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Toxico-Biological Estimation Of Meat Of Broiler Chickens After The Use Of Feed Additive And Antibacterial Preparations.

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ABSTRACT

The purpose of the study was to study the influence of "Probix" feed additive (FA) and antibacterial preparations of pharmazin and tilotsiklinvet on the toxicity of poultry meat during their use. In the first stage, the influence of the "Probix" feed additive on the relative biological value and toxicity of broiler chickens' meatwas studied, which included the Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus, Enterococcus faecium strains. At the second stage of the study, the influence of pharmazin and tilotsiklinvet on the toxicity of white and red muscles of chicken broilers was studied. According to the results of the first stage of the study, no negative effects were found in the meat samples of chicken broilers of the control and experimental groups, which were fed with "Probix" FA, on morpho-physiological characteristics of Ciliate Tetrachymena pyriformis, and hence, the said meat is not toxic. It was found, that the meat of poultry from experimental groups, which were fed with pharmazin and tilotsiklinvet, compared to the control group, is weakly toxic, which does not allow it to be used for food purposes without any restrictions. In the experimental group receiving pharmazin, within 24 hours the death rate of ciliate Tetrachymena pyriformis in white muscles was about 20 %, slowing of ciliates moving was observed in 72 % of individuals, active and mobile – 6 %, pathological changes in forms – up to 2 %, growth inhibition was not observed. In red muscles in the experimental group, receiving tilotsiklinvet, the death of ciliates was about 14 %, cells with unnatural movements – 70 %, active and mobile – 16 %, pathological changes in form and growth depression were not found.

Keywords: poultry, probiotics, pharmazin, tilotsiklinvet, toxicity, relativebiological value

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INTRODUCTION

Poultry farming, as a livestock sector, is progressive and is being under the improvement constantly. It differs from other branches of animal husbandry with a high coefficient of reproduction of the livestock and fast grow, which makes it the main provider of protein of animal origin for population. In modern broiler production, the observance of scientifically substantiated technological norms of feeding and keeping broilers and enriching their diet is essential not only for the main nutrients, but also for some biologically active components that influence the physiological processes in the body of the bird, increase its preservation and productivity.

In addition, one need to consider, that the important factor that may have a significant influence on human health is not only the quality of animal products, food and biological value, but also its safety [1,13]. When using any veterinary drugs in the poultry industry, it is mandatory to carry out an appropriate study to determine the parameters of safe meat and to exclude adverse effects on human organs during the consumption of this meat [14].

After the prohibition of the use of fodder antibiotics in animals and poultry feeding, the urgent problem for producers becomes the search for new chemicals – alternative to antibiotics, which would ensure a more efficient use of nutrients of forage, increase productivity, bird's health, the quality of the products of its slaughter and would be safe. Pro- and prebiotics are considered to be quite effective for this [4,8,10,11]. One of these chemicals is a newly developed "Probix" feed additive, which is recommended for use, including in poultry farming.

During the biotransformation of antibiotics in the body, more toxic compounds can be formed different from the starting substance. These compounds often are not detected by existing chemical methods, and therefore do not allow to determine the degree of toxicity of metabolites. It is possible to detect this effect only by means of an appropriate test on the test organism – the simplest ones – the Ciliate Tetrachymena pyriformis, which is similar to animals in terms of the main parameters of the metabolism. This culture gives us a possibility, along with chemical methods, to determine pollutants and more fully and reliably assess the safety and quality of poultry products, and various environmental objects, which are involved in the raising and processing of poultry, – so that has both scientific and practical value.

`The aim of the study is to study the influence of the "Probix" feed additive containing probiotics and antibacterial preparations of pharmazin and tilotsiklinvet on the toxicity of broiler chickens' meat.

MATERIALS AND METHODS

For the research, they used broiler chickens of "Ross 380" crossbreed of the slaughter age of 42 days (in two stages). At the first stage, the influence of the "Probix" feed additive on the relative biological value and toxicity of broiler chickens' meat was studied. For the experiment, one experimental group and one control group (6 chickens each) were formed. The feed additive "Probix" is a mixture of probiotics and prebiotics. The probiotic component of the premix is made using specially selected original strains of lactic acid microorganisms Lactobacillus acidophilus (20 %), Lactobacillus helveticus (5 %), Lactobacillus bulgaricus (5 %), Lactobacillus lactis (5 %), Streptococcus thermophilus (5 %), Enterococcus faecium (20 %), which are isolated from natural sources, dairy products or selected by other methods, without the use of genetic modifications. The prebiotic component of the premix is made using polydecrosis (10 %) and inulin (20 %). Calcium Carbonate $CaCO_3$ (10 %) is used as a stabilizer.

The additive was fed with a feed at a rate of 600 g/ton of feed from the 5th to the 27th day, and 300 g/t – from the 28th to the 42nd day. Chickens in the control group received only the main diet. All broiler chickens, both control and experimental groups, had free access to a water and a feed. At the end of the study, they performed euthanasia in compliance with the generally accepted principles of bioethics.

At the second stage of the study, the influence of pharmazin and tilotsiklinvet on the relative biological value and toxicity of white and red muscles of chicken broilers was studied. For the experiment, four groups of broiler chickens of the daily age were formed: two control and two experimental (12 broiler chickens each). For the chickens of the first experimental group, they used pharmazin containing the tylosin tartrate

active substance (AS) of 500 mg in 1 g; and for the second – tilotsiklinvet containing the tylosin tartrate and doxycycline glycate AS of 100 mg per 1 g of powder. Pharmazin and tilotsiklinvet were fed orally with a water at a dose of 1 g per 1 dm^3 of water and according to the instructions for use ("HUVEPHARMA" JSC, Bulgaria, LLC "Vetsyntez", Ukraine). Antibiotic preparations were given to broiler chickens with a prophylactic purpose for the first 3 days of life, on the 28−29 and 38−42 days of the experiment. At the end of the antibiotics use, 6 broiler chickens from each group were slaughtered at the beginning of the elimination period (after 3 h) and after the end of the withdrawal period (after 5−8 days), respectively, after the last pharmazin and tilotsiklinvet use.

To determine the content of toxic compounds in broiler chickens' meat they used the three days old culture of Tetrachymena pyriformis of WH14 strain. They prepared homogenized samples of a meat of broiler chickens, adding them into vials at a dose of 50, 100, 200 mg, and adding the same amount of 0.56 % sea salt and Ciliates Culture. Vials with a 0.56 % solution of pharmaceutory sea salt served as the control; with an average sample of meat obtained from poultry in control groups. The vials were sealed with cotton-gauze plugs, shaken and placed in thermostat at 25 ºC for 24 hours. During the day, the vials were shaken 3 times to aerate the environment and evenly distribute the particles that settled to the bottom of the vials. After 24 hours, cultures from each bottle were looked at under a small magnification of the microscope. The toxicity of samples of broiler chickens' meat from experimental groups was determined by the presence of dead ciliates, changes in the shape, nature of the movement and inhibition of growth of Tetrachymena pyriformis. Death ciliate were those individuals who did not show any signs of mobility and had signs of destruction. Changes in the shape were expressed in the formation of various sprains, deformation, elongation or shortening of cells of ciliates. Changes in the nature of the movement were determined by the presence of cells with rotational, spindle-shaped or circular motions. Inhibition of growth of ciliates was determined by the degree of reproduction of individuals, compared to the control. The presence of dead or deformed cells, slowing and changing the nature of the movement, inhibition of growth and reproduction of ciliates, compared to the control, showed us the toxicity of the test material. The absence of death ciliates or other pathological changes within 24 hours showed a lack of acute and subacute toxicity of the product.

Relative biological value of broiler chicken meat was determined by the intensity of reproduction of ciliate on a nutrient substrate, which contained experimental samples of meat as a source of protein. The number of infusions was calculated using a hemocytometer. Preparation of the average samples was carried out in the same way as described above. An indicator of the relative biological value of meat was the number of ciliate appeared in 3 days at 25 ºC in the experimental sample, as compared to the number of cells in the control. Everyday they performed 3 times shaking of vials. Each day the vials were visually permeated in passing light and their contents were microscopically investigated. The study of each vial was performed in triple repeats.

RESULTS

The studies on the determination of the relative toxicity and biological value of meat of broiler chickens receiving feed additive "Probix" found, that after 24 hours of incubation in the experimental and control groups the culture of Tetrachymena pyriformis ciliates stayed alive, clean, and their growth is active. Ciliates were of a natural form, mobile, had normal movement. The absence of negative influence of samples of meat of the experimental group on the morpho- and physiological indicators of ciliates testify that the meat examined is not toxic. The relative biological value of the broiler chickens' meat of the experimental group receiving FA "Probix" was somewhat higher compared to the control; hence, there is no negative impact of FA "Probix" on this indicator (Table 1).

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Table 1: Toxico-biological estimation of meat of broiler chickens after the use of "Probix" feed additive, (M±m, n=6)

Motile behaviour of ciliate	Control group	Experimental group FA "Probix"	Control group	Experimental group FA "Probix"	Relative biological value compared to the control, %
	index of mobility of ciliate, %		Ciliates number		
mobile, %	100	100	$34,9±1,05×10^4$	$35,8±1,05×10^4$	2,58 %
sessile, %	–	–	–	–	–
with pathological changes in form	–	–	–	–	–
dead, %	–	–	–	–	–

The results of the study on the influence of pharmazin and tilotsiklinvet on the toxicity of broiler chickens' meat showed, that in the experimental groups of broiler chickens, receiving pharmazin and tilotsiklinvet, there was no inhibition of growth of Tetrachymena pyriformis ciliates, as within 24 hours they actively reproduced; however, some characteristic pathological changes of culture cells were observed (Table 2).

Table 2: Results of the study of the toxicity of broiler chickens' meat after the antibiotics use, n=12

Groups	material under study	State and behavior of the *Tetrachymena pyriformis* culture				
		active and mobile	unnatural moves	inhibition of growth	pathological changes	presence of dead cells
First control group	white muscles	+	-	-	-	-
	red muscles	+	-	-	-	-
First experimental group (pharmazin)	white muscles	-/+	+	-	+	+
	red muscles	+	+	-	-/+	+
Second control group	white muscles	+	-	-	-	-
	red muscles	+	-	-	-	-
Second experimental group (tilotsiklinvet)	white muscles	+	+	-	-	+
	red muscles	+	+	-	-	+

Note: "+" signs inherent to the Tetrachymena pyriformis culture; "–" these signs are absent; "–/+" the signs are absent or inherent to the ciliate culture at the beginning and at the end of the elimination period.

At the beginning of the withdrawal period, in the first experimental group in white muscles the active and mobile individuals of culture were not detected, the inhibition of movements was detected in 63 % of cells, with pathological changes in the form – 2 %, dead cells – 35 %, compared to the control. At the end of the withdrawal period, 13 % of the active and mobile ciliate were observed in white muscles, 80 % – with slow movements, 2 % – with pathological changes, and 5 % – dead individuals.

In red muscles of the poultry of the first experimental group, receiving pharmazin, they observed about 13 % of active and mobile cells at the beginning of the antibiotic withdrawal, 85 % – with unnatural movements, 2 % – of dead cells, pathological changes in the infusion forms were absent. At the end of the elimination period, 15 % of the active individuals were detected, 75 % – with ravenous and oscillatory movements, 3 % – with the changes in forms, and 7 % – dead cells, compared to the control.

In the second experimental group of broiler chickens, receiving tilotsiklinvet, about 57 % of active and mobile cells were found in white muscles at the beginning of the withdrawal period, with unnatural movements – 30 %, dead cells – up to 13 %. At the end of the elimination period, they observed 53 % of active individuals, with slow movements – 40 %, dead cells – 7 %, as compared to the control. In red muscles, at the beginning of the half-withdrawal period, about 20 % of active and mobile cells were observed, with slow movements – 65 %, dead individuals – up to 15 %. On the 9th day after the last antibiotic use, the number of active and mobile cells was about 12 %, with unnatural movements – 75 %, dead cells – up to 13 %. After the use of tilotsiklinvet, no pathological changes in forms were not detected in white and red muscles.

As a result of the research conducted, in the first experimental group in white muscles at the beginning of withdrawal period the ciliates of Tetrachymena pyriformis culture were characterized by a fairly high degree of death or unnatural movements existing. In red muscles, ciliates had a low level of death, a moderate amount of active and mobile cells. In the experimental group broiler chickens, receiving tilotsiklinvet, ciliates were more active and mobile in white muscles and had a lower death indicator, compared to the cells of ciliates in red muscles.

The results of the study can be explained by the fact that pharmazin is mostly accumulated in white muscles, and tilotsiklinvet – in red ones. A movement slowing, change in the nature of movements, the presence of dead cells or those with unnatural forms, is the evidence that the muscle tissue of broiler chickens of experimental groups contains toxic elements or compounds, that actually are the products of decomposition of pharmazin and tilotsiklinvet, respectively. Therefore, according to the results of the research conducted, the meat of broiler chickens is weakly toxic.

The results of the research on the relative biological value of meat indicate that it is reduced with the use of antibiotics (Table 3).

Table 3: Relative biological value of meat of broiler chickens after the use of antibiotics at the end of the withdrawal period (M±m, n=12)

Groups	material under study	Ciliates number	Relative biological value compared to the control, %
First control group	white muscles	$31,9±0,81×10^4$	–
	red muscles	$32,1±0,15×10^4$	–
First experimental group (pharmazin)	white muscles	$8,7±0,11×10^{4*}$	27
	red muscles	$14,04±0,17×10^{4*}$	44
Second control group	white muscles	$33,7±0,13×10^4$	–
	red muscles	$32,8±0,16×10^4$	–
Second experimental group (tilotsiklinvet)	white muscles	$14,10±0,19×10^{4*}$	43
	red muscles	$10,45±0,21×10^{4*}$	31

P ≥ 99,9%

The relative biological value of meat of broiler chickens after the use of antibiotics decreased significantly at the end of the withdrawal period. So the biological value of white muscles of the experimental group, receiving pharmazin, decreased by 73 % compared to the control, and the red ones – by 56 %; the biological value of white muscles of the experimental group, receiving tilotsiklinvet, decreased by 57 % compared to the control, and the red ones – by 69 %; which confirms the accumulation of pharmazin in white muscles, and the tilotsiklinvet – in red ones.

DISCUSSION

Biotesting is one of the methods that gives an opportunity to determine the complex effect of various properties of the product or individual components – chemical composition, nutritional value and toxicity, by registering changes in the biological parameters of test objects. As a test object they use some sensing element that is able to respond to external factors and meets the following criteria: sensitivity, versatility, productivity and reproducibility of results, quick response to anabolic component of the product, the

opportunity to trace the changes at the genetic level, the simultaneous study of a large number of samples, low cost, safe for the environment and operator himself.

All organisms of all taxonomic groups can serve as test objects. However, the use of eutherian and plants for biotesting is difficult sometimes or even impossible because of number of reasons (ethical, economic). Along with this, there is a world trend of alternative alive models usage – unicellular organisms of ciliates (Ciliophora). The use of the latter, as a biotesting material, enables the use of commonly accepted indexes – LD_{50}, LC_{50}, ID_{100}, IC_{100}, LOED/LOEC, TLm etc.

Nowadays, the use of protozoans is performed in three areas of scientific studies: assess the toxicity of new chemical compounds; determine the toxic contamination of soils, waste and natural reservoirs; evaluate the toxicity of feed and food. Among the wide variety of organisms of the Ciliophora type (about 6 thousand), Paramecium caudatum, Tetrahymena pyriformis, Stylonychia mytilus, Colpoda steinii have become the most widespread for biotesting.

The results of the comparative evaluation and the establishment of the relationship between the toxicity parameters of various substances for Ciliates and white rats are worth discussing. To assess the parameters of toxicity, they used potassium permanganate, ferrum sulfate, sodium nitrite, potassium nitrite, lithium carbonate, potassium sulfate, cadmium sulfate, cobalt chloride, phenol, chloroform, acetonitrile, ethanol, resorcinol, furatsilin, dimethylsulfoxide, citridine, chlorophos, dichlorovinyl dimethyl phosphate (DDVP), diacetoxyscirpenol, T-2 toxin at doses that cause 50 %-lethal effect if they are injected directly into the stomach of experimental animals (white rats) – LD_{50}, since this indicator is one of the most common in toxicological studies. Comparative evaluation of toxic parameters indicated a high degree of their correlation connection. The correlation coefficient (r) between LD_{50} and the Tetrahymena growth inhibiting concentrations (IC_{min}, IC_{50} i IC_{100}), was 0.99, 0.94 and 0.77, respectively; between LD_{50} and similar concentrations that inhibit chemotaxis – 0.99, 0.98, and 0.91. This indicates the occurrence of the toxico-biological reaction for both eutherian and ciliates, and indicates the similarity of the basic stages of their metabolism. Based on the data obtained, one managed to develop mathematical formulas that allow determining the LD_{50} index for white rats based on the determining the concentration of substances that inhibit the growth and behavioral response of ciliates; and that has both scientific and practical importance during toxicological studies performance [5].

To understand the toxic effects and the mechanism of influence on the cellular level of metals (Fe, Cu, Zn, Ag, Ni, Mo, W), metal oxides (CuO, ZnO, Fe_3O_4 (I), Fe_3O_4 (II), Al_2O_3, NiO, MoO_3) and metal based composites (FeCo, CuZn (I), CuZn (II), the wild strain of Stylonychia mytilus was used. The analysis of the data showed a high toxic action of metal oxides. Maximum toxicity was observed in iron oxides (Fe_3O_4, Fe_2O_3), copper (CuO) and molybdenum (MoO_3) (0,1; 0,025; 0,0125 M) in 24 hours of incubation with a biotesting object; the maximum toxic effect of metals was observed under the influence of Cu, Fe, Ag (0,025–0,0015625 M). The statistical analysis showed a high correlation ($P \leq 0,001$) between the concentration of the substances studied and the incubation time [9].

In the course of the research "To develop an express methods of assessment of the harmlessness of dietary supplements that are the sources of amino acids, vitamins and mineral substances" (2013−2017 years) it has been determined the toxicity and biological value of dietary supplement (a mixture of dietary fiber and chromium) using T. pyriformis. It has been found that the samples tested had a lower biological value comparing to the egg protein by 38 % ($p<0,05$) [15].

Scientists of the Institute of Bacteriology and Mycology in Leipzig studied the use of T. pyriformis for the detection of botulinum neurotoxin. During the studies on the growth of Tetrahymena pyriformis ciliates, in the presence of culture broth from 27 strains of Gram-positive and Gram-negative bacteria – grown on peptone yeast glucose medium, it has been found that bacterial metabolites can both stimulate and inhibit the growth of ciliates. This phenomenon depended on the species and strain of bacteria. It has been found, that humic acids, as well as products of bacterial life, can stimulate and inhibit the growth of ciliates [7].

T. pyriformis is a sensitive indicator of the biological value of meat raw material obtained from diseased animals. During biotesting of meat of cattle, the growth of biological value was between 7.4 % and 20.7 %, depending on the pH increase from 5.33 to 6.7. It has been found, that when the animal is suffering with pseudomonosis, the relative biological value of broiler chickens' meat is reduced by 8.9 % compared to

healthy chickens; and if with leptospirosis – by 9.8 %−10.7 %, respectively, and such meat is considered to be dangerous. During the study on the influence of residual pharmacological agents' quantity in a meat, they have observed the ciliates growth inhibition, influenced by negative 0.35 mg/kg of furazolidone and of the similar residual amount in the liver – 0,011 mg/kg, – which is explained with the biotransformation of furazolidone. Aminazine in the residual concentration of 0.1−0.3 mg/kg in muscle tissue inhibits the growth by 15−20 % compared to controls [6].

As a result of the studies conducted, the effectiveness of the use of ciliates for the evaluation of the toxicity of meat of slaughtered animals has been proved. In samples that exceeded the maximum permissible levels (MPL) Zn^{2+} in 1,5−2 times, they observed the instant separation of all the test organisms from the prototype, circular movements around its axis of about of 65 % of ciliates [12].

Assessing the toxic effects of the most common preservatives in the food industry – potassium sorbate (E202), sodium benzoate in (E211) – each separately and together, using T. pyriformis, it has been found that those biological effects are dose-dependent. Adding to the nutrient medium of 0,02 % E211 and 0.188 % E202 to the studied substances causes the intracellular changes with the subsequent death of ciliates [2]. Combined action of a mixture of sodium nitrite, potassium iodide and mycotoxin ochratoxin A for its toxicity (LD_{50}) is characterized by a potential toxicity in 1,52 times, and for a functional indicator (inhibition of the generative function in a chronic experiment) – by a reduction of the toxic effect on the stage and interphase activity of the T. pyriformis population. When adding this mixture into a nutrient medium in the amount corresponding to the average level of alimentary load, there was a decline in biological and adaptive capacity by 11 % and 9 %, respectively ($p<0,05$); and if in the amount exceeding the level of alimentary load – a reduction of the biotic and adaptive potential of the population by 24 % and 13 % ($p<0,05$) [3].

Thus, the analyzed studies of domestic and foreign scientists indicate that T. pyriformis organisms are used to determine the biological value of biologically active additives, meat of fish, cattle, toxicity of heavy metals, food preservatives, and pharmacological preparations.

The results obtained show that when using T. pyriformis one can assess the biological value and toxicity of fodder (biological additives) for poultry as well as a toxicity of a meat of broiler chickens. The biological value of broiler chickens' meat did not decrease and it was not toxic after the use of "Probix" FA. The weak toxicity of meat has been registered after the use of antibiotics of the macrolide group (the meat has been studies at the beginning of the withdrawal period of these drugs). The results obtained give the reasons to prolong the timing of tilotsiklinvet and pharmazin withdrawal from the poultry organisms and, consequently, the dates of slaughter.

CONCLUSIONS

The results of the conducted research on the determination of the relative biological value and toxicity of broiler chickens' meat after the use of "Probix" FA show, that the negative effect of the samples of meat of broiler chickens of control and experimental groups on the morphological and physiological indicators of ciliates Tetrachymena pyriformis has not been established, and therefore, this meat is not toxic.

Based on the results of the experiments, it was found, that the meat of a poultry of experimental groups, receiving pharmazin and tilotsiklinvet, as compared to the control group, is weakly toxic, which does not allow it to be used for food purposes without restrictions.

In the experimental group, receiving pharmazin, the number of dead Tetrachymena pyriformis ciliates within 24 hours was about 20 % in white muscles, the slowing in movement was observed in 72 % of individuals, active and mobile were 6 % of them, with pathological changes in forms – up to 2 %, growth inhibition was absent. In red muscles in the experimental group, receiving tilotsiklinvet, the death of ciliates was about 14 %, cells with unnatural movements – 70 %, active and mobile – 16 %, pathological changes in form and growth inhibition were absent. This confirms the fact of a higher accumulation of pharmazin in white muscles, and tilotsiklinvet – in red ones.

The biological value of white muscles of the experimental group, receiving pharmazin, decreased by 73 % compared to the control, and by 56 % – for red ones. The biological value of white muscles of the

experimental group, receiving tilotsiklinvet, reduced by 57 % compared to the control, and of red ones – by 69 %.

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