Quality Assessment of Biological Product of Microbial Origin

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Abstract*: Today, the use of microbial origin biological products in poultry farming is widespread. However, it was found that the use of living bacteria in such preparations is not always effective due to their inactivation under the action of enzymes located in the gastrointestinal tract. In this connection, it is promising to use their destroyed forms (lysates), which also have high biological properties. The aim of the scientific work was to assess the quality of the developed hydrolyzate of lactic acid bacteria and its compliance with regulatory documents. It was established that the studied lactobacillus hydrolyzate meets the requirements of the regulatory documentation for this biological product, does not have toxic and irritating effects on the body of laboratory animals, and therefore can be offered in industrial poultry farming to increase the safety and productivity of farm poultry.

Keywords* : bacterial hydrolyzate, microbiological purity, quantification, shelf life, safety.

I. INTRODUCTION

Immunostimulating activity is one of the most important functions of the normal microflora of the gastrointestinal tract, associated with the participation in the maintenance of the working state of specific and non-specific, humoral and cellular mechanisms of immunity, with local and general manifestation. And one of the main, if not the main, role in this is played by lactobacilli, which are an integral part of the intestinal normal flora [6].

The immunostimulating effect of lactic acid bacteria is primarily associated with the presence of peptidoglycans and teichoic acids in their cell wall. The main immunostimulant is glucosaminyl muramyl dipeptide (GMDP), which is the main structural unit of the peptidoglycan of the bacterial cell wall. It acts on the cells of the innate immune system, causing stimulation of the effector functions of phagocytes and the production of cytokines, which, in turn, induce proliferation, activation and differentiation of acquired immunity cells - T and B lymphocytes. Through increased production of colony-stimulating factors, GMDP induces leukopoiesis. As a result of its action, all parts of the immune system are activated with an increase in anti-infection immunity [1, 10, 11, 12].

Preparations for which lactic acid bacteria are used are widely used in veterinary practice. However, experience shows that the use of these bacteria in their native state is often ineffective due to their inactivation by the action of gastric acid enzymes. Obviously, and this is shown by practice, it is more advisable to use hydrolysates of lactic acid bacteria, since they are easier to digest and easier to be involved in biochemical processes [7].

The aim of the scientific work was to assess the quality of the developed bacterial hydrolyzate, as well as to study its toxicological effect on laboratory animals.

II. MATERIALS AND METHODS

The object of research was a biological product obtained by the thermoacid method of lactic acid bacteria.

The quality of the developed bacterial hydrolyzate was studied in accordance with OST 91500.05.001-00 and the following indicators were selected: description, dry residue, authenticity, pH, microbiological purity, quantification, and shelf life [5].

A description of the lactobacillus hydrolyzate was carried out visually in daylight scattered light.

To determine the dry residue, 1.0 ml of the biological product was placed in a pre-calcined and finely weighed porcelain crucible. Then the crucible was carefully heated at a low temperature, allowing the substance to evaporate. Then they were calcined in a muffle furnace at 500 ° C to constant weight. At the end of the process, the crucible was cooled in a desiccator and weighed.

As a criterion for the authenticity of the tested biological product, low molecular weight peptides were chosen, since the presence of their complex is the active principle of the hydrolyzate. But also to determine the authenticity of a biological product, the presence of its main active component among low molecular weight peptides, glucosaminyl muramyl dipeptide (GMDP), was studied. The presence in the hydrolyzate of the sum of low molecular weight peptides was studied by comparing the absorption spectra of the standard solution of GMDP and the microbial hydrolyzate obtained by the method of determining the total content of low molecular weight peptides with Benedict's reagent.

The active acidity (pH) of the biological product was determined by the potentiometric method on the device Ionomerp H-meter I-500.

Revised Manuscript Received on October 05, 2019

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Tests to determine the microbiological purity of the developed biological product were carried out in accordance with the requirements of OFS.1.2.4.0002.15 “Microbiological purity” [4]. Evaluation of the results was carried out according to the recommended requirements for category 6.1, which includes the studied bacterial hydrolyzate.

The quantitative content included the determination of the following biologically active substances: total protein, peptides with M.m. <1500 D, protein and peptides with M.m. > 1500 D, amino acids, and GMDP.

The total protein was determined spectrophotometrically in the visible region using Benedict's reagent, and bovine serum albumin (BSA) was used as a standard sample.

The determination of low molecular weight peptides was carried out as in the case of determining the total protein, however, GMDP (the drug for animals "Glycopin" or for humans "Lycopid") was used as a standard sample.

The determination of amino acids was carried out according to the standard method for the quantitative content of amino acids in microbial lysates, and glutamic acid was used as a standard sample.

The content of protein substances with Mm> 1500 D was determined by the difference between the content of total protein and low molecular weight peptides.

The determination of GMDP was carried out by the micellar variant of capillary electrophoresis.

The study of the shelf life of the hydrolyzate was carried out according to previously published scientific papers [1; 6].

III. RESULTS AND DISCUSSION

Description. The hydrolyzate of lactic acid bacteria is a clear solution of dark brown color. The color of the biological product is due to the formation of hydrolysis of colored products - melanoidins. Melanoidins are compounds formed by the interaction of amino acids and carbohydrates under the influence of high temperature. A number of studies have shown the ability of these compounds to possess antioxidant, antimicrobial, as well as immunostimulating activity [7; 8; 9]. In the study of 5 series of biological product, the studied indicator in all samples did not change. All 5 series of the hydrolyzate looked like a clear dark brown solution.

Dry residue. An important indicator reflecting the quality of the biological product is the determination of ash, and in our case the dry residue, showing the total content of all biologically active compounds that make up the hydrolyzate. The hydrolyzate of lactobacilli in the amount of 5 series, each in three replicates, was subjected to quality assessment for the dry matter content according to the methodology of Global Fund XI (Issue 2) [3]. The results on the amount of solids (%) are presented in table 1.

Table - I: Dry residue quality assessment results

Series No.	Dry residue,%
1	6,74
2	6,71
3	6,69
4	6,72
5	6,70

The research results showed that the quality of the obtained biological product can be considered satisfactory, in the case of a dry matter content in it - at least 6.6%. Everything below this indicator is rejected.

Authenticity. The results of comparing the absorption spectra of the standard solution of GMDP and the studied bacterial hydrolyzate are presented in Figure 1.

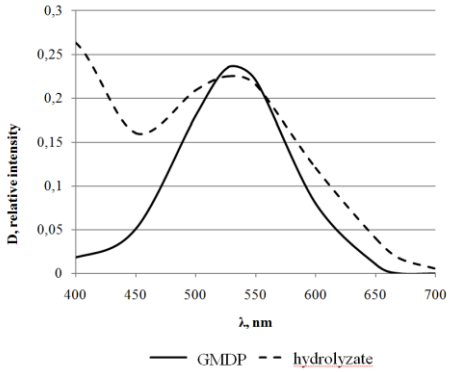

Fig. 1. Absorption spectrum of a standard solution of GMDP and hydrolyzate

The graph shows that the absorption spectra of the studied samples coincided. It was also found that the additional introduction (additive method) of a standard sample of GMDP to the developed hydrolyzate caused an increase in its optical density at the used wavelength without changing the nature of the spectrum, which confirms the content of peptides with M.m. in the biological product <1500 D.

To determine the authenticity of GMDP, it is necessary to use the method of capillary electrophoresis. Figure 2 shows the electrophoregram of the studied bacterial hydrolyzate and a standard solution of GMDP.

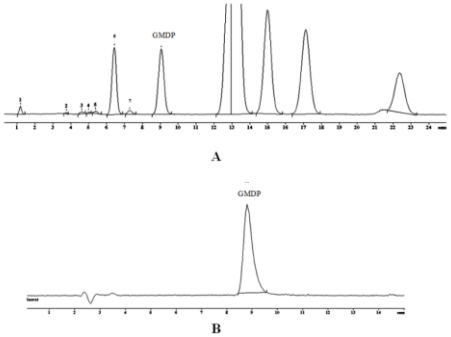

Fig. 2. Electrophoregram: A− hydrolyzate of lactobacilli; B - standard sample GMDP

From electrophoregrams it is seen that the time of migration of GMDP in both cases was the same. However, to confirm the authenticity of GMDP on an electrophoregram, in addition to the studied hydrolyzate, a standard GMDP sample was added and the analysis was repeated. It was found that the peak area of the studied low molecular weight peptide in the

hydrolyzate on the electrophoregram increases without changing its shape (asymmetry), which confirms the presence of GMDP.

PH value. Active acidity (pH) is characterized by the concentration of free hydrogen ions in solution. The pH of the bacterial hydrolyzate during the storage period (2 years) at 4–6 °C was in the range from 2.9 to 3.1 units (table 3).

Microbiological purity. The results of a study of the microbiological purity of the bacterial hydrolyzate are presented in table 2.

Table - II: Microbiological purity of the studied lactobacillus hydrolyzate

Indicator	Actual result	Recommended requirements according to OFS.1.2.4.0002.15
The total number of aerobic microorganisms	Not found	Not more than 50 CFU in 1 ml
Enterobacteria	Not found	Lack of
Escherichia coli	Not found	Lack of
Bacteria of the genus Salmonella	Not found	Lack of
Pseudomonas aeruginosa	Not found	Lack of
Staphylococcus aureus	Not found	Lack of
The total number of yeast and molds	Not found	Less than 10 yeast and molds in 1 ml

The study of the total number of aerobic microorganisms was carried out by plate agar methods, in particular, deep and surface. As a result of the experiment revealed the absence of bacterial growth. In none of the cases were microbial colonies recorded.

To restore the activity of enterobacteria, pre-growing a sample in a liquid nutrient medium is used. If typical colonies of enterobacteria are identified on the Mossel agar, morphological and tinctorial properties representing gram-negative non-spore-forming bacilli that do not have cytochrome oxidase, it is believed that the test sample is contaminated with enterobacteria resistant to bile. However, in our case, enterobacteria were not detected.

The method for determining Escherichiacoli is based on the ability of these bacilli to ferment lactose in the nutrient medium with the formation of acid and gas at (37 ± 1) ° C for 24 hours. However, gas formation was not detected in our studies, which indicated the absence of these types of pathogenic bacteria.

The method for detecting salmonella is based on the use of the Vidas device for the automatic immunoconcentration of salmonella in the Vidas ICS kit after preliminary non-selective enrichment followed by automated identification of microorganisms in the Vidas SLM kit. The test results showed the absence of bacteria of the Salmonella genus in the studied biological product.

To detect Pseudomonasaeruginosa bacteria, the studied hydrolyzate diluted 1:10 with a sterile buffer solution was transferred in a volume of 10.0 ml in 100.0 ml of soya-casein broth. They were mixed and grown under standard conditions for 24 - 48 hours. After the incubation, the presence of bacterial growth was not recorded.

The method for determining Staphylococcusaureus without preliminary enrichment by seeding on agarized selective media is based on seeding the product on the surface of a dense medium, incubation, and counting of typical colonies. The research results showed that after incubation on the Petri dishes no growth of colonies characterizing Staphylococcus aureus was detected.

The method for determining yeast and mold is based on the seeding of a biological product in nutrient media, determining the affiliation of the isolated microorganisms to mold fungi and yeast by the characteristic growth on nutrient media and by cell morphology. As a result of studies on a nutrient medium after cultivation with a hydrolyzate, no growth of colonies and the appearance of mycelium, characterizing yeast and mold fungi, were revealed.

Quantitation. The results of the norms for the quantitative content of biologically active substances in the developed biological product are presented in table 4.

Determination of the shelf life of the hydrolyzate. When studying the shelf life of a biological product, its quality was controlled by the following indicators: total quantitative content of amino acids, total protein, peptides with a molecular weight of more and less than 1500 D, GMDP, pH, and the amount of solids. The hydrogen indicator of the hydrolyzate was determined potentiometrically, the quantitative content of low molecular weight peptides, amino acids, total protein was determined by the photometric method, GMDP using the method of capillary electrophoresis, and the dry residue, respectively, according to the previously described methods. The results of the study of the studied parameters depending on the shelf life of hydrolyzate-lactobacilli at a temperature of 4-6 ° C are presented in table 3.

Table - III: Dependence of the studied quality indicators on the shelf life of the bacterial hydrolyzate

Shelf life, months	Amino acids, g / 100 ml	Total protein, g / 100 ml	Peptides with M.M. <1500 D, g / 100 ml	Peptides with M.M.> 1500 D, g / 100 ml	Number of GMDP, g / 100 ml	pH, un.	Dry residue,%
At the time of release	3,51	5,21	2,71	2,50	0,083	2,94	6,71
1	3,50	5,22	2,72	2,50	0,081	2,98	6,70
3	3,51	5,20	2,71	2,49	0,082	2,95	6,69
6	3,52	5,19	2,70	2,49	0,082	2,98	6,71
9	3,49	5,20	2,69	2,51	0,082	2,97	6,68
12	3,51	5,21	2,68	2,53	0,081	3,02	6,65
15	3,48	5,19	2,66	2,53	0,083	3,05	6,68

18	3,51	5,19	2,68	2,51	0,080	3,10	6,67
21	3,49	5,18	2,69	2,49	0,081	3,07	6,69
24	3,50	5,19	2,67	2,52	0,081	3,08	6,68

As a result of studies over 2 years, it was found that the studied bacterial hydrolyzate remained in a stable state. No significant changes were observed in the pH value and it was in the range from 2.9 to 3.1 units. The solids content met the requirements. Similar values were recorded when assessing the quantitative content of amino acids, total protein, low molecular weight, and GMDP. In general, it was proposed to take this expiration date (2 years) as a normative value.

Generalized quality indicators of the studied microbial hydrolyzate are presented in table 4.

Table - IV: Quality Indicators of Lactic Acid Hydrolyzate

Indicator	Research method	Standard value
Description	Visually in daylight diffused light	Dark brown clear solution
Authenticity:		
The sum of peptides with M.m. <1500 D	Visible spectrophotometry	The studied and standard solutions should have a maximum absorption spectrum at a wavelength of 527 nm
GMDP	Capillary Electrophoresis	On the electrophoregram of the studied solution there must be a peak corresponding to the peak in electromotive mobility on the electrophoregram of the standard solution (GMDP)
Dry residue	GF XI (Issue 2)	Not less than 6.6%
pH	Potentiometry	2.9–3.1 units
Microbiological purity:	OFS.1.2.4.0002.15 - “Microbiological purity”	
The total number of aerobic microorganisms		Not found
Enterobacteria		Not found
Escherichia coli		Not found
Bacteria of the genus Salmonella		Not found
Pseudomonas aeruginosa		Not found
Staphylococcus aureus		Not found
The total number of yeast and molds		Not found
Quantitation:		
Amino acids, g / 100 ml, not less	Visible spectrophotometry	3,48
Total protein, g / 100 ml, no more	Visible spectrophotometry	5,22
Peptides with M.m. <1500 D, g / 100 ml, not less than	Visible spectrophotometry	2,67
Peptides with M.M.> 1500 D, g / 100 ml, not less than	By the difference between the content of total protein and low molecular weight peptides	2,49
GMDP, g / 100 ml, not less	Capillary Electrophoresis	0,080
Shelf life, not less	2 years	
Toxicity	Is safe	

An important indicator of the developed biological product for both medical and veterinary use is its safety, which, when studying the toxicological properties of the hydrolyzate, showed the absence of signs of toxicosis in laboratory animals. The studied hydrolyzate according to the set of experiments can be attributed according to GOST 12.1.007-76 to the 4th hazard class (low toxic substances) [2].

IV. CONCLUSION

The developed lactobacillus hydrolyzate meets the requirements of the regulatory documentation for this biological product, does not have toxic and irritating effects on the body of laboratory animals, and therefore can be offered in industrial poultry farming to increase the safety and productivity of poultry.

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