

ORIGINAL ARTICLE

The evaluation of safety of broiler chickens' meat with organoleptic indicators and it's toxicity after the use of fluoroquinolone antibiotics

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The negative effect of the residual amounts of antibiotics in poultry slaughter products on human health is that they cause sensitizing effects, the risk of allergic reactions, may cause a dysbiosis, superinfections, the formation of resistant strains of pathogenic microorganisms and a decrease in the therapeutic effectiveness of antibiotics. Antibiotics worsen the sanitary qualities and technological properties of a poultry meat; distort the results of bacterial insemination. The article deals with the experimental application of the Danoksan-50 antibacterial preparation for broiler chickens of the Cobb-500 crossbreed of a slaughter age. Danofloxacin is a broad-spectrum chemotherapeutic drug that belongs to the 4th generation of fluoroquinolone antibiotics group. It is active against gram-positive and gram-negative microorganisms, mycoplasmas. The influence of fluoroquinolone antibiotics on the microbiological parameters and toxicity of femoral and pectoral muscles of broiler chickens is not sufficiently studied. Therefore, the aim was to study the influence of Danoksan-50 on the safety of broiler chickens' meat. The use of fluoroquinolone antibiotics for broiler chickens do not affect the level and species composition of microflora in the studied samples of poultry, as all the microbiological parameters in experimental groups meet the requirements of current regulations. Basing on microbiological indicators, it was found that the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), *Escherichia coli* bacteria, *Proteus*, *Salmonella* and *S. aureus*, *L. monocytogenes* bacteria in femoral and pectoral muscles of broiler chickens from 24 to 120 hours, after the last oral feeding with Danoksan-50, does not exceed the permissible levels. According to the toxico-biological assessment results, using *Tetrachymena pyriformis* ciliates, in pectoral and femoral muscles in 24 hours – after the last use of Danoksan-50 in a dose of 0.1 ml/kg of a body weight for 5 days – no toxic effects on broiler chickens' meat have been found; and that is confirmed with a ciliates death rate of 0.2-1.0% and an increase in their total number.

Keywords: Broiler chickens; Danoksan-50; microbiological indicators; toxicity

Introduction

The problems of food safety of the country have become especially urgent nowadays; one of the most important among those is the production of food products that would meet the requirements of safety and quality indicators (Krysanov, 2010, Iakubchak et al., 2017). The ecological and biological safety of food products is a major scientific and practical problem for food producers and sellers. In this regard, the implementation of sanitary-microbiological control of indicators of the safety of poultry meat in compliance with veterinary and sanitary requirements is relevant (Kasianenko, 2015). At the same time, there is a problem of residual amounts of antibiotics of different groups (Tetracycline, Aminoglycoside, β -lactam, Fluoroquinolone, Macrolide antibiotics, etc.) in food products of both domestic production and imports. According to the monitoring results, 45% of antibiotic residues were found in poultry slaughter products of domestic production, and 47.5% – in imported products (Fotina, 2007, Kaniuka&Pavliv, 2009, Holovko, 2014). Scientists from different countries of the world have proved that there is a common problem of residual amount of antibiotics in poultry products (Bokma-Bakker, 2014, Yamaguchi, 2015, Offiah, 2015). The use of antibiotics during the poultry raising, their long-term intake into the body – even in small doses, may

lead to changes in the organism's resistance or to the development of antibiotic-resistant strains of microorganisms. Danoksan-50 is a veterinary drug registered in 2015 (Registration Certificate № AB-05875-01-15 from June 10, 2015), the active substance is Danofloxacin. Danofloxacin is a broad-spectrum chemotherapeutic drug that belongs to the 4th generation of fluoroquinolone antibiotics. It is active against gram-positive and gram-negative microorganisms, mycoplasmas. Danofloxacin has a high level of distribution. Thus, its concentrations in tissues and organs are much higher than in serum. The organs in which the greatest concentration may be observed are the lungs, liver, kidneys, intestines and muscles (Goudah&Mounair, 2009, Er et al., 2013, Muylaert&Mainil, 2013). The influence of fluoroquinolone antibiotics on the microbiological parameters and toxicity of pectoral and femoral muscles of broiler chickens are not studied enough, therefore, the purpose of our study is to conduct an experimental study about the impact of Danoksan-50 drug on the indicators of the safety of broiler chickens' meat.

Materials and methods

Broiler chickens of the Cobb-500 crossbreed of a slaughter age were taken as a material for the study. They determined the influence of fluoroquinolone antibiotics on the microbiological parameters and toxicity of broiler chickens' meat. 2 groups of broiler chickens were formed for the research, one experiment and one control, 6 chickens in each (n=12). The chickens were fed with dry, complete fodder of the Kiev-Atlantic Ukraine brand; for birds from 21 to 35 days-they used Grower, and from 36 to 42 days – Finisher. Birds from experimental groups were fed orally with Danoksan-50 in a dose of 0.1 ml/kg of a body weight for 5 days. Birds of the control group had a purified water. Broiler chickens of the experimental and control groups were slaughtered in 5 days after the last use of the drug, guided by the "European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes" (Strasbourg, 1995). Definition of indicators of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and the *Escherichia coli* bacteria in products of slaughter of broiler chickens from control and experimental groups was performed according to GOST 7702.2.1- 95 (Poultry meat. By-products and semi-finished products of a poultry. Methods of selection and samples preparation for microbiological studies), GOST 10444.15-94 (Food products. Methods of mesophilic aerobic and facultative anaerobic microorganisms quantity determination) and DSTU ISO 7251: 2006 (General guidelines for the enumeration of presumptive *Escherichia coli*). *Salmonella*, *Listeria monocytogenes* and *Proteus* bacteria were identified according to DSTU ISO 6579:2006 (Microbiology of food and animal feeds. *Salmonella* spp. detection method), DSTU ISO 11290-1:2003 (Microbiology of food products and animal feeds. Horizontal method for the detection and enumeration of *Listeria monocytogenes*) and GOST 7702.2.7-95 (Poultry meat, by-products and semi-finished products. Methods for identifying *Proteus* bacteria.) *Staphylococcus* bacteria – *Staphylococcus aureus* – were determined according to GOST 10444.2-94 (Food Products. Methods for identifying and determination of *Staphylococcus aureus* quantity). Toxicological assessment of pectoral and femoral muscles of broiler chickens of experimental and control groups has been performed using *Tetrachymena pyriformis* ciliates of a WH-14 strain. The research has been conducted in accordance with "Methodological guidelines on the toxicological assessment of meat, meat products and milk, using *Tetrachymena pyriformis* ciliates (Express method)" They also performed variational and statistical digital data processing using "Microsoft Excel" computer software packages. The probability was determined according to Student's criterion, taking into account the significance levels: $p \leq 0.05$; $p \leq 0.01$, $p \leq 0.001$.

Results and discussion

The results of the research on the microbiological indicators of the products of slaughter of broiler chickens from the experimental group, which were fed with Danoksan-50, indicate that the quantity of MAFAnM in pectoral muscles of broiler chickens of the experimental group tended to increase compared to the same period in the control group. It was also found, that the quantity of MAFAnM in femoral muscles of broiler chickens in the experimental group tended to decrease compared with the same indicator in the control group (Table 1). Based on the results of the study, it was found that the number of microorganisms of the *Proteus* genus in femoral and pectoral muscles of broiler chickens in experimental groups tended to increase in comparison with similar indicators in control groups. Comparing the data obtained with the requirements, specified in the "Mandatory minimum list of researches on raw materials, products of animal and plant origin, fodder raw materials, mixed fodders, vitamin products, and others, which should be carried out in the state laboratories of veterinary medicine and the results of which are issued a veterinary certificate (F-2)" (2004), it was found that the indicators of QMAFAnM, CGB, bacteria of the *Proteus*, *Salmonella* та *L. monocytogenes*, *S. aureus* genus in the meat of broiler chickens of the experimental group, which were fed with Danoksan-50, did not exceed the established norms.

Table 1. Microbiological indicators in pectoral and femoral muscles of broiler chickens, CFU/g, M \pm m, n=6.

Indicators	Pectoral muscles		Femoral muscles	
	Experimental	Control	Experimental	Control
MAFAnM, in 1g	(1.33 \pm 0.09)*10 ³	(1.20 \pm 0.10)*10 ³	(1.23 \pm 0.11)*10 ³	(1.31 \pm 0.65)*10 ³
CGB, in 1 g	(1.20 \pm 0.21)*10 ²	(1.11 \pm 0.28)*10 ²	(1.35 \pm 0.22)*10 ²	(1.29 \pm 0.19)*10 ²
<i>Proteus</i> , in 1 g	Not found	Not found	Not found	Not found
<i>L. monocytogenes</i> , in 25 g	Not found	Not found	Not found	Not found
<i>S. aureus</i> , in 0.01 g	Not found	Not found	Not found	Not found
<i>Salmonella</i> , in 25 g	Not found	Not found	Not found	Not found

Table 2 shows the results of toxico-biological assessment of pectoral and femoral muscles of broiler chickens in compliance with the express method using *Tetrachymena pyriformis* ciliates.

Table 2. Toxicity of broiler chickens' meat, M ± m, n=6.

Group	Experimental samples	<i>Tetrachymena pyriformis</i> quantity				
		Alive (active/mobile)	Unnatural movements	Growth inhibition	Forms change	Dead ciliates
Control	femoral muscles	(42.29 ± 0.84)*10 ⁴	–	–	–	(1.35 ± 0.40)*10 ³
	pectoral muscles	(35.48 ± 1.00)*10 ⁴	–	–	–	(3.58 ± 0.47)*10 ³
Experimental	femoral muscles	(42.16 ± 0.73)*10 ⁴	–	–	–	(2.15 ± 0.29)*10 ³
	pectoral muscles	(35.87 ± 0.46)*10 ⁴	–	–	–	(0.76 ± 0.61)*10 ³

Note: "–" indicators, which are not inherent to *Tetrachymena pyriformis*.

Table 2 shows that in femoral muscles of broiler chickens of the control group, in 120 hours after the last Danoksan-50 use, and in 24 hours after the start of the experiment, no signs of unnatural movements, inhibition of growth or pathological changes in the form, inherent to *Tetrachymena pyriformis*, were found. They observed a death of 0.2% of ciliates, and of 99.8% of active mobile ciliates. A similar situation was observed in pectoral muscles of broiler chickens in the control group; in particular, cells of *Tetrachymena pyriformis* with unnatural movements, pathological changes in form or inhibition of growth were not found. They also observed a death of 1.0% of ciliates, and of 99.0% of actively moving ciliates (Table 2). In pectoral and femoral muscles of broiler chickens from the experimental group, no cells with unnatural movements, pathological changes, or sedentary (depressed mobility) were found. In femoral muscles of broiler chickens from the experimental group, 99.5% of ciliates were alive and active, 0.5%-were dead. In femoral muscles of broiler chickens from the experimental group, 99.7% of ciliates were alive and active, 0.3%-were dead. The relative biological value of pectoral muscles of broiler chickens of the experimental group tended to increase by 0.58% compared to the index of the pectoral muscles in the control group. A similar situation was observed when comparing the relative biological value of the femoral muscles of broiler chickens of the control and experimental groups. Thus, the relative biological value of femoral muscles of the poultry from the experimental group tended to increase by 0.43% compared to the control group of broiler chickens. After the toxico-biological assessment of broiler chickens' meat by express method using *Tetrachymena pyriformis* ciliates in 24 hours after the last Danoksan-50 use, they observed in pectoral and femoral muscles, both in experimental and control groups of broiler chickens, a death of 0.2-1.0% of ciliates and their dense growth by 99.5-99.7%. Such results show us that there is no toxic effect of the drug on broiler chickens' meat from the experimental groups.

The relative biological value of pectoral and femoral muscles of broiler chickens of the experimental and control groups was almost the same. The indicator of the relative biological value of pectoral muscles of broiler chickens from the experimental groups tended to increase compared with those in the control groups by 0.58%, and the index of biological value of femoral muscles-by 0.43% (Table 2).

Conclusions

The use of fluoroquinolone antibiotics for broiler chickens do not affect the level and species composition of microflora in the studied samples of poultry meat, as the microbiological indicators (QMAFAnM, CGB, bacteria of the *Proteus*, *Salmonella* та *L. monocytogenes*, *S. aureus* genus) in femoral and pectoral muscles of the experimental groups, from 24 to 120 hours after the last use of Danoksan-50 drug, meet the requirements of the current normative legal acts. After the toxico-biological assessment of broiler chickens' meat using *Tetrachymena pyriformis* ciliates in femoral and pectoral muscles, in 24 hours after the last Danoksan-50 drug use in a dose of 0.1 ml/kg of a body weight for 5 days-no toxic effects on broiler chickens' meat have been found, which is confirmed by the death of 0.2-1.0% of ciliates and their quantity increase.

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