CREATION OF A PROBIOTIC OF A BROAD SPECTRUM OF ACTIVITY ON THE BASIS OF LACTIC ACID AND PROPIONI ACID BACTERIA

Abstract. Information about development of complex probiotics on the basis of lactic acid and propionic acid bacteria of a broad spectrum of activity for veterinary science are provided. During creation of probiotics considered antagonism of the bacteria to clinical strains of causative agents of diseases also ability to biosynthesis of biologically active agents - hydrolytic enzymes, vitamins, irreplaceable amino acids, resistance to low values pH, bile, sublimation drying was considered. It is shown that the created drugs have the immunostimulating action, increase proliferation of T-lymphocytes, granulocytes, monocytes, increase synthesis of immunoglobulins of class A, increase nonspecific immunity by increasing in lizotsimny activity of serum of blood and also increase concentration of hemoglobin in blood of animals.

The effectiveness of probiotics against causative agents of the mixed intestinal infection, coccidiosis, helminthiasis, pasteurellosis of farm animals and birds, of beediseases, mastitis at the lactating cows is proved. The presented data indicate the prospects of creation of complex probiotics with a broad spectrum of activity for prevention and treatment of diseases of farm animals and birds.

Keywords: lactic acid and propionic acid bacteria, probiotics, antagonism, spectrum of action, intestinal infections, pasteurellosis, mastitis.

Infections are the most frequent cause of death of young growth of farm animals and birds, decrease in their productivity. The complexity of treatment of infectious diseases consists in emergence at pathogenic microorganisms of polyresistance to antibiotics. Besides, application of antibiotics, unbalanced feeding and other negative factors lead to significant changes in the microorganism of the gastrointestinal tract of animals, and residual amounts of therapeutic drugs in animal products negatively influence the human body [1-3]. This proves the need for wider use in the treatment of infectious diseases of probiotics on the basis of the symbionts of the gastrointestinal tract, harmless to humans and animals.

Probiotics in veterinary medicine are used to correct the intestinal biocenosis, immune, hormonal and enzyme systems of young animals. Besides, a probiotics has relevance not only for animal breeding, but also for public health. They reduce the risk of morbidity of people and increase the environmental safety of agricultural products [4-6].

The basis for application of probiotics in veterinary medicine are the positive effects caused by them in an organism of animals. The main effects of probiotics include improved digestion, immunostimulatory effects and increased animal productivity.

Improvement of the digestive processes occurs due to colonization of the intestine with microorganisms of probiotics, which are antagonists of opportunistic enterobacteria, produce biologically active substances. This increases the synthesis of microbial protein and vitamins, increases the absorption of nutrients [7-9].

Literature data indicate the enormous role of the normal intestinal microflora as a factor of nonspecific resistance, which is realized not only through microbial antagonism, but also nonspecific activation
of macrophage phagocytic and cytostatic activity, stimulation of lymphoid tissue, and impact on immunocompetent T and B cells [10].

Now the large number of the probiotics for animal breeding consisting of lactic acid and bifidum bacteria, being the main protective group of the microorganisms of intestines harmless to the person and animals are known [11-14].

However the known probiotics did not become an alternative to antibiotics as have insufficiently broad antimicrobial spectrum of action and is used mainly for restoration of normal intestinal microflora.

In this regard, the most important task for receiving effective probiotics against gastrointestinal diseases is the directed selection of strains on their antagonistic activity. Preference should be given to antagonists against pathogens of specific diseases by the selection of strains in the composition of therapeutic drugs. Inclusion in composition of treatment-and-preventive preparations not only antagonists to the mixed intestinal infections, but also producers of biologically active substances will promote increase in efficiency of their action.

We have developed the probiotic of Laktotvit-K on the basis of Lactobacillus brevis B-3 and of Propionibacterium shermanii 15 differing from known by a wide spectrum of antimicrobial action against the most often found pathogenic and opportunistic microorganisms [15]. The treatment and preventive activity of preparation is established on 10-day goslings, intraperitoneally infected with E.coli serotype 04 at a dose of 1 ml of culture washes with MPA per head. Giving the drug to infected goslings at a dose of 4-6% of the daily feed rate promoted to the recovery of goslings for 6-7 days. The use of the drug as a preventive agent in a dose of 2-4% of the daily feed rate for 5 days made it possible to avoid disease and the death of goslings after infection. In the control group that did not receive the drug, death was observed on days 4-5 after infection and in the following days. At necropsy, congestive hyperemia of the liver, inflammation of the small and large intestines, pericarditis, purulent-fibrinous and myocardial dystrophy were registered.

The high treatment-and-protective efficiency of preparation against intestinal infections is proved also in farms of Almaty region on 64.5 thousand chickens, 250 calves and 22 pigs. It is established that preparation can be used against a colibacteriosis, salmonellosis, coccidiosis and other infections [16-18].

The ability of association from lactic acid and propionic acid bacteria also is established to suppress growth of causative agents of diseases of bees: enterobacteriosis, European and American rotteness, ascosporasis [19].

Tests of the Laktotvit-K association on bees showed its high efficiency. Thus, when using the preparation in combination with carbohydrate – protein supplementation, a decrease in the incidence of bees by an American rotteness was noted by 90.7%, and prevalence a tick of Varroa jacobsoni - for 74.1% [20].

Our researches showed need of constant selection of strains antagonists to again allocated pathogens as because of variability of the last the strains of lactic acid bacteria which are selected earlier can be insufficiently effective.

Under the ISTC grant K-673, the Polylaktotvit association was created from the lactic acid bacteria Lactobacillus plantarum 2b, Lactobacillus brevis B-3/21 and the propionic acid bacteria Propionibacteriumshermani -5, which is effective in the treatment of mixed enteric infection [21], as well as having a preventive effect against the virus of Newcastle disease [22].

One of the factors of efficiency of probiotics is their resistance to the reactogenic environment of a stomach and upper intestines. In this regard, adaptation of the lactic acid and propionic acid bacteria, belonging to the complex probiotic developed by us, were adapted to low pH and bile values. The variants of bacteria surpassing initial cultures in antagonistic activity, resistance to low values pH and bile are selected [23].

It is established that the studied strains of lactic acid bacteria are steady to pH 3 in the presence of the majority of the tested protective components and adsorbsents. Resistance to bile at strains of microorganisms is increased by pectin, food fibersand and starchentered into composition of nutrient medium in number of 0.5%.

It is shown that preparations from lactic acid and propionic acid bacteria possess the immuno-stimulating action, increase proliferation of T-lymphocytes, granulocytes, monocytes, increase synthesis
of immunoglobulins of class A, increase nonspecific immunity due to increase in lizocinmy activity of serum of blood and also increase concentration of hemoglobin in blood of animals [24].

It is established that the resistance to antibiotics at the studied strains of probiotic bacteria is controlled by a chromosome and it cannot be transferred to other microorganisms. The absence of translocation and colonization of the blood and internal organs of experimental rabbits by probiotic microorganisms after giving them developed probiotics has been confirmed. This indicates their safety [25, 26].

Selection of variants of the lactic acid and propionic acid bacteria surpassing initial cultures by 2-3 times in survival at sublimation drying and also on antagonistic activity is made [28, 29].

The new association on the basis of selected variants of Lactobacillus brevis B-3/75, Lactobacillus plantarum 2v/2 and 14d/A-17, Lactobacillus acidophilus 27w and Propionibacterium shermani S-8 was compiled. It surpasses the association from initial cultures in survival during freeze-drying and antagonistic activity.

The production technology of a liquid and dry probiotic of «Polilaktovit» [30, 31] having high treatment-and-protective efficiency against the mixed intestinal infection and also pasteurellosis is developed.

The efficiency of the received dry and liquid preparation is tested on the basis of LLP «Kaz. RIVS» in vivo on the rabbits infected with salmonellosis and pasteurellosis.

Rabbits of all experimental and control groups were infected with washout of daily agar cultures of reference test strains of Salmonella typhimurium 371, Pasteurella multocida 216 hypodermically to the area of a backart the dscE.Dso. 24 hours after infection began treatment of animal experimental groups with a probiotic in comparison with an antibiotic. The working solution of a dry probiotic was prepared just before the use. For this purpose 25 g of a dry probiotic dissolved in 100 ml of the boiled and cooled up to 30°C drinking water. The liquid probiotic was used without dilution.

As a result of the conducted researches the high preventive and therapeutic efficiency of the probiotic «Polilaktovit» both in combination with antibiotic, and without it is established. The control rabbits infected with Salmonella typhimurium 371 or Pasteurella multocida 216 and not treated fell on 3 - the 4th day after infection. The experimental rabbits receiving for treatment only per os probiotic on 5 ml 3 times a day at salmonellosis and on 10 ml 3 times a day on 15 minutes prior to feeding at pasteurellosis had an improvement of the general state on third day, recovery occurred on the third and fourth day.

At the experimental rabbits receiving within 10 days to infection a dry or liquid probiotic of per os and continuing to receive it on 10 ml 3 times a day in 15 minutes prior to feeding during the experiment without interruption (14 days), insignificant oppression only within the first day after infection was noted. For the 2nd day of symptoms of a disease of salmonellosis and pasteurellosis it was not noted, rabbits did not get sick.

At the rabbits receiving an antibiotic after infection, the state was normalized for the 5th day, the good appetite was noted, symptoms of a disease were not observed.

The best result is noted at treatment of salmonellosis and pasteurellosis at the rabbits receiving at the same time an antibiotic and a probiotic "Polilaktovit". After treatment by an antibiotic of the rabbits (2 injections) infected with salmonellosis and the probiotic set by per os on 5 ml 3 times a day in 15 minutes prior to feeding, animals recovered on the 2nd – the 3rd day after infection. The rabbits infected with pasteurellosis after treatment by an antibiotic (3 injections) and a liquid probiotic on 10 ml 3 times a day recovered for the fourth day.

All rabbits of experimental groups receiving a probiotic during an experiment considerably put on the weight (from 3.3-3.5 to 4.5-6.0 kg). More than others rabbits put on weight (up to 6 kg), a long time receiving a probiotic, beginning in 10 days prior to infection and until the end of experience (24 days).

On the 14th day after infection with salmonellosis and pasteurellosis, 36 rabbits of all experimental groups were slaughtered. As a result of bacteriological research, it was established that after treatment of animals with probiotic in dry and liquid form, the persistence of pathogens in the internal organs of rabbits was not observed.

Test of the dry preparation «Polilaktovit» as treatment-and-prophylactic means against dyspepsia of newborn calves was carried out in country economy of "Habit".
For tests 30 heads at the age of five days, from them 10 heads of the patients with dyspepsia, and 20 heads for the purpose of prevention are selected. Working solution of a dry probiotic was prepared just before application. The daily dose of preparation for sick animals made 100 ml. To sick calves received the preparation twice on 50 ml in the morning and in the evening on a hungry stomach within 2-3 days. All calves recovered, fallen among them was not.

In order to prevent diseases, the preparation was given to calves in 50 ml once a day (in the morning on a hungry stomach), no cases of diseases among them were found.

In the same farm tested the effectiveness of the dry probiotic «Polylaktovit» as a therapeutic and prophylactic agent against dyspepsia in newborn lambs. The studies involved 20 newborn lambs, from whom 10 animals received a probiotic in dose of 15 ml per head for 15 minutes before the first feeding for 3 days. Other 10 animal probiotics did not receive and served as control. As a result of tests it is established that in group of the newborns receiving «Polylaktovit» with the preventive purpose, the incidence was not registered. In control group the number of sick animals made 30%. The probiotic was given to sick lambs 3 times a day on 15 ml on the head before feeding to an absolute recovery. Recovery occurred for 2-3 days.

By results of tests the conclusion is made that the probiotic «Polylaktovit» can be used as treatment-and-prophylactic means against dyspepsia of calves and lambs. At the same time need for application of antibiotics and other antibacterial means for treatment of sick animals disappears.

It is established that the probiotic «Polylaktovit» can be used also for prevention and treatment of mastitis at cows [32].

Research and production experiment was made in the farm «Alipov-T» on 2 groups of the lactating cows of the Alatau breed: the experimental group consisted of 24 heads, the control group included 6 heads. The animals were kept in different bases, the duration of the experience - 2 weeks.

In the experimental group a probiotic solution was applied for the irrigation of the nipples using a spray bottle after removing the milking machine. In the control group sanitary processing of an udder was carried out on the technology used in economy (processing of nipples after milking by the medicine «Dawn»).

It is established that the bactericidal effect of probiotic bacteria after processing is shown in a significant decrease in the number of potential mastitis pathogens – Staphylococcus aureus (by 78%) and Escherichia coli (by 68.9%). This is comparable with similar indicators in the experiment with the chemical agent «Dawn», which is widely used in the farms of the Republic of Kazakhstan and is one of the most effective means for treating the udder.

At the same time, an increase in the total bacterial contamination of the skin of the udder nipples in the experimental group of cows due to the tested probiotic cultures was established. The advantage of probiotic preparation is their environmental safety, low cost, positive biological effect on the skin of the nipples and udder for various damages.

In milk of skilled cows decrease in number of somatic cases which in number over 500 thousand/cm³ are symptom of the latent mastitis is noted. There is an increase in milk of lactose, fat, casein, SOMO. The offered way of prevention of mastitis surpasses in these indicators of quality of milk opposed.

In Amiran LLP and JSC agrarian and industrial complex Adal the therapeutic efficiency of probiotic means at treatment of subclinical forms of mastitis at cows is studied. To cows of experimental group with subclinical mastitis a probiotic enteredparavaginal in a dose of 10 ml on an animal daily 1 time a day before recovery. The cows of control group treated by the traditional method accepted in farms – injection of mastisan-A to the udder in a dose of 5 ml 2 times a day after morning and evening milking before an absolute recovery.

All animals were treated before double negative reaction of tests of milk with pro-mastitis.

When using with the medical purpose of a probiotic in experimental group of cows the therapeutic efficiency was 90%, the average duration of treatment - 5.2±0.15 days. In control group applying medicine mastisan-A, from 10 cows recovered 8, that made 80%. Recovery of animals happened on average in 6.0±0.82 days. Besides, at 2 cows of control group the latent mastitis in the course of treatment passed into clinical expressed.

The physical and chemical structure of milk was estimated on the content of fat, protein, SOMO and density. Sanitary and hygienic indicators of quality of milk estimated on acidity, a bacterial seeding by quantity of somatic cases.
At cows of experimental group density and acidity of milk conformed to requirements of state standard, a mass fraction of fat and protein – basic norm. At application of a probiotic milk in the first day after recovery had negative reaction to the inhibiting substances therefore it could be realized without restrictions. When using of medicine mastisan-A containing antibiotics, the last were allocated with milk all term of treatment and four days after recovery.

Thus, the probiotic possesses more expressed therapeutic action at subclinical mastitis at cows in comparison with a widespread traditional way of treatment – introduction of medicine mastisan-A. At the same time duration of treatment of cows of experimental group in comparison with control is reduced by 0.8 days, the therapeutic efficiency increases by 10%. The probiotic normalizes physical and chemical, sanitary and hygienic indicators of milk and increases its biological value.

Conclusions. The provided experimental data demonstrate prospects of creation of complex probiotics of a broad spectrum of activity for prevention and treatment of diseases of farm animals and birds.

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НЕГІЗІ СУТ ЖОНЕ ПРОПИОНО КЫШКЫЛЫ БАКТЕРИЯЛАРЫНДЫН ТУРАТЫН ЕСЕР ЕТЕ СПЕКТРІ КЕҢ ПРОБИОТИКТІ ДАЙЫНДАУ

Аннотация. Herizі сут жоңе пропион нышқалы бактерияларының туратын есер ету спектрі кен, кешенді пробиотиктер дайындау және аралас және метіметтер кетірілген. Пробиотиктерді даярдау кезінде, оның құрылымы қөретін бактериаларды және бір аударын клиникалық штамдарының қозыңдығына қарсы антагонизм, сондықтан қатар биологиялық белсенді заттарды жаттығу – гидролитикалық ферменттердің, дарумендердің, алмаспайтын амин қышқылдарының аурусын қабылдайтын оларға, ортак рең ғының бөлігінен, құрал және құрал қоюы көрсетілді.

Анықтау: сұпина және пропион қышқыл бактерияларының, пробиотиктер, антагонизм, есер ету спектрлі, ішек инфекциялары, пастерелез, мастьт.

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СОЗДАНІЕ ПРОБИОТИКА ШИРОКОГО СПЕКТРА ДЕЙСТВИЯ НА ОСНОВЕ МОЛОЧНОКИСЛЫХ И ПРОПИОНОВОКИСЛЫХ БАКТЕРИЙ

Аннотация. Представлена информация о разработке комплексных пробиотиков на основе молочно-кислых и пропионовокислых бактерий широкого спектра действия для ветеринарии. При создании пробиотиков учитывалась антагонизм входящих в их состав бактерий к клиническим штаммам возбудителей конкретных заболеваний, а также способность к биосинтезу биологически активных веществ - гидролитических ферментов, витаминов, незаменимых аминокислот, устойчивость к низким значениям pH, жёлчи, сублимационному высушиванию. Показано, что созданные препараты обладают иммунностимулирующим действием, увеличивают пролиферацию Т-лимфоцитов, гранулоцитов, моноцитов, увеличивают синтез...
иммуноглобулинов класса А, повышают неспецифический иммунитет за счет увеличения лизоцимной активности сыворотки крови, а также увеличивают концентрацию гемоглобина в крови животных.

Доказана эффективность пробиотиков в отношении возбудителей смещенной кишечной инфекции, кокцидиоза, гельмитозов, пастереллеза у сельскохозяйственных животных и птиц заболеваний пчел, массита у лактирующих коров. Представленные данные свидетельствуют о перспективности создания комплексных пробиотиков широкого спектра действия для профилактики и лечения заболеваний сельскохозяйственных животных и птиц.

Ключевые слова: молочнокислые и пропионовокислые бактерии, пробиотики, антагонизм, спектр действия, кишечная инфекция, пастереллез, массит.

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