

Determination of the Photoditazine photosensitizer solution effect on the productivity and microflora of broiler chickens

Vadim Shlenskiy^{1,*}, Tatiana Pashnik¹, and Andrey Koshchaev²

¹ Moscow State Academy of Veterinary Medicine and Biotechnology – MVA named after K. I. Scriabin, city of Moscow, Russia

² FSBEI HE Kuban State Agrarian University, Russia

Abstract. The scientific work analyzes the effectiveness of the Photoditazine photosensitizer for rearing broiler chickens to prevent infectious diseases, in particular salmonellosis. In the conducted studies, the presence of antimicrobial activity in photosensitizers in experiments on bacterial cultures was confirmed by the example of a second-generation photosensitizer - Photoditazine, the active substance of which is dimeglumine chlorin E6. Comparisons of weight gain in broiler chickens from experimental groups were carried out, the composition of the microflora of experimental groups was determined. The harmlessness of the oral use of the Photoditazine photosensitizer was revealed by measuring the main hematological and biochemical parameters of experimental bird groups. Based on the results obtained, it can be concluded about the effectiveness of the use of Photoditazine photosensitizer to maintain the well-being of production poultry livestock.

1 Introduction

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 [1, 2]. One of the solutions to this global problem may be the development of antibacterial photodynamic therapy methods. According to the results of many studies, pathogenic microorganisms are unable to develop resistance to this type of treatment [3, 4, 5]. The principle of photodynamic therapy is based on the use of special substances, photosensitizers, irradiated with light using a special emitter [6].

The organic origin of photosensitizers makes it possible to obtain food-safe products during processing with them [7]. In this regard, the study of the effectiveness of photosensitizers as an alternative to antibacterial preparations is relevant.

Purpose of the work: To determine the preventive effectiveness of the use of the photosensitizer Photoditazine in poultry farming on the example of experimental groups of broiler chickens.

Objectives:

* Corresponding author: shl-vadim@yandex.ru

1. To determine the bactericidal activity of photosensitizers using the example of Photoditazine with the measurement of the minimum suppressive concentration of these preparations on archival strains of microorganisms.

2. To study the effect of oral use of the Photoditazine photosensitizer on the quality indicators of primary poultry products by measuring zootechnical, hematological, and biochemical parameters.

3. To draw conclusions about the effectiveness of the use of the Photoditazine photosensitizer solution to maintain well-being in the production poultry livestock.

Scientific novelty. The use of the Photoditazine photosensitizer solution in poultry farming as a preventive drug is justified: to normalize the intestinal microflora, prevent salmonella infection and increase productivity. For the first time, the optimal scheme of the Photoditazine photosensitizer solution application, which ensures well-being in the livestock of industrial poultry farming, is substantiated.

2 Materials and methods of research

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.

Scientific and production experiments and approbation of scientific research were carried out at the poultry farm of the Moscow region, poultry meat processing plant of the Krasnodar Territory, in the bacteriological laboratory of the research Institute of the FSBI "All-Russian State Center for Quality and Standardization of Medicines for Animals and Feed (VGNKI)".

Microbiological, biochemical, physiological, morphological, immunological, clinical, pharmacological, zootechnical research methods, and methods of variation statistics were used in the set-up of experiments. The objects of laboratory research were: washes from the cloaca, droppings, poultry carcasses, blood.

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°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. As well as a broad-spectrum antibiotic Enroflon.

The experiments were carried out on broiler chickens of the Ross-308 cross from the 1-day to the 38-day age in the amount of 400 heads. The studies were conducted on 4 groups, 100 chickens each: group 1: received a solution of the Enroflon antibiotic, group 2: a solution of the activated Photoditazine photosensitizer, group 3: a solution of the inactive Photoditazine photosensitizer, group 4 was used for control, intact.

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 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.

In experiments to determine the antimicrobial activity of Photoditazine with the determination of its minimum suppressive concentration, sequential tenfold dilutions of this preparation in concentrations from 1×10^0 to 1×10^{-8} were used. To determine the minimum suppressive concentration of Photoditazine for *Enterococcus faecalis*, *Staphylococcus aureus*, and *Salmonella enteritidis* bacteria, archival strains from the collection of the FSBI "VGNI" were used. From test tubes containing crops with archival strains on a semi-liquid agarized medium, crops were made on dense nutrient media with differential diagnostic properties for each strain: on Slanetz-Bartley agar for the *Enterococcus faecalis* strain, on Baird-Parker agar for the *Staphylococcus aureus* strain, and on Rambach agar for the *Salmonella enteritidis* strain, respectively. After a day of cultivation in a thermostat at 37°C, colonies grown on these dense media were examined for the manifestation of typical enzymatic properties for the corresponding types of microorganisms and for the homogeneity of the grown colonies to confirm the purity of the experimental cultures. For the greatest reliability, the analyzed colonies from dense media were identified on a MALDI Biotyper mass spectrometer, which finally confirmed that the experimental archival strains belonged to the corresponding species. After these experiments, colonies were seeded from dense nutrient media into meat-infusion broth to confirm the species. Test tubes with these cultures of microorganisms of each species were incubated for a day at 37°C to obtain a daily culture suspension for further experiments. The obtained daily culture suspensions of microorganisms of each species were brought to 0.5 McFarland standard with sterile saline solution and added to pre-diluted Photoditazine solutions in concentrations from 1×10^0 to 1×10^{-8} from the calculation of 0.5 ml of suspension corresponding to 0.5 McFarland standard, to 0.5 ml of Photoditazine solution. After that, the resulting mixtures of bacterial suspensions of microorganisms of each species and Photoditazine solutions were incubated for a day at 37°C. After incubation, the absence of growth was assessed visually, and the obtained experimental mixtures were sieved into meat-infusion agar to confirm the assumption that the studied concentration of the Photoditazine solution leads to the lysis of vegetative forms of experimental microorganisms.

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.

Table 1. Scheme of experiments

Experiment No. 1 Determination of the minimum suppressive concentration of Photoditazine to archival strains	
Name of microorganism	Measures
<i>Enterococcus faecalis</i>	1. Bac. study on the effect of Photoditazine dilutions from 1×10^0 to 1×10^{-8} on the growth of the isolate
<i>Staphylococcus aureus</i>	

Salmonella enteritidis			
Experiment No. 2 Determination of the effect of Photoditazine on the weight gain and microflora of the gastrointestinal tract of broiler chickens			
Group	Amount of heads	Duration, day	Feeding conditions
1-O	100	21	OD + activated Photoditazine with water at the rate of 0.0002 ml per 1 kg of weight
2-O	100	21	OD + Enroflon with water
3-K	100	21	OP

3 Results of own research

3.1 Determination of the minimum suppressive concentration of Photoditazine to archival strains

In our studies, we determined the minimum suppressive concentration of the Photoditazine photosensitizer for archival strains of sanitary-indicative microorganisms. When exposed to daily cultures of the *Enterococcus faecalis* microorganism, the absence of growth persisted until the preparation concentration of 1×10^{-6} , in test tubes with *Staphylococcus aureus* inoculum, growth was also absent before preparation dilution of 1×10^{-6} . In turn, for gram-negative bacteria of the *Salmonella enteritidis* species, the maximum preparation dilution, in which the absence of growth was maintained, was 1×10^{-4} (Table 2).

Table 2. The minimum suppressive concentration of Photoditazine in relation to *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella enteritidis* bacteria

Concentration of Photoditazine in 0.5 ml, mg \ Species of microorganism	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Salmonella enteritidis</i>
1	No growth	No growth	No growth
1×10^{-1}	No growth	No growth	No growth
1×10^{-2}	No growth	No growth	No growth
1×10^{-3}	No growth	No growth	No growth
1×10^{-4}	No growth	No growth	No growth
1×10^{-5}	No growth	No growth	Growth
1×10^{-6}	No growth	No growth	Growth
1×10^{-7}	Growth	Growth	Growth
1×10^{-8}	Growth	Growth	Growth

The difference in the sensitivity of gram-positive and gram-negative bacteria to the photosensitizer observed in this experiment is due to fundamental differences in the structure of the cell walls of these two groups of bacteria. Gram-positive bacteria contain a large number of peptidoglycans in the cell wall, which can be a substrate for a photochemical reaction. It is generally accepted that the most important structure responsible for the overall resistance of gram-negative bacteria to various external agents (antibiotics, detergents, dyes) is the outer membrane, which is part of the cell wall.

Thus, on cultures of archival strains, by determining the minimum suppressive concentration, it was proved that the bactericidal effect of Photoditazine persisted to a concentration of 1×10^{-4} mg of the preparation in a concentration of 0.5 ml of solution.

3.2 Determination of the Photoditazine photosensitizer solution effect on the productivity and microflora of broiler chickens

According to the results of weighing broiler chickens from experimental groups on laboratory scales, the average daily weight gain and the average weight of one head of an experimental chicken were calculated, the data are presented in Table 3.

Table 3. Live weight of chickens, g

Indicators	1-O (Chickens receiving activated Photoditazine, solution concentration 1×10^{-4})	2-O (Chickens receiving antibiotic)	3-K (Chickens raised with regular feeding)
Av. weight of 1 head (g), at 35 days	1 907 \pm 11.7*	1 667 \pm 11.3	1 517 \pm 10.9
Livestock (head)	100	100	100
Viability (%)	99	99	98
Cost of antibacterial treatment per 1 head (rub)	0.39	0.64	-

*Differences between the experimental and control groups are significant at $p < 0.05$

By the end of the experiment, the live weight in group 1 was 390 g more than in the control group and 240 g more than in the second group.

It can be concluded that the use of the Photoditazine photosensitizer solution contributes to livestock viability, increases average daily weight gain, live weight, and has an advantage in price over the use of an antibiotic.

In the experiments on the study for the determination of acute and chronic toxicity, methods were used to determine the indicators of the general hematological and biochemical analysis of the blood of experimental chickens.

Indicators of hematological and biochemical analyses of experimental birds are presented in Tables 4 and 5.

Table 4. Hematological parameters of experimental and control groups of broiler chickens

Indicators	1-O (Birds received Photoditazine)	2-K (Intact birds)
Hemoglobin, g/100 ml	11.2 \pm 0.21	10.9 \pm 0.21
BSR, 1h	4.8 \pm 0.01	4.3 \pm 0.01
Red blood cells, M/ul	3.81 \pm 0.02	3.11 \pm 0.02
White blood cells, K/ul	22.8 \pm 0.11	21.37 \pm 0.15
Platelets, K/ul	88.6 \pm 1.03	91.72 \pm 1.01
Hematocrit, %	36.8 \pm 0.14	37.4 \pm 0.11

According to the results of the study of general clinical blood parameters of experimental broiler chickens, it can be concluded that the use of a photosensitizer does not lead to a change in blood homeostasis.

Table 5. Biochemical parameters of the experimental and control groups of poultry

Indicators	1-O (Birds received Photoditazine)	2-K (Intact birds)
ALT, u/l	7,34 \pm 0,24	6,19 \pm 0,21
AST, u/l	358 \pm 0,43	331 \pm 0,51
ALP, u/l	112,1 \pm 1,1	125 \pm 1,1
Protein, g/l	33,61 \pm 0,25	39,22 \pm 0,25

Urea, mmol/l	1,9±0,15	12,1±0,15
Creatinine, umol/l	16,8±1,1	17,6±1,4
Glucose, mmol/l	4,63±0,11	4,51±0,11
Cholesterol, mmol/l	4,14±0,01	4,79±0,01
Total bilirubin, umol/l	19,24±0,014	19,3±0,014

By the absence of differences in biochemical parameters in experimental groups of broiler chickens, it can be concluded that Photoditazine is harmless, and indirectly it can be judged that there is no acute body intoxication.

The intestinal microflora of chickens from the experimental groups was studied by taking washes from the cloaca. The results of bacteriological studies of the microflora of experimental birds are presented in Table 6.

Table 6. The effect of Photoditazine on the microflora of the gastrointestinal tract of chickens

Indicators	1-O (Chickens receiving activated Photoditazine, solution concentration 1×10^{-4})	2-O (Chickens receiving antibiotic)	3-K (Chickens raised with regular feeding)
QMA&OAMO, CFU/g	0.27×10^5	0.9×10^6	0.2×10^9
Coliform bacteria	Detected before the second dilution	Detected before the fourth dilution	Detected before the fifth dilution
Microorganisms of the genus Salmonella	Not found	Not found	Not found
Microorganisms of the genus Listeria	Not found	Not found	Not found

The results of the microflora study showed that the content of mesophilic bacteria in the gastrointestinal tract of broiler chickens of the 1st group is 1000 times less than in the control group and an order less than in the second group. The coliform bacteria indicators correspond to physiological norms in the experimental groups, in the control group, the contamination of with coliform bacteria exceeds by an order of magnitude. Microorganisms of pathogenic genera Salmonella and Listeria have not been isolated.

4 Conclusions

- 1) The Photoditazine photosensitizer has bactericidal activity, the minimum concentration of the photosensitizer to obtain the best bactericidal effect is 1×10^{-4} , at this concentration the absence effect of growth of the studied cultures of microorganisms is achieved.
- 2) The live weight of chickens receiving a photosensitizer for preventive purposes was significantly higher by 21.8% than in the group receiving feed antibiotics, without changes in hematological and biochemical parameters. Bacterial contamination of the gastrointestinal tract of chickens treated with a photosensitizer is 46.7% less than in the control group, without changing the microflora composition.
- 3) Based on the data obtained in the experiments, it can be concluded about the effectiveness of oral administration in poultry farming of the solution of the Photoditazine photosensitizer once every day at a dosage of 0.0002 ml per 1 kg of weight to achieve the antibacterial effect of the preparation – an analogue of antibiotics.

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