Veterinary and sanitary examination of poultry meat contaminated with pseudomonosis

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Abstract. The article presents materials on veterinary and sanitary examination and assessment of broiler meat experimentally contaminated with pseudomonosis. The clinical and pathological manifestation of the disease was studied in experiments on chickens of 40-55 days of age. The results identify the deviations in the commodity and organoleptic parameters of meat, in the physicochemical properties and chemical composition of muscles, in microbial contamination and harmlessness of meat infected with pseudomonosis of broilers, in comparison with the meat of healthy chickens. Proposals have been developed for veterinary and sanitary assessment and the most rational use of poultry meat contaminated with pseudomonosis.

1. Introduction

In our country, of all branches of the agro-industrial complex, poultry farming is developing most successfully. Intensive breeding of broilers made it possible to solve the problems of poultry meat deficit in the domestic market and determined the possibility of exporting poultry meat raw materials to other countries.

The pace of development of poultry farming in many regions of the Russian Federation exceeds those of other branches of the agro-industrial complex. According to literature data, the world production of poultry meat by 2021 will exceed 95-96 million tons, of which 85-86% will be broiler chicken meat, turkey meat will amount to about 7%, 4% will be duck meat, 3% falls on goose meat and 1% will be guinea fowl and quail meat. Growing and fattening meat broilers is characterized by lower production costs and lower production costs. Broiler meat is characterized by high nutritional properties and financial affordability for all age groups of the population.

The increase in broiler meat production is achieved through the use of highly productive linear and hybrid crosses, their intensive fattening at a high concentration of livestock in a limited area. However, when growing chickens in such conditions, the natural resistance of their body decreases, the accumulation of conditionally pathogenic and pathogenic microflora in the workshops of the poultry
house occurs. Therefore, even with minor violations of the technology of keeping and feeding poultry, outbreaks of various infectious diseases are possible: salmonellosis, pasteurellosis, escherichiosis, tuberculosis, etc., including those caused by opportunistic microflora. Diseases caused by opportunistic microflora, including pseudomoniasis, began to acquire great importance for broilers.

The causative agent of pseudomonosis (Pseudomonas aeruginosa) is widespread in nature. It is highly resistant to various factors of physical and chemical attack. The causative agent of this disease creates stable foci on farms, which can be maintained for a long time by rodents and farm animals. In chickens, this disease can be accompanied by an acute course and death of livestock or a chronic form of the infectious process, reducing daily weight gain and live weight of broilers. In adult poultry, pseudomonosis often occurs in an asymptomatic form with long-term bacterial carriage, which contributes to the mass contamination of chickens and the accumulation of the pathogen in the meat and an increase in the risk of toxic infection in humans [1, 2, 3].

The incidence rate of pseudomoniasis in chickens is 0.27-0.39%. Broiler meat infection by Ps. aeruginosa reaches 7.1-14.4% of the number of carcases studied. Microorganisms of the genus Pseudomonas are cold-resistant, can accumulate in meat at low temperatures and cause accelerated spoilage of carcases. Such poultry meat poses a certain danger to humans, primarily for children and the elderly. In many regions of the country, cases of toxic infections of pseudomonous etiology are reported annually after eating poultry meat contaminated with Ps. aeruginosa [4, 5].

It is believed that pseudomonosis in poultry has not yet been sufficiently studied. The issues of veterinary sanitary examination and veterinary and sanitary assessment of meat in this disease are still not reflected in the regulatory documents. This served as the basis for studying pseudomonosis of broiler chickens during experimental infection, determining the clinical manifestation of the disease and veterinary and sanitary parameters of broiler meat, and developing proposals for the veterinary and sanitary assessment of products of slaughter of chickens with pseudomonosis.

2. Materials and Methods
Broiler chickens were infested at 40 days of age and clinically observed for 10 days, after which they were sent for slaughter. to infect the chickens, Ps. aeruginosa strain was used isolated from broiler chickens, having previously confirmed its pathogenic properties in experiments on white mice. Broilers were injected intramuscularly and intraperitoneally with a two-day culture of Ps. aeruginosa at a dose of 1.0 million and 10 million cells per chicken. Uninfected chickens of the same age, which received sterile saline, served as control. The conditions of keeping and feeding the experimental and control chickens before slaughter remained the same.

When studying the clinical status of broiler chickens, in the morning and in the evening, they were subjected to a veterinary examination with measurement of body temperature and weighing the infected and control livestock. Blood samples for research were taken before infection, 5 days after infection, and during slaughter.

The laboratory analysis of meat included a commodity assessment of carcases and organs, a study of the organoleptic and physicochemical properties of meat, the chemical composition of the pectoral and thigh muscles, microbiological and histological parameters of chicken meat immediately after slaughter and when stored refrigerated for 5 days. Additionally, the total biological value and safety of broiler meat were determined in experiments on growing laboratory rats and on protozoa (Tetrahymena pyriformis). When studying broiler meat, we used the generally accepted research methods defined by GOST for poultry meat, Veterinary Sanitary Expertise Rules (1988) and SanPiN 2.3.2. 1078-01 [5].

A total of 45 broiler chickens were used, of which 30 were infected with Ps. aeruginosa, 15 broilers were the control group. The data obtained were tabulated and analyzed taking into account the results of statistical processing.

3. Results and Discussion
It was found that all broiler chickens infected with pseudomonosis showed changes in blood and clinical status. At the same time, the most pronounced deviations from control were noted in chickens that
received 10 million microbial cells. At the same time, signs of a decrease in physical activity, an increase in the rhythm of respiration and heartbeat, a disorder of the function of the digestive system, a deterioration in feed intake, signs of thirst were observed. Daily weight gain decreased, plumage lost its shine, combs and mucous membranes turned pale and, in some cases, acquired cyanosis. Body temperature 1–2 days after infection increased by 1.2–1.7 °C. The most pronounced deviations from the norm were detected for the first time 5-7 days of the experiment.

The study of blood revealed a decrease in the content of erythrocytes by 1.5–2.2×10^3 μl and decrease of hemoglobin by 3.5–3.6 g/dl, an increase in the number of leukocytes by 1.2–1.4×10^3 μl (due to lymphocytes. by 55.8–56.2%), an increase in the total protein content by 0.6–1.2 g/l, (due to globulins, by 3.1–3.6 g/l), the bilirubin content by 2.1–2.4 μmol/l, and the bactericidal activity of the blood by 1.4–3.4%. These data are presented in Table 1.

### Table 1. Results of a clinical study of chickens

| Indicator                               | Chicken test results | Deviation |
|-----------------------------------------|----------------------|-----------|
| Live weight before infection, g         | 1460-1540            | ±5-10     |
| Live weight before slaughter, g         | 1806-1905            | ±72-98    |
| Average daily weight gain over 40-55 days, g | 26.1-28.3           | -4.4-6.4  |
| Body temperature 5-7 days after infection, °C | 41.1-41.7           | +1.6-12   |
| erythrocytes in the blood after infection, x10^3 μl | 6.3-7.2             | -2.2-1.5  |
| hemoglobin after infection, g/dl        | 21.2-21.3            | -3.5-3.6  |
| leukocytes, x10^3 μl                    | 10.4-10.7            | +1.2-1.4  |
| eosinophils, %                          | 8.0-8.7              | -12.8-13.2|
| lymphocytes, %                          | 66.0-66.9            | +55.8-56.2|
| segmented leukocytes, %                 | 26.0-26.7            | -34.4-43.8|
| total protein in serum, g/l             | 35.2-36.1            | +0.6-1.2  |
| albumin, g/l                            | 20.0-20.4            | -1.3-3.0  |
| Bactericidal activity of blood, %       | 46.2-48.6            | +1.4-3.4  |

Ten days after infection, in most broilers, no changes in the clinical status of chickens were noted; however, the hematological parameters still differed when compared with the blood parameters of control broilers.

The increase in body weight of pseudomonosis-infected broilers was lower compared to the increase in body weight of control chickens. While in control chickens the daily weight gain (on average for 10 days of observation) was 31.5–34.7 g, in infected broilers it was only 26.1–28.3 g, i.e. less by 19%.

After slaughter, in chickens infected with pseudomonosis, changes were revealed in the lungs, liver, kidneys, intestines, spleen and heart in the form of numerous or single hemorrhages on the serous integument.

The body condition indicators of the experimental chickens were lower than those of the control broilers. At the same time, the slaughter yield of broiler carcasses in the control group was 66.8-69.1%, in the experimental group, it amounted to 64.3-64.8%, and the yield of edible parts of the carcass was 54.9-56.3% and 52.7–53.8%, respectively.

Laboratory analysis of white and red meat of experimental and control broilers revealed differences in chemical composition in some physicochemical, microbiological, histomorphological and organoleptic parameters, as well as in terms of the general biological value and harmlessness of meat. These data are presented in Table 2.
Table 2. Results of laboratory analysis of meat

| Indicator                                      | Results of the study of carcasses of broiler chickens with pseudomonosis |          |          |          | Deviation |
|------------------------------------------------|------------------------------------------------------------------------|----------|----------|----------|-----------|
|                                                | Infected | Control | Infected | Control | Infected | Control | Infected | Control |
|                                                | white meat | red meat | white meat | red meat | white meat | red meat | white meat | red meat |
| Slaughter output, %                           | 64.3-64.8 | 64.3-64.8 | 66.8-69.1 | 66.8-69.1 | -2.5% | -4.3% |
| Output of edible parts of the carcass, %      | 52.7      | 53.8      | 54.9      | 56.3      | -2.2% | -2.5% |
| Water, %                                      | 75.5      | 75.9      | 72.3      | 72.0      | +3.2% | +3.9% |
| Protein, %                                    | 20.1      | 17.7      | 20.8      | 18.5      | -0.7% | -0.5% |
| Fat, %                                        | 1.8       | 2.3       | 3.8       | 5.5       | -2.0% | -2.2% |
| Ash substances, %                             | 1.04      | 1.03      | 1.05      | 1.04      | 0.01% | -0.01% |
| Extractive substances, %                      | 1.6       | 2.1       | 2.1       | 3.3       | -0.5% | -1.1% |
| Amount of essential amino acids               | 35.9      | 33.8      | 36.7      | 34.9      | -0.8% | -1.1% |
| Amount of nonessential acids                  | 64.1      | 67.2      | 63.2      | 65.8      | +0.9% | +1.4% |
| pH of meat                                    | 5.9       | 6.2       | 5.7       | 5.9       | +0.2 | +0.3 |
| Reaction CuSO4                                | -         | -         | -         | -         | -     | -     |
| Peroxidase reaction                           | +         | +         | +         | +         | +     | ±     |
| Reaction to H2S                               | -         | ±         | -         | -         | -     | -     |
| Volatile fatty acids, KOH/g                   | 3.3       | 3.5       | 3.2       | 3.3       | +0.1% | +0.2% |
| Ammonia and ammonium salts with Nessler's reagent | -         | -         | -         | -         | -     | -     |
| Bacterioscopy of smears, cells in the field of view | 3         | 6         | 0         | 1         | 3     | 5     |
| QMA&OAMO, CFU/g                               | 2.4x10^2  | 2.9x10^2  | 0.3x10^2  | 0.7x10^2  | +2.1x10^2 | +2.1x10^2 |
| Water binding capacity                        | -         | -         | -         | -         | -     | -     |
| Acid number of fat, mg KOH/g                  | 0.54      | 0.57      | 0.47      | 0.49      | +0.07 | +0.08 |
| Peroxide number of fat, % of iodine           | 0.02      | 0.03      | 0.01      | 0.01      | +0.01 | +0.02 |
| Organoleptic assessment (on a 5-point scale): | - broth   | 3.6       | 3.8       | 4.2       | 4.5     | -0.6 | -0.7 |
| - meat                                        | 3.6       | 4.0       | 4.7       | 4.3       | -0.5 | -0.3 |
| Indicators of harmlessness (growth of cells of ciliates) | 4.74x10^5 | 4.56x10^5 | 5.12x10^5 | 4.86x10^5 | -0.38x10^5 | -0.28x10^5 |
| OBC, %                                        | 96.9      | 95.7      | 100       | 100       | -3.1% | -4.3% |

For example, the water content in the white and red muscles of infected broilers increased by 3.2 and 3.9%, respectively, compared to the meat of control chickens, the protein content, on the contrary,
decreased by 0.7-0.8%, fat dropped by 2.0-2.2%, extractives dropped by 0.5-1.1%, ash elements dropped by 0.01%. The pH values of the meat of sick chickens were 0.2-0.3 higher. At the same time, the reactions with copper sulfate, for peroxidase, for ammonia or ammonium salts did not have regular differences in all the samples under study. But in some samples of fresh meat from infected chickens, these indicators differed from the control for the worse. At the same time, the water-binding capacity of the meat of infected broilers was lower by 2.1-2.5%, the acid and peroxide values of fat were higher by 0.07-0.08% and 0.01-0.02%, respectively.

The meat of broilers infected with pseudomonosis had an increased microbial contamination. At the same time, the indicators of QMA&QAMO (CFU/g) of such meat were 4-8 times higher than the microbiological indicators of control meat and amounted to 2.4–2.9x10^2 g^-1, in the control group it reached 0.3–0.7x10^2 g^-1. In addition, microbial cells of the genera Ps. aeruginosa and coliforms were detected in individual muscle samples from sick chickens.

In histosections of white and red muscles of infected broilers, accelerated processes of autolysis and destruction of muscle fibers were noted, and in the parenchyma of the liver, kidneys and spleen, signs of mild dystrophy were observed.

Indicators of the total biological value of the meat of chickens infected with pseudomonosis in experiments on growing laboratory rats were 3.7-4.3% lower than in the meat of control broilers. The harmlessness of meat infected with pseudomonosis broilers in experiments on infusoria was also lower by 5.8-7.5 in comparison with the meat of control chickens. The extract from the meat of infected broilers negatively influenced the accumulation rate of ciliate cells and their motility.

When assessing the organoleptic indicators by the tasting commission on a 5-point scale, the samples of white muscles of infected broilers received an average of 3.6 points, red muscles - 4.0 points, and samples of control broilers - 4.1 and 4.3 points, respectively, i.e. 0.3-0.5 points higher. The organoleptic assessment of broth from meat of infected broilers was also lower by 0.6-0.7 points.

When storing carcasses of experimental and control broilers in a refrigerated state (0-4 °C) for 5 days, the most pronounced changes, starting from 3-4 days, were noted in the meat of infected chickens.

4. Conclusions
Pseudomoniasis in broiler chickens has a certain distribution. This disease is detected in 1.13-3.10% of the livestock sold, and the causative agent of the disease is isolated from carcasses more often. Pseudomonosis in broilers is manifested by changes in the blood and clinical status, as well as the development of inflammatory and dystrophic processes in the parenchyma of internal organs.

With this disease in broilers, meat productivity decreases by 19%, indicators of fatness and slaughter meat yield deteriorate.

In case of pseudomonosis, the indicators of OBC and meat harmlessness decreased by 3.7-7.5%, a higher microbial contamination is found in it, microorganisms of the Escherichia coli group and the causative agent of the disease (Ps. aeruginosa) are detected.

The meat of broilers infected with pseudomonosis is less well stored in a refrigerated state, spoilage processes develop faster and more intensively in it, it becomes more dangerous for the consumer.

These data allow us to conclude that with pseudomonosis broiler meat cannot be used without restriction, it must be sent for processing at elevated temperatures (boiled sausages, canned food) or boiled carcasses in a chopped form for 1-1.5 hours, followed by use in production of various meat products.

When slaughtering poultry with an elevated temperature or with signs of exhaustion and dystrophy of muscle tissue due to pseudomonosis, carcasses and organs must be sent to the manufacture of dry animal feed or other technical products.

Sick chick carcasses should not be kept refrigerated for more than 3 days. They should be sent for fresh processing or frozen at least at minus 15-18 °C.

Internal organs (offal) and bone by-products of sick birds are sent for scrap or for animal feed after boiling. Feathers and down are subject to thermal disinfection.
In the premises after the slaughter of broiler chickens with signs of pseudomonosis, a complex of veterinary and sanitary measures should be carried out to reduce the contamination of equipment, containers and implements with the causative agent of the disease.

References
[1] Bolotskiy I A, Shipitsyn A G, Vasiliev A K, Prutsakov S V, Vasiliev V F 2010 Animal pseudomoniasis (Moscow: Kolos)
[2] Matveichuk A V, Seryogin I G, Loginov I A 2011 Experimental pseudomonosis Proceedings of the International scientific-practical conference “Actual problems of infectious diseases of young animals” (Moscow)
[3] Seryogin I G, Baranovich E S, Kurmakaeva T V, Gusarova M L 2019 Infectious diseases detected during the growing and processing of poultry BIO 6(225) 14-17
[4] 1988 Rules for veterinary examination of slaughter animals and veterinary and sanitary examination of meat and meat products (Agropromizdat) 62 p
[5] Seryogin I G, Usha B V, Nikitchenko D V, Nikitchenko V E 2013 Laboratory methods in veterinary and sanitary examination of food raw materials and finished products (Moscow: RUDN) 252 p