Immunocorregating properties of propolis and their composite forms

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Abstract. Gastrointestinal and respiratory diseases of young farm animals account for the largest percentage of all diseases. Currently, vaccines have been developed and successfully used for the specific prevention of diseases of infectious etiology, but it is not always possible to develop immunity of sufficient intensity, since vaccination without immunostimulation does not contribute to sufficient antibody formation. One of the important tasks of practical veterinary medicine was to find environmentally safe immunocorregulating substances that do not have a suppressive effect on the body. It was established that the drug ferran activated hematopoietic reactions of the body. Ferran in combination with enterozyme restores immunopoiesis and erythropoiesis to the indicators of physiological norms. The combined use of enterozyme with propolis has a high immunostimulating effect, enhanced the productive phase of the immune response, removed the suppressive effect of the vaccine and increased the factors of natural and colonization resistance of the intestine.

1. Introduction

Diseases of young cattle in the first months of life in the livestock farms of the country exceed 35%. Gastrointestinal and respiratory diseases account for the largest percentage of all diseases of young animals [1]. Currently, vaccines have been developed and successfully used for the specific prevention of diseases of infectious etiology, but it is not always possible to develop immunity of sufficient intensity, since vaccination without immunostimulation does not contribute to sufficient antibody formation [2].

According to statistics in the Republic of Bashkortostan in recent years, the incidence of young cattle in the first months of life exceeds 35%, with a mortality rate of up to 20% and higher. The leading place among them is given to gastrointestinal diseases of infectious etiology, including salmonellosis. Despite the fact that there are a number of anti-salmonella vaccines, it is not always
possible to develop immunity of sufficient intensity, because a whole complex of reasons slow down the normal development of the immune reactivity of newborn calves [3]. Therefore, vaccination without immunostimulation does not promote sufficient antibody production [4].

On the other hand, a wide variety of immunocorrecting substances has been proposed today. Their use without taking into account the immune status of newborn animals can lead to the development of suppressive reactions in the body. Frequent oral administration of chemotherapeutic agents induces the development of intestinal infections [5]. The inclusion of antibiotic growth stimulators in the feed prolongs the period of salmonella carrier, because antibiotics suppress the growth of a part of the microflora that plays a protective role, which makes it possible for pathogenic microflora to multiply [6]. Violation of colonization resistance of the intestine causes a violation of the immune balance of the entire body [7].

One of the important tasks of practical veterinary medicine is to find environmentally safe immunocorregulating substances that do not have a suppressive effect on the body [8]. In connection with the above, the search for ecological principles of influence on the growth and development of animals, including immunocorrecting substances that do not have a suppressive effect on the body, is an important task of immunology [9].

The aim of the work was to study the immunocorregating properties of propolis [10] and their composite forms with Enterozyme, the organic compound Ferran iron against the background of immunization of calves against salmonellosis.

2. Materials and methods

The studies used 36 calves of black-and-white breed, from 5 days of age, which were divided into 6 groups, 6 heads in each, according to the principle of analogues. All animals were vaccinated with a concentrated formol-alum vaccine against calf salmonellosis. The calves of the group 1 were controls, the animals of the group 2 were used enterozyme, the group 3 – propolis, the group 4 – enterozyme in combination with propolis, the group 5 – ferran, the group 6 – ferran in combination with enterozyme. Vaccination with a concentrated pharmaco-alum vaccine against salmonellosis was performed twice at intervals of 10 days, intramuscularly, at a dose of 2 ml. The first time is at 10 days old, the second time is at 20 days old.

20% alcohol extract of propolis was prepared by layering in 96° ethyl alcohol at room temperature for three days, with daily stirring. The extract is a clear brown liquid. The dry matter content was determined by evaporation over a water bath to a constant weight. It was 115-120 mg/ml. Propolis milk was prepared at the rate of 5 ml of 20% alcohol extract of propolis per 1000 ml of boiled and chilled water. The dose was 10 ml per head. Enterozyme and the drug "Ferran" were used according to the induction for use.

Drinking of propolis solution from a plastic syringe was carried out at the specified doses, enterozyme in the appropriate doses 2 times a day before feeding for 10 days and OSJ "Ferran" twice for 5 ml at intervals of 15 days from 5 days of age. Before the start of the experiments, and then after 7, 10, 20, 30, 60 days, blood and feces were taken for immunological and bacteriological studies.

Hematological studies were carried out according to generally accepted methods: hemoglobin was determined on a Sali hemometer, red blood cells and white blood cells were counted in the Goryaev counting chamber. To isolate the leukocyte formula, blood smears were stained according to Romanovsky – Giemsa and Main – Grunwald.

The complement activity in the blood serum was determined by titration in the hemolytic system of RSC, in a volume of 0.5 ml by 100% hemolysis of red blood cells. Sheep red blood cells were washed twice in Hanks solution by centrifugation for 10 min at 1000 rpm. A 2.5% suspension of red blood cells was prepared in an isotonic sodium chloride solution. An equal volume of hemolysin in the working titer was added to the suspension of red blood cells. Sensitization was carried out in a thermostat at 37 °C for 30 min. To successive dilutions of serum in the volume of 0.3 ml, 0.2 ml of sensitized red blood cells were added. The reaction was placed on plexiglassed plates. The control wells were filled with 0.3 ml of isotonic sodium chloride solution and 0.2 ml of sensitized red blood
cells. The reaction was taken into account after 45 min of standing in a thermostat for 100% hemolysis of red blood cells.

Heparinized blood was used to determine the phagocytic activity of neutrophils. The object of phagocytosis was daily cultures of *Staphylococcus aureus* grown on Hottinger agar. Two volumes of heparinized blood and one volume (500 million/ml) of daily microbial culture suspension in 0.9% sodium chloride solution were added to the test tubes. After mixing, the test tubes with the contents were incubated in a thermostat at 37 °C for 30 min, then placed in cups with running water for 5 min. Then the smears were prepared, fixed and painted according to Romanovsky-Giemsa. In each smear, the phagocytic microbes of the extra-neutrophils were counted and the phagocytic number was calculated.

To determine bactericidal activity of blood serum, 4.5 ml of Hottinger broth was poured into ordinary microbiological test tubes. In the test tubes, 1 ml of the test serum was added, and in the control tubes, Hottinger broth was added in the same amount. All test tubes were filled with 0.1 ml of 2.5 billion/ml each. weigh the test microbe. After adding all the components of the reaction, the test tubes were shaken and 2 ml of the contents were taken from each with a sterile pipette to measure the optical density on the FEC-M, that is, the determination of D1. The measurements were carried out in cuvettes with a working length of 5 mm with a green light filter against distilled water. The test tubes with the remaining contents were closed with stoppers and placed in a thermostat for 2 h and 15 min. Then the optical density was determined again, i.e., D2 was found. The bacterial activity of the test serum was expressed as a percentage, calculated by the formula (1):

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BIBS = \left( \frac{D_{2\text{experience}} - D_{1\text{experience}}}{D_{2\text{control}} - D_{1\text{control}}} \right) \times 100\% \tag{1}
\]

To obtain lymphocytes, blood samples were taken from calves in tubes from the jugular vein with the preliminary addition of heparin at the rate of 25 units per 1 ml of blood. Lymphocytes were isolated by separation in the ficoll-verografin density gradient (density 1.007 g/ml). The gradient was prepared as follows: to 100 ml of 9% ficoll (Pharmacia, Uppsala), 42 ml of 36.17% verografin raster was added, the density was adjusted to 1.007 g/ml with water, measured with a densintoliter. Blood in a volume of 2 ml, diluted twice with a balanced Hens solution, was layered on an 8-10 ml gradient and subjected to centrifugation. After that, the lymphocytes were carefully collected from the interphase with a Pasteur pipette, washed twice with medium 199 by centrifugation at 1000 rpm for 5 min. The cell sediment was resuspended in 0.1 ml of medium 199, the concentration of lymphocytes was calculated in the Goryaev chamber and brought to 2x10⁶ in 1 ml by the medium.

One of the methods for determining B-lymphocytes is to detect receptors for the activated third component of the complement on them. To do this, an antiserum is prepared against the red blood cells of an animal, the red blood cells are treated with this antiserum in a subagglutinating titer – an "antigen–antibody" complex is formed. Then a complement is added, which attaches to this complex, forming aggregates "antigen-antibody-complement". The aggregated third component of the complement is able to bind to the corresponding receptor on the B-lymphocyte, forming "rosettes". Isolation of B-lymphocytes was performed with bovine erythrocytes.

Antiserum against erythrocytes was obtained from sheep immunized intravenously at two-day intervals with 25, 50 and twice with 75% erythrocyte sediment. A week after the last immunization, blood was taken from the calves, antiserum was obtained, and the titer was determined. The antiserum was stored in the refrigerator at 4 °C. To obtain the complex (erythrocytes, antiserum, complement), 2.5% of the erythrocyte suspension in medium 199 was mixed with antiserum in a subagglutinating solution. The mixture was incubated for 30 min at 37°C. Then it was washed twice with medium 199 at 1000 rpm. For 10 min and to the sediment, 2 ml of medium 199 and 2 ml of complement were added in the desired dilution (0.2 ml of mouse serum + 1.8 ml of medium 199) and incubated at 37 °C for 30 min. The red blood cells loaded with antiserum and complement were washed three times with medium 199 (at 1000 rpm, 10 min). A 0.5% concentration of red blood cells was prepared. Then 0.1 ml of blood lymphocytes were added to 0.1 ml of this suspension, incubated in a thermostat at
37°C for 5 min and centrifuged for 5 min at 1000 rpm. The mixture prepared in this way was left at room temperature for 1 h, then resuspended in medium 199 and the number of rosette-forming cells was counted in the Goryaev chamber. B-lymphocytes were taken as cells that had joined ≥3 sensitized red blood cells.

The population of T-lymphocytes in the blood of calves was determined by the method of spontaneous rosette formation of calves' lymphocytes with sheep red blood cells. To set up this reaction, a suspension of lymphocytes and a suspension of indicator red blood cells was prepared. For this purpose, the blood of a 1:1 ram was placed in a preservative solution of Alsver. Concentrated red blood cells at 4°C in the refrigerator, used for 2-3 weeks. Before each reaction, the red blood cells were thoroughly washed with saline solution. A 1% suspension of red blood cells in the medium 199 was prepared from the sediment. 0.2 ml of lymphocyte suspension (2 \times 10^6/ml) was poured into small test tubes. Then 0.4 ml of erythrocyte suspension and 1.2 ml of medium 199 were added to the calves' lymphocytes. The mixture was incubated for 30 min at 37°C, then centrifuged at 1000 rpm for 1 min and the test tubes were placed in the refrigerator at 4°C for 1-1.5 h. The rosette-forming marks were counted in the Goryaev chamber. T-cells were taken to be lymphocytes that attached three or more corresponding red blood cells.

Qualitative studies of the intestinal microflora were carried out according to the method developed by the G N Gabrichevsky Research Institute. Fecal sampling from the rectum was carried out in a sterile dish with 9-10 ml of isotonic sodium chloride solution with glycerin. In the laboratory, they were thoroughly mixed and left for 10 to 15 min at room temperature. Inoculation of 1-2 drops of fecal suspension was carried out on a number of elective and differential media. The materials were seeded on media used for isolation of intestinal bacteria (Endo, Levina, MPA, BCH). To differentiate other bacteria of the Enterobacteriaceae family, motility (–) was studied, reactions to lactose (+), mannitol (+), inositol (–), gelatin (–), urea (–), indole (+), hydrogen sulfide (–), with methylrote (+), citrate salt assimilation (–), Voges-Plosauer (–), and milk coagulation (+) were set in RA.

To isolate staphylococci, selective media were used – saline blood MPA (with 8-10% sodium chloride and 5% dextranized blood), blood MPA. From a pure culture grown on MPA, reactions to plasma coagulation, fibrinolysin, litsinase, DNA-aza, and latent hemolytic activity were determined. From the biochemical properties, gelatin dilution (+), milk coagulation (+), reactions to mannitol (+), lactose (+), sucrose (+), ammonia (+), and hydrogen sulfide (+) were determined.

The isolation of anaerobic bifidobacteria was carried out by sowing large dilutions of feces in the Blaurocca medium. In test tubes with 13-15 ml of regenerated Blaurocca medium for 40 min, 1 ml of feces was sown in a dilution of 10^-9. The crops were incubated at 37°C for 24 h. To isolate clostridium, cultures were carried out on special nutrient media for anaerobes: Kitt-Tarozzi meat – peptone – liver broth (MPPB), Wilson Blair dense medium, and Zeisler glucose – blood agar. Lactobacilli were determined on a medium consisting of glucose 0.5, tomato juice 10.0, yeast water 2.0, cystian 0.05, agar 1.5.

Before the start of the production experiment and at the end of it, animals were weighed to calculate the average daily gain in live weight and determine the percentage of livestock safety. The obtained data were subjected to biometric processing mathematically by the method of variation statistics with the calculation of the arithmetic mean for the group (M), the error of the arithmetic mean (m) and the confidence (P). The confidence criterion (P) was determined using the standard values of the Student's criterion.

3. Results and discussion
The analysis of the obtained research results showed that the calves, against the background of vaccination against salmonellosis without preliminary immunostimulation, develop immunity of insufficient intensity. The maximum bactericidal activity of the blood serum reached an indicator of 40.4 ± 0.92% by day 30, the phagocytic activity of blood leukocytes – 46.3%, and the complementary activity on day 20 of the experiment was 15.2±2.65%. Indicators of natural resistance and phagocytosis indicated a weak activity of the body's protective reactions. This was confirmed by
anemia in calves of group 1. The level of red blood cells and hemoglobin also corresponded to the lower limit of physiological norms, amounting to 3.8–4.5 million/µl and 49.9–54.6% according to Sali. The low parameters of the T- and B- immune systems were also indicative of the reduced immune status of group 1 calves. Thus, the level of T-E-ROCK lymphocytes ranged from 53.7 to 55.7%, T-helper cells – from 25.7 to 28.8%, B- EAC lymphocytes – from 16.5 to 28.7%. At the same time, the level of T-suppressors was in the range from 17.3 to 19.4% and exceeded the parameters of the experimental groups.

Low indicators of the immune status of calves of group 1 also corresponded to the disturbed balance of normal and conditionally pathogenic microorganisms in the intestine. It was accompanied by a reduced level of bifidobacteria and lactobacilli to the level of 7.9 to 9.5 Lg CFU/g and 5.5 to 8.8 Lg CFU/g and an increased content of staphylococci - from 7.0 to 9.3 Lg CFU/g, Escherichia - from 7.8 to 9.5 Lg CFU/g, clostridium - from 7.01 to 7.8 Lg CFU/g.

Thus, vaccination against salmonellosis of calves with insufficient immune balance does not restore the violation in the autoflora of animals, which is manifested by negative shifts in the bifid and lactoflora. Against the background of a deficiency of bifidobacteria and lactobacilli, the ratio between the regular intestinal microorganisms is violated, causing an increase in the level of staphylococci, Escherichia, Clostridium. This, according to a number of authors, has an adverse effect on the secretory function of the intestine, the absorption processes and indicators of carbohydrate, lipid, protein and mineral metabolism, vitamin synthesis and enzymatic functions and create conditions for dysbiosis.

The reduced immune reactivity in the body of group 1 animals can be explained by a number of reasons. First of all, this is due to the fact that the calves were obtained from mothers with a disturbed immune balance due to insufficient exercise, unbalanced in all the main parameters of the diet, lack of data that meet the requirements of zoohygienic conditions of detention; violations in the frequency of giving colostrum. To these causes are added violations of the conditions of keeping and feeding the calves themselves from the first days of their life. These factors are predisposing to the fact that the newborn young animals are not fixed at the proper level of immune mechanisms and develop an immune deficiency state.

The titer of anti-salmonella antibodies in the blood serum of calves of group 1 was the lowest in all the study periods, and was inferior to the parameters of animals of the other experimental groups by 3.7-9.9 times. The average daily increase in live weight was inferior to the parameters of the animals of all other experimental groups and amounted to 478.3 g.

More favorable effect on the immunogenesis of calves was the use of the drug enterolzyme against the background of vaccination against salmonellosis. Here, the maximum parameters of bactericidal and complementary activity of blood serum exceeded the level of animals of group 1 by 1.09 and 1.21 times, red blood cells and hemoglobin in the blood were higher by 1.13 and 1.01 times, and the phagocytic activity of white blood cells by 1.04 times. In the blood, T-E-ROCK lymphocytes exceeded the indicators of group 1 calves by 1.08 times, T-helper cells by 1.14 times, and B-EAC lymphocytes – very slightly. The response of T-suppressors was reduced by 1.04 times.

Enterolzyme had a good effect on colonization resistance of the intestine. Here, bifidobacteria exceeded the index of group 1 animals by 1.05 times, and lactobacilli – by 1.22 times. The level of conditionally pathogenic microorganisms, on the contrary, was lower, in comparison with the data of animals of group 1: staphylococci by 1.17 times, Escherichia-by 1.06 times, clostridium-by 1.20 times.

Despite a significant increase in the parameters of the immune balance in animals of group 2, compared with the data of calves of group 1, we believe that the data obtained indicate the need to find more effective methods and ways to correct the immune status because the titer of anti-inflammatory antibodies was still not high enough and prolonged. The average daily increase in live weight in the group was 535.0 g, which exceeded the parameters of calves of the 1st and 5th experimental groups.

High levels of immune resistance were recorded in the body of calves of groups 3 and, especially, 4. Here, the maximum level of bactericidal activity of blood serum was higher than their values in calves of group 1, 1.26 and 1.68 times, and 1.36 and 2.42 times, and the phagocytic activity of blood
leukocytes – 1.12 and 1.38 times. The maximum values of red blood cells and hemoglobin exceeded the control figure by 1.19 and 1.26 times, and by 1.08 and 1.06 times. In the blood of calves of groups 3 and 4, T-E-ROCK lymphocytes had the highest values, exceeding the level of animals of group 1 – by 1.16 and 1.21 times, T-helper cells-by 1.01 and 1.28 times. T-suppressors were 1.08 times lower than their level in group 1 calves.

Despite the significant progress made in the study of the microflora of the digestive tract, the problem of dysbiosis remains one of the main ones. Violation of the developed evolutionary-harmonic relationships between individual representatives of the microbial ecosystem, as a result of environmental changes, the increasing role of stress factors, uncontrolled and unsystematic antibiotic therapy, and others lead to a weakening of the ecological barrier and a decrease in colonization resistance, followed by the development of various pathological conditions.

In the course of evolution, the intestinal microflora was divided into two large groups: conditionally pathogenic and non-pathogenic normal microorganisms. The indicator system of the well-being of the symbiotic complex of the intestinal microflora is the normoflora. Therefore, the search for means that contribute to maintaining the correct balance between normal and conditionally pathogenic intestinal microflora is relevant. In this regard, we attempted to study the effect of propolis on the state of intestinal microbiocenosis against the background of immunization of calves against salmonellosis.

A study of the dynamics of bifidobacteria and lactobacilli showed that propolis contributed to an increase in the activity of the intestinal normoflora. The maximum level of bifidoflora recorded on the 30th day of the experiment in the intestines of calves of groups 3 and 4 exceeded the indicator of animals of group 1 by 1.34 and 1.53 times, lactobacilli-by 1.55 and 1.62 times. An increase in the level of bifidoflora is a positive thing. The predominance of bifidoflora in the intestine, as a rule, prevents the manifestation of the pathogenic action of opportunistic microbes. However, not only eubiosis with a dominant number of bifidobacteria, but also the lysozyme of digestive secretions, as well as secretory immunity with a predominance of JgA, play an important role in the mechanism of the protective barrier. Eubiosis stimulates the synthesis of lysozyme, lysozyme increases the lytic and anti-adhesive properties of secretory JgA, which in turn contributes to the normal state of the intestinal microbiocenosis. The violation of at least one link in this closed ring of interaction inevitably entails the violation of other links.

The positive effect of lactobacilli in the intestine is attributed to the metabolic products of homo- and heterofermentative lactobacilli-lactic and acetic acid. The antimicrobial activity of lactic acid produced by lactobacilli depends on the combined presence of lactic, acetic and propionic acids. The synergism of this combination provides inhibition of the growth of pathogenic and opportunistic microorganisms.

Another product of the metabolism of lactobacilli is CO₂, which contributes to the maintenance of anaerobic conditions in the intestine, high partial pressure and acts as hydrogen acceptors in the biosynthesis of hexose acetate by intestinal microorganisms. As a result of the activation of oxygen by lactobacilli under the influence of flavin-containing enzymes or NADH-peroxidase, hydrogen peroxide is formed. Its presence in the intestine activates the antibacterial effect of the lactoperoxidase system of milk and colostrum. The next product of lactobacil biosynthesis is diacetyl, which, at a low pH value, slows down the growth rate of Escherichia and some Gram-positive intestinal bacteria.

In parallel with the active increase in the intestines of calves of groups 3 and 4 of bifidobacteria and lactobacilli, we recorded a significant inhibition of the activity of opportunistic microorganisms: staphylococci, Escherichia, Clostridium. The minimum level of staphylococci was observed on the 30th day of the experiment. By this time, their level was lower, compared with the content in the intestines of calves of group 1 in 1.2 and 1.48 times. The reduction of staphylococci to the level of their physiological norms is in our case a favorable factor. In a healthy body, staphylococci are saprophytic, but when environmental conditions change in the gastrointestinal tract, pathogenic homolytic forms multiply among them, which produce lethal, hemolytic, dermonecrotic and enterotoxin, leucocidin, hyaluronidase, coagulase, fibrinolysin.
The content of Escherichia in the intestines of calves of group 4 by day 30 also corresponded to the physiological level, amounting to 5.3 Lg CFU/g, which was 1.5 times lower than its level in the intestines of animals of group 1. In group 3, their level remained high. Escherichia produce more than 20 types of colosans in the intestine, which inhibit the growth of other homologous strains of microbes. Enteropathogenic escherichia causes diarrhea and escherichia, such as sepsis, cystitis, cholecystitis, peritonitis, pancreatitis, and pneumonia. Non-pathogenic Escherichia have an antagonism to the causative agents of dysentery, tuberculosis, proteus, cocci forms and synthesize B vitamins and a number of amino acids.

Clostridia in the intestines of calves of groups 3 and 4 also tended to decrease to the level of physiological norms. Their content decreased by the 30th day of the experiment, in comparison with the indicator of the animals of the group 1, by 1.41 and 1.84 times. Clostridia have the ability to saprophytic existence in the gastrointestinal tract. They synthesize vitamins (pantothenic, nicotinic, folic acids, riboflavin) and are involved in maintaining the body's nonspecific resistance. The pathogenicity of Clostridium depends on the formation of toxins and various enzymes. In the state of dysbiosis, the pathogenicity of clostridium is sharply reduced.

Thus, the highest indicators of the immune status and colonization resistance of the intestine are recorded in animals of the 3 (propolis), and especially 4 (propolis + enterozyme) groups. The average daily weight gain for 3 months from the beginning of the experiments here was 575.0 and 678.3 g. In the body of calves of group 5, and especially group 6, there were also deep immunological changes. The parameters of the animals of group 5 in terms of immunological parameters were at their level in calves of group 2.

In calves of group 6, the factors of natural resistance, T- and B-systems of immunity and colonization resistance were high and were second only to those of calves of group 4. Thus, by the 30th day of the experiment, the bactericidal activity of blood serum exceeded the indicator of animals of group 1 by 1.48 times, the complementary activity-by 2.19 times, and the phagocytic activity of white blood cells by 1.29 times. The level of T-E-ROCK lymphocytes in the blood was 1.18 times higher than that of the animals of group 1, T- helper cells – 1.22 times, suppressors were at their level in the animals of groups 3 and 4.

In the intestines of calves of group 5, the state of natural microbiocenosis did not have significant positive changes and the parameters of normal and conditionally pathogenic microorganisms did not have sharp differences from their level in the intestines of animals of group 1. This indicates that Ferran can not be recommended as a drug that prevents the development of dysbiosis. However, its use in combination with enterozyme, against the background of vaccination (group 6) significantly changed the intestinal microbiocenosis in the direction of recovery. By the 30th day of the experiment, bifidobacteria exceeded the index of group 1 animals by 1.38 times, and lactobacilli-by 1.57 times. At the same time, conditionally pathogenic microorganisms decreased to the level of physiological norms and were inferior to the indicators of calves of group 1: staphylococci - by 1.42 times, Escherichia – by 1.48 times, clostridium – by 1.76 times.

Significant changes occurred under the influence of the drug Ferran-5 group and Ferran with enterozyme-6 group in the content of red blood cells and hemoglobin in the blood of animals. On the 30th day of the experiment, red blood cells in the blood of animals of group 5 and 6 exceeded the index of calves of group 1 by 1.41 and 1.51 times, hemoglobin-by 1.29 and 1.3 times. The preparation Ferran belongs to ferromagnetic compounds with a developed active surface. The drug is an organoelement compound similar in structure to blood hemoglobin. For the first time, its hemostimulating and immunostimulating properties were studied in piglets. For 3 months from the beginning of the experiments, the daily increase in live weight in groups 5 and 6 was 516.6 and 611.6 g.

The analysis of studies allows us to conclude that the immunization of calves obtained from cows weakened by the immune status with an anti-salmonella vaccine does not contribute to the creation of immunity of sufficient tension, the maintenance of the immune balance of the entire body and colonization resistance of the intestine. Immune stimulation with propolis on the background of
therapy with enterozyme, as well as the introduction of enterozyme in combination with OSJ Ferran, restore the immune status and natural microbiocenosis, preventing the development of secondary immunodeficiency and dysbiosis.

As a result of research and analysis of the data obtained, it was established that the drug ferran (group 5) aktivizes the hematopoietic reactions of the body. Ferran in combination with enterozyme (group 6) restores immunopoiesis and erythropoiesis to the indicators of physiological norms. The combined use of enterozyme with propolis (group 4) has a high immunostimulating effect, enhances the productive phase of the immune response, removes the suppressive effect of the vaccine and increases the factors of natural and colonization resistance of the intestine.

4. Conclusion
Immunization of calves obtained from cows weakened by the immune status with an anti-salmonella formolquacal vaccine does not contribute to the formation of immunity of sufficient intensity. Enterozyme and propolis milk, against the background of vaccination against salmonellosis, enhance the productive phase of the immune response, remove the suppressive effect of the vaccine, increase the factors of natural resistance of the T- and B-systems of immunity and colonization resistance of the intestine to the level of the lower limit of physiological norms. The drug Ferran, against the background of vaccination against salmonellosis, activates the hematopoietic reactions of the body (the level of red blood cells increases by 1.48 (by 2.03%), hemoglobin – by 1.31 times (by 16.2%)). OSJ Ferran in combination with enterozyme, against the background of vaccination against salmonellosis of calves, restore immunopoiesis and erythropoiesis to physiological norms (the level of red blood cells increases by 1.60 times (by 2.48%), hemoglobin – by 1.38 times (by 19.3%).) High immunostimulating effect on the body of calves, against the background of vaccination against salmonellosis, has a complex application of enterozyme with propolis, which manifests itself in the form of:

- increase in natural resistance factors (bactericidal and complementary activity by 1.89 (by 28.1%) and 4.27 times (by 19.6%), phagocytic activity of white blood cells-by 1.62 times (by 20.3%);
- increase and maintain a high physiological level in the blood of T-E-ROCK lymphocytes – 1.13 (by 7.2%), T-helper cells – 1.41 (by 10.2%), B-EAC lymphocytes – 1.02 times (by 16.9%);
- normalization of the reaction of T-suppressors;
- activation of antibody formation;
- restoration of the natural microbiocenosis of the intestine (an increase in bifidobacteria by 1.76 (by 5.5 LgKOE/g), lactobacilli – by 2.50 times (by 8.1 LgKOE/g), with a decrease in staphylococci, Escherichia and Clostridium);
- increase in the average daily increase in live weight to 678.3 g. and the safety of livestock 100%.

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