixture was kept in a water bath for 10 minutes. Then the test tube was cooled and a 0.04% alcohol solution of phenolphthalene was added, and then sodium hydroxide 0.2 N was added drop by drop until the medium acquired a pale pink color. Next, the resulting mixture was centrifuged, and the sedimentary liquid was used to carry out the precipitation reaction.

Carefully, 0.3 ml of immune sera obtained from standard strains of group C and B streptococci were added into Ulengut tubes along the wall. Then 0.3 ml of the liquid we prepared from epizootic strains of streptococci was added to the same test tube. The result was assessed by the turbidity between the two liquids.

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Treatment of lambs was started according to three schemes; lambs of the first group were treated with amoxicillin for therapeutic purposes, once a day, at a dose of 1 ml/10 kg of weight intramuscularly, and also once a day, a mixture of zinc oxide, streptocide and natural sorbent was applied to the umbilical cord area after cleaning zeolite, which had the ability to absorb and firmly retain various contaminants, the lambs of the second group were treated according to a scheme in which only amoxicillin was administered intramuscularly once a day, at a dose of 1 ml/10 kg of weight, intramuscularly, the lambs of the third group were treated with streptomycin, 20,000 units /kg three times a day intramuscularly.

On the first day of treatment, lambs of the first group noted lethargy, pain in the umbilical cord and swelling in the umbilical area, purulent exudate was released from the umbilical cord.

On the second day of treatment, the lambs showed an improvement in their general condition, pain in the navel area decreased somewhat, and the discharge of purulent exudate stopped.

On the third day of therapy, the lambs felt normal, there was no pain in the navel area, and there was no purulent exudate.

In the lambs of the second group, on the first day of therapy there was lethargy, pain in the umbilical cord, swelling in the umbilical area and purulent exudate was released from the umbilical cord.

On the second day of therapy, the lambs showed an improvement in their general condition, but pain in the umbilical area was still felt, upon palpation the animals were restless, and purulent exudate was released from the umbilical cord.

On the fifth day of treatment, the condition returned to normal, the pain was felt to a lesser extent, and the discharge from the navel stopped.

In the lambs of the third group, on the first day of therapy there was lethargy, pain in the umbilical cord, swelling in the umbilical area and purulent exudate was released from the umbilical cord.

On the second day of therapy, the lambs showed a slight improvement in their general condition and pain in the umbilical area was still felt; upon palpation, the animals became restless, and purulent exudate was released from the umbilical cord.

On the fifth day of treatment, the condition remained satisfactory, pain was felt, and there was slight discharge from the navel.

On the seventh day of treatment, the condition returned to normal, no pain was observed.

Research results. As a result of laboratory tests, gram-positive cocci were identified, growth on the MPB was weak, and a slight gray-white precipitate was observed at the bottom of the tube. Small, sparse colonies grew on the MPA.

When growing on a liquid medium with 0.5 - 1% glucose and 3 - 5% normal blood serum, rapid growth was observed. The broth became cloudy evenly and intensely during the first 8 hours, after which a fine cottony sediment formed at the bottom of the test tube. The flakes fell to the bottom in the form of lumps of cotton wool. After 19 hours, the growth of the culture completely ended. By this time, the medium above the sediment became clear or slightly cloudy. When shaken, the sediment easily broke up and turned into a uniform turbidity. On glucose meat-peptone agar, small, round, transparent, non-merging colonies, like dew drops, grew. On blood agar, streptococci grew in the form of small, transparent colonies with smooth edges. The quality of hemolysis was determined after 24 hours by the zone of clearing around the colonies; complete clearing around the colonies was observed - beta hemolysis.

The virulence of the isolated cultures was studied on white mice. For infection, a broth culture of streptococci was used, containing 10,000 billion bodies in 1 ml, according to the optimal turbidity standard. White mice were infected intraperitoneally, which resulted in 100% death of mice.

In order to identify the isolated strains, we used a set of streptococcal group diagnostic sera obtained for various serological groups [A, B, C] in the precipitation reaction [RP]. Epizootic strains of streptococci obtained by extraction with perchloric acid were used as the antigen. It was found that all isolated strains of streptococci belong to serogroup C, the species Streptococcus zooepidemicus.

In order to study the immunobiological parameters of the blood of lambs with streptococcosis, the bactericidal activity of blood serum was studied,

dynamics of changes in the phagocytic activity of leukocytes, lysozyme activity of blood serum.

The bactericidal activity of the blood serum of lambs with streptococcosis was slightly lower than that of the

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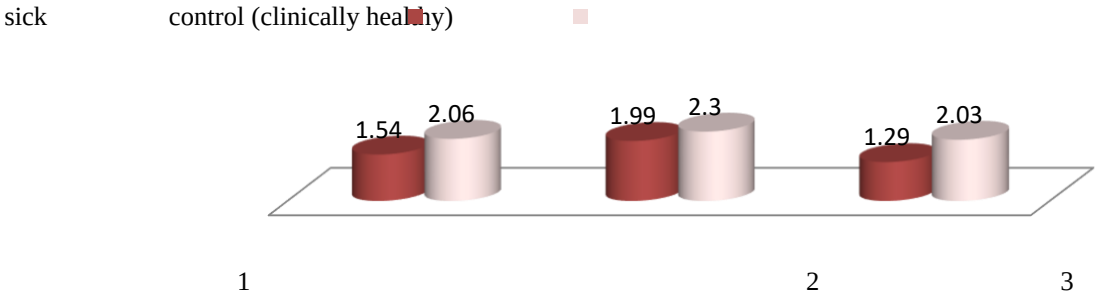
control group and was: in group 1 - 34.8 ± 1.26% versus 51.2 ± 0.50 (P<0.001); in group 2 - 26.3 ± 0.42% versus 52.5 ± 0.34 (P<0.001); in the 3rd group - 26.1 ± 0.63% versus the 4th control - 52.9 ± 0.49 (p < 0.05).

Bactericidal activity of blood serum of patients



Picture 1 . Bactericidal activity of blood serum of lambs with streptococcosis

Note:

1- lambs
2- 1-5 days of age.
3- 2 lambs 5-6 days old.
4- 3 lambs 6-10 days old.

Phagocytic activity, phagocytic index and phagocytic number of lambs with streptococcosis are presented in the figures.

From these data it is clear that the indicators of phagocytic activity, phagocytic index and phagocytic number of blood serum of sick lambs were lower than those of control ones and were: phagocytic activity in group 1 -28.7 ± 0.71% versus 42.8 ± 1 .01% (p < 0.05), in group 2 - 26.3 ± 0.42% versus 44.2 ± 1.06%; in group 3 - 26.1 ± 0.63% versus 35.8 ± 1.49% (p < 0.05); phagocytic index in group 1 - 1.54 ± 0.16 versus 2.06 ± 0.16; in group 2 - 1.99 ± 0.06 versus 2.30 ± 0.08; in group 3 - 1.29 ± 0.10 vs. 2.03 ± 0.06, (p < 0.05); phagocytic number in group 1 - 0.22 ± 0.01 versus 0.62 ± 0.04 (p < 0.05), in group 2 - 0.34 ± 0.03 versus 0.54 ± 0 .10, in group 3 0.36 ± 0.05 versus 0.53 ± 0.11 (p < 0.001).

Thus, studies have shown that in case of streptococcosis in lambs, phagocytic activity, phagocytic index and phagocytic number changed and were slightly lower than the control ones. These indicators are also depicted in histograms.

Phagocytic activity, %

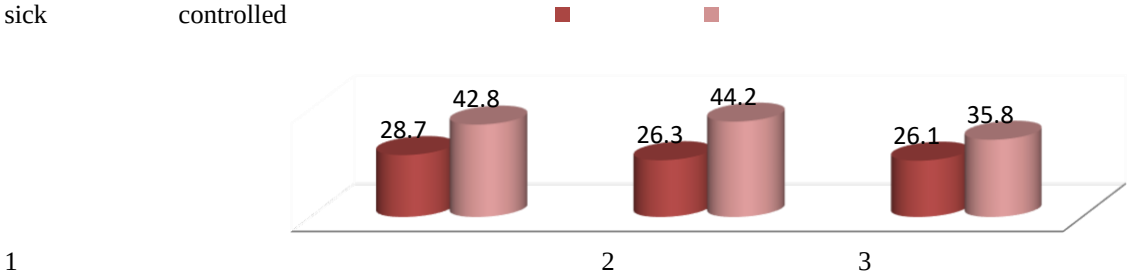


Figure 2. Phagocytic activity of lamb blood serum

Note:

1- lambs 1-5 days of age.
2- 2 lambs 5-6 days old.
3- 3 lambs 6-10 days old.

Phagocytic index

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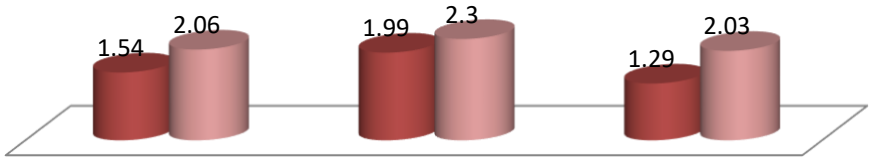

Figure3. Phagocytic index of lamb blood serum

Note:

1- lambs 1-5 days of age.

2 lambs 5-6 days old.

3 lambs 6-10 days old.

Phagocytic number

sick control (clinically healthy)

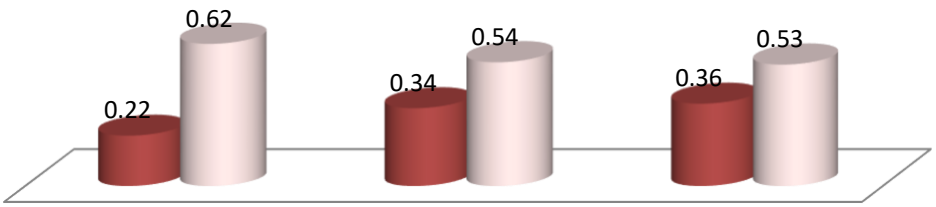

Figure 4. Phagocytic number of lamb blood serum

Note:

1- lambs 1-5 days of age.

2 lambs 5-6 days old.

3 lambs 6-10 days old

Based on our experiments, we identified changes in the lysozyme activity of lambs with streptococcosis.

The data are presented in Figure 5.

From Table 3.10 it is clear that in the blood serum of lambs with streptococcosis, lysozyme activity was lower than the level of the control groups and amounted to 28.8 ± 1.14% in the first group versus 45.9 ± 0.82%, ($p < 0.05$), in the second group - 27.4 ± 1.21% versus 47.3 ± 0.46%, ($p < 0.05$), in the third group - 26.8 ± 1.26% versus 48.0 ± 0 ,51%, ($p < 0.001$).

Thus, the lysozyme activity of the blood serum of lambs with streptococcosis was slightly lower compared to the control group

Lysozyme activity

sick control (clinically healthy)

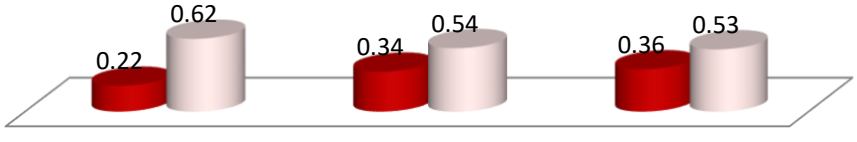

Figure 5. Dynamics of lysozyme activity in lamb blood serum

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Note:
1- lambs 1-5 days of age.
2- 2 lambs 5-6 days old.
3- 3 lambs 6-10 days old

As a result of the therapeutic measures, the general condition of the lambs which were treated according to the first scheme using zeolite improved, pain in the umbilical cord area was not observed and the animals recovered within 3 days of therapy. In animals that were treated according to the second scheme, without the use of natural zeolite sorbent, pain on palpation was observed for some time,
the animals were worried, treatment continued for another 5 days.
In animals that were treated according to the third regimen, pain on palpation was observed for some time, the animals became restless, treatment continued for another 7 days.

Below are the immunobiological parameters of the blood serum of lambs after treatment.

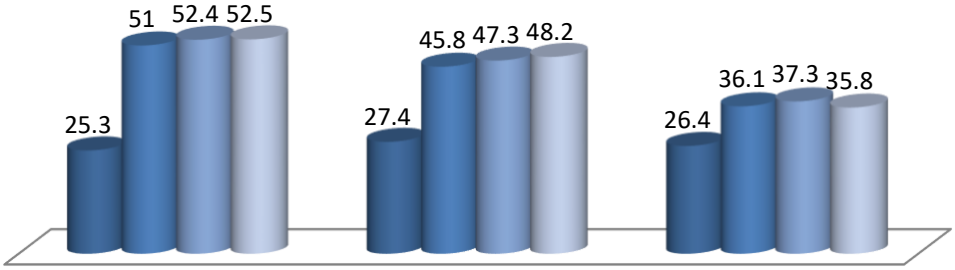

Figure 6. Bactericidal activity of lamb blood serum before and after treatment

Note:
1- group of lambs, treatment according to the first scheme;
2- group of lambs, treatment according to the second scheme;
3-control – treatment according to the third scheme.

Thus, the indicator of bactericidal activity of lambs of the first group after treatment increases almost 2 times, which is not observed in lambs of the second and third groups. In these groups, a slight increase in this indicator was observed.

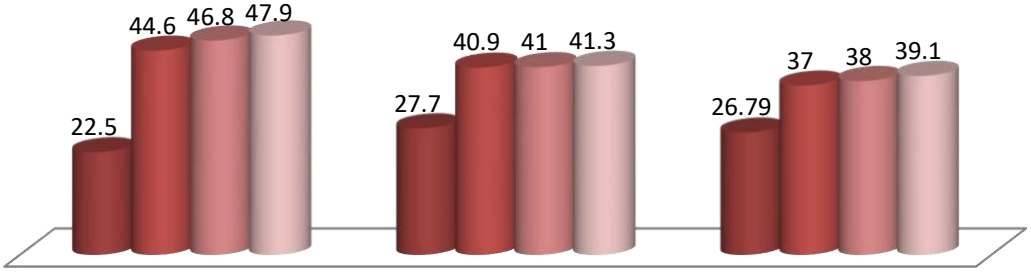

Figure7. Lysozyme activity of blood serum of lambs before and after treatment

Note:

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1st group of lambs, treatment according to the first scheme
2- group of lambs, treatment according to the second scheme;
3- control – treatment according to the third scheme.

Analysis of the results obtained allows us to conclude that the lysozyme activity of the blood serum of lambs of the first group increases sharply by almost 2 times, which is not observed in the other groups of lambs.

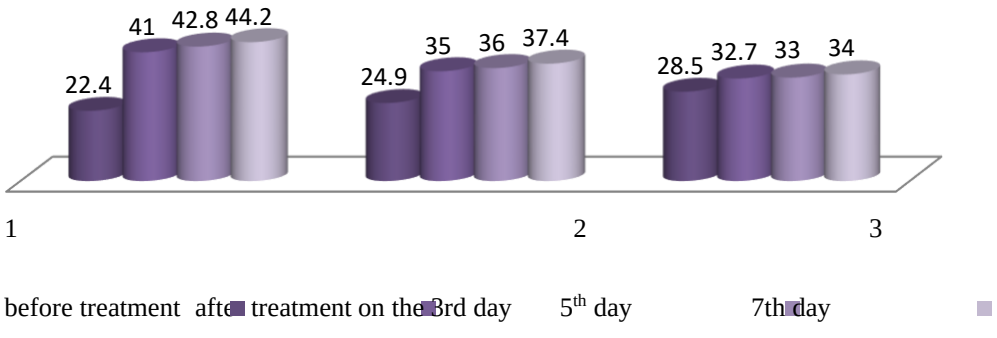

Figure 8. Dynamics of phagocytic activity in the blood of lambs before and after treatment
Note:
1st group of lambs, treatment according to the first scheme
2nd group of lambs, treatment according to the second scheme;
3rd control – treatment according to the third scheme.

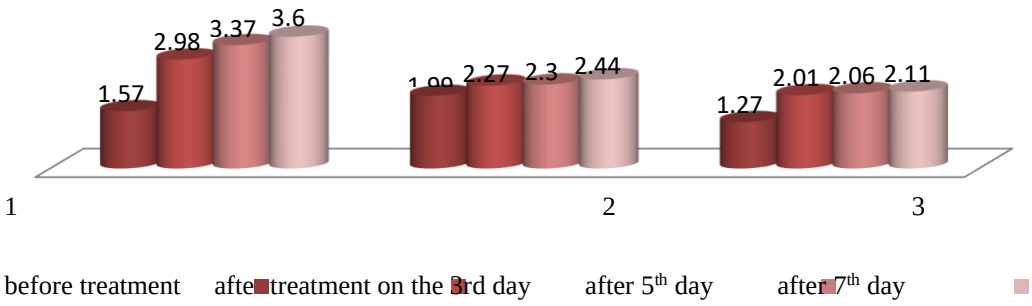

Figure 9. Dynamics of the phagocytic index of lamb blood before and after treatment
Note:
1st group of lambs, treatment according to the first scheme
2nd group of lambs, treatment according to the second scheme;
3rd control – treatment according to the third scheme.

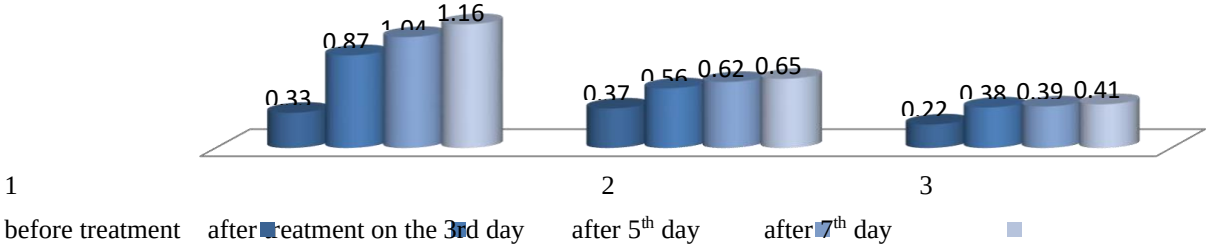

Figure 10. Dynamics of the phagocytic number of blood of lambs before and after treatment

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Note:
1st group of lambs, treatment according to the first scheme
2- group of lambs, treatment according to the second scheme;
3- control – treatment according to the third scheme.
Summarizing the data obtained after treatment, we can conclude that the phagocytic activity of blood leukocytes of lambs of the first group doubled during the short period of treatment and was higher than the indicators of the second and third groups.
The results of the studies showed that the immunological parameters of lambs of the first group that received amoxicillin with a mixture of zeolite, zinc oxide and streptocide were higher than in lambs of other groups. And an increase in these indicators was achieved in a short period of treatment.
Conclusion. Thus, it was revealed that the difference between the treatment effects of the first, second and third groups was significant. During the treatment period, significant differences in the recovery of animals were revealed. In animals of the first group, immunobiological indicators were higher than in other groups; already on the third day of therapy, the lambs felt normal, there was no pain in the navel area, there was no purulent exudate. In animals of the second and third groups, the condition remained satisfactory, pain was felt, There was a slight discharge from the navel.

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