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***Pharmacology***

# Tilmicosin Pharmacokinetics and Tissue Residues in Healthy and Naturally Respiratory Infected Broilers

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## ABSTRACT

The goal of this study was to clarify the tilmicosin pharmacokinetics in healthy and naturally infected broiler with respiratory disorders (NRI). Tilmicosin achieved its $C_{max}$ after 3.01 and 2.63 h ($T_{max}$) of administration, with values of 1.21 and 0.93 µg/ml, respectively, following single intracrop dose (25 mg/kg BW). Tilmicosin $t_{0.5ab}$ was 0.59±0.034 h & 0.59±0.02 h and $t_{0.5el}$ was 011.32 & 8.89 h, sequentially. Tilmicosin attained its $C_{max}$ following a single intramuscular injection (25 mg/kg BW, IM) after 1.51 & 1.56 h ($T_{max}$), respectively, with values of 1.20 and 1.28 µg/ml. The $t_{0.5ab}$ (0.29 & 0.30 h) and $t_{0.5el}$ (7.07 & 5.04 h) of tilmicosin showed a clear reduction relative to intracrop dosing, serially. Tissue residues after repeated oral (75 mg/L of drinking water for 3 consecutive days) administration revealed a lower significant plasma tilmicosin concentration after all times of sampling in NRI chickens related to those of healthy ones. Tilmicosin residues was assayed in lung, kidney, liver, thigh and breast muscles, after "2h, 24 h, 3d, 5d, 7d, 9d and 11 days post tilmicosin stoppage. The highest residue values were found in the lungs, followed by the liver and kidneys, while the lowest irrelevant values were found in the thigh and breast muscles. Finally, tilmicosin has a fast absorption rate, a long elimination half-life, and substantial blood-to-tissue penetration, particularly in the lungs. Following repeated oral tilmicosin administration, the required withdrawal period is 9 d for thigh and breast muscles and 10 d for internal organs.

**Keywords**: *Pharmacokinetic, Tilmicosin, Tissue residues, Broilers, HPLC.*

## INTRODUCTION

The broiler is a meat-producing livestock that is quite capable of providing people's animal protein requirements. In addition, broiler meat is inexpensive and easy to gain relative to other animal proteins. Broilers grow quickly and metabolize feed into meat economically. Also, broilers more sensitive and more liable to disease outbreaks, which are reflected on lower performance and higher mortality rates (Ensminger *et al*., 2004). Mycoplasma can cause economic collapse and is still the main cause of poultry production losses that should be considered in the poultry industry (Kleven, 1990).In eradication programs, the use of new medications, both in prophylaxis and therapy, is still preferred above the use of sanitary measures or vaccines (Jordan *et al*., 1999).

Tilmicosin is a bacteriostatic, semisynthetic macrolide antibiotic used widely inclinical veterinary problems. Because of its varied tissue distribution, long half-life, and mostly accumulated in the lungs, the tilmicosin is a drug of choice for the treatment and control of respiratory illnesses caused by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacteriumrhinotracheale and Pasteurella multocida* (Debono *et al*., 1989; Varga *et al*., 2001; Abu-Basha *et al*., 2007).

Administering veterinary drugs to animals without giving them enough time to recover may leave large residues in their tissues. Despite the fact that tilmicosin is widely used in the poultry market, little is known about its tissue residues in chickens with varieties of dosing. The primary goal of this study was to explore the tilmicosin pharmacokinetic profile after single intracrop and intramuscular treatment, as well as tissue residues after repeated oral dosages in healthy and NRI broilers, by HPLC technology.

## MATERIALS and METHODS

### *Tilmicosin*

Tilmicosin phosphate was obtained as a powder (Shandong Faming Bangjia Pharmaceutical Co. Ltd., NO. 1666, Kunming oad, Peony HIGH-TECH INDUSTIAL GAREN, Heze, Shandong-CHINA, Code: BJ- CP058- 20033). Each 33.33g of tilmicosin phosphate will add to 100 ml of distilled water (Eq. to 25.0 g tilmicosin base) which applied orally by intracrop administration or prepared for IM injection according to Co. instruction.

### *Experimental Chickens*

Two hundred and twenty-two "111, apparently clinically healthy and 111, apparently CRD-infected" Hubbard chickens weighting 1800 to 2000 g. Birds were chosen randomly from poultry farm in Menofia province, Egypt. Chickens were of both sexes. Individual cages with a 12-hour dark/light cycle was employed for broilers housing. With free access to balanced feed and water, the temperature was kept at 25±2 °C and the humidity at 45–65 percent. Before receiving the medications, the birds were examined for a week in the experimental unit of Fac. Vet. Med., Sadat City University, Menofia governorate, Egypt, for any obvious clinical signs and adaption. The Ethical Committee of the Fac. Vet. Med., Sadat City University, Egypt (Ethical approval number: VUSc-016-1-20), accepted this work.

### *Experimental design*

To determine pharmacokinetics parameters of tilmicosin (25 mg/kg BW) in healthy and NRI broilers, the birds were divided into 4 groups (180 birds):

**Group 1 & 3**: Clinically healthy and NRI broilers were orally administered (intracrop) a single tilmicosin dosage.

**Group 2 & 4**: Clinically healthy and NRI broilers were given a single tilmicosin injection intramuscularly (in left thigh muscle).

To determine tilmicosin tissue residues**,** repeated oral administration of 75.0 mg tilmicosin/L of drinking water for 3 consecutive days [equivalent approximately 15 mg tilmicosin/kg BW/day- according to EMEA (1998)] in forty-two broilers of control healthy and NRI broilers were done. Three chickens were randomly selected and slaughtered for each time "2h, 24 h, 3d, 5d, 7d, 9d and 11 days post last dose of tilmicosin. From each slaughtered chicken, plasma and tissue (lung, liver, kidney, breast muscle and thigh muscle) samples were collected for assaying of tissue tilmicosin concentration using the HPLC method. Samples were stored at -80°C until analysis after tissue homogenization**.**

### *Collection of samples*

Heparinized blood samples (4 ml) were taken from the brachial veins of 5 birds/ time at "15 min, 1h, 2h, 4h, 6h, 8h, 12h and 24 h" after dosing for determination of tilmicosin concentration using HPLC assay. Plasma were separated by centrifugation at 600 g for 15 min. Plasma were stored at -80°C until assay.

### *Tissue homogenization*

The collected organs were washed, weighed (2 g) and homogenized (By Tissue lyser LT, SN 23, 1001/ 06076, QIAGEN Hilden, Germany) in 2 ml of PBS (pH 7.4). After homogenization, both plasma and tissue homogenate samples were kept at −80 ˚C until analysis.

### *Plasma concentration of tilmicosin in broilers by HPLC*

Perchloric acid (50µL) were added to broilers plasma (950µL) and tissue homogenate, vortexed for 30 seconds, then centrifuged at 3500 rpm for 5 minutes. The supernatant was collected in a separate glass tube, and 20µL of each sample

were injected into the HPLC apparatus for analysis (Abu-Basha *et al*., 2007).Tilmicosin concentrations were determined using Clark *et al.* (2009) and Eraslan (2007) modified techniques. A mobile phase of 0.2 M ammonium acetate (pH 5), water, acetonitrile, and methanol (20:32:24:24) was used for HPLC analysis. A 0.45µm membrane filter was used to filter the mobile phases under vacuum. UV detection at 291nm was used to produce chromatographic separation at a flow rate of 1 mL/min.

***Pharmacokinetic analysis***

According to Baggot (1978 a; b), the pharmacokinetic parameters were estimated. Non-compartmental analysis based on statistical moment theory, as described by Gibaldi and Perrier (1982), was used to analyze the data for pharmacokinetics.

***Statistical analysis***

The data were calculated as mean ± standard error (S.E) of observation for PK and residue analysis. Snedecor and Cochran (1980) were used to conduct all statistical analyses.

## RESULTS

The plasma concentrations of tilmicosin after a single intracrop dose were measured in healthy and CRD-infected broilers. In both broiler kinds, several pharmacokinetic parameters, tissue distribution, and residues of tilmicosin were evaluated.

***Comparison between tilmicosin after single intracrop administration in healthy and NRI broilers:***

The mean plasma concentrations of post single intracrop administration in healthy and NRI broiler chickens at different time intervals at a dosage level of 25 mg/kg BW were recorded in the table 1 and depicted in figure 1. Tilmicosin was firstly detected at 15 minutes post single intracrop administration in both healthy and NRI broilers in a concentration of 0.26 and 0.19 µg/ml, respectively. The peak plasma level (1.33 and 1.0 µg/ml) was achieved 2h post intracrop administration then tilmicosin decreased gradually reached a concentration of 0.29 and 0.195 µg/ml at 24h post single intracrop tilmicosin administration, respectively. Lower plasma concentrations were detected in NRI chickens at different time intervals compared to that value detected in healthy ones (Table 1). Different kinetic parameters post oral administration of tilmicosin in both controls healthy and NRI broilers were significantly different *($p \leq 0.001$)* and presented in table 2. Data revealed that the apparent first order absorption rate constant ($K_{ab}$) and the maximum plasma concentrations ($C_{max}$) and the area under the plasmatilmicosin concentration curve (AUC) were significantly decreased in infected chickens than in healthy broilers following drug administration. The absorption half-life ($t_{0.5ab}$) and the elimination half-life ($t_{0.5el}$) were significantly decreased in infected chickens than in normal ones after intracrop administration.

***Comparison between tilmicosin after single intramuscular injection in healthy and NRI broilers:***

The mean plasma concentrations post single **tilmicosin** IM administration in healthy and NRI broilers at different time intervals were tabulated in the table 3 and depicted in figure 2. Tilmicosin was firstly detected at 15 minutes post single IM administration in both types of broilers in a concentration of 0.57 and 0.41 µg/ml, respectively. The peak plasma level (1.59, and 1.20 µg/ml) was achieved 1.0 h post IM administration then tilmicosin decreased gradually reached a concentration of 0.20 and 0.17 µg/ml at 24h post tilmicosin injection in healthy and diseased broilers, respectively.

Different kinetic parameters post tilmicosin injection in both healthy and diseased broilers were significantly different *($P \leq 0.001$)* and presented in table 4. The maximum plasma level ($C_{max}$) was 1.20 and 1.28 µg/ml, ($T_{max}$) was 1.51 and 1.56 h in both broilers kinds, respectively. Tilmicosin was rapidly absorbed in both broilers classes with absorption half-life ($t_{ab}$) of 0.29 and 0.30, respectively and rapidly eliminated in both healthy ($t_{el}$ 7.07 h) and diseased ($t_{el}$ 5.04) broilers, correspondingly.

***Plasma and tissue tilmicosin concentrations in healthy and NRI broilers after repeated oral administration for 3 successive days.***

Tissue concentrations of tilmicosin in slaughtered healthy and NRI broilers following the repeated oral dosage program of 75 mg/L of drinking water for three consecutive days, were detailed in table 5. The data revealed a significant decrease in tissue concentrations of tilmicosin in infected broilers relative to healthy ones. Lungs had the uppermost concentrations of the drug

followed by liver and kidney, while the lowermost concentrations were determined in muscles of thigh and breast. This suggests that lung should be the target tissue for tilmicosin residues in broiler chickens. In addition, on day 9 tilmicosin concentration in all tissues of either healthy and NRI broilers was found to be below the MRL ((MRL, 75, 1000, and 250 µg/Kg for muscle, liver, kidneys, respectively).

**Table 1**: Mean plasma tilmicosin concentrations (µg/ml) after single intracrop administration in healthy and NRI broilers (n= 5).

| Time (Hours) | Mean ±S.D | |
|---|---|---|
| | Healthy | Diseased |
| 0.25 | 0.26±0.01 | 0.190±0.020*** |
| 0.5 | 0.42±0.02 | 0.310±0.010*** |
| 1 | 0.75±0.04 | 0.600±0.020*** |
| 2 | 1.33±0.10 | 1.000±0.060** |
| 4 | 1.11±0.04 | 0.860±0.010*** |
| 6 | 1.07±0.02 | 0.730±0.020*** |
| 8 | 0.81±0.04 | 0.660±0.010** |
| 12 | 0.67±0.02 | 0.490±0.048*** |
| 24 | 0.29±0.01 | 0.195±0.008*** |

***Significant at P ≤ 0.01, *** Significant at P ≤ 0.001.*

**Table 2:** Pharmacokinetic parameters of tilmicosin in healthy and NRI broilers after single intracrop (25 mg/kg BW) administration (n= 5).

| kinetic parameters | Unit | healthy | Diseased |
|---|---|---|---|
| $K_{ab}$ | $h^{-1}$ | 1.1800±0.073 | 1.18±0.04** |
| $t_{0.5(ab)}$ | h | 0.5900±0.034 | 0.59±0.02*** |
| B | ug | 1.4900±0.080 | 1.20±0.077*** |
| $C_{max}$ | µg/ml | 1.2100±0.020 | 0.93±0.03*** |
| $T_{max}$ | h | 3.0100±0.150 | 2.63±0.07*** |
| $K_{el}$ | $h^{-1}$ | 0.0614±0.001 | 0.08±0.01** |
| $t_{0.5(el)}$ | h | 11.3200±0.270 | 8.89±0.089*** |
| AUC $_{(0-\alpha)}$ | µg. h/ml | 20.2600±0.380 | 15.88±1.29*** |
| MRT | h | 13.5800±0.550 | 14.44±1.58** |

***Significant at P ≤ 0.01, *** Significant at P ≤ 0.001.*

**Table 3:** Mean plasma concentrations of tilmicosin (µg/ml) after single intramuscular injection in healthy and NRI broilers (n= 5).

| Time (hours) | Mean ± S.D | |
|---|---|---|
| | Healthy | Diseased |
| **0.25** | 0.57±0.07 | 0.31±0.07*** |
| **0.5** | 0.84±0.03 | 0.62±0.03*** |
| **1** | 1.59±0.09 | 1.20±0.06*** |
| **2** | 1.39±0.04 | 1.10±0.04*** |
| **4** | 1.16±0.04 | 0.93±0.038** |
| **6** | 0.92±0.03 | 0.7±0.01*** |
| **8** | 0.82±0.04 | 0.59±0.01*** |
| **12** | 0.58±0.02 | 0.30±0.02*** |
| **24** | 0.20±0.01 | 0.17±0.01** |

***Significant at P≤0.01, *** Significant atP≤0.001*

**Table 4:** Pharmacokinetic parameters of tilmicosin (25 mg/kg) after single intramuscular injection in healthy and NRI broilers (n= 5).

| kinetic parameters | Unit | Control healthy | Diseased |
|---|---|---|---|
| $K_{ab}$ | $h^{-1}$ | 2.35±0.060 | 2.34±0.200 |
| $t_{0.5(ab)}$ | h | 0.29±0.010 | 0.30±0.020 |
| B | ug | 1.70± 0.055 | 1.42± 0.057* |
| $C_{max}$ | µg/ml | 1.20±0.060 | 1.28±0.030* |
| $T_{max}$ | h | 1.51±0.030 | 1.56±0.040 |
| $K_{el}$ | $h^{-1}$ | 0.10±0.010 | 0.14±0.010** |
| $t_{0.5(el)}$ | h | 7.07±0.450 | 5.04±0.300*** |
| $AUC_{0-\alpha}$ | µg. h/ml | 18.15±0.300 | 14.90±0.360*** |
| MRT | h | 10.63±0.650 | 10.30±0.580 |

**Table 5:** Plasma and tissue tilmicosin concentrations in healthy and NRI broilers after repeated oral administration (75.0 mg/L of drinking water) for 3 successive days (n=3).

| *Time | Broilers classes | Tilmicosin Concentration (µg/ml or µg/g) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plasma | Lung | Liver | Kidney | Thigh m. | Breast m. |
| 2 h | Healthy | 2.70±0.21 | 17.50± 1.32 | 9.50±1.12 | 6.30±0.37 | 2.90±0.34 | 2.60±0.17 |
| | Diseased | 2.10±0.10 | 11.20 ±0.18 | 6.50±0.59 | 4.10±0.37 | 1.90±0.21 | 1.60±0.17 |
| 1 day | Healthy | 1.70±0.12 | 9.70±1.02 | 6.40±0.93 | 3.50±0.26 | 1.67±0.23 | 1.30±0.11 |
| | Diseased | 1.40 ±0.01 | 7.40± 0.78 | 4.80±0.37 | 2.70±0.19 | 0.95±0.13 | 0.88±0.11 |
| 3 day | Healthy | 0.60±0.05 | 5.04 ±0.93 | 3.01 ± 0.48 | 1.70±0.19 | 0.72±0.10 | 0.59±0.099 |
| | Diseased | 0.45±0.001 | 5.04±0.42 | 2.70±0.16 | 1.30±0.012 | 0.51±0.05 | 0.49±0.014 |
| 5 day | Healthy | 0.21±0.013 | 2.70±0.22 | 1.60±0.21 | 0.84±0.22 | 0.25±0.02 | 0.22±0.03 |
| | Diseased | 0.012±0.001 | 2.10±0.22 | 1.10±0.09 | 0.67±0.04 | 0.13±0.001 | 0.12±0.01 |
| 7day | Healthy | N.D | 1.50±0.13 | 0.74±0.04 | 0.22±0.06 | 0.072± 0.002 | 0.07±0.001 |
| | Diseased | 1.10±0.78 | 0.61±0.04 | 0.18±0.002 | 0.055± 0.001 | 0.048± 0.0015 | 1.10±0.78 |
| 9 day | Healthy | N.D | 0.65±0.080 | 0.34±0.02 | 0.15±0.01 | N.D | N.D |
| | Diseased | 0.25±0.06 | 0.08±0.01 | 0.06±0.002 | N.D | N.D | 0.25±0.06 |
| 11 day | Healthy | N.D | N.D | N.D | N.D | N.D | N.D |
| | Diseased | N.D | N.D | N.D | N.D | N.D | N.D |

* **Time=** Post Tilmicosin Stopping, **N.D**= Not Detected

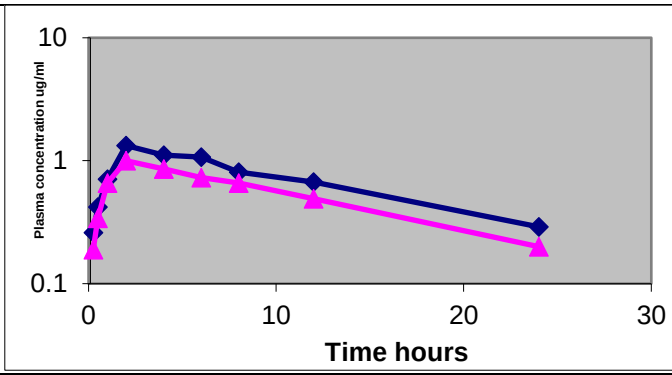


**Figure 1**: Mean plasma tilmicosin concentrations (µg/ml) in healthy (•) and NRI broilers (Δ) after a single intracrop (25 mg/kg BW) administration.

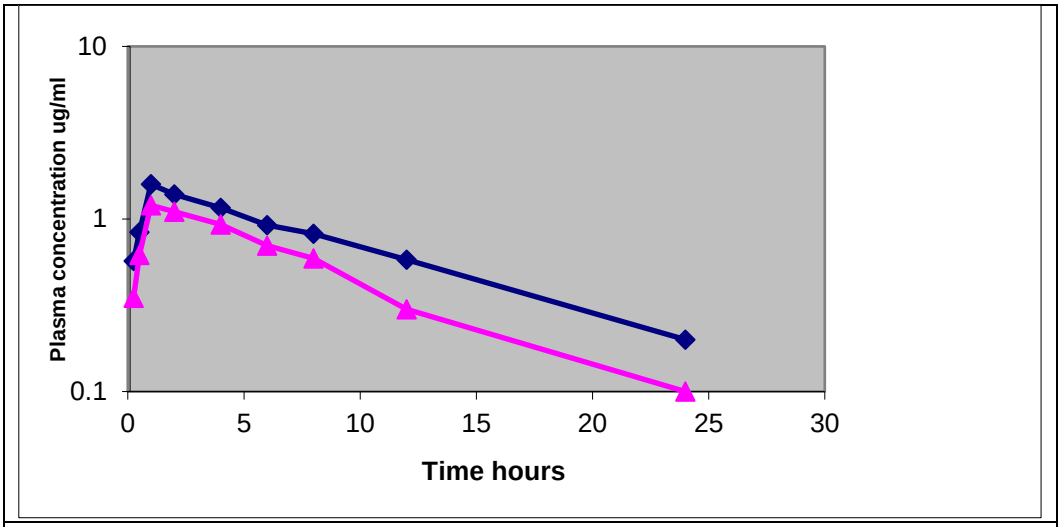


**Figure 2**: Mean plasma concentrations (µg/ml) of tilmicosin (25 mg/kg BW) in healthy (•) and NRI chickens (Δ) after a single intramuscular injection
***Significant at P≤0.01, *** Significant atP≤0.001*

## DISCUSSION

Numerous methods have been reported for the determination of tilmicosin such as microbiological assay (Pol Hofstad *et al.*, 2008), fluorescence immunoassay (Wei *et al.*, 2013), thin-layer chromatography coupled to microbiological detection (Vincent *et al.*, 2007). However, the majority of these techniques are time-consuming, expensive, and necessitate specialist technical people and complex sample pre-treatment. In the meantime, a few studies have been published on an HPLC assay for detecting tilmicosin in plasma (Foster et al., 2017), which is simple, fast, and capable of achieving high sensitivity with a small sample volume; the brave use of this method in determining tilmicosin in plasma and tissues of broilers.

***Pharmacokinetic of single intracrop tilmicosin administration in healthy and NRI broilers***

Regarding of tilmicosin pharmacokinetic after single intracrop administration, the drug was firstly detected at 0.25 h after administration, peaked at 2h post- administration, and decreased gradually till reaching the lowest drug concentration achieved at 24 hr post drug administration. The result of $C_{max}$ (1.49 µg/ml), reported in the present study was found to be closely connected to that testified by Attia *et al.*(2018*)* for tilmicosin in broilers (1.06 µg/ml) and for tilmicosin in rabbits (1.31µg/ml) at 12.5 mg/kg but lower than that stated by Abu-Basha *et al.*(2007) for tilmicosin (Pulmotil AC®) in broilers (2.12 µg/ml) at 30 mg/kg, and that reported by Gallina *et al.*,(2010).

This discrepancy could be attributed to dose, species differences, and/or the drug assaying process.In contrast, time to peak plasma level ($t_{max}$ 3.01) was longer compared to that determined by McKay *et al.* (1996) for tilmicosin in rabbits (2h), Abu-Basha *et al.* (2007) for tylosin in chicken (2.36 h), Attia *et al.* (2018) and Shaban *et al.* (2019) for tilmicosin in broilers (2.56h and 2.1h, respectively) but lower than that estimated by Abu-Basha *et al.* (2007) in broilers for tilmicosin (Pulmotil AC®) (5.82h) at 30 mg/kg. The cause of these differences could be attributed to species and dose variation, medication administration routes, and the presence of food in the chicken crop, which would alter crop motions, as well as the consistency of the feed, which could affect crop emptying. Furthermore, the presence of lactobacillus flora in the crop, which may be responsible for macrolide inactivation (Cerda *et al.*, 2010).Tilmicosin was rapidly absorbed, with a short absorption half-life ($t_{0.5ab}$, 0.59 h), similar to what Abo-El-Sooud *et al.* (2012) found for azithromycin in broiler chickens ($t_{0.5ab}$ 0.57h), Attia *et al.*, (2018) for tilmicosin in broiler chickens (0.52 hr) but shorter than those stated by Li (2003) for tilmicosin in broilers (0.948h and 0.757h) and that reported by Shu *et al.* (2004) for tilmicosin in chicken (0.66h)

which could be due to individual differences (Cerda *et al.*, 2010). Tilmicosin has been gradually eliminated with elimination half-life ($t_{0.5el}$ 11.32 h).This result is closely connected to that reported by Shaban *et al.* (2019) for tilmicosin in broilers (13.49 hours), but lower than those recorded for tilmicosin in chicken (30.18, 24.18, 47.4, and 21.86 hours) as determined by Keles *et al.* (2001); Li (2003); Abu-Basha *et al.*, (2007); Attia *et al.*, (2018), respectively.

In the present study, the calculated area under plasma concentration-time curve (AUC) was 20.26 µg.h/ml which come in agree with that detected in chicken (21.82 µg.h.ml$^{-1}$) for tilmicosin (Abu-Basha *et al.*, 2007), but higher than that recorded by Shaban*et al.* (2019) for tilmicosin in broilers (16.4 µg.h.ml$^{-1}$) post oral dose of 20 mg/kg. BW and in pigs (9.68 µg.h.ml$^{-1}$) by Dimitrova *et al.* (2011). The inter-species and dose dissimilarity, differences in the formulations, the interval between blood samples and/or health state, live body weight, , age of the animal in addition to climatic conditions related to experimental design could be responsible for the noteworthy difference in the calculated ACU.

Following single intracrop administrations of the current investigation, plasma tilmicosin levels in NRI broilers were significantly lower than those in healthy ones. These lower concentrations in infected broilers were consistent with the result formerly stated for tilmicosin by Attia *et al.* (2018) and might be due to the drug's increased penetrating capacity into diseased tissues (Baggot, 1980).Tilmicosin has good tissue penetration and reaches a high concentration and mount up in the lungs of rats than plasma and infection/inflammation further improve its tissue penetration (Modric *et al.*, 1999). This is reliable with the rapid elimination of the drug in diseased birds indicated by the shorter elimination half-life in diseased birds ($t_{½el}$ 8.89h) as compared with the value for healthy ones ($t_{½el}$ 11.32h). Maximum plasma concentrations of tilmicosin in healthy and diseased broilers (1.3 and 1.0 µg ml$^{-1}$) were achieved 2h post intracrop administration of the drug. In this investigation, the computed $C_{max}$ and $t_{max}$ for healthy broilers (1.21 µg.ml$^{-1}$ and 3.01 h, respectively) were greater than those for NRI broilers (0.93 µg.ml$^{-1}$ and 2.63 h), respectively. Furthermore, ACU of control healthy (20.26 µg.h/ml) were recorded higher values than calculated ones in NRI broilers (AUC, 15.88 µg.h/ml).The stated short distribution and elimination half-lives ($t_{½}\alpha\&\beta$); in diseased birds is consistent with the observed lower plasma concentrations of tilmicosin in NRI broilers. Similar results have previously been reported for certain antibiotics in NRI broilers **(**Aziza Amer, 1987) and chloramphenicol in chickens suffering from *E. coli* infection (Atef *et al.*, 1991).

***Tilmicosin pharmacokinetic after single IM injection in healthy and NRI broilers***

The tilmicosin was firstly detected (0.57µg/ml) at 0.25h after single IM administration, peaked at 1.0h (1.59 µg/ml) post administration and decreased gradually till reach the lowest drug concentration (0.20 µg/ml) achieved at 24h post IM administration. The calculated value of $C_{max}$ (1.46 ± 0.05µg/ml), reported in the present study was found to be similar to that reported post intracrop administration in the present study (1.49 µg/ml) and for tilmicosin in rabbits (1.31 µg/ml) at a single dose of 12.5 mg/kg but lower than that stated by Abu-Basha *et al.* (2007)for tilmicosin (Pulmotil AC®) in broilers (2.12 µg/ml) at 30 mg/kg, and that reported by Gallina *et al.* **(**2010).This variability might be attributed to dose, species variations, assaying method of the drug and/or administration method. In contrast, time to peak plasma level ($t_{max}$ 1.51) was closely related to the determined by McKay *et al.* (1996) for tilmicosin in rabbits (2 h), Abo-ElSooud *et al.* (2012) for azithromycin (1.9 h) in broiler, Abu-Basha *et al.* (2007) for tylosin in chicken (2.36 h) and Attia *et al.* (2018) ; Shaban *et al.* (2019) for tilmicosin in broilers (2.56 h and 2.1 hours, respectively) post oral administration.

Tilmicosin was rapidly absorbed with a short absorption half-life ($t_{0.5ab}$ 0.29 h) post IM injection compared to the value found after intracrop administration in the present study ($t_{0.5ab}$ 0.59 h). Tilmicosin has been gradually eliminated with elimination half-life ($t_{0.5el}$ 7.07 h) compared to values considered after oral administration ($t_{0.5el}$ 11.32 h). These values were lesser than that described by Shaban *et al.* (2019) for tilmicosin in broilers (13.49 h), and lower than those recorded for tilmicosin in chicken post oral administration (30.18, 24.18, 47.4, and 21.86 h) determined by Keles *et al.* (2001); Li (2003);

Abu-Basha *et al.* (2007); Attia *et al.*, (2018), respectively. In the present study, the calculated area under plasma concentration-time curve (AUC) was found to be 18.15 µg.h/ml which come in agree with that then that reported here post oral administration (20.26 µg.h/ml) and closely in favor with that detected in chicken (21.82 µg.h.ml$^{-1}$) but higher than that recorded by Shaban *et al.* (2019) for tilmicosin in broilers (16.4 µg.h.ml$^{-1}$) post oral dose of 20 mg/kg BW and in pigs (9.68 µg.h.ml$^{-1}$) by Dimitrova *et al.* (2011).

Plasma tilmicosin levels determined in NRI broilers were considerably lower than those in healthy ones following a single intracrop or IM administration. These lesser concentrations in naturally infected broilers were consistent with the finding previously reported for tilmicosin by Attia *et al.* (2018) and might be attributed to the higher penetrating power of the drug to the diseased tissues (Baggot, 1980) post intracrop administration. Tilmicosin has good tissue penetration and extents a high concentration and accumulates in the lungs of rats than plasma and infection/inflammation further increase its tissue penetration (Modric *et al*., 1999). This is consistent with the rapid elimination of the drug in diseased birds indicated by the shorter elimination half-life in diseased birds ($t_{½el}$5.04 ± 0.3 h) as compared with the value for healthy ones ($t_{½\ el}$7.07 ± 0.45 h). Maximum plasma concentrations of tilmicosin in healthy and diseased broilers (1.59 and 1.2 µg.ml$^{-1}$) were achieved 1.0 h post IM administration. The calculated $C_{max}$ and $t_{max}$ for healthy broilers (1.2 µg.ml$^{-1}$ and 1.56h**,** respectively) recorded in this study were similar to values recorded in NRI broilers ($C_{max}$1.28 µg.ml$^{-1}$, and 1.56 h). In addition, ACU calculated in healthy (18.15 µg.h/ml) was significantly higher than values calculated in NRI broilers (AUC14.90 µg.h/ml). The reported short distribution and elimination half-lives ($t_{½}\alpha$ and β); in diseased birds is consistent with the observed lower plasma concentrations of tilmicosin in NRI chickens post IM injection. Corresponding findings have been previously recorded post oral administration for certain antibiotics in NRI chickens **(**Aziza Amer, 1987) and chloramphenicol in chickens suffering from *E. coli* infection (Atef *et al*., 1991)

***Plasma and tissue tilmicosin concentrations after repeated oral administration in healthy and infected broilers.***

In the current work, following repeated oral administrations of tilmicosin, the obtained tilmicosin plasma concentration in NRI broiler was significantly lesser than those in healthy ones. These lower concentrations of tilmicosin in NRI broiler might be attributed to the infection/inflammation, improves its tissue penetration **(**Modric *et al*., 1999) and these records were parallel to the recorded data of Abo El-Ela *et al.* (2015) and El-Komy *et al*. (2016). The obtained results of all routes of administration showed that the $C_{max}$ was significantly lower in diseased broilers than in healthy ones. These findings were comparable to those of El-Komy *et al.* (2016), who observed that a single subcutaneous injection of 10 mg tilmicosin/kg BW resulted in a substantial decrease in $C_{max}$ in experimentally *Pasturella multocida* infected lactating goats compared to healthy ones. Furthermore, the mean peak plasma concentration of tilmicosin (1.25 µg/ml) in healthy and diseased boilers (1.21& 0.93 µg/mL, respectively) is higher than MICs for *Mycoplasma synoviae* (0.013-0.100 µg/mL) and lower than MICs for *Clostridium perfringens* strains isolated from commercial broiler farms (Watkins *et al*., 1997).Due to its prolonged stay in the lung tissues at therapeutic concentrations (Papich and Riviere, 2001), tilmicosin at the indicated dosage is effective for the treatment of respiratory disease in numerous animal species (Moore *et al.*, 1996; Christodoulopoulos *et al.*, 2009).

In the current study, the obtained results of plasma and tissue residues of tilmicosin following its repeated oral administrations of 75.0 mg/l for 3 successive days revealed a good distribution of tilmicosin in plasma and other tested tissues (lung, liver, kidney and muscles). Tilmicosin concentrations in lung, liver, kidney, and muscles were highest on day 1 after stoppage of tilmicosin medication in both healthy and NRI broilers. The residue levels were significantly higher in the lung than in liver, kidney or muscle in both classes of broilers. Similar findings to those obtained were previously reported by Zhang *et al*. (2004).According to the veterinary drug

residue regulations of the European Union, the maximum residue levels (MRLs) of tilmicosin in broiler chicken muscle, liver, and kidney are 75, 1000, and 250 µg/Kg, respectively (EMEA, 1998).

In this study tilmicosin residue in all tissues (muscle, liver, and kidney) decreased to the accepted level after 7 days of withdrawal. These results were slightly different from those obtained by Zhang *et al*. (2004) who found that tilmicosin residues in muscle decreased to the approved level after 2 days of withdrawal, in the liver after 9 days, and in the kidney after 5 days. In the present study tilmicosin residue in the muscle decreased to 75 µg/Kg and 66 µg/Kg on day 7 in healthy and NRI birds. The minimum withdrawal time of 9 days was indicated for residue levels in muscle, liver, and kidney tissues below the maximum residue level (MRL). While EMEA (1998) recommended withdrawal time is 10 days following the repeated oral administration of tilmicosin at 75.0 mg/l in drinking water for 3 successive days.

## CONCLUSION

Plasma and tissue concentrations of tilmicosin in healthy and NRI broilers could be detected at a therapeutic level following oral or IM administration, exceed minimum inhibitory concentration (MIC) of tilmicosin for *Mycoplasma gallisepticum*. Tilmicosin was rapidly absorbed and slowly eliminated after oral administration in broilers. The needed withdrawal time is 9 d (for thigh and breast muscles) and 10 d (for visceral organs) following the repeated oral administration of 75.0 mg tilmicosin /l in drinking water for 3 successive days (equivalent approximately 15 mg tilmicosin/kg BW/day).

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