Normalization of the immune system of large cattle with the help of “Immunopheron” and “Ribotan” medicines

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Abstract. This article presents the results of studying the effect of immunostimulants "Imunopheron" and "Ribotan" on the indices of specific and non-specific resistance of calves. To this end, according to the principle of analogues, 3 groups of calves were formed, with 5 animals each. Calves of the third group served as a control. In the blood of calves, the content of immunoglobulins A, M, G was determined on the analyzer "Uniplan". The number of T-lymphocytes in the blood was determined by the method of spontaneous rosette formation with erythrocytes of a ram, B-lymphocytes - by the method of complementary rosette formation using standard rabbit hemolysin and as a complement - fresh serum of cows. The bactericidal activity of the blood serum (BASK) was determined by O.V. Smirnova and TA. Kuzminoy, lysozyme activity of blood serum (LASK) - according to KA. Kagramanova and Z.V. Ermolieva, phagocytic index and number (FH and FI) - according to V.S. Gostevu. Experiments showed that the calves of the experimental groups showed an increase in serum levels of immunoglobulin A, M and G, as well as bactericidal and lysozyme activity of blood serum, a phagocytic number and an index. The relative and absolute levels of B-lymphocytes and T-lymphocytes were also significantly higher in the calves of the test groups than in the control group. And the most significant changes were noted with the use of the drug "Imunopheron".

1. Introduction
Immunity is one of the most important characteristics for all living organisms created in the process of evolution [1]. The immune system protects the body from infections [2–4]. The state of the immune system, like any other organ, is characterized by a complex of morphological, functional and clinical indicators inherent in the immune system in the norm and they determine the immune status [5, 6]. The change in any of these parameters indicates violation of the immune status, that is, a deviation from the norm, and it might be treated as an immunodeficiency [7].

The problem of immunodeficiency is of interest to veterinarians in connection with the steady growth of infectious and inflammatory diseases prone to chronic course against the background of low effectiveness of the basic therapy, bacterial and viral diseases that cause a high incidence rate, and even mortality [8–10].
The purpose of this research was to study the effect of immune stimulants “Immunoferon” and “Ribotan” on cows.

2. Experimental research
The studies were carried out in the conditions of a cattle farm of LLC “Dairy products” of the Soviet district of the Republic of Mari El.

The experiment was conducted on cows of black and motley breed in the second half of the pregnancy, 3 groups of 5 animals were formed. The animals of the first group were injected with medicine “Immunoferon”, intramuscularly in a dose of 10 ml per animal, once. The second group was injected subcutaneously with “Ribotan” twice a dose of 5 ml per animal and an interval of 48 hours. The third group served as a control and was kept on a regular diet.

The blood contained immunoglobulin A, M, G, T-lymphocytes and B-lymphocytes; there was bactericidal and lysozyme activity of blood serum, phagocytic index and number.

3. Results and discussion
During the research, it was noted that the level of immunoglobulin A in the blood of cows increased significantly on the 15th day in the first group by 4% (p <0.05), in the second group by 12.8% (p <0.01). On the 45th day of the experiment, their level in the first group was 2.28 ± 0.05 mg / ml (p <0.01), in the second group it was 2.16 ± 0.06 mg / ml (p <0.01). On the 60th day, the level of immunoglobulin A was higher (p <0.01) by 14% (2.14 ± 0.04 mg / ml) than the initial data in the first group, in the second - 17% (2.09 ± 0.02 mg / ml), and in the control group the level was 1.86 ± 0.04 mg / ml.

The concentration of immunoglobulin M increased by 9% (p <0.05) on the 15th day, in the second group increased by 18.5% (p <0.05). On the 60th day, in the first group the level was 2.65 ± 0.08 mg / ml (p <0.01), in the second group - 2.59 ± 0.05 mg / ml (p <0.01), which is higher than the background indicator by 13 and 28.5%, respectively.

A similar pattern was noted in the analysis of the level of immunoglobulin G in serum. At the end of the study the level was 18.01 ± 0.73 mg / ml (p <0.001) in the first group, in the second group it was 17.95 ± 0.68 mg / ml (p <0.001).

In the control group, the level of immunoglobulin M and G was 2.30 ± 0.05 mg / ml and 16.95 ± 0.33 mg / ml on the 60th day.

Bactericidal and lysozyme activity of blood serum significantly increased throughout the research in all experimental groups (table 1). Thus, bactericidal activity of blood serum on the 60th day in the first group was 46.16 ± 1.05% (p <0.05), in the second group it was 48.35 ± 2.32% (p <0.05), in the third - 48.09 ± 1.19% (p <0.05). A lysozyme activity of blood serum on the 60th day was 38.02 ± 1.14% (p <0.05) in the first group, in the second group it was 39.31 ± 0.63% (p <0.05) and in the third group - 40.06 ± 0.99% (p <0.05), which is higher than the background indicator, respectively, in 1.08; 1.09 and 1.14 times.

The phagocytic number significantly (p <0.05) increased by 6.7% (48.11 ± 1.44) in the first group on the 15th day and in the second group increased by 11.2% (49.05 ± 2.05). On the 60th day of the study, the phagocytic number was 49.43 ± 1.04 (p <0.01) in the first group, 49.11 ± 1.56 (p <0.01) in the second group.

The phagocytic index also increased in all experimental groups and was 4.26 ± 0.09 and 4.12 ± 0.04, respectively, in the first, second and third groups of the experiment (p <0.01) on the 60th day of the research. In the control group, the phagocytic index and the phagocytic number also remained at a low level.

On the 15th day of the research the relative number of T-helpers increased by 1.13 times (p <0.01) in the first group compared to the background indicator in the first group, 1.13 times in the second group (p <0.001). On the 45th day, their number was 56.6 ± 1.77% (p <0.05) in the first group and 60.1 ± 1.62% (p <0.01) in the second group. On the 60th day of the study, the relative number of T-helpers was 1.17 times higher in the first group (p <0.001) and 1.15 times in the second group (p <0.001). The
absolute number of T-helpers also increased on the 60th day in comparison with the background in the first group 1.17 times (p < 0.001), in the second group increased 1.16 times (p < 0.001).

Table 1. Dynamics of Changes in Bactericidal and Lysozyme Activity of Blood Serum, Phagocytic Number and Index in Blood of Cows, n = 5.

| Group   | Research Term, Days | Bactericidal Activity of Blood Serum, % | Lysozyme Activity of Blood Serum, % | Phagocytic Number | Phagocytic Index |
|---------|---------------------|----------------------------------------|-------------------------------------|-------------------|-----------------|
|         | Background          | 15                                     | 45                                  | 60                |                 |
| First   | 43.89±2.11          | 47.11±1.77**                           | 46.00±2.19*                         | 46.16±1.05**      |                 |
| Second  | 44.05±1.98          | 48.02±1.41*                           | 47.98±1.03*                         | 48.35±2.32*       |                 |
| Control | 44.19±1.95          | 43.74±1.34                           | 43.55±1.93                         | 43.81±2.24        |                 |
| First   | 35.02±1.54          | 39.14±1.91*                           | 38.21±0.94*                         | 38.02±1.14*       |                 |
| Second  | 36.11±1.10          | 40.23±1.32**                          | 41.08±1.01**                        | 39.31±0.63*       |                 |
| Control | 34.08±2.28          | 35.01±2.03                           | 33.12±0.88                         | 34.14±1.01        |                 |
| First   | 45.07±0.70          | 48.11±1.44**                          | 47.08±1.06**                        | 49.43±1.04**      |                 |
| Second  | 44.10±1.43          | 49.05±2.05**                          | 48.13±2.11**                        | 49.11±1.56**      |                 |
| Control | 45.18±1.09          | 44.32±0.97                           | 44.00±0.71                         | 43.25±0.92        |                 |
| First   | 3.22±0.06           | 3.93±0.04*                            | 4.31±0.04*                         | 4.26±0.09**       |                 |
| Second  | 2.96±0.03           | 4.01±0.03**                           | 4.18±0.11**                         | 4.12±0.04**       |                 |
| Control | 3.31±0.05           | 3.27±0.07                            | 3.36±0.09                          | 3.34±0.04         |                 |

Note: + - p < 0.05; ++ - p < 0.01; * - p < 0.001 the level of significance of the reliability criterion in comparison with the control group (similarly for subsequent tables).

On the 15th day of the research the relative amount of T suppressors increased by 1.15 times in the first group (p > 0.05) and 1.15 times in the second group (p > 0.05). On the 45th day, their relative number was 29.1 ± 0.88% (p < 0.01) and in the second group it was 31.5 ± 0.56% (p < 0.001). On the 60th day, their level in the first group (p > 0.05) was 1.17 times higher than the background indicator, and in the second group - 1.24 times (p < 0.001). The absolute number of T-suppressors on the 60th day was significantly (p < 0.001) higher than the background in the first group 1.38 times (0.90±0.14-10^9/l), in the second group - 1.30 times (1.01±0.06-10^9/l).

In the control group, the absolute and relative number of T-helpers and T-suppressors remained at a low level throughout the entire experiment.

The relative number of T-lymphocytes also increased during the course of the studies and was 62.1 ± 1.19% (p < 0.001) in the first group on the 60th day, and 59.4 ± 2.05% in the second group (p < 0.001), which is 1.18 and 1.21 times higher than the original indicator.

On the 60th day the absolute number of T-lymphocytes was 1.21 and 1.19 times higher (p < 0.001) in the first and second groups than in the control group. On the 15th day the relative number of B-lymphocytes was 30.7 ± 2.03% (p < 0.01) in the first group, 29.1 ± 1.12% in the second group (p < 0.01). On the 45th day, their number was 1.35 times higher in the first group (p < 0.001), and in the second group it was 1.35 times (p < 0.001) higher. On the 60th day, their number in the first group was 33.5 ± 1.21% (p < 0.001), and in the second group it was 34.6 ± 2.11% (p < 0.001).

On the 60th day the absolute number of B lymphocytes was 1.26 times (p > 0.05) higher than the initial level in the first group and in the second group it was 1.39 times higher (p < 0.05). In the control group, the relative and absolute number of B-lymphocytes was, on the 60th day, 25.5 ± 0.19% and 0.99±0.02-10^9/l, respectively.

The content of immunoglobulins in the blood serum of calves obtained from experimental and control cows is presented in table 2. It can be seen from the table that on 8-10th days the concentration of immunoglobulins A was higher by 65.2% (p < 0.01) and 72.3%, in the first and second groups than
in the control group. On 30-32\textsuperscript{th} days, the concentration of immunoglobulins A was significantly higher by 85.3\% (p <0.01) in the first group, in the second group - 91.1\% compared to the control group. The level of immunoglobulin M was also higher in calves obtained from experimental cows during the research.

| Group | Terms of the Study, the Calves’ Age in Days | 8-10 | 15-17 | 30-32 |
|-------|--------------------------------------------|------|-------|-------|
| Immunoglobulin A, mg / ml | First | 1.85±0.02** | 1.90±0.01** | 1.89±0.03** |
|       | Second | 1.93±0.04** | 1.96±0.02** | 1.97±0.04** |
|       | Control | 1.12±0.03 | 1.03±0.02 | 1.02±0.04 |
| Immunoglobulin M, mg / ml | First | 2.13±0.03* | 2.17±0.02* | 2.15±0.01* |
|       | Second | 2.16±0.02* | 2.14±0.04* | 2.19±0.03* |
|       | Control | 1.32±0.03 | 1.24±0.03 | 1.28±0.04 |
| Immunoglobulin G, mg / ml | First | 12.08±0.54* | 13.11±0.45* | 13.22±0.63* |
|       | Second | 11.58±0.33* | 12.26±0.27* | 12.31±0.40* |
|       | Control | 7.27±0.26 | 7.92±0.21 | 7.21±0.24 |

On the 8-10\textsuperscript{th} days the bactericidal activity of serum was significantly higher 1.27 and 1.20 times, respectively, in the first and second groups (p <0.01) than in the control group. On the 15-17\textsuperscript{th} days, the bactericidal activity of blood serum was 59.1 ± 3.95\% (p <0.01) in the first group, and 57.5 ± 2.64\% (p <0.01) in the second group. On the 30-32 days, this index was 59.9 ± 1.88\% (p <0.01) in the first group and 58.2 ± 2.35\% (p <0.01) in the second group, and in the control group - 43.2 ± 2.85\%.

The lysozyme activity of the serum of the experimental calves was also higher in comparison with the control group. On the 30-32\textsuperscript{th} days, the lysozyme activity of blood serum was 1.42 times higher (p <0.01) in the first group, in the second group - 1.33 times, as compared to the control group.

On the 8-10\textsuperscript{th} days, the phagocytic number and phagocytic index were 6.22 ± 0.45 (p <0.001) and 2.65 ± 0.03 (p <0.01) in the first group. In the second group, they were 6.38 ± 0.50 (p <0.001) and 2.80 ± 0.06 (p <0.01). On the 15-17\textsuperscript{th} days of the experiment, these parameters were higher in the first group in comparison with the control group, respectively, in, and in the control group they were 3.97 ± 0.27 and 1.87 ± 0.02 on the 30-32\textsuperscript{th} days.

The relative level of T-lymphocytes on the 30-32\textsuperscript{th} days was 1.43 and 1.37 times higher (p <0.001) in the first and second groups than in the control group. On the 30-32\textsuperscript{th} days the absolute level of T-lymphocytes was 2.94±0.01·10\(^9\)/l (p<0.001) in the first group, in the second group it was 2.90±0.03·10\(^9\)/l (p<0.001), and in the control group – 2.41±0.01·10\(^9\)/l.

On the 30-32\textsuperscript{nd} days the relative and absolute levels of B-lymphocytes were 1.22 and 1.27 times in the first group; 1.22 and 1.23 times higher (p <0.05) in the second group than in the control group.

On the 30-32\textsuperscript{nd} days relative levels of T-helpers and T-suppressors in the blood of calves were also significantly higher in comparison with the control group and made 52.0 ± 1.06\% (p <0.05) and 35.6\% in the first group ± 1.11\% (p <0.05), in the second - 48.1 ± 2.00\% (p <0.05) and 33.3 ± 0.68\% (p <0.05) and in these indicators were 40.0 ± 1.96\% and 27.8 ± 0.77\%.

4. Conclusion
Thus, the results of the research showed that the use of “Ribotan” and “Imunoferon” increases the specific and non-specific resistance of the organism of both cows and calves obtained from them.

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