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PRODUCTION OF RECOMBINANT β-SUBUNIT OF CHOLERA TOXIN

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Abstract: *Prevention of severe intestinal infections, including cholera, does not lose its relevance due to episodic outbreaks. Creation of a new variant of the cholera vaccine based on a genetically modified non-toxigenic V. cholerae strain supplemented with a recombinant cholera toxin β subunit (CtxB) is a promising approach. The β subunit elicits an immune response but has no toxic effect without the a subunit. The world experience in obtaining recombinant CtxB using bacterial cultures has been studied, approaches have been chosen for the production and purification of CtxB in the laboratory. The literature devoted to the peculiarities of the interaction of CtxB with intestinal epithelial cells was also analyzed [1]. Using standard molecular biological methods, a line of plasmids was obtained that allows random triggering of ctxB expression in E. coli cells. The constructed vectors differ in their encoded signal sequence fused to the gene of the target protein. When expression is activated, a chimeric polypeptide molecule containing CtxB and a fragment of the E. coli OmpA protein or Erwinia carotovora PelB protein is synthesized [2]. These sequences ensure the release of the produced toxin into the culture medium and make it possible to simplify the process of its purification.*

Keywords: *β-subunit of cholera toxin, CtxB, expression, recombinant protein.*

Purpose of the work: To create genetic constructs for the production of cholera toxin β-subunit, to determine the optimal conditions for the induction of the target protein (temperature, duration of induction, composition of the medium), the place of its accumulation (cytoplasm, periplasm, cultivation medium) and the preferred method of its isolation.

Materials and methods: Standard molecular biological methods (amplification, restriction, ligation, transformation) were used to create the expression construct. Isolation of the target CtxB protein from the *E. coli* BL21DE3 culture transformed with the modified pET22b+ plasmid was carried out by Immobilized Metal Affinity Chromatography (IMAC) using Ni-NTA-agarose as a carrier. Features of the β-subunit of cholera toxin make it possible to use the method of metal-chelate chromatography for its purification even in the absence of polyhistidine sequences [3].

Results: A number of genetic episomal constructs (plasmids) have been created that allow arbitrary triggering of the expression of the cholera toxin β-

subunit (CtxB) gene in *E. coli* cells. The analysis of the efficiency of CtxB production using various variants of plasmids was carried out, and the most promising ompA clone was selected. It was found that the optimal yield of the product is observed when using the M9 medium with the addition of glycerol and induction at 20°C. Under these conditions, CtxB accumulates mainly in the medium and, to a lesser extent, in the periplasm. Based on the results of selection of conditions by metal-chelate chromatography (IMAC), a trial purification of recombinant CtxB from the culture liquid (supernatant) of the *E. coli* culture grown on M9 medium with glycerol was carried out; 48 hours after induction, the concentration of the target product in the medium can reach 50 mg/l of the β-subunit of cholera toxin with a purity of about 96%, while the protein is stable and retains the pentameric structure, which allows us to consider the created system as a promising tool for the industrial synthesis of recombinant CtxB for medical and research purposes.

Conclusion: As a result of the work performed, a genetic system for the production of recombinant CtxB protein in *E. coli* cells was created and a method for purifying CtxB by metal-chelate chromatography (IMAC), was developed. The obtained groundwork can be used as a basis for the development of industrial approaches to the production of CtxB, including as a component of the cholera vaccine.

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OVERVIEW OF THE EFFICIENCY USING THE PROBIOTIC BASULIFOR IN FEEDING POULTRY AND LIVESTOCK

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Abstract. *Recent studies have shown that probiotics can have an immunomodulatory effect and stimulate natural resistance factors. Probiotics belonging to different types and strains of bacteria have different effects on immunological processes and have high enzymatic activity and antagonistic activity in relation to pathogenic and conditionally pathogenic intestinal microflora. They are also considered technologically advanced in production and*

do not affect the quality of poultry products. Furthermore, they are safe and stable during storage and as a part of compound feed [4]. In this regard, the paper conducts an overview study of recent studies, which described using the probiotic antibacterial drug "Basulifor" for feeding poultry and livestock and considered its impact on them.

Keywords: *probiotic Basulifor, productivity, immunoglobulins, protein, feeding.*

Introduction. The demand for food products of animal origin is still growing today to meet the nutritional needs of the growing world population and the growing financial capabilities of the population of many countries that can now afford more animal proteins. The importance of poultry is that it is one of the main products that have nutritional value both in eggs and meat, as meat provides a high amount of protein with a low percentage of fat, and eggs are a source of proteins, salts, and vitamins. Also, the speed of the production cycle, especially in broiler chickens, makes poultry products cheaper than livestock products. They are also easily digestible, as well as easily available and bred.

This continuous growth of the market for food products of animal origin encouraged the expansion of intensive agriculture around the world to increase production and meet the growing demand. The majority of breeders wanted to get profit quickly and at the lowest cost, and this led to a clear imbalance between the progress of the poultry industry and poor sanitary conditions and the quality of services provided for animal care. The increasing volume of poultry industry has led to the spread of many diseases, especially those that cause a significant economic loss to the poultry industry. Moreover, the loss may increase in the future if the provision of inequality and unbalanced feed without enough protein, energy, vitamins, and mineral salts is made, and without providing technical and veterinary services, conducting periodic tests, and monitoring hatches.

Probiotic bacteria have many benefits, as they raise the rate of food conversion and increase weight by opening the appetite and increasing the bird's ability to absorb food more, reduce the death rate as a result of treating all types of microbial diarrhea, improve the health and immune status of the herd, increase resistance to diseases, improve the quality of the carcass to give a safer product for the consumer, increase egg productivity and improve its quality. In relation to the above, we see the importance of developing and introducing a new domestic probiotic feed supplement for dietary poultry meat processing technology, and here we will do a literature study to see the importance and impact of using Basulifor in nutrition. This work aims at describing the importance of using Basulifor in the poultry industry as a good alternative to antibiotics, its positive impact on poultry farming and bird health, and increasing productivity by checking the results of the works [1, 2, and 3].

Main Work. Ivan Alekseev and others [1] conducted an experiment in which they used a probiotic supplement to confirm its ability to increase

nonspecific resistance and the productivity of small quail birds. In the experiment, the birds were divided into three groups taking into account gender, age, and weight. The first experimental group was fed with Basulifor 0.2 grams of feed, and the second group with 0.3 grams; the control group did not contain any feed additives. The experiment's results were as follows: increase in the number of erythrocytes in the blood of birds is by 5.20-5.69% (P<0.05), hemoglobin concentration – by 3.80-4.48 (P<0.05), the amount of total protein in serum blood – by 3.89-6.66% (P<0.05), gamma globulins – by 7.29-8.03% (P<0.01), class A immunoglobulin – by 11.50-15.30% (P<0.01), IgM – by 7.14-10.70% (P<0.01), IgG – by 6.54 6.80% (P<0.01), birds' preservation – 93.0 to 97.34% (P<0.01). The live weight of the birds increased in comparison with the control group by 5.30 and 10.50 g (P<0.05); the average daily gain was 0.08 and 0.17 g. The killing yield of quails against the background of the feed supplement was higher by 1.97% and 3.76% (P<0.05) than in the control group.

Ivan Alekseev and Roman Egorov, in their work on calves titled (Immunological indices of blood and viability of calves when using a probiotic feed additive «BASULIFOR») [2], conducted an experiment on calves to study the effect of Basulifor on immunity and increase natural resistance and obtained the following results: compared to the background of the use of probiotic feed additives, the number of erythrocytes in the blood of experimental animals increased by 4.54% (P<0.05), hemoglobin – by 8.58% (P<0.01) in comparison with the control variant. The introduction of this supplement into the calves' diet contributed to the activation of protein metabolism in the body of experimental animals. This is confirmed by an increase in the total protein level in the blood serum of calves by 3.28% (P<0.05), albumins – by 3.80% (P<0.05), globulins – by 4.20% (P<0.05), gamma globulins – by 12.97% (P<0.01), immunoglobulins: Ig "A" – by 5.00% (P<0.05); Ig "M" – by 5.64% (P<0.01); Ig "G" – by 5.90% (P<0.05); safety of calves – by 5.55% (P<0.05) in comparison with the control variant.

Maxim Zebelina and colleagues experimented [3] on two groups of chickens of meat cross "Cobb 500" (control and experimental groups) formed at the age of one day, with 100 chickens in each group. We can summarize their results in the following main points: it was found that broiler chickens receiving the probiotic Basulifor had a higher pre–slaughter live weight (2451.1 g), the mass of the gutted carcass (1784.4 g) and slaughter yield (72.8%) compared to analogues that did not receive a feed additive. So, by weight of the gutted carcass, their advantage was 126 g, or 7.6%; by slaughter yield 1.6%. Furthermore, as a result, it was found that in the pectoral muscles of broiler chickens of the experimental group, there was an increase in protein content by 6.1% and a decrease in fat content by 2.4%, ash by 2.2%. Thus, an increase in protein content and a decrease in fat and ash content in muscle tissues indicate an increase in the nutritional value of meat, which is achieved as a result of the inclusion of the probiotic Basulifor in the poultry diet at the rate of 200 g/t of compound feed for the entire period of research.

Conclusion. We can summarize the main results of the previous works in the following main points:

1. Under the influence of the probiotic feed additive Basulifor, the body's physiological, hematological, biochemical, and immunological blood serum parameters in animals and birds are activated.
2. On using the probiotic feed additive, there is an increase in the meat productivity of animals and birds, which is expressed by stimulation of the intensity of the average daily weight gain.

Future Plan for our experiments: Our experiments aim to specify the recommended amount of Basulifor in the broiler parent stock's mixed feed to improve the chickens' safety and reproductive qualities.

1) The scientific and economic experiment will be carried out on chickens of the parent flock of broilers of the Cobb-500.
2) The duration of the experiment will be 42 weeks. Three groups of 100 animals each will be formed using pairs-analogs by live weight.
3) The birds of each group will be kept in a separate house. Chickens of the control group will receive the main diet adopted in the economy.
4) The hens of the experimental groups will be given the primary diet of 0.3 kg/t and 0.5 kg/t of probiotic compound feed (Basulifor).

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ANALYZING SOME DESIGN AND PERFORMANCE PARAMETERS OF A COMBINE HARVESTER IN TERMS OF ITS AUTOMATION