Modern concept of probiotics and principles of development of new-generation symbiotic preparations

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Abstract. The purpose of this work is to review data on the biological effectiveness of probiotics, their mechanisms of action, and the prospects for the development of new-generation preparations. Scientific and practical justification of symbiotic drugs of a new generation – metabiotics as a continuation of the probiotic concept is given. The authors discuss the literature data and their own results, which allow them to propose new approaches to the creation of symbiotic drugs. The authors scientifically substantiate the main scientific provisions of the probiotic concept for the development of new-generation symbiotic drugs:
- study of aspects of the cultivation of the metabolic probiotic *B. bifidum* strain No. 1 in appropriate nutrient media;
- determination of the quantitative and qualitative composition of metabolites in the probiotic producer;
- scientific and practical substantiation of the qualitative and quantitative composition of the experimental probiotic suspension as a biologically active additive;
- the effect of probiotic suspension on the metabolism, the formation of microbiocenosis and nonspecific immunity. The substantiation for the creation of new feed probiotic preparations in the form of a composition of living microorganisms with molasses-based metabolites is given, which has a certain scientific and practical significance, and is promising for use in animal diets. Studies have found that to stabilize normal microflora in suckling pigs and weanlings, it is advisable to use various drugs in the form of biologically active additives of probiotic agents and their metabolites. The article provides a scientific and practical justification of a dietary supplement for correcting the microbiocenosis of the gastrointestinal tract and some factors of cellular immunity in growing young pigs based on the probiotic microorganism *B. bifidum* when cultivated on a nutrient medium from molasses.
Key words: probiotics, metabolism, concept, metabiotics, microbiocenosis, nonspecific immunity, symbiotic dietary supplement, piglets.

1 Introduction

The development of fundamental studies of the symbiotic interactions of the body and biologically active substances determines a new refinement in the definition of metabolism. It should be noted that metabolism is a process that takes place in any living organism. Metabolism allows the body to grow, reproduce, heal damage and respond to external and internal stimuli, therefore, the response of the immune system to certain changes in the body when using biologically active substances in animal feeding is included in the concept of "metabolism".

Feed enzyme, probiotic preparations, are widely used in the practice of animal husbandry, allow to improve the existing systems of breeding and feeding of farm animals, become an important component of modern rational animal feeding.

Probiotics are live microbial supplements that have a beneficial effect on the animal's body by improving the intestinal microbial balance, stimulating metabolic and immune processes. Probiotics refer to correctors of animal productivity, energotropics (means that direct the energy of nutrients to increase the productivity of animals). These drugs are not vital for the body, but they can increase and stabilize the productivity of animals [1,2,3,4,5].

The positive effect of probiotics based on living microorganisms on the host body is carried out through the normalization of its microbial ecology due to the following factors: a) inhibition of the growth of potentially harmful microorganisms as a result of the production of antimicrobial substances; b) stimulation of the growth of endogenous flora as a result of the production of vitamins and other growth-stimulating factors, normalization of pH, neutralization of toxins;

c) changes in microbial metabolism, manifested in an increase or decrease in the activity of enzymes.

Probiotic drugs are harmless to the macroorganism even in concentrations significantly higher than recommended for use, and some strains can significantly increase the nonspecific resistance of the macroorganism.[6,7,8,9]

Some strains of bacilli are characterized by the following properties: antagonistic activity to many pathogenic and conditionally pathogenic microorganisms; high enzymatic activity, which allows to significantly regulate and stimulate digestion; anti-allergic and antitoxic effect.

As indicated by numerous studies [10,11,12], the range of indications for the use of probiotics is wide, they are used:

- to stimulate cellular and humoral factors of immunity;
- activation of metabolic processes and normalization of digestion;
- treatment and prevention of disbiosis;
- treatment of gastrointestinal diseases of infectious and alimentary etiology;
- normalization of the microflora of the digestive tract after treatment with antibiotics and other antibacterial chemotherapeutic agents;
- to stimulate the growth of young animals, accelerate the adaptation of animals to high-energy diets and non-protein nitrogenous substances;
- increase the efficiency of feed use.

In modern research, the development of dietary supplements is carried out in the following areas: search for new highly active strains of bifidobacteria and lactobacilli; development of probiotics based on associated microbial cultures; the use of auto-and donor strains of endogenous microorganisms for the design of new drugs, the production of antibiotic-resistant strains and the creation of pharmaceutical preparations based on them to prevent the development of chemotherapeutic disbiosis, the optimization of production processes, the development of effective and economical nutrient media and dosage forms of drugs [13,14,15].

It should be noted that probiotic drugs affect such processes as correction of microflora, stabilization of intestinal permeability, growth of pathogenic bacteria and their competition, elimination, strengthening of the body's immune responses and the metabolic activity of microflora. According to most experts, when developing probiotics, the fundamental principle is to create preparations based on microorganisms - representatives of the normal human microflora: bifidobacteria, coli -, lactobacilli, bacterioids [16,17,18,19].

Taking into account all the positive qualities and widespread use of probiotic drugs, they are not always highly effective, which is explained by the mass death of probiotic cultures when they enter the gastrointestinal tract, the viability of about 5% of the injected cells is preserved. To effectively solve the problem in modern science, the direction of metabiotics is considered as a continuation of the probiotic concept, it is a new generation of symbiotic drugs based on the metabolites of probiotic cultures. [20,21,22,23]

Thus, the creation of new symbiotic drugs, the study of their interaction with the macroorganism, for the correction and maintenance of the microecology of the animal body (in the formation of immunometabolic reactions and the state of the microbiocenosis of the gastrointestinal tract) is a significant direction in the strategy of scientific development of the probiotic concept by obtaining metabolites of probiotic cultures in different nutrient media.

Among the variety of representatives of probiotic cultures, the bacteria of the genus Bifidobacterium are widely used. Bifidobacteria are known as classical probiotic microorganisms. Bifidobacteriumbifidum is a species of Gram-positive anaerobic bacteria. They participate in enzymatic processes, perform a vitamin-forming function (synthesis of B vitamins, vitamin K, folic and nicotinic acids), improve the indicators of protein, lipid and mineral metabolism, as they enhance the hydrolysis of proteins, ferment carbohydrates, saponify fats, dissolve fiber, stimulate intestinal peristalsis, promote normal intestinal cleansing, and also promote the synthesis of essential amino acids, better absorption of calcium salts, vitamin D, have anti-anemic, antirachitic and anti-allergic effects, stimulate the lymphoid apparatus. The beneficial properties of bifidobacteria include the ability to effectively absorb lactose, stimulate the immune system, reduce cholesterol in the blood, and have an anti-carcinogenic effect. [24,25,26]

When cultivating probiotic cultures, the nutrient medium is very important, since the quantity and quality of the obtained metabolites depend on it. Bifidobacteriumbifidum is very demanding on the composition of the nutrient medium [27,28,29].

As a nutrient medium, the corresponding composition for the cultivation of B. bifidum corresponds to both carbohydrate-based beet molasses and protein-based grain. Our research is aimed at creating biologically active supplements with a live culture of probiotics in combination with metabolites in a liquid nutrient medium.

We have identified the main scientific provisions of the probiotic concept for the development of a new generation of symbiotic biologically active additives (metabiotics) for the correction of metabolism in piglets.

1. study of aspects of cultivation of the metabolic probiotic B. bifidum strain No. 1 in appropriate nutrient media (grain nutrient medium based on naked oats and beet molasses);
2. scientific and practical substantiation of the qualitative and quantitative composition of the experimental probiotic suspension;
3. the effect of probiotic suspension on the metabolism, the formation of microbiocenosis and nonspecific immunity in piglets of early weaning;
4. scientific substantiation of the relationship of microbiological producers of metabolites with the macroorganism.

The aim of the research is to provide a scientific and practical substantiation of the probiotic concept of B. bifidum in a nutrient medium from beet molasses and naked oats.

2 Materials and methods

The research was carried out in the Laboratory of Agrobiotechnologies of Kursk Federal Research Center in the period of 2019-2021. The research material was sugar beet molasses of 5% concentration and grain culture medium based on naked oats prepared according to the author's method and probiotic microorganisms of B. bifidum strain No. 1. When obtaining the bacterial concentrate, a daily culture of microorganisms was used as a culture medium, which was standardized to 1x106 CFU / cm^3. After standardization, the bacterial culture was added to the culture medium from molasses and

naked oats were cultured for 14 days in a KBCG-100/250 thermostat at 37±1 C0. In the experimental period of cultivation, the amount of CFU in the culture liquid was taken into account when calculating their amount in the Goryaev chamber using a Levenhuk 740T, M1400 Plus microscope.

3 Results and Discussion

1. Study of aspects of cultivation of the metabolic probiotic B. bifidum strain No. 1 in appropriate nutrient media (grain nutrient medium based on naked oats and beet molasses)

The analysis of the information, the obtained research results and scientific assumptions allow us to note the validity of the probiotic concept in the development of dietary supplements for the correction of metabolism in animals.

Sugar beet molasses is taken as the basis of the nutrient medium due to the sufficient content of carbohydrate components, and grain based on naked oats as protein-carbohydrate.

In the process of cultivation of B. bifidum on a nutrient medium from molasses, a certain metabolic activity of their vital activity was established, which is expressed in a decrease in the mass fraction of the amount of fermented sugars in the range of 53.7% to 23.7%, the hydrogen index from 6.70 to 3.77.At the same time, a certain variability in the growth dynamics of colony-forming units (CFU 106) during the cultivation of probiotic culture was established. In the first 3 days there is an increase in CFU in the range from 0.4-6.6x106 CFU/cm^3, starting from 5 days. the growth decreases to 3-4x106 CFU / cm3, and from 7 days. an intensive growth of CFU is observed until the end of the experiment and reaches 5.9 x106 CFU / cm3. It should be assumed that the active growth of probiotic microorganisms in the first day of the experiment is due to the nutrition of easily fermented sugars, and the repeated increase in the number, from 7 days it is associated with the transition of bifidobacteria to a diet of more complex sugars.

Dynamics of the number of bifidobacteria growth on the grain nutrient medium in the first 3-4 days it reached up to 4.9-5.0x106 CFU /cm3 after a decrease was observed until the end of the experimental period to 1. 9x106 CFU/cm3.Active growth of probiotic microorganisms in the first 4 days it is associated with the fermentation activity of the nutrient medium.

2. Scientific and practical substantiation of the qualitative and quantitative composition of the experimental probiotic suspension.

It should be noted that with an increase in the number of probiotic microorganisms in experimental nutrient media, their metabolites in the form of amino acids, organic acids and vitamins are simultaneously released. There is a significant increase in the synthesis of essential amino acids in relation to the control.

In studies, when cultivating a probiotic culture on a molasses culture medium, a significant increase in Lysine 26 times, phenylalanine 14 times, Leucine+isoleucine 6.4 times, and other non-replaceable and conditionally replaceable amino acids was found, which indicates a significant activity of probiotic microorganisms in the culture medium. At the same time, the importance of individual protein-forming amino acids for the animal body and the resulting complex of amino acids in protein metabolism should be taken into account.

The metabolic composition of the probiotic microorganisms produced in the grain culture medium from naked oats had characteristic features. The lysine content was 2.9 times higher, for tyrosine 2.5 times, for phenylalanine 3.3 times, for Leucine+isoleucine 1.7 times, for glycine 3.5 times.

Thus, in the comparative analysis of the metabolic composition of the nutrient media produced by the probiotic culture of B. bifidum, the amino acid composition is preferred by the nutrient medium from beet molasses, which is confirmed by an increase in the synthesis of amino acids: Lysine, Leucine+isoleucine 3 times, threonine by 7 times, and other non-replaceable and conditionally replaceable amino acids. The metabolic composition of organic acids has a certain variability and a positive tendency to increase in the grain nutrient medium.

When cultivating B. bifidum in nutrient media based on naked oats and molasses, the activity variability for amylolytic and proteolytic enzymes has different dynamics. The enzymatic activity directly depends on the amount of CFU. Characteristic increases in proteolytic and amylolytic enzymes are observed on days 2 and 3, in the range of 0.85-0.62 mg / ml / sec in the nutrient medium of beet molasses and 0.93-0.78 mg/ml/sec in the medium of naked oats. A decrease in these indicators is also noted from day 5 and reach values in the range of 0.73-0.56 mg / ml/ sec, a repeated increase in activity is observed from day 7 and reaches 0.78-0.60 mg / ml / sec in a nutrient medium of beet molasses. A decrease in the dynamics of the activity of proteolytic and amylolytic enzymes in the nutrient medium from naked oats is observed on day, and until the end of the experiment reaching values of 0.68-0.56 mg / ml / sec.

Thus, the amount, of enzymes produced in the experimental probiotic suspension depends on the probiotic culture and the composition of the nutrient medium. The obtained metabolites of the probiotic culture of B. Bifidum have different variability, which is associated with the multicomponent nutrient media. The obtained experimental probiotic suspensions are characterized by a certain biologically active composition and can be used for further development as a biologically active additive for the correction of metabolism in animals.

3. The effect of probiotic suspension on the formation of microbiocenosis and nonspecific immunity in piglets of early weaning.

It should be noted that in fact, the entire metabolic potential of the intestinal microflora is localized in the large intestine and it determines its functional significance for the animal body [30,31,32,33]. Therefore, one of the factors of a new approach to the creation of dietary supplements based on probiotic microbial metabolites was the study of the intestinal microbiocenosis and the relationship with cellular immunity.

As a result of clinical trials of the biological product, it was found that piglets at 30 days of age had a fairly high content of bifidobacteria and lactobacilli and typical escherichia. At the same time, the presence of Clostridium and other opportunistic enterobacteria in the intestinal contents was noted. It should be assumed that the resulting composition of the microbiocenosis corresponds to the normal state of the intestinal microflora.

It should be noted that the normal microflora is a competition for many pathogens, and the mechanisms for suppressing their growth are quite diverse. The main one is the selective binding of cell surface receptors, especially epithelial ones [34,35,36]. Most representatives of the resident microflora show a pronounced antagonism, directed, among other things, against pathogenic species. Such properties are especially pronounced in bifidobacteria.

The studies conducted at the age of 60 days allow us to note that there is a tendency to change the intestinal microflora with the phenomena of relative disadvantage of the microbiocenosis. There was a decrease in CFU / g of lactobacilli 10^3, an increase in staphylococci and Clostridium to 10^6 and 10^7, and a slight increase in other opportunistic microflora. At the same time, the content of bifidobacteria 10^9 is maintained at a sufficient level.

No less important is the role of normal microflora as an antigenic stimulator of the immune system, the absence of a normal microbial biocenosis causes its numerous disfunctions, and in suckling pigs, the underdevelopment of the main immunocompetent organs is noted. During normal colonization, numerous microbes induce the formation of low titers of antibodies, the target of which is unlikely to be specific species [37,38,39]. These antibodies are mainly Class AIg, released on the surface of the mucous membranes.

Considering the finished dynamics of T-and B-lymphocytes, it should be noted that in the first days of piglets' life, the bulk of the lymphocytes were T-cells. As the age of piglets increases, there is a significant increase in the absolute number of B-lymphocytes and a positive tendency to increase T-helper cells. At the same time, cellular immunity factors provide an objective picture of the relationship between microbiocenosis and indicators of nonspecific immunity.

Thus, it should be assumed that the change in microbiocenosis is associated with feeding piglets with vegetable compound feeds, while the additional introduction of a biological product based on *Bifidobacteriumbifidum* strain - No. 1 in an amount of at least 10^9 CFU/head allows you to correct the state of the intestinal microflora and cellular immunity of growing piglets.

4 Conclusions

Thus, the scientific concept in research is an integrated approach to solving the problem of creating a biologically active supplement. The main scientific provisions of the probiotic concept for the development of new-generation symbiotic drugs are determined:

- study of aspects of the cultivation of the metabolic probiotic *B. bifidum* strain No. 1 in appropriate nutrient media.
- determination of the quantitative and qualitative composition of metabolites in the probiotic producer;
- scientific and practical substantiation of the qualitative and quantitative composition of the experimental probiotic suspension as a biologically active additive;
- the effect of probiotic suspension on metabolism, the formation of microbiocenosis and nonspecific immunity in early weaning piglets.

The results of the studies suggest that after weaning, piglets develop disbiotic changes in the intestinal microflora, manifested in a decrease in the number of representatives of the normoflora: bifidobacteria and lactobacilli, typical escherichia, and an increase in the number of facultative and conditionally pathogenic microorganisms. To stabilize the normal microflora in suckling pigs and weanlings, it is advisable to use various drugs in the form of biologically active additives, probiotic agents and their metabolites. The research carried out a scientific and practical justification of a biologically active supplement for the correction of the microbiocenosis of the gastrointestinal tract and some factors of cellular immunity in growing young pigs based on the probiotic microorganism *Bifidobacteriumbifidum*when cultured on a nutrient medium from molasses**.**

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