The application of the Photoditazine photosensitizer for the sanation of industrial poultry products

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Abstract. The scientific work analyzes the effectiveness of the Photoditazine photosensitizer for the sanation of poultry products, namely broiler chicken carcasses and chicken eggs to reduce bacterial contamination of the final product. The quality of the products processed with the Photoditazine photosensitizer was assessed from the point of view of veterinary and sanitary expertise and microbiology. Specific and quantitative identification of microflora of chicken carcasses and chicken eggs was carried out during treatment with Photoditazine photosensitizer. Based on the results obtained, it can be concluded about the effectiveness of the Photoditazine photosensitizer in industrial poultry farming for the sanation and improvement of food safety of final products.

1 Introduction

The main task of industrial animal husbandry and poultry farming is to supply the population with high-quality food. In accordance with the Doctrine of Food Security of the Russian Federation, approved by Decree of the President of the Russian Federation No. 20 dated January 21, 2020, food security is one of the key areas of ensuring country security, a factor in preserving its statehood and sovereignty, a necessary condition for the implementation of a strategic national priority – improving the quality of life of citizens of the Russian Federation [1]. Effective, safe means of preventing infectious diseases ensure the production of environmentally friendly products of high sanitary and hygienic quality and guarantee the protection of the population from diseases common to humans and animals. Such means include photosensitizers.

Photosensitizers are substances capable of transferring light energy to other substances, thereby triggering a chain of physical and chemical processes, of which the reactions that lead to the formation of free radicals and reactive oxygen species are of the greatest interest. According to the data from the article of Bondarenko V.M., Konovalov G.N., Nikolaeva E.V. and co-authors "The effect of photodynamic effects of metal complexes of E6 chlorin derivatives on conditionally pathogenic bacteria using ultra-bright cold white light LEDs", published in 2008, the photosensitizer may have bactericidal properties [2]. Currently, the method of photodynamic therapy (PDT) is used in veterinary surgery for

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oncology treatment. This method is based on the interaction of photosensitizer molecules with the cell membrane.

Currently, there is an increase in the resistance of microorganisms to antibiotic preparations, which makes it necessary to search for new preparations with bactericidal effect [3]. Photosensitizers used in photodynamic therapy, due to the characteristics of the reactions they enter into, contribute to the absence of the risk of resistance in bacteria. The organic origin of photosensitizers makes it possible to obtain food-safe products during processing with them [3,4]. In this regard, the study of the effectiveness of photosensitizers as an alternative to antibacterial preparations is relevant.

The purpose of the work: To determine the effectiveness of the Photoditazine photosensitizer for the sanation of poultry products.

Objectives:

1. To determine the effect of Photoditazine photosensitizer on the microflora of primary poultry products - carcasses of broiler chickens.
2. To study the effect of Photoditazine photosensitizer on the quality indicators of primary poultry products by conducting veterinary and sanitary examination of organoleptic and biochemical parameters.
3. To determine the effect of the Photoditazine solution on the bacterial contamination of eggs during surface treatment.

Scientific novelty. For the first time, the effect of the use of the Photoditazine photosensitizer solution on the microflora of broiler chicken meat and chicken egg was studied. For the first time, an optimal scheme for the use of the Photoditazine photosensitizer solution, providing a reduction in bacterial contamination of the final poultry products, has been substantiated.

2 Materials and methods of research

The work was carried out in the period from 2018 to 2021 at the Department of Microbiology, Epizootology, and VO of the Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Scriabin and in the Department of Sanitary and Clinical Microbiology of the FSBI "VGNKI".

The work includes a microbiological study of the sensitizer antimicrobial activity, the development of indications for use, the determination of the preventive effectiveness of the domestic sensitizer of the 2nd generation in poultry farming.

Microbiological, biochemical, and organoleptic research methods and methods of variation statistics were used in the experiment set-up. The objects of laboratory research were: poultry carcasses, chicken egg.

The work was carried out in the "dirty" zone of the Department of Sanitary and Clinical Microbiology of the FSBI "VGNKI" in aseptic conditions. To conduct bacteriological experiments, laminar boxes of the abacterial air environment of the II protection class were used, in which washes were previously taken from the working surfaces to confirm the absence of microflora in them. All dilutions of the experimental preparation Photoditazine were carried out using sterile distilled water, and one test tube of each dilution from all experiments was placed in a thermostat at 37^{o}C for incubation for 3 days to confirm the impossibility of the concomitant microflora development in them. All work was carried out in compliance with safety regulations and aseptic rules.

The material for the study was the test preparation Photoditazine - dimethylglucamine chlorin E6 salt, a second-generation photosensitizer, a water-soluble chlorophyll derivative proposed for use for medical purposes. This preparation belongs to the pharmacological group of photosensitizers and has the ability to absorb light in the visible region, resulting in its photoactivation and subsequent relaxation of the excited state with the transfer of

energy to molecular oxygen dissolved in tissues and organic substrates. After administration, the preparation enters the liver, and then the blood, after which it is redistributed into the organs and tissues of animals. Photoditazine was activated by a 0.8 W laser with a wavelength of 662 nm.

Microbiological studies included the following:

- GOST R ISO 20776-1-2010 "Clinical laboratory studies and diagnostic test systems in vitro", used for in vitro experiments to determine the antimicrobial activity of Photoditazine and its minimum suppressive concentration [13];

- GOST R 50396.0-92 "Poultry meat, offal, and semi-finished poultry products. Sampling methods and preparation for microbiological studies" and GOST 31468-2012 "Poultry meat, offal and semi-finished products from poultry meat. Salmonella detection method" were used for sampling and conducting bacteriological studies in the experiment to determine the minimum Photoditazine concentration necessary for the sanitation of poultry products from bacteria of the genus Salmonella [14, 15];

- GOST 31468-2012 "Poultry meat, offal, and semi-finished products from poultry meat. Methods of organoleptic and physico-chemical studies" was used in the set-up of a benzidine test for peroxidase activity [16];

- GOST 10444.15-94 "Food products. Methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms" and SanPiN2.3.2.1078-01 "Hygienic requirements for the safety and nutritional value of food products" were used to determine the total microbial contamination of the studied samples and to evaluate the results of these studies.

As well as the choice of nutrient media to confirm the species identity of experimental cultures of microorganisms Enterococcus faecalis, Staphylococcus aureus, Salmonella enteritidis, and Salmonella gallinarum was based on the following regulatory documents: GOST 28566-90 "Food products. Method of detection and determination of the enterococci amount", GOST 31746-2012 "Food products. Methods of detection and determination of the number of coagulase-positive staphylococci and Staphylococcus aureus" and MU 4.2.2723-10 "Laboratory diagnostics of salmonellosis, detection of salmonella in food and environmental objects", respectively.

The following nutrient media were used to isolate microorganisms in experiments:

1) Meat-infusion broth is a medium for the cultivation of heterotrophic microorganisms. It was used to accumulate experimental cultures after their exact species identification.

2) Meat-infusion agar is the main dense nutrient medium used for growing chemo-organotrophic bacteria. It was used to confirm the absence of growth in experiments to determine the Photoditazine minimum suppressive concentration and to determine the total microbial contamination in the slaughter products of experimental chickens.

3) Slanetz-Bartley agar is a highly selective medium for enterococci. Sodium azide in its composition suppresses the growth of gram-negative microorganisms. The triphenyl tetrazolium chloride included in it is reduced by bacteria to insoluble formazan, which is deposited in the cell wall, causing the dark red color of the colonies. All maroon colonies that appeared at elevated incubation temperatures (44-45°C) presumably can be attributed to enterococci. The medium was used to confirm the purity of enterococcal cultures.

4) Baird-Parker agar is a selective agar for isolation and quantitative accounting of Staphylococcus aureus. The medium contains lithium chloride and potassium tellurite to suppress the growth of concomitant microflora. Sodium pyruvate and glycine selectively stimulate the growth of staphylococci. The medium was used to confirm the purity of staphylococcal cultures.

5) Rambach agar is a chromogenic differential diagnostic medium for the identification of salmonella in food, raw materials, and clinical material. Sodium deoxycholate inhibits concomitant gram-positive microflora. Salmonella produce acid from propylene glycol,

resulting in a change in the pH environment, and salmonella colonies turn red. To differentiate salmonella from coliform bacteria, a chromogenic mixture is included in the medium, which reveals the presence of the enzyme β–galactosidase, a characteristic enzyme of coliform bacteria. Coliform bacteria grow in the form of blue-green or blue-purple colonies. This medium provides unambiguous differentiation of Salmonella from other bacteria.

6) Buffered peptone water - serves as a primary enrichment medium, which is used to increase the seeding of damaged salmonella from food products (before selective enrichment and isolation). It is also recommended by MU 4.2.2723-10 "Laboratory diagnostics of salmonellosis, detection of salmonella in food and environmental objects".

7) RVS broth (Rappaport-Vassiliadis medium) - this medium is recommended for enriching salmonella under conditions of high osmotic pressure, low pH, low nutrient content and temperature of 43°C according to MU 4.2.2723-10 "Laboratory diagnostics of salmonellosis, detection of salmonella in food and environmental objects".

8) Endo medium is a differential diagnostic nutrient medium designed for the isolation of enterobacteria. It was used to confirm the purity of cultures of the genus Salmonella bacteria.

Organoleptic studies of poultry meat were conducted in accordance with GOST R 53747-2009 "Poultry meat, offal, and semi-finished products from poultry meat". Physico-chemical studies of meat: benzidine peroxidase test to find out whether Photoditazine affects the maturation of meat and reaction with copper sulfate to detect products of shallow protein breakdown in accordance with GOST 31470-2012 "Poultry meat, offal, and semi-finished products from poultry meat. Methods of organoleptic and physico-chemical research" [12].

Table 1. Scheme of experiments

Experiment No. 1 Determination of Photoditazine effect on the microflora of broiler chicken carcasses			
Group	Number of samples	Multiplicity of treatments, exposure	Feeding conditions
1-О	25	Once, 3 min	Surface treatment with a solution of the activated Photoditazine photosensitizer
2-К	25	-	Without treatments
Experience No. 2 VSE of broiler chicken meat			
Group	Researches		
1-О	Organoleptic, biochemical, bacteriological		
2-К			
Experiment No. 3 Determination of the Photoditazine solution effect on the egg microflora			
Group	Number of samples	Multiplicity of treatments, exposure	Measures
1-О	100	Once, 2h	Surface treatment with a solution of the activated Photoditazine photosensitizer
2-К	100	-	Without treatments

3 Results of own research

3.1 Determination of the Photoditazine photosensitizer solution effect on the microflora of broiler chicken carcasses

The microflora of chicken carcasses from the experimental groups was studied by taking washes from the surface. The results of bacteriological studies of the microflora of experimental birds are presented in Table 2.

Table 2. The effect of Photoditazine on the microflora of broiler chicken carcasses

Indicators	1-O (Carcasses treated with Photoditazine solution)	2-K (Carcasses without treatment)	SanPiN2.3.2.1078-01 Hygienic requirements for the safety and nutritional value of food products
QMA&OAMO, CFU/g	$0.27x10^5$	$0.9x10^6$	$1x10^5$
Coliform bacteria	Detected before the second dilution	Detected before the fourth dilution	Allowed up to the fifth dilution
Microorganisms of the genus Salmonella	Not found	Not found	Not allowed
Microorganisms of the genus Listeria	Not found	Not found	Not allowed

The results of the microflora study showed that the content of mesophilic bacteria in the meat of the carcasses of broiler chickens of the 1st group is 10 times less than in the control group, which is an order less. The indicators of coliform bacteria correspond to the normative documentation in the experimental groups, in the control group the contamination with coliform bacteria exceeds by an order of magnitude. Microorganisms of pathogenic genera Salmonella and Listeria have not been isolated.

3.2 Results of organoleptic, biochemical parameters of carcasses of broiler chickens

As can be seen from the data given in the table, the use of the Photoditazine photosensitizer reduces the number of mesophilic bacteria in the obtained primary products, unlike the 2nd and control groups. The amount of mesophilic bacteria from the 1st experimental group is 10 times less than in the 2nd and 100 times less than in the control group. According to the current regulatory documentation, the meat of experimental broiler chickens from the 1st experimental group meets current quality standards.

The results of the organoleptic study of chicken meat from the experimental groups are shown in Table 3.

Table 3. Organoleptic parameters of the studied samples

Samples of poultry meat	Appearance, points	Smell, aroma of broth, points	Taste (test boiling), points	Consistency (tenderness, hardness), points	Juiciness, points	Total quality assessment, points	Average score, points
1-O (Samples of carcass meat processed with Photoditazine)	Very pleasant 9	Very pleasant and strong 9	Very tasty 9	Tender 8	Juicy 8	Exl. 9	8.67
2-K (Control)	Very pleasant 9	Very pleasant and strong 9	Very tasty 9	Tender 8	Juicy 8	Exl. 9	8.67

As can be seen from the table, the organoleptic parameters of chicken meat samples from the experimental groups do not differ in quality. The taste, color, and smell were the same, the consistency of chicken meat of all groups were identical, without loss of elasticity. The boiling test showed that the broth turned out with single flakes with small drops of fat in all experimental groups, which corresponds to the norms.

The results of biochemical studies of broiler chicken meat are presented in Table 4.

Table 4. Biochemical parameters of the studied samples

Group	Benzidine test for peroxidase activity	Reaction with copper sulfate
1-O (Samples of carcass meat processed with Photoditazine)	Blue-green color, turning into greyish-brown after 1.16 minutes	No sediment
2-K (Control)	Blue-green color, turning into greyish-brown after 1.22 minutes	No sediment

In biochemical studies of meat, the activity of peroxidase in the benzidine test was preserved in all samples, as well as no traces of primary protein breakdown were found during the test with copper sulfate.

3.3 Determination of the Photoditazine photosensitizer solution effect on the microflora of eggs during surface treatment

The results of bacteriological analysis after treatment with Photoditazine photosensitizer solution by spraying with a solution at a concentration of $1x10^{-4}$ 2 hours before taking bacterial washes from the surface of eggs showed that the bacterial contamination of eggs from the experimental group is 2 times less than in the control group. The QMA&OAMO indicators of the experimental groups correspond to the regulatory documentation.

Table 5. Microflora of eggs during surface treatment with Photoditazine solution

Indicators	***SanPiN 2.3.2.1078-01 Hygienic requirements for the safety and nutritional value of food products***	**1-O (Treated egg, Photoditazine, solution concentration $1x10^{-4}$)**	**2-K (Egg without treatment)**
QMA&OAMO, CFU/g	*$5x10^2$*	$1.8x10^2$	$4.2x10^2$
Coliform bacteria (coliforms)	*Allowed up to 2 dilution*	Detected up to 1 dilution	Detected up to 2 dilution
Bacteria of the S. aureus species	*Not allowed*	Not detected	Isolated colonies are found on EYA
Pathogenic microorganisms, incl. of the genus Salmonella	*Not allowed*	Not detected	Not detected

According to the data from the table, it can be seen that treatment with photosensitizer solution helped to eliminate gram-positive microflora, including S. aureus and reduce bacterial contamination of eggs without causing organoleptic changes in the final product.

4 Conclusions

1) The Photoditazine photosensitizer has bactericidal activity, the minimum concentration of the photosensitizer to obtain the best bactericidal effect is $1x10^{-4}$, at this concentration the absence effect of growth of the studied cultures of microorganisms is achieved.
2) Bacterial contamination of chicken meat treated with photosensitizer is 46.7% less than in the control group. Microflora of carcasses of broiler chickens treated with Photoditazine photosensitizer complies with regulatory documentation, without changing the meat quality.

The quality of poultry meat treated with a photosensitizer does not differ from the meat obtained from the control group.

From the point of view of veterinary and sanitary expertise, it can be concluded that meat processed with Photoditazine can be used on a general basis without restrictions.

3) Bacterial contamination of eggs treated with a photosensitizer solution is 40% lower than that of not treated eggs. Also, treatment with photosensitizer solution helped to eliminate gram-positive microflora, including S. aureus.

Practical suggestions

We recommend treating carcasses and eggs with Photoditazine photosensitizer solution by surface treatment method with a solution in a concentration of $1x10^{-4}$, which reduces bacterial contamination.

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