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# Tilmicosin Intake and Distribution in Healthy Broiler Chickens' Organisms

Anna Mykhailivna Tyshkivska[1]*, Volodymyr Bogdanovych Dukhnytskyj[1] , Vadym Dmitrovich Ishchenko[1], Mykhailo Yaroslavovych Tyshkivsky[2], Natalia Vasylivna Tyshkivska[2], Raisa Volodymyrivna Shahanenko[2], and Tetiana Ivanivna Bakhur[2]

[1]*National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine Heroyiv Oborony St., 15/3, 03041, Ukraine*
[2]*Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine pl. Soborna, 8/1, 09117, Ukraine Tserkva National Agrarian University, Bila Tserkva, Ukraine*
*Corresponding author's Email: annatyshkivska@gmail.com ORCID: 0000-0003-4419-2174

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## ABSTRACT

Detection of the time required to reach the maximum concentration in the organs promotes better prediction of antibiotics activity for the treatment of infectious diseases in broiler chickens. The current article presented the research results of the intake, distribution, and elimination of the antibiotic Tilmox 25% (the active ingredient is tilmicosin phosphate (TPh)) from the body of healthy broiler chickens (cross COBB-500) during oral administration. The findings of the current study indicated the rapid absorption of TPh from the digestive tract of a fowl and its intake into the internal organs. The maximum TPh content was observed in the lungs and liver 2 hours after the start of the Tilmox solution using which amounted to 17.02 ± 0.24 and 12.78 ± 0.22 µg/g, respectively. The maximum values of 8.25 ± 0.19µg/g were recorded for the kidneys after 26 hours, and for the pectoral muscles and heart after 52 hours (6.19 ± 0.28 and 5.23 ± 0.39 µg/g, respectively). The content of TPh in the lungs, liver, and kidneys did not depend on the duration of Tilmox watering when clinically healthy broiler chickens were watered with 25% Tilmox solution. In some periods of the experiment, the TPh content increased in the pectoral and cardiac muscles, compared with the indicators 2-4 hours from the beginning of watering. The highest content of TPh was observed in the broiler chickens' lungs during 96 hours of watering with the Tilmox solution which indicated its organ affiliation. After the poultry stopped drinking the 25% Tilmox solution, there was a significant decrease in the concentration of the active substance (TPh) within the organs. Thus, 24 hours after the cessation of drinking a 25% Tilmox solution (for 120 hours of the experiment), the content of TPh in the lungs was 1.9 times less than the previous indicators (for 96 hours), and it was estimated as 1.6, 1.4, 1.7, and 1.3 times in the liver, kidneys, pectoral muscles, and heart, respectively. Moreover, 5 days after the cessation of watering broiler chickens with Tilmox solution, the residual amounts of TPh in the organs under study were estimated as 1.20 ± 0.03 µg/g in the lungs, 1.01 ± 0.02 µg/g in the liver, and 0.91 ± 0.03 in kidneys. In the course of the research, the smallest content of TPh was detected only in one heart sample as 0.02 µg/g, and the drug was not detected in the pectoral muscles.

**Keywords:** Broiler chickens, Bioavailability, Distribution, Pharmacokinetics, Tilmox 25%, Withdrawal period

## INTRODUCTION

Among a large number of chemotherapeutic agents, macrolides occupy a prominent place. It is such a group of broad-spectrum antibiotics, natural and semi-synthetic origin, which have a large molecule lactone ring associated with carbon residues. Macrolides are widely used in veterinary and human medicine to treat patients with local and systemic infections.

Tilmicosin (20-deoxo-20-(3,5 dimethylpiperidin-1-yl) desmycosin), a relatively new chemically modified macrolide antibiotic, was first developed by the American pharmaceutical company Elanco Animal Health in the 80s. Tilmicosin was chemically synthesized from tylosin by consequent hydrolysis (Creemer et al., 2003; Rasheed et al., 2018). It is highly active against Gram-negative bacteria, such as *Pasteurella* spp. *Ornithobacterium rhinotracheale*, *Micoplasma* spp, and *Actinobacillus* spp (Shixin Xu, 2008). In recent years, tilmicosin has been actively used in European countries for the treatment of fowl with respiratory diseases. It was found that the pharmacokinetic properties of tilmicosin are characterized by rapid absorption after oral administration, good penetration into the respiratory tract tissues, and

concentration in the lung tissue (Xiong et al., 2019; Huang et al., 2019). The pharmacokinetics of tilmicosin has been studied in animals and poultry of various species. The maximum concentration in blood plasma is usually recorded 2 hours after the start of drug administration (Abu-Basha et al., 2007; Elsayed et al., 2014). One of the main features of the antibiotic is the ability to accumulate in the lung tissue, where the concentration is already four times higher than the concentration in the blood plasma 12 hours after a single use (Li et al., 2016; Shaban et al., 2019). The drug bioavailability is determined by the degree of binding with blood plasma proteins. As far as it is known, only small molecules can penetrate through the endothelium of capillaries. Therefore, the drug molecule's property to bind to the blood plasma main protein fraction (albumin) determines the property of drugs to penetrate tissues, where the infection focus is located (Elkomy & Eltanany, 2018*)*. Tilmicosin has a high ability to bind to blood plasma proteins and accumulate rapidly in body tissues in effective concentrations (Gallina et al., 2010). However, with an infectious process, the drug distribution in the organs may differ significantly. The profile of the drug pharmacokinetic parameters is influenced by pathophysiological changes that occur in the body during the pathological process. The studies have indicated that the deformity of physiological functions and biochemical processes in the body, which are accompanied by changes in body temperature, the coefficient of binding to blood plasma proteins, blood pressure, anemia, liver functional state can affect the distribution and accumulation of the drug in organs (Ludden, 1985; Scorneaux and Shryock, 2001). Therefore, to determine the optimal treatment regimen and an objective assessment of the drug pharmacokinetic profile, studies should be carried out on healthy and sick poultry.

The goal of the research for authors was to investigate the tilmicosin phosphate (TPh) distribution when it had been used in the form of the drug Tilmox 25% in the healthy broiler chickens' bodies.

## MATERIALS AND METHODS

The research was conducted in 2019-2020, on the basis of the chemical-analytical sector of the Expert Center for Diagnostics and Laboratory Support of Biolights (Baryshivka, Kyiv region, Ukraine), accredited in ISO/IEC 17025:2017 for №201864.

### Ethical approval

All stages of research were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (Strasbourg, 1986) and approved by the Commission on Bioethics of Bila Tserkva National Agrarian University, Kyiv region, Ukraine (Approval number: №10 from 28.01/2021).

### Experimental animals

The studies were carried out on 75 clinically healthy broiler chickens of the COBB-500 cross at the age of 16 days. The samples were kept in compliance with all sanitary and hygienic standards on deep bedding. Prior to research, poultry was vaccinated against Gumboro disease, Newcastle disease, and infectious bronchitis. For feeding the poultry, the full-feed compound feed was used, considering the technological scheme of cultivation.

### Drugs

For the research, a solution of the drug Tilmox 25% of the AVICO trademark (each 1 $cm^3$ solution containing the active ingredient is TPh 250 mg) was used, which was mixed in an amount of 0.3 ml with 1 liter of drinking water. This dosage is recommended by the pharmaceutical manufacturer (EMEA, 1998).

The manufacturer of tilmicosin recommends its use in sick birds for 3 days (Tilmox 25%. Solution for oral administration). However, some scientists have investigated the effects of this antibiotic as a result of its five days use (Elsayed et al., 2014). In the conditions of Ukrainian broiler farms, for higher efficiency in respiratory infections, veterinarians use Tilmox 25% for 4 days, maintaining the dosage. To achieve the goal of the study, it was important to determine the distribution of the antibiotic in organs under the condition of industrial use. Therefore, a standardized dosage of Tilmox 25% over a 4-day course was used in current studies (Tyshkivskaya et al., 2020).

All reagents used for extraction and analysis were analytical or high-performance liquid chromatography (HPLC) grade.

### Multiple-dose study

Internal organs were taken from broiler chickens to control the TPh intake and distribution in their body. For controlling TPh content in the internal organs and establishing its elimination period from the body, organ selection was performed after 2, 4, 8, 12, 24, 26, 28, 32, 36, 48, 52, 72, 76, and 96 hours from the beginning of the Tilmox solution's administration, and after 24, 48, 72, 96,

and 120 hours after stopping the Tilmox solution's intake (that is after 120, 144, 168, 192 and 216 hours after the start of the experiment). Each time, organ selection was performed from three chickens. For this purpose, the chickens were killed by decapitation under light ether anesthesia according to AVMA Guidelines for the Euthanasia of Animals (2020). Decapitation was performed quickly with a sharp knife. The selected organs were pectoral muscles, heart, lungs, liver, and kidneys. The collected samples were frozen and stored separately at -20°C until analysis.

**Standard solutions**

Tilmox was used as an analytical standard. 1 ml of Tilmox was diluted with 249 ml of distilled water. Then, the standard solution was prepared by weighing 10.0 ± 0.1 mg of these substances and dissolving them in 10 ml of methanol. Working standard solutions in water were prepared on the day of analysis (Gajda et al., 2014).

**Working solutions**

To prepare a working solution, 1.5 ml of acetonitrile was mixed with 1.5 ml of distilled water and 2 ml of 1% acetic acid was added. (Gajda et al., 2014).

**Extraction and clean-up**

One gram of muscle, livers, kidneys, heart, and lungs was homogenized with a 5 ml extraction solution and was followed by centrifugation at 9000 × g for 20 min. After centrifugation, the liquid was placed in a refrigerator for settling at a temperature of +4℃ for 24 hours. In the next step, 2 ml of the supernatant was diluted evaporated acetonitrile, and filtration through a 0.22μm polyvinylidene difluoride filter. Finally, 20 μl of the filtrate was taken for HPLC analysis.

**Analytical procedure**

For developing the analytical methodology, a unique technique based on the related studies (Horie et al., 2003; Gajda et al., 2014; Anker et al., 2018) on the isolation of antibiotics from the organs of birds was created. The technique has been successfully tested and meets the requirements of European Decision 2002/657/EU. It is described below in this and the next section "Validation".

The determination of the residual amount of TPh was carried out using the method of high-performance liquid chromatography with mass detection (Horie et al., 2003; Anker et al., 2018). Tilmicosine was quantified using a Waters LC-MS-MS and a Waters 2587 UV detector set at a wavelength of 285 nm (Waters, USA). The tilmicosin concentration was linear over the range of 0.02-10 μg/ml with a correlation coefficient of 0.999. The limit of quantification (LOQ) was 0.05 μg/ml.

The mass spectrometer was operated in electrospray positive ionization mode (ESI+). MS data acquisition was performed in the multiple reaction monitoring mode, selecting one precursor ion to two product ion transitions. The result of mass spectrometry parameters included resolution Q1 and Q3: unit, curtain gas = 20 psi, gas nebulizer = 40 psi, collision gas = 3 psi, auxiliary gas = 50 psi, and ion sputtering voltage = 5500.

**Validation**

Samples of muscle, kidney, and liver were spiked with the Tilmicosine working solution to levels corresponding to 0.5, 1, and 1.5 × maximum residue limits (MRL). The recovery was determined by comparing peak area ratios (Tilmicosine /internal standard) from fortified matrix samples with peak area ratios (Tilmicosine /internal standard) from direct injections of equivalent quantities of standards.

The method was validated by repeatability and reproducibility. For this purpose, 2 samples with identical concentrations of tilmicosin at different times were examined for three days (n=6). The concentration in each of the days was different. Based on the fact that the results of the two-day measurements were identical, a conclusion was made about the accuracy and reproducibility of the method.

Linearity was tested by preparing a matrix-matched calibration curve on six levels corresponding to 0.1, 0.5, 1.0, 1.5, 2.0, and 5.0 × maximum residue limits (MRL). During the validation process, the decision limit (CCα) and detection capability (CCβ) were calculated. To evaluate the limit of quantification (LOQ) of the developed method, six samples were spiked at the concentration of 5 μg/g, which was the lowest point of a matrix-matched calibration curve.

**Statistical analysis**

Statistic for analysis of experimental data carried out by conventional methods of variation statistics and using the computer program Microsoft Excel 2019. The significance of the difference in the average concentration of the drug in lungs and other organs during the period of use was determined (n = 42). Statistical processing was performed by multiple comparisons of variances using the Fisher distribution (ANOVA). The results were statistically processed using the Statistica 13.3 IT application. The obtained data was assessed using

Duncan's Multiple Range Test at the significance level of $p < 0.05$.

## RESULTS

The feeding of broiler chickens with the preparation (Tilmox 25%) was accompanied by the rapid distribution of its active ingredient TPh in the internal organs of the fowl. After 2 hours from the beginning of drinking Tilmox, the highest content of tilmicosin was found in the lungs 17.07 ± 0.24 µg/g, while in the liver, kidneys, heart its content was less than in the lungs in 1.3, 2, 1.5 times and amounted to 12.78 ± 0.22, 8.11 ± 0.07 and 3.08 ± 0.06 µg/g, respectively. In the breast muscles of broiler chickens, TPh was not found during this period of research (Table 1). After 4 hours from the beginning of drinking the «Tilmox 25%» solution, its active ingredient TPh was found in the broilers' breast muscles in the amount of 2.72 ± 0.30 µg/g. The pattern of the TPh quantitative distribution in other organs was the same, as in the previous period of research, but its content in the kidneys, liver, and lungs has already decreased. After 8 hours, the content of TPh in the broiler chickens' organs decreased significantly and amounted to14.35 ± 0.65 (lungs), 9.81 ± 0.23 (liver), 6.42 ± 0.14 (kidneys), and 2.65 ± 0.47 µg/g (heart) in the liver, kidneys, and, which respectively is 16, 23, 21, and 14% less than the indicator established after 2 hours. During this research period, the TPH content in the lungs was the highest, while this content was 1.5, 2.2, 4.0, and 5.4 times less in the liver, kidneys, heart, and pectoral muscles, respectively. In the pectoral muscles, the content of tilmicosin increased and amounted to 3.79 ± 0.07 µg/g.

The TPh's lowest content in the broiler chickens' organs when watering with 25% Tilmox solution on the first day was revealed after 12 hours. However, in the lungs, compared with those in other organs for this period, its content was the highest and amounted to 12.98 ± 0.40 µg/g. Liver and kidneys had significantly low amounts 7.24 ± 0.28 and 5.50 ± 0.30 µg/g, respectively, and the observed content in the heart was only 3.48 ± 0.27 µg/g. In the pectoral muscles during the research period, the tilmicosin content continued to increase and amounted to 4.07 ± 0.08 µg/g. After 24 hours, the tilmicosin content increased in the lungs by 19%, liver by 14%, kidneys by 3%, heart by 15%, pectoral muscles by 4% and amounted to 15.47 ± 0.78, 8.31 ± 0.09, 5.98 ± 0.15, 3.95 ± 0.15, and 4.26 ± 0.05 µg/g, respectively, compared with the indicator at 12 hours (Table 1).

**Table 1.** Tilmicosin phosphate content in the broiler chickens' organs when drinking a Tilmox 25% solution (µg/g, n = 3)

| Time (hours) | Organ (µg/g) | | | | |
|---|---|---|---|---|---|
| | **Muscles** | **Kidneys** | **Liver** | **Lungs** | **Heart** |
| 2 | – | 8.11 ± 0.07 | 12.78 ± 0.22 | 17.02± 0.24 | 3.08 ± 0.06 |
| 4 | 2.72 ± 0.30 | 7.13 ± 0.08 | 12.00 ± 0.40 | 16.59 ± 0.33 | 3.09 ± 0.04 |
| 8 | 3.58 ± 0.30 | 6.42 ± 0.14 | 9.81 ± 0.23 | 14.35 ± 0.65 | 2.65 ± 0.47 |
| 12 | 4.07 ± 0.08 | 5.50 ± 0.30 | 7.24 ± 0.28 | 12.98 ± 0.40 | 3.43 ± 0.27 |
| 24 | 4.26 ± 0.05 | 5.98 ± 0.15 | 8.31 ± 0.09 | 15.47 ± 0.73 | 3.95 ± 0.15 |
| 26 | 5.90 ± 0.22 | 8.25 ± 0.19 | 10.24 ± 0.07 | 15.69 ± 0.29 | 4.89 ± 0.02 |
| 28 | 5.75 ± 0.20 | 7.16 ± 0.13 | 9.70 ± 0.26 | 14.66 ± 0.29 | 4.76 ± 0.04 |
| 32 | 4.27 ± 0.24 | 6.15 ± 0.39 | 8.16 ± 0.20 | 14.10 ± 0.12 | 4.20 ± 0.10 |
| 36 | 4.05 ± 0.12 | 6.12 ± 0.21 | 8.46 ± 0.10 | 13.88 ± 0.16 | 4.43 ± 0.27 |
| 48 | 4.25 ± 0.06 | 5.60 ± 0.45 | 7. 90 ± 0.06 | 14.23± 0.12 | 4.55 ± 0.55 |
| 52 | 6.19 ± 0.28 | 7.79 ± 0.25 | 10.47 ± 0.15 | 15.79 ± 0.25 | 5.23 ± 0.39 |
| 72 | 4.32 ± 0.04 | 6.32 ± 0.06 | 8.46 ± 0.10 | 15.21 ± 0.49 | 5.09 ± 0.04 |
| 76 | 5.83 ± 0.14 | 7.22 ± 0.05 | 10.00 ± 0.39 | 15.62 ± 0.27 | 5.08 ± 0.20 |
| 96 | 3.77 ± 0.34 | 5.79 ± 0.29 | 7.62 ± 0.52 | 15.47 ± 0.73 | 4.63 ± 0.33 |
| The average value for the application period (n = 42) | | | | | |
| Mean | 4.54 ± 0.18* | 6.68 ± 0.19* | 9.37 ± 0.21* | 15.08 ± 0.36 | 4.22 ± 0.20* |

Note: *$p < 0.05$ regarding the content in lungs.

The increase in the tilmicosin content in the broiler chickens' internal organs in the period from 12 to 24 hours is explained by a decrease in fowl activity in the evening and at night, since the sampling (after 24 hours) fell on 8 a. m. In our opinion, a decrease in fowl activity during this period of the day is accompanied by a weakening of the biotransformation processes and tilmicosin excretion from the body. After 26 hours from the beginning of drinking a Tilmox 25% solution, an increase in the TPh content was revealed in all broiler chickens' internal organs and breast muscles, although with different intensities. So, in comparison with the previous period (after 24 hours), the TPh content in the kidneys and pectoral muscles increased by 38% in the liver, 23% in heart, and 1% in the lungs, but it was the highest in comparison with indicators in other organs. In the period from 28 to 36 hours, the TPh content decreased in all broiler chickens' organs and ranged from 4.05 ± 0.12 μgr/g in the pectoral muscles to 13.98 ± 0.16 μg/g in the lungs. After 48 hours, the TPh content in the broiler chickens' internal organs did not differ significantly from those established at 36 hours.

It is emphasized that during the second day of the 25% Tilmox solution application (24-48 hours), the content of its active substance (TPh) in the lungs was consistently high, and its indicators ranged from 13.98 ± 0.16 μg/g per 36 hours at 15.69 ± 0.25 μg/g at 26 hours.

At 52 hours (after 4 hours from the beginning of 25% Tilmox solution watering on day 3), an increase in the TPh content was observed in all studied organs, in particular in the pectoral muscles by 45%, kidneys by 39%, liver by 33%, compared with the indicators set at 48 hours. In the lungs and heart, the TPH content also increased, but only by 11 and 15%, respectively. The TPh content in the pectoral muscles was the highest in comparison with the indicators for the previous study periods and amounted to 6.19 ± 0.28 μg/g.

A significant increase in the TPh content (the active substance is Tilmox 25%) in the poultry's internal organs at 26 and 52 hours indicates the active antibiotic solution consumption by the poultry with the beginning of a new research day (they were given a fresh drug solution from 8 a.m. every day) and its high bioavailability. After 72 hours (3 days from the beginning of 25% Tilmox solution drinking), the tilmicosin content in the heart and lungs decreased by 3 and 4%, respectively, compared with the previous study indicator (52 hours), while its content in the liver and kidneys was lower by 19%, in pectoral muscles by 30%.

At 76 hours (after 4 hours from the start of Tilmox 25% drinking on day 4), its active ingredient content (TPh) was at the level of the previous indicator (72 hours) in the heart, exceeding by 3% in the lungs, while its contents in the kidneys, liver and pectoral muscles were larger than the previous one by 14, 16 and 35%, respectively. After 96 hours (4 days) from the beginning of broiler chickens' feeding with 25% Tilmox solution, its active ingredient's highest content was found in the lungs – 15.47 ± 0.73 μg/g, much less in the liver and kidneys 7.62 ± 0.52 and 5.79 ± 0.29 μg/g, respectively. The TPh lowest contents in this research period in the heart and pectoral muscles were 4.63 ± 0.33 and 3.77 ± 0.34 μg/g, respectively. The research studies indicate that after drinking broiler chickens with 25% Tilmox solution for 96 hours, its active ingredient TPh is rapidly absorbed from the intestinal tract and after 2 hours reaches its maximum amounts in the lungs and liver, while in the kidneys after 26 hours. In the broiler chickens' breast muscles after 2 hours, no TPh was shown, which, in our opinion, is due to the lower blood supply intensity to them. The highest TPh content in the pectoral muscles and heart was found only after 52 hours.

The specific nature of the TPh distribution in the broiler chickens' body is that the significantly highest level of its content for 96 hours was in the lungs, which, in our opinion, is explained by the phenomenon of the drug's affinity to this organ and a sufficiently high blood supply to the lungs. On the other hand, the TPh's affinity and organ affiliation to the lung tissues is of great practical importance in the case of infectious diseases, the causative agents of which are localized in the lung tissues.

TPh accumulation level in the broiler chickens' internal organs had the following decreasing pattern: lungs> liver> kidneys> heart. During all research periods, TPh content in the broiler chickens' pectoral muscles was lower than in the lungs, liver, and kidneys, while at 8, 24, 26, 28, 32, 52, and 76 hours its content in the heart was lower than in pectoral muscles.

After the cessation of feeding broiler chickens with a 25% Tilmox solution, the content of its active ingredient, TPh, in the organs under study decreased significantly. Specifically, at 120 hours of the experiment (a day after a Tilmox 25% solution cessation drinking), the TPh content was lower than in the previous indicators (96 hours) by 1.9 times in lungs,1.6 times in the liver, 1.4 times in kidneys, 1.7 times in chest muscles, and 1.3 times in the heart (Figure 1).

At 144 hours of the experiment (2 days after the end of drinking 25% tilmox solution), the TPh content in the lungs, liver, kidneys, heart, and pectoral muscles of poultry were 5.86 ± 0.26 μg/g, 3.00 ± 0.14 μg/g, 2.86 ±

0.14 µg/g, 2.12 ± 0.05 µg/g, and 2.02 ± 0.16 µg/g which is less than the indicators set at 96 hours in 2.6, 2.5, 2.0, 2.2, and 2.3 times, respectively. In subsequent periods of research (168 and 192 hours of the experiment), the process of the studied organs releasing from TPh somewhat slowed down, and its content for 192 hours was 2.65 ± 0.16 µg/g in lungs, 0.35 ± 0.05 µg/g in the liver, 1.26 ± 0.05 µg/g in kidneys, 1.19 ± 0.05 µg/g in the heart, and 1.41 ± 0.15 µg/g in pectoral muscles (Figure1).

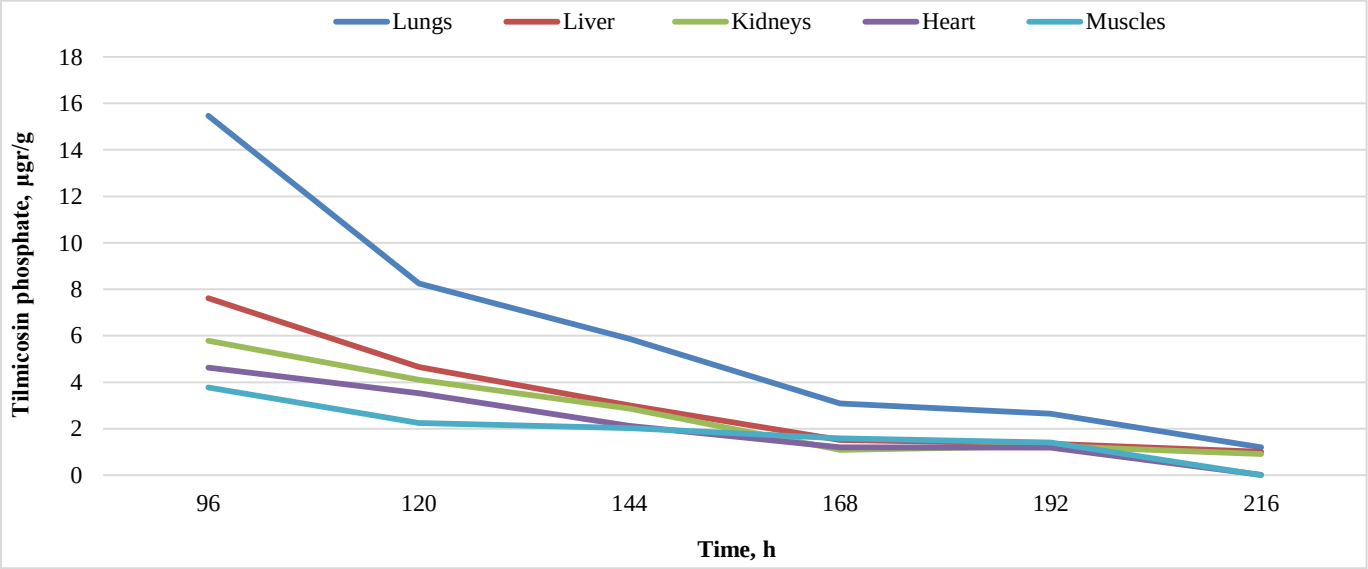


**Figure 1.** The content of tilmicosin phosphate in the organs of broiler chickens after stopping feeding tilmicosin solution

At 216 hours of the experiment (5 days after the cessation of feeding broiler chickens with Tilmox solution), the TPh residual amounts in the organs under study were reported as 1.20 ± 0.03 µg/g, 1.01 ± 0.02 µg/g, and 0.91 ± 0.03 µg/g in the lungs, liver, and kidneys, respectively. The lowest TPh content, during this research period, was shown only in one heart sample (0.02 µg/g) while the drug was not shown in the pectoral muscles.

To conclude, TPh was applied to healthy broiler chickens in the composition of the drug (Tilmox 25%) in accordance with the recommended scheme (with drinking water for 4 days). The research results showed that it was excreted from the body in maximum quantities in 5 days after the drug feeding cessation. The absence of TPh residual amounts in the broiler chickens' pectoral muscles on the 5th day after discontinuation of Tilmox 25% gives reason to consider this term to establish the withdrawal period.

## DISCUSSION

The results of the study showed that TPh is highly bioavailable as indicated by the obtained results of Tilmox 25% indicated the concentration of the antibiotic reaches a maximum after 2 hours in the lungs and liver, 26 hours in the kidneys, and 52 hours in the chest and heart muscles. The rapid release of TPh in large amounts into internal organs and muscles indicates its ability to easily penetrate the intestinal mucosa and blood vessel walls and enter the bloodstream. The high bioavailability of Tph is indicated by the research results obtained by Attia et al. (2018) obtained also on healthy broiler chickens. They found that with a single oral Tph administration to healthy broiler chickens at a dose of 25 mg/kg of body weight in the form of a solution, its maximum concentration was in the blood serum after 2.56 hours and was 1.06 µg/ml, the lowest was established after 24 hours and was 0.63 µg/ml. In healthy broiler chickens, which were experimentally infected with *Mycoplasma gallisepticum* and *Escherichia coli*, and after the onset of clinical symptoms, tilmicosin was given orally at a dose of 25 mg/kg body weight once a day for 5 days, the maximum amount of tilmicosin in the blood serum was 0, 69 µg/g and the time maximum was 2.81 hours. In healthy broiler chickens, who were given TPh at a dose of 25 mg/kg body weight once a day for 5 days, and its content was determined in blood serum, internal organs, and thigh muscles 2 hours as well as1, 2, 5, 7, 9 and 13 days after the last application. Residual amounts of tilmicosin were observed in the liver and kidneys after 5 days and in blood serum and fat after 2

days. The largest residual amounts, regardless of the time of the study, were found from 30.67 ± 0.67 μg/g after 2 hours to 15.20 ± 2.00 μg/g on the 5th day in lungs;,; from 19.20 ± 0.00 after 2 hours to 5.73 ± 0.67 μg/g on the 5th day in the liver; from 13.20 ± 0.00 after 2 hours to 3.44 ± 0.48 μg/g on the 5th day in kidneys; from 6.24 ± 0.53 after 2 hours to 0.58 ± 0.04 μg/g on the 5th day in the spleen; from 5.73 ± 0.67 after 2 hours to 0.55 ± 0.03 μg/g on the 5th day in muscles of the thigh;– from 5.73 ± 0.67 to 3.20 ± 0.24 and from 2.00 ± 0.29 after 2 hours to 0.91 ± 0.08 on the second day in fat and serum respectively (Attia et al., 2018).

The obtained results of the current research established a similar pattern in the content of Tph residual amounts. In particular, it was detected in the liver, kidneys, lungs, and heart 1, 2, and 5 days after discontinuation of Tilmox 25%. However, it was not detected in the pectoral muscles only after 1 and 2 days, and after 5 days. In addition, the presence of tilmicosin in the pectoral muscles of chickens was not indicated 2 hours after drinking Tilmox 25%. The largest residual amounts, regardless of the time of the study, were found from 8.25 ± 0.29 μg/g on the first day to 1.20 ± 0.03 μg/g on the fifth day in lungs, from 4.65 ± 0.08 to 1.01 ± 0.02 μg/g in the liver, from 4.11 ± 0.26 to 0.91 ± 0.03 μg/g in kidneys, from 3.52 ± 0.34 to 0.02 ± 0.00 μg/g in cardiac muscle, while in pectoral muscles it was 2.24 ± 0.18 μg/g on the first day, 2.02 ± 0.16 μg/g on the second, and there was no report of that after 5 days.

The TPh is also an active ingredient in Pulmotil AC (powder for solution) and Provitil (ready-made aqueous solution). The study of TPh's pharmacokinetics was carried out on broiler chickens (Abu-Basha et al., 2007). The maximum TPh concentration in blood plasma was 2.09 ± 0.37 μg/ml for Pulmotil AS and 2.12 ± 0.40 μg/ml for Provitil, and the time to reach the maximum concentration in blood plasma was 3.99 ± 0.84 and 5.82 ± 1.04 hours, respectively. The research results indicate bioequivalence and bioavailability of TPh-preparations in the form of a ready-to-drink solution and the powder. The TPh's absorption rate and level in the form of a ready-made Provitil solution were slightly higher, compared to Pulmotil AS powder. However, the difference remained insignificant that allows us to assert a high TPh's bioavailability in various dosage forms.

The obtained results of a study conducted by Abu-Basha et al. (2007) correspond to the current study, in particular in terms of bioavailability, as indicated by the TPh's rapid intake into the internal organs and blood in the composition of Tilmox 25%, Provitil and Pulmotil AS preparations. The current research also showed a slow TPh's elimination in the composition of Tilmox 25% from the broiler chickens' body because its residual amounts were shown in the lungs, liver, kidneys, and heart muscle even 5 days after the cessation of use.

The TPh's distribution indices were similar to those reported by Attia et al. (2018) when clinically healthy and *Mycoplasma gallisepticum*-infected broiler chickens were watered for 3 days. It was found that the TPh content in the blood serum 15 minutes after its application was higher in healthy chickens, and amounted to 0.25 ± 0.020 mg/ml, while it was 0.18 ± 0.01 μg/ml in sick chickens. The highest concentration in blood serum was found 2 hours after application and was 1.23 ± 0.062 μg/ml in healthy chickens, and 0.80 ± 0.05 μg/ml in sick chickens. The data obtained convincingly indicate the effect of the pathological process on the decrease of Tph intake into the blood of the chickens. The TPh content in the internal organs of clinically healthy and sick chickens 24 hours after the last application showed a similar tendency to distribution in the current studies, which is in line with a study performed by Attia et al. (2018).

In particular, a high TPh concentration in clinically healthy chickens and chickens infected with *Mycoplasma gallisepticum* was calculated as 9.45 ± 0.34 and 8.30 ± 0.25 μg/g in lungs, 5.32 ± 0.16 and 4.56 ± 0.14 μg/g (less) in the liver, 4.53 ± 0.12 and 3.88 ± 0.17 μg/g (even less) in kidneys, and 4.24 ± 0.17 and 3.41 ± 0.16 μg/g (the least) in the heart, respectively. It should be noted that the tendency for the TPh distribution persists in the body of sick chickens although antibiotic concentrations were lower in all organs (Elkomy et al., 2018).

In a study performed by Elsayed et al. (2014), it was also found that after oral TPh administration to clinically healthy chicken broilers for 5 days, its highest concentration 24 hours after the start of watering was found as 8.76 ± 0.08 μg/g in lungs, 4.61 ± 0.07 μg/g (less) in the liver, and 3.47 ± 0.09 μg/g (the least) in kidneys. Tilmicosin was not detected in the pectoral and femoral muscles, as well as in the heart muscle, fat, and skin after 24 hours (Elsayed et al., 2014). The research results of the TPh's distribution patterns in the broiler chickens' internal organs obtained by Elsayed et al. (2014), are consistent with the current study and the ones carried out by Attia et al. (2018) and Elkomy et al. (2018).

In previous studies, the pharmacokinetic parameters of doxycycline hyclact (the active substance of the Polyodoxin drug), which are commonly used in broiler chickens (the KOBB-500 cross) had significant differences also. In particular, the maximum amounts of

doxycycline hyclact in the lungs, liver, kidneys, cardiac and pectoral muscles were manifested after 2-4 hours from the start of application on the first day. During the entire watering period of the Polyodoxin preparation (within 96 hours), the doxycycline hyclact maximum levels were manifested 9 times (by 2, 4, 8, 12, 26, 28, 32, 36, and 56 hours) in the liver, 4 times (at 48, 72, 76, and 96 hours) in kidneys, and once in lunge during 24 hours. This is important for a number of poultry diseases (ornithobacteriosis, mycoplasmosis, and pasteurellosis), the causative agents of which are localized in the lungs. Residual amounts of doxycycline were shown in the internal organs and pectoral muscles even 5 days after the discontinuation of Polyodoxin while residual amounts of tilmicosin for the use of Tilmox 25% were not shown in the broiler chickens' pectoral muscles (Tyshkivska et al., 2020).

## CONCLUSION

When healthy broiler chickens were administered with Tilmox 25% (the AVICO trademark) in accordance with the recommended regimen for diseases accompanied by respiratory damage, the studied pharmacokinetic parameters (the active substance of Tilmox) of Tilmicosin phosphate (TPh) had the following characteristics. The TPh exhibits high bioavailability, rapidly distributed to internal organs and skeletal muscles, and reaches maximum amounts in the lungs and liver after 2 hours, after 26 hours in the kidneys, and after 52 hours in the pectoral muscles and heart. During the application period (96 hours) to healthy broiler chickens, a solution of Tilmox 25%, TPh was distributed in the largest quantities to the lungs, much less to the liver, and the least to the kidneys, heart, and skeletal muscles. The TPh distribution in maximum amounts to the lungs indicates its organ affiliation, or selective tropism of the drug, which is important upon the infectious agents' localization in the lungs of broiler chickens (ornithobacteriosis and mycoplasmosis). The TPh excretion from the body of healthy broiler chickens occurs intensively within 48 hours after the cessation of the use of 25% Tilmox solution (in the period from 96 to 144 years of experience), further the process slows down. The TPh's residual amounts in the internal organs upon cessation of watering the 25% Tilmox solution (from 96 to 216 hours of the experiment) indicate a long period of its excretion and, due to this, the simultaneous provision of an antimicrobial effect. The absence of TPh's residual amounts in the breast muscles of healthy broilers at 216 hours is an important indicator for assessing the safety of broilers' meat. Taking into account a number of factors, including the chemical structure of the antibiotic, its' ability to penetrate the biological barriers of the body, form complexes with blood plasma proteins, as well as the influence of the pathological process, the next stage of our research will be the study of the TPh's pharmacokinetic parameters in the broiler chickens' body with ornithobacteriosis.

## DECLARATIONS

### Authors' contributions

All authors have contributed significantly to this work. DVB, IVD, and TAM developed the concept of work. TAM, TMYa, TNV, ShRV, and BTI participated in the collection, processing, and analysis of data. TAM and SRV prepared the manuscript, and DVB, IVD, TMYa, TNV, and BTI then critically edited the manuscript for intellectual content and an adequate description of the research process, as well as the results obtained. The final text of the manuscript was approved by all authors before publication.

### Competing interests

The authors declare that this article does not have any financial or non-financial conflict of interest.

### Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors before the submission.

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