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# Immunomorphological indicators in calves after vaccination of cows against colibacillosis and their stimulation with apiphytopreparation "Vita-Force M"



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## ABSTRACT

Young livestock more often suffers from gastrointestinal diseases, especially colibacillosis. Growing healthy young livestock and their preservation from diseases and death are one of the main tasks in animal husbandry and veterinary medicine. Diseases and death of young livestock, especially in the first month of their lives, cause significant economic loss as a result of deaths and forced slaughter, low level of productivity and breeding abilities of animals, financial and labor costs for treatment and prevention. Currently, the best way to fight these diseases is active immunization of pregnant cows and sows. At present there is a growing trend towards developing and taking preparations made from natural raw materials, many of which possess diverse biological activity, capable of stimulating the immune system and at the same time harmless for the body. These include preparations made from bee products. There are data on the influence of bee products on the immunogenesis in the cases of severe clinical diseases of various etiologies of infectious and noninfectious origin. Colibacillosis developed in critical periods of calves' lives causes the development of pronounced

immunodeficiency in the organism, accompanied by a decrease in the number of T-lymphocytes in the peripheral blood of 17.25% in the thymus – by  $3,15 \times 10^6$  cells.

On livestock farms where there are a lot of cases of colibacillosis vaccination of cows with the SP4PV vaccine against colibacillosis after stimulation with apiphytopreparation "Vita-Force M" leads to a balanced immune system. Using a similar stimulation for 7-day-old calves during 5 days leads to the increase in the number of T-lymphocytes in the peripheral blood by 3.98%, in the thymus - by  $1,01 \times 10^6$  cells, that contributes to a balanced immune system.

After immunization of cows with the SP4PV vaccine against colibacillosis, apiphytopreparation "Vita-Force M" displays adjuvant properties activating changes in T- and B-dependent areas of lymphoid organs and cytological composition of mesenteric lymph nodes by increasing the area of the cortex in the thymus, strengthening the productive phase of the immune response, improving immunogenic properties of the vaccine, modulating T- and B-cell cooperation of lymphocytes.

**Keywords:** colibacillosis, thymus index, vaccine, apiphytopreparation "Vita-Force M", immunomorphology, dysbiosis, immunodeficiency, immunostimulation

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## INTRODUCTION

Young livestock more often suffers from gastrointestinal diseases, especially colibacillosis. The analysis of statistical data shows that morbidity of calves and piglets on the first days of their lives is over 70% and mortality – 80–90% of the disease.<sup>5</sup>

Currently, the main way to fight these diseases is active immunization of pregnant cows and sows. As a result, specific antibodies enter the organism of newborns from immunized mothers with colostrum and milk and provide protection.<sup>2,4</sup>

However, some newborns do not receive the required amount of this natural protection because of poor absorption of immunoglobulins in the colostrum by the bowels of newborns. This may be caused by poor nutrition of pregnant animals, harmful effects of environmental factors (radiation, change in the microclimate), ecotoxicants,

biological agents (parasites, viruses, bacteria, fungi) on them.<sup>6</sup>

The imbalance in the interaction between the individual representatives of the microbial ecosystem as a result of changes in the environment and stress factors leads to a weaker natural barrier and lower colonization resistance with subsequent development of various pathologies, in particular, dysbiosis.

At present, researchers pay great attention to the study of the impact of biologically active bee products (BABP) on the body, which activates its protective forces, stimulate the phagocytic activity of neutrophils, regulate the metabolism of globulins, positively affect the immune- and hemopoiesis, as well as metabolism.<sup>1</sup> Thus, it is necessary to intensify factors of natural resistance and immune

system with immunostimulating drugs of zoogenic origin, which are environmentally safe and have a natural origin.

Taking into account the urgency of the issue, we have developed the SP4PV vaccine against colibacillosis and multifunctional prophylactic drug "Pabisorb" on the basis of bee products (propolis, pollen, dead bees, drone milk), metabolic products of *B. bifidum* and *B. subtilis*, as well as microparticles of natural mineral sorbent - montmorillonite.<sup>2,3</sup>

## MATERIALS AND METHODS

The study was carried out in 110 calves from the age of 1 day. The first group (control Group) with the calves from healthy unvaccinated cows. In the second group, the calves were sick with colibacteriosis. The calves in the third, fourth, fifth and sixth groups were born by cows immunized with the SP4PV vaccine against colibacillosis. The vaccine was administered 2 times (45 and 30 days before calving) at a dose of 10 cm<sup>3</sup>. After the second vaccination, the cows that were going to give birth to the calves for the fourth group were stimulated with apiphytopreparation "Vita-Force M", for the fifth group — with oxymethyluracil, for the sixth group – with oxymethyluracil in combination with apiphytopreparation "Vita-Force M" during 5 days. 7-day-old calves of these groups were stimulated with the same drugs during 5 days. The 0.2% extract of the apiphytopreparation "Vita-Force M" was given with drinking water 2 times a day at a dose of 10 ml/h. Oxymethyluracil was given with water orally at a dose of 50 mg/kg. Immunological, bacteriological, and serological studies in the dynamics, blood samples were taken from the jugular vein of control and experimental animals aged 1, 3, 7, 30, 45, 60 and 90 days. Absolute and relative growth rate was calculated with a conventional method. To take material for immunological, immunomorphological, bacteriological, serological, histological studies, the animals were slaughtered: in the first group (control, healthy) at the age of 1, 3, 7, 45, 90 days, 3, 6, 9, 12, 19 months; in the second group (control, sick) at the age of 1–30 days, in the third, fourth, fifth and sixth groups at the same age as in the first group. The index of the thymus was determined with the method of Leflkovits and Pernis (1979). Phagocytic activity of neutrophils (phagocytic number, phagocytic index and the completeness of phagocytosis) was determined with standard methods and NBT-test. To estimate T-E-RFC and B-EAC-RFC- lymphocytes in the blood and lymphoid organs and to perform RLBT (Reaction of Lymphocyte Blast Transformation), lymphocytes were isolated in a density gradient

phenol-verografin (1.007 g/ml) then a rosette test was performed. The study of subpopulations of lymphocytes was conducted with the technique developed by FGBU "FCTRB-VNIVI".

## RESULTS AND DISCUSSION

Comparative analysis of the results of mass metric studies in the dynamics showed that the weight of the animals in the control group increased by 3.45 times by the end of the experiment, the weight of the thymus – by 2.45 times. The thymus index showed a good immune status in the calves. The absolute number of T-lymphocytes in the thymus was steadily increasing, and by the 90th day of the experiment, their number was 1.09 times more than the basal value.

Mass-metric indicators in the calves of the second group showed great pathological changes in the calves as they were affected by the colibacillosis pathogenic agent. By the 30th day of the research, the body weight increased by 1.16 times (in the control group – by 1.56 times), the weight of the thymus increased by 1.01 times. The number of thymocytes in the organs was lower than at the beginning of the experiment by 1.94 times and in comparison with the control value – by 2.05 times. The thymus index which was 0.42 indicated the development of severe immune pathologies in the body.

Body weight of the calves in the fourth group was slightly higher than in the third group, and at the end of the experiment, it was 3.27 times more than the basal value, 1.103 times more than in the control group. The weight of the thymus – by 5.46 and 1.06 times, respectively.

The results of the cytometric study showed that the content of thymocytes reached a maximum value by the 30th day and was 1.13 times higher than the control value. By the end of the experiment, the number of T-lymphocytes was of the control value. The thymus index was gradually growing from the 30th day of the experiment, and by the end of the research, it exceeded the 30-day index by 1.05 times indicating the immune balance in the body.

Indicators of body weight, the weight of the thymus, the number of T-lymphocytes in the thymus and the thymus index were about the same as those in the control group by the end of the experiment.

The used parameters were most pronounced in the calves of the sixth group. By the end of the research, the body weight of the animals exceeded the weight of the control calves by 1.05 times and the basal value – by 3.56 times. By the 90th day, the weight of the thymus exceeded the control value

by 1.13 times, the basal value – by 5.32 times. The number of thymocytes in the thymus was maximum by the 45th day; it was 1.17 times higher than the control value. By the end of the experiment, this figure was 1.07 times higher than the control value. The thymus index in the calves of the sixth group was equal to that in the control group from the 30th day and remained the same until the end of the experiment.

The results of the immunomorphological study showed that during the whole experiment there were changes in the structure of the thymus in the calves of the control group -- the area of the cortex was reduced and the area of the medulla was expanded. By the 90th day of the research, the cortex of the thymus of the calves in the first group took 1.16 times less area on the histology specimen than at the beginning of the research. The medulla of the thymus in the control calves, on the contrary, was expanded and by the end of the experiment its area exceeded the basal value by 1.35 times. In the calves sick with colibacteriosis, the area of the cortex of the thymus was reduced, by the 30th day of the research it was 1.55 times less than in the control group and 1.73 times less than the basal value. The medulla of the thymus occupied the area 1.59 times more than in the control group and 2.07 times more than the basal value.

Until the 7th day of the experiment, the area of the cortex was decreasing while medulla was expanding in the histological structure of the thymus in the animals of the third group. From 30th to 45th days of the research the cortex of the thymus was expanding, it exceeded the control value by 1.12 times while medulla reduced by 1.25 times. By the end of the experiment, the cortex of the thymus of the calves in the third group was equal to that of the calves in the control group.

Parallel histological analysis of the thymus structure in the calves of the fourth, fifth and sixth groups showed that until the 7th day of the research the area of the cortex was increasing, it was 1.15 times more than the control value in the fourth group, 1.12 times more - in the fifth group, 1.18 times more - in the sixth group. The medulla of the thymus was decreasing. Later the area of the cortex was decreasing, but in all those groups that indicator was higher than in the control group (by 1.05 times in the fourth group, by 1.03 times in the fifth group and by 1.13 times in the sixth group). The medulla of the thymus, on the contrary, decreased by 1.05, 1.03, and 1.26 times, respectively.

## CONCLUSION

Colibacillosis developing in critical periods of calves' lives leads to the development of pronounced

immunodeficiency accompanied by a decrease in T-lymphocytes by 17.25% in the peripheral blood, by  $3,15 \times 10^6$  cells in the thymus.

On livestock farms where there are a lot of cases of colibacillosis vaccination of cows with the SP4PV vaccine against colibacillosis after stimulation with apiphytopreparation "Vita-Force M" leads to a balanced immune system. Using a similar stimulation for 7-day-old calves during 5 days leads to, increase in the number of T-lymphocytes in the peripheral blood by 3.98%, in the thymus - by  $1,01 \times 10^6$  cells, that contributes to a balanced immune system.

After immunization of cows with the SP4PV vaccine against colibacillosis apiphytopreparation "Vita-Force M" displays adjuvant properties activating changes in T- and B-dependent areas of lymphoid organs and cytological composition of mesenteric lymph nodes by increasing the area of the cortex in the thymus, strengthening the productive phase of the immune response, improving immunogenic properties of the vaccine, modulating T- and B-cell cooperation of lymphocytes.

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