Study of the reproductive toxicity of probiotic strains on laboratory animals

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Abstract. The objective of this study was to determine the effect of probiotic preparations contained Bacillus subtilis 10⁹ CFU / ml, Propionibacterium 10⁷ CFU / ml, Lactobacillus plantarum 10⁷ CFU / ml on the physiological state, reproductive functions and the mortality rate of white rats and their offspring. The results of the analysis of the reproductive toxicity of the suspension of strains of probiotics and producers of prebiotics showed that long-term oral administration of the studied doses did not lead to a decrease in the reproductive function of laboratory animals during the experimental period, corresponded to the norm and did not differ significantly from the indicators of the control group. Based on the results obtained, the investigated form of the probiotic can be classified as a drug of low toxicity for reproductive function and, in accordance with GOST 12.1.007-76, referred to the IV hazard class.

1. Introduction
The use of probiotics can increase the productivity of animals to 10-20%, the effectiveness of treatment of gastrointestinal diseases - to 25-40% and reduce the incidence of young animals to 15-30% [1]. The use of probiotics in animal husbandry is becoming an integral part of this process, since the growth and health of animals depends on the work of the intestines, the synthesis of vitamins and amino acids, the digestion of food and the assimilation of all necessary vitamins and minerals from the feed, and the utilization of metabolic products [2].

Probiotics are becoming increasingly popular as an alternative to antibiotic use to prevent and control intestinal pathogens in animals. Although probiotics have now been determined to be beneficial, the results are highly variable and information on their mode of action and effect on the gastrointestinal microbial ecology is limited [3].

There is evidence in the literature that the use of probiotics prevents the development of dysbacteriosis, promotes the stimulation of cellular and humoral factors of immunity, increases the nonspecific resistance of the body, stimulates regeneration processes in the body, and normalizes metabolism [4-11].

Evaluation of the reproductive toxicity of medicinal products, including probiotics, in laboratory animals makes it possible to identify harmless doses of drugs and its side effects. The objective of this
study was to determine the effect of probiotic preparations on the physiological state, reproductive functions and the mortality rate of white rats and their offspring (in the first generation). This makes it possible to assess the toxic properties of the drug and to assume the degree of safety of its use in veterinary medicine.

2. Materials and methods

2.1. Evaluation of reproductive toxicity of probiotic strains on laboratory animals.

We used complex of probiotic bacteria KPM 2, contained \( B. \text{subtilis} \) \( 10^9 \text{ CFU/ml} \), \( \text{Propionibacterium} \) \( 10^7 \text{ CFU/ml} \), \( \text{Lactobacillus plantarum} \) \( 10^7 \text{ CFU/ml} \).

The experiments were carried out on 27 white rats (males and females, 2 months, live weight 150.1 ± 13.1 g), which were kept on a normal diet in a vivarium in accordance with GOST 33044-2014 "Principles of Good Laboratory Practice". During the study, the requirements of regulatory legal acts on the procedure for experimental work with the use of animals were fulfilled [12]. The experiment took clinically healthy animals that had passed a 14-day quarantine before the start of the experiment.

The experimental animals were divided into three equal groups:

1. Control group - intact animals, 3 males and 6 females.
2. Experimental group 1 - animals, which were daily injected (1 ml) of the tested suspension, 3 males and 6 females.
3. Experimental group 2 - animals that were injected daily (1 ml) of the tested suspension, 3 males and 6 females.

The animals were dosed with the test substance daily, seven days a week, orally through a metal tube at the above doses. On such rations, rats were kept before and during crossing, during pregnancy and lactation. Laboratory feed and water were always available throughout the experimental period for all animals. Before the introduction of the probiotic suspension, each animal was weighed and the dose was calculated taking into account the individual body weight of the rat. During the experiment, the rats were clinically examined and weighed. After birth, the female rats were placed in separate cages. The parameters of weight and size of rat pups from different experimental groups born at the same time (1-2 days) were measured two weeks after birth.

At the end of the study, the animals of the control and experimental groups were subjected to autopsies for morphometric analysis of internal organs.

2.2. Hematological and biochemical blood tests

Studies of the effect of probiotic drugs on the hematological parameters of rat blood included: hemoglobin content, the number of erythrocytes, leukocytes, and% of lymphocytes using the Mythic 18 hematological analyzer (C2 Diagnostics, France).

Studies of the effect of probiotic drugs on the biochemical parameters of the blood of rats included a study of blood serum for: glucose, urea, total protein, AsAT, AIAT using a Stat fax 3300 biochemical analyzer (Awareness Technology, USA) and a set of reagents "Olvex" (Olvex Diagnosticum, Russia).

3. Results of the study

The data obtained showed a low mortality rate among rat pups born from treated with probiotics and producers of prebiotics KPM 2 during 3 weeks after birth, in the experimental and control groups. The number of pups in a litter of females who were given the KPM 2 preparation with a titer of \( 10^{12} \text{ CFU/ml} \) was on average 7.2, with a titer of \( 10^{11} \text{ CFU/ml} \) - 7.0 rat pups per female (table 1). Fewer rats were born from female rats from the control groups, 6.7 pups per female.

Surviving rat pups born to females receiving CPM2 preparations were approximately the same in size and weight compared to rat pups born to females from control groups (table 2).
The state of all groups of animals during the experiment remained satisfactory: they noted a good pronounced appetite, upon external examination, the coat was shiny, the rats were mobile, the reaction to external stimuli remained the same. No violations of the functional activity of the organs of the digestive and urinary systems have been established.

| Groups                | Number of rat pups per female | Number of newborn rat pups | The number of dead rats pups | Dead rat pups / total births (%) |
|-----------------------|-------------------------------|----------------------------|------------------------------|---------------------------------|
| Control               | 6.7                           | 40                         | 4                            | 10.0                            |
| Experimental group 1  | 7.0                           | 42                         | 3                            | 7.1                             |
| Experimental group 2  | 7.2                           | 43                         | 3                            | 7.0                             |

| Groups                | 50-40g                        | 40-30g                     | 30-20g                       | 20-10g                         |
|-----------------------|-------------------------------|----------------------------|------------------------------|--------------------------------|
| Control               | 8.2%                          | 38.8%                      | 40.8%                        | 12.2%                          |
| Experimental group 1  | 8.4%                          | 39.0%                      | 40.6%                        | 12.0%                          |
| Experimental group 2  | 8.5%                          | 39.2%                      | 40.6%                        | 11.9%                          |

After 6 weeks of administration of the liquid form of the probiotic preparation, euthanasia and pathomorphological dissection of the control and experimental groups of animals were performed. Autopsy of rats showed no significant changes in internal organs. The following picture was observed. Corpses of animals of the correct constitution, average fatness. Natural openings: the mouth is closed, the tongue is in the oral cavity, the mucous membrane of the lips, gums are pale pink, smooth, shiny. Nasal openings - mucous membrane pale pink, dry, no outflows, good patency. The auricles are unchanged, the external auditory canal is clean. The anus is closed, the mucous membrane is pale pink. The coat is well kept, the coat is shiny. The skin is elastic, subcutaneous tissue is well expressed, yellowish, elastic. The muscles are reddish in color, well developed, the tendons and ligaments are white, elastic and strong. The configuration of bones and joints is not disturbed. The position of the organs of the chest and abdominal cavities: anatomically correct. There is no fluid in the chest and abdominal cavities. The patency of the pharynx and esophagus is not impaired. The heart is not changed in volume. In the cavities of the heart there is a small amount of non-coagulated blood, the endocardium is smooth, shiny. Lungs from pale pink to red, unevenly colored, well-pronounced lobulation. The spleen is not enlarged, the edges are sharp, oblong, elastic, red-brown in color. The liver is not enlarged, the edges are sharp, the shape is not changed, the consistency is dense, cherry color. The stomach is filled with fodder. The gastric mucosa is not hyperemic, without hemorrhage. The mucous membrane of the small and large parts of the intestine is unchanged. The kidneys are bean-shaped, dark brown in color, the perineal tissue contains a moderate amount of fat, the capsule is easily separated, the border between the cortical and cerebral zones is pronounced. The mucous membrane of the bladder is pale pink. Genital organs without deviations. In males, the testes are of an elastic consistency, are located in the cavity of the scrotum, and have an elliptical shape. In females, the ovaries and uterus are normal. The brain is not edematous, the medulla has an elastic consistency, without hemorrhages.

Studies of the hematological parameters of the blood of rats during the experiment showed that the leukocyte content did not change significantly in all groups of the studied rats and was within the physiological norms in the range of 11 - 12 × 10⁹ / L. The optimal number of leukocytes is demonstrated when there are no infections of bacterial or viral origin in the body. In particular, this means the absence of the infectious effect of the applied probiotic preparation KPM 2 on the rat
organism. In addition, a stable level of leukocytes indicates the absence of allergens and diseases associated with the lymphoproliferative process, internal bleeding. % of lymphocytes, also, usually grows against the background of infections. Studies have shown that % of lymphocytes did not change significantly in all groups of rats, which indicates the absence of infections. Hemoglobin and erythrocytes slightly increased during the experiment in the section "Control - No. 1 - No. 2 group", within 2%, but were also normal.

Investigations of biochemical parameters of rat blood were carried out during the experiment. High transaminase levels may indicate myocardial infarction, hepatitis, cirrhosis, metastatic carcinoma and other liver diseases [13], muscular dystrophy, acute pancreatitis, and other organ damage. An increase in ALAT and ASAT indicates necrotic changes in the body, therefore it is widely used to quickly determine the severity of the disease. Normally, the activity of ALAT and ASAT in the blood is low. The results of the study show that the activity of ALAT and ASAT in intact rats and animals receiving the drug was comparable, which indicates the absence of any toxic effect of the probiotic drug.

Urea in the blood increased during the experiment in all groups of rats, but in the experimental groups it grew faster. Moreover, in experimental group No. 2 it was faster than in experimental group No. 1. At the end of the experiment, in experimental group No. 1, the urea content in blood was higher than in the control group to 2.5%, and in experimental group No. 2 to 5.7%. Thus, there is a correlation between the increase in the dose of KPM 2 and urea in the blood of rats. An increase in the concentration of urea in the blood may be a consequence of an increase in the intake of protein from food or an increased protein catabolism. But an increase in protein catabolism would cause an increase in the activity of ALAT and ASAT, which was not found. This means that an increase in the concentration of urea in the blood occurs due to an increase in the intake of protein, which in turn occurs as a result of improved digestion with an increase in the dose of the KPM 2 drug.

Glucose and total blood protein in all groups of rats were stable in the zone of normal physiological values and did not correlate with the dose of KPM 2.

4. Conclusions
The results of the analysis of the reproductive toxicity of the suspension of strains of probiotics and producers of prebiotics showed that long-term oral administration of the studied doses did not lead to a decrease in the reproductive function of laboratory animals during the experimental period, corresponded to the norm and did not differ significantly from the indicators of the control group.

Based on the results obtained, the investigated form of the probiotic KPM 2 can be classified as a drug of low toxicity for reproductive function and, in accordance with GOST 12.1.007-76, referred to the IV hazard class (low hazard substances).

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