Review of studies on the use of synbiotic feed additives in compound feeds

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Abstract. The article discusses feed additives with probiotic activity, the composition and methods of their preparation, an assessment of the situation of the use of synbiotic additives in compound feeds. Generalised literature data on the use of synbiotics in animal husbandry and aquaculture are presented, their advantages and disadvantages are revealed. A review of studies on the use of feed with probiotic activity in the diet of animals, birds and fish proves the high effectiveness of their use: there is an increase in survival, a decrease in feed conversion, an improvement in the microbiota and the general condition of the animal. Based on the review, a new substrate for growing bacterial strains based on a grain heap of wheat in the early stages of ripeness is proposed.

1 Introduction

The agro-industrial complex is a dynamically developing industry with significant potential in terms of the country's food security strategy. Nevertheless, technological problems stand in the way of a stable increase in production volumes in the country and providing the population with high-quality, diverse and affordable agricultural products. First of all, they include:

- diseases of cultivated objects that reduce productivity, marketability of goods;
- lack of effective control systems and rapid diagnosis of pathogens;
- environmental problems related to the quality of the growing environment of hydrobionts;
- the lack of a sufficient assortment and the high cost of specialised compound feeds of domestic production and others [1].

Modern applied biotechnology is focused on the development and implementation of fundamentally new multifunctional feed additives in a complex form. Currently, a search is underway for new approaches to the maintenance of animals and fish under the influence of environmental factors based on the use of biologically active additives. One of the main

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problems of biomedicine in recent years is the widespread spread of pathogenic microorganisms resistant to certain antibiotics.

Intensive antibiotic therapy has become one of the causes of disruption of normal bacteriocenosis and a decrease in the immunobiological reactivity of animals and fish, the emergence of resistant strains of pathogens, which reduces the therapeutic effect of antibacterial drugs. The use of antibiotics leads to the accumulation of microorganisms with complex antibiotic resistance in the environment. Unlike agricultural technologies, when using antibiotics in aquaculture, it is almost impossible to prevent them from entering natural reservoirs.

One of the promising ways to solve these problems is the use of drugs based on probiotic strains of bacteria. In fisheries, they are successfully used to prevent morbidity and improve water quality [2].

The quality of the feed affects the growth rate, maturation of sexual products and survival of fish. Full-fledged compound feed fully covers all the needs of animals and poultry in nutritious, biologically active and mineral substances. Such a diet is used in feeding fish, poultry, rabbits, pigs, horses and other species. One of the main problems for agricultural enterprises is to find high-quality compound feed that would meet all the requested requirements.

For normal growth and development, fish need a certain amount and ratio of essential nutrients. Protein (with a certain set of essential amino acids), fat, carbohydrates, minerals, vitamins and other biologically active substances should be contained in the feed in accordance with the needs of fish. Such a need varies depending on age, size, water temperature and other factors. The more the composition of the feed corresponds to the biological needs of each age group of fish, the more effective the use of nutrients by fish [3].

Synbiotic refers to dietary supplements that combine a prebiotic and a probiotic in the form of synergy. The main purpose of using synbiotic is that a real probiotic without prebiotic food does not take root well in the digestive system. It was found that a combination of probiotic and prebiotic, i.e. synbiotics, can be more effective than a probiotic or a prebiotic alone [4-8]. In addition, synbiotic is a mixture of probiotics and prebiotics that have a beneficial effect on the animal's body, increasing its survival and implantation of living microorganisms in the gastrointestinal tract [9].

The Food and Agriculture Organisation of the United Nations (FAO) recommends using the word "synbiotic" only if the net health benefits are synergistic [10, 11]. Synbiotic is intended not only to increase the population of beneficial microorganisms in the gastrointestinal tract, but also to promote the proliferation of autochthonous-specific strains in it [12]. Studies on the effects of synbiotics on metabolic health are still limited. It is worth noting that the healing effect is likely to depend on the combination of synbiotics. Therefore, synbiotics seem promising for modulating the composition of the intestinal microbiota [13-16].

2 Synbiotic feed additives overview

According to modern ideas, in order to improve the quality and safety of products for the consumer, as well as to improve fish-breeding and biological indicators, preparations based on synbiotic cultures of viable synbiont bacteria are increasingly used in feed formulations.

A known feed probiotic additive for fish, consisting of a bacconcentrate, which is a mixture including bacteria strains *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus lactis subsp. lactis* and *Propionobacterium freudenreichii subsp. Shermanii*, and yeast mushrooms, previously freeze-dried to the state of freeze-dried powder. At the

same time, the feed additive additionally contains fillers: milk powder whey, skimmed cow's milk powder and fulvic acid [17].

Also known is a probiotic feed additive used in the manufacture of compound feeds containing probiotics for farm animals, poultry and fish, and containing a biomass of spore–forming bacteria *Bacillus subtilis B–2250* and/or *Bacillus licheniformis B-2252*, and a sorbent carrier containing hydrophilic aerosil grade A and hydrophobic grade AM, as well as a moisture-intensive filler [18]. The disadvantages of this additive include the absence of a prebiotic substrate in its composition that promotes the growth of the introduced probiotic component.

Feed additive with probiotic activity for farm animals, birds, horses and fish - patent RU 2652836, containing in its composition the biomass of *Enterococcus faecium 1-35* bacteria complex with a titer of live bacteria $3{,}8x10^7$-$2{,}2x10^8$ CFU and *Bacillus megaterium bacteria* with a titer of live bacteria $3{,}8x10^7$-$3{,}3x10^8$ CFU, deposited on filler, which is used as bran or sunflower meal, or diatomite, or trepel. The disadvantage of this feed additive is the insufficiently high titer content of living bacteria and the method of obtaining bacterial biomass is liquid-phase fermentation, which does not ensure the formation of a biofilm by probiotic bacteria.

Probiotics colonising a solid substrate form a biofilm, which makes them more resistant to various stresses and aggressive agents, including antibiotics. At the same time, probiotics grown in the solid phase more effectively displace pathogenic microorganisms. Synbiotics can produce various antagonistic compounds, including antibiotic-like substances.

Primix-Biororm P(K) is a well–known complex synbiotic for the prevention and treatment of gastrointestinal infections and dysbiosis of various etiologies, restoration of intestinal microflora, prevention of oncological diseases [21]. Synbiotic supplement contains a combination of *Lactobacillus acidophilus, Bifidobacterium lactis* and fructooligosaccharides (FOS). This combination in Primex-Bionorm improves digestion, reduces inflammation and strengthens the immune system of animals.

The synbiotic Lactosubtil-Forte is also known - a complex of preparations Avilact-1K, Avisubtil and Cerevet containing *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus* and inulin.

Avilact-1K is a liquid or dry probiotic containing live cells of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Enterococcus faecium* and mannanoligosaccharides (MOS), improving intestinal health, as well as reducing the risk of diarrhea and increasing egg production in poultry;

Avisubtil is a liquid or dry probiotic containing live cells of *Bacillus subtilis*, *Saccharomyces cerevisiae* and fructooligosaccharides (FOS). This synbiotic supplement is designed specifically for livestock, and helps to improve digestion, reduce the risk of respiratory infections and increase weight gain in livestock.

Cerevet is a protein feed additive containing dry biomass of inactivated cells of the *S. cerevisiae (diastaticus)* bacterial strain (deposit number of the All-Union collection of industrial microorganisms VKPM-u-1218). It is used to increase the effectiveness of vaccination of broiler chickens against Newcastle disease [22, 23].

It has been shown that probiotics in Lactrosubtil Forte improve intestinal health, reduce the risk of diarrhea and strengthen the immune system in animals.

The most commonly used synbiotic and probiotic feed additives in Russia are additives of the following brands: "Bacell" containing bacterial strains *Bacillus subtilis, Lactobacillus acidophilus, Ruminococcus albus*; «Monosporin» – *Bacillus subtilis*; "Prolam» – *Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus 43c, Lactococcus*

lactis subsp. lactis 574, Lactococcus lactis subsp. lactis 1704-5, Bifidobacterium animalis 83 [19, 20].

Synbiotic supplements have a positive effect on the health of animals during feeding. The combination of probiotics and prebiotics in these supplements improves intestinal health, reduces the risk of diarrhea and respiratory infections, strengthens the immune system, improves weight gain and egg production in animals. The main parameter is the choice of the right synbiotic supplement for the specific needs of an agricultural animal, because different supplements are designed for different types of animals and contain specific necessary combinations of probiotics and prebiotics for a certain type of animal.

3 Overview of compound feeds with the use of synbiotic additives

Also known is the production feed for rainbow trout fry RGM-5B - patent RU 2777768 C1. This compound feed is used only for growing fingerlings and yearlings of trout weighing from 5 to 50 g. The essence of the invention is the additional use of the fat of the Black Soldier fly larva *Hermetia illucens* in the formulation of the production compound feed. The advantages of this formulation is a high content of biologically active substances, due to which an increase in the immunological reactivity of fish follows. According to the results of the experiments, it is proved that the composition of the production feed increases the survival rate of trout, and also contributes to an increase in the mass of fish without negatively affecting its general condition.

There is a fish feed with a probiotic feed additive - patent RU 2652833 C1. The probiotic additive contains dried biomass of two strains: *Enterococcusfaecium 1-35* bacterial strain with a live bacteria titer of 1.3×10^8-1.8×10^8 CFU and *Bacillus megaterium B-4801* bacterial strain with a live bacteria titer of 1.0×10^8-3.3×10^8 CFU, applied in the mixture in equal amounts on the filler. In the first variant, sunflower meal containing live bacteria *Enterococcus faecium 1-35* 1.8×10^8 CFU and live bacteria *Bacillus megaterium B-4801* 1.0×10^8 CFU in 1 g of feed additive is used as a filler. In the second variant, trepel containing live bacteria *Enterococcus faecium 1-35* 1.8×10^8 CFU and live bacteria *Bacillus megaterium B-4801* 1.0×10^8 CFU in 1 g of feed additive is used as a filler. Such a feed probiotic supplement in the diet of fry allows to increase their safety and increase the gain of live weight [24].

Synbiotic is known under the trademark “Synbiosorb-1”, used in compound feeds for broiler chickens, which has a positive effect on the production indicators of their cultivation. It was found that feeding broiler chickens with synbiotic “Synbiosorb-1” at a dosage of 0.05 increases their live weight at 14 days of age by 1.1%, at 28 years of age - by 5.5% and at 37 years of age - by 1.8%. The synbiotic itself contains 10^8 CFU microorganisms of lactic acid bacteria. Also included in the preparation is a unique mineral-organic complex as a prebiotic and sorbent, as well as lactulose and fructose. The mineral sorbent of the preparations is monomeric nanometer-sized silicon particles sequentially grouped into agglomerates [25].

The effect of the synbiotic additive of the brand “ProStor”, which includes cultures of microorganisms Bacillus subtillis, Bacillus licheniformis, lactic acid bacteria, vitamins, amino acids, as well as extracts of medicinal herbs (echinacea and milk thistle) immobilised on a phytosorbent, was evaluated as part of a production feed for juvenile sturgeon. The rate of introduction of probiotic into the production feed for sturgeon fish is 4 g/kg of compound feed. The addition of this drug to the production feed for fish OT-7 contributes to obtaining higher fish-biological and hematological indicators. Milk thistle extract and echinacea, which are powerful immunomodulators, increase the resistance of fish to aggressive environmental factors [26].

Probiotic drugs “Monosporin”, “STF-1/56” are also known in the diet of carp feeding. “Monosporin” consists of a microbial mass of spore-forming bacteria *Bacillus subtilis*, beet molasses, soy hydrolysate and water. 1 cm^3 of the preparation contains at least 1x108 CFU of spore-forming bacteria. It does not contain GMOs. It is a liquid suspension with suspended particles from light brown to cream colour with shades of different intensity, with the smell of the nutrient medium. *Bacillus subtilis* bacteria used for the manufacture of the drug, multiplying in the intestines of animals, secrete biologically active substances, under the influence of which the digestive processes are activated, nonspecific immunity is enhanced, as a result of which the average daily increments increase, the safety of livestock and the efficiency of raising young farm animals increases. “Monosporin” is recommended to be used for the treatment of eggs and larvae in an amount of 0.2% of their weight. Processing of fertilised eggs should be carried out during its de-gluing. After hatching the pre-larvae and switching them to exogenous nutrition, it is also necessary to treat them before planting in ponds.

The drug “STF-1/56” is manufactured by LLC Biotechagro for the purposeful improvement of the therapeutic and prophylactic properties of the probiotic *Enterococcus faecium* - enterococci in the process of their vital activity participate in the processes of metabolism in the intestine, help digestion, absorption of vitamins, etc. This probiotic drug has shown high efficiency in animal husbandry as a method of suppressing colibacteriosis and salmonella infection without disturbing the intestinal microflora and the physiological balance of the body as a whole. *Enterococcus faccium* bacilli are able to continue functioning in reduced temperature conditions in organisms of poikilothermic animals and fish. In order to treat infected fish, a therapeutic course with a probiotic drug should be carried out for 10 days. In the prevention of salmonellosis in fish, it is necessary to use a therapeutic feed containing the drug "STF-1/56" for 3-5 days.

“Monosporin” + “STF-1/56” (in equal amounts), due to its high activity in suppressing pathogens, can be used both as a preventive and therapeutic purpose. The enzymes produced by the bacterium *B.subtilis* break down feed components in the form of fats, proteins and carbohydrates, which improves digestion. However, their functional activity is controlled by a number of factors, the main of which are the composition of food, the intensity of nutrition, age, etc. It is a well-known fact that the digestibility (or physiological availability) of carbohydrates, especially vegetable ones, by cyprinid fish is insignificant. In compound feeds, their number mainly reaches 30%. Amylolytic, lactic acid and cellulolytic groups of microorganisms participate in the breakdown of carbohydrate-containing food substrates.

4 The use of a grain heap of wheat of the early stages of ripeness in compound feeds

Synbiotics are grown on nutrient substrates containing both prebiotic compounds and probiotic microorganisms. Examples of prebiotic compounds that are commonly used as nutrient substrates for synbiotics include fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), and lactulose. Probiotic microorganisms used in synbiotic formulations include *Lactobacillus, Bifidobacterium, Streptococcus* and other beneficial bacterial strains. It is believed that the combination of prebiotics and probiotics in synbiotics promotes the growth and survival of probiotic microorganisms in the intestine, which leads to improved intestinal health and overall well-being.

The most common substrates used for growing synbiotic supplements are carbohydrates. These include lactose, glucose, fructose and various types of oligosaccharides. Advantages and advantages of using different substrates:

Lactose is a widely used substrate for growing probiotics such as *Lactobacillus* and *Bifidobacterium*. It is known that these probiotics contribute to the normalisation of the intestinal microflora, reduce inflammation and strengthen the immune system. Lactobacilli have been shown to reduce the symptoms of lactose intolerance, and bifidobacteria have been shown to reduce the symptoms of irritable bowel syndrome.

Glucose and fructose are simple sugars that are easily metabolised by probiotics - streptococci and enterococci. It is known that these probiotics, as well as lactobacilli, contribute to the normalisation of the intestinal microflora.

Oligosaccharides are complex carbohydrates that are not digested by humans, but are a food source for probiotics. They are often obtained from plant sources such as chicory root, jerusalem artichoke and soybeans. They have a beneficial effect on the animal's immune system, and also improve the absorption of minerals such as calcium and magnesium.

To obtain a feed additive with probiotic activity for feeding animals and fish, bacteria are grown directly on a moist substrate with pronounced prebiotic properties [28, 29]. In this case, the solid particles of the substrate serve as the basis for growth and a source of nutrients for probiotic bacteria, that is, it is prebiotic.

An example of the manufacture of a plant-based substrate is also the use of oil cakes of essential oil plants. Screening of essential oil plant cakes for prebiotic properties was carried out for further development of prebiotic feeds for farm animals based on them. As plants potentially possessing prebiotic properties, the following types of cake and whole plants were selected: ginger seed cake (*Camelina sativa (L.) Crantz typus*), nigella seed cake (*Nigella sativa*), Sarepta mustard cake (*Brassica juncea*), narrow-leaved lavender cake (*Lavándula angustifólia*), cake and whole milk thistle plant (*Silybum marianum*), a whole plant of the soul cat (*Calamintha nepeta*), cake and a whole plant of mountain savory (*Satureja montana*). Essential oil plants were selected based on the high content of biologically active substances. Studies have shown that some of these species enhance the acid-forming properties of lactic acid bacteria, thereby reducing the pH of the medium, which leads to a decrease in the number of *Enterococcus, E. coli* and lactose-positive bacteria, as well as complete suppression of Proteus [36].

As a nutrient medium for cultivating bacterial strains of a synbiotic additive, a grain heap of wheat of the early stages of ripeness can be used (Figure 1). The use of a substrate from a grain heap allows preparing raw materials for production with a higher content of protein, fats, macro- and microelements, and nutrients. The substrate is a source of plastic and biologically active substances: proteins, polyunsaturated and short-chain fatty acids, oligosaccharides, phytates, flavonoids. Its use can increase the level of bifidobacteria and lactobacilli and change the ratio of microbiota towards reducing the populations of pathogenic bacteria in the intestine, thereby reducing the risk of diseases and having a beneficial effect on the health of animals and fish [30, 31].

The main biogenic elements are carbon, nitrogen, phosphorus, oxygen, hydrogen, sulfur. These are components of proteins, carbohydrates and fats, as well as nucleic acids. These elements are required in significant quantities. Biogenic elements must be present in the nutrient medium in a form accessible to microorganisms. As a rule, metal ions, sulfur, phosphorus and trace elements are added to the medium in the form of mineral salts. The mineral basis of the medium (mineral background) is almost the same for most microorganisms [32].

Fig. 1. Grain of winter wheat of early ripeness phases

The substrate from the grain heap of wheat of the early stages of ripeness refers to natural, loose, universal media for the cultivation of microorganisms. Natural media are used to accumulate microbial biomass and are widely used for primary isolation from natural substrates, since their composition allows satisfying the nutritional needs of many groups of microorganisms. They contain products of animal or vegetable origin rich in various organic substances, having a complex and unstable composition. As bulk media, sets of long-stored dry components are used, which are dissolved or moistened with water before work. It can be grain, bran, solid waste from agriculture and the food industry. Universal media are used for the accumulation of microbial cells and the initial identification of the species diversity of microorganisms in mixed populations. They make it possible to support the growth of a significant number of microorganisms [33-35].

5 Conclusion

A review and analysis of scientific papers reflecting the results of the use of probiotic feed additives in the diet of animals, birds and fish showed that probiotic microorganisms have a high therapeutic potential. The most promising is the use of biotherapeutic formulas containing both probiotic microbial strains and synergistic prebiotics. Such synbiotic products are more effective than separate probiotics and prebiotics. The use of synbiotic feed additives in the diet of livestock and aquaculture facilities ensures the favorable development and restoration of beneficial intestinal microflora, and also allows them to strengthen their resistance to adverse environmental factors, thereby increasing growth rates, improving metabolism, increasing the digestibility of nutrients in the diet. Probiotic strains are grown mainly on bean cakes, oilseeds, as well as on grain fibers: soy, sunflower meal, wheat fibers and others. A grain heap of wheat of the early stages of ripeness can be considered as a substrate, since it contains a large amount of nutrients, as a result of which probiotic strains can show good growth and survival. Nevertheless, further research is needed to substantiate the preventive and therapeutic health benefits, optimal consumption, duration of animal feed with probiotic activity and the choice of the best synbiotic (specific probiotic strains and substrates) to achieve the desired result in farm animals, birds and fish.

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