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Effect of Amprolium and Toltrazuril on Disposition Kinetics of Tylvalosin in Infected Broiler Chickens with *Mycoplasma Gallisepticum* Ahmed M. El Mahdy* And Zinab R. Mohamed**

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ABSTRACT

After a single oral treatment, the pharmacokinetic characteristics of tylvalosin alone and in combination with either amprolium or toltrazuril in *Mycoplasma gallisepticum*-infected broiler chickens were evaluated. 18 chickens were divided into 3 equal groups, group one was given tylvalosin only, group two was given tylvalosin and amprolium and group three was given tylvalosin and toltrazuril. The best model for describing tylvalosin serum levels in our study was found to be a two-compartment open model. The maximum serum concentration of tylvalosin was 1.62 ± 0.03 , 1.2 ± 0.02 , and 1.41 ± 0.01 $\mu\text{g/ml}$ reached at 2.95 ± 0.014 , 2.8 ± 0.01 , and 2.83 ± 0.02 h in group one, group two, and group three respectively. A significant ($P < 0.001$) increase in V/F and Cl/F 10.19 ± 0.11 (mg)/($\mu\text{g/ml}$), 1.54 ± 0.04 (mg)/($\mu\text{g/ml}$)/h and 8.56 ± 0.07 (mg)/($\mu\text{g/ml}$), 1.31 ± 0.03 (mg)/($\mu\text{g/ml}$)/h were found in group two and group three, respectively in comparison with group one 7.24 ± 0.06 (mg)/($\mu\text{g/ml}$), 1.09 ± 0.02 (mg)/($\mu\text{g/ml}$)/h. The absorption and elimination half-lives of tylvalosin were 1.69 ± 0.016 h, 10.63 ± 0.330 h and 1.56 ± 0.01 h, 9.63 ± 0.41 h and 1.58 ± 0.02 h, 9.64 ± 0.35 h in group one, group two, and group three respectively. The area under the curve was significantly ($P < 0.001$) decreased in group two and group three which were 13.6 ± 0.06 and 15.98 ± 0.14 $\mu\text{g/ml/h}$ respectively, in comparison with group one which was 18.7 ± 0.093 $\mu\text{g/ml/h}$. It was found that the serum concentration of tylvalosin in group one after twenty-four hours is higher than MIC for the used *Mycoplasma gallisepticum* (0.25 $\mu\text{g/ml}$) unlike the second and third group, so it is recommended to give tylvalosin every twenty-four hours if it is given alone while when it is given with amprolium and toltrazuril it should be given every twelve hours.

INTRODUCTION

In veterinary medicine, pharmacokinetic medication interactions are extremely important. Drug interactions with simultaneous

administration of drugs with the same metabolic route are common (Abdelsalam and Ford 1986). This is a critical issue in poultry farming as many compounds as more than 13 have

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been commonly used as feed additives in poultry regimens (Jones and Ricke 2003) which may interfere with any therapeutic drug administered. From this point of view, antibiotic doses in chicken farms must be determined according to their interactions with these drinking water additives.

In the field of veterinary medicine in Egypt, particularly in the chicken industry, the concurrent use of many drugs is feasible, such as the concurrent use of anticoccidials, antibiotics, and other drugs to fight diseases and growth promoters at the same time (Jones and Ricke, 2003). The subsequent application of various drug interactions can occur when two or more drugs are taken together. Interactions between drugs can cause variations in concentrations of drug in the body, which may exaggerate or impair the proposed viability of antibiotics used to treat bacterial diseases.

The antibiotic tylvalosin is a novel macrolide. It was originally referred to as (acetyl isovalerylTylosin). Factor A Tylosin was fermented with *Streptomyces thermotolerans* to produce it. The highly active 16-member lactone ring is acetylated as a result of this fermentation (Huang et al. 2001).

Tylvalosin inhibits protein synthesis in Gram-positive bacteria (e.g., Staphylococcus, Micrococcus, Microbacterium, Bacillus, Corynebacterium, Aerococcus, Arthrobacter and Streptococcus, Campylobacter, Enterococcus, and Clostridia) and mycoplasma by reversibly binding to the 50S ribosomal component. It was shown to be ineffective against the vast majority of gram-negative bacteria (EMA 2009). On the other hand, It is approved for the prevention and treatment of Mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*, and other Mycoplasma species), *Ornithobacterium-rhinotracheale*, and *Clostridium perfringens*-related illnesses in chickens, replacement pullets, and turkeys. Furthermore, Tylvalosin has an anti-inflammatory effect due to its direct antibacterial activity (Zhao et al. 2014)

Amprolium is a thiamine analog that is used to treat and prevent coccidiosis in poultry. (McDougald and Reid 1997). Toltrazuril, a derivative of triazinetrione, is used to prevent

and cure coccidiosis in chickens and turkeys. (Vertommen et al. 1990).

In the present work, the researchers aim to study the pharmacokinetics of tylvalosin in *Mycoplasma gallisepticum* infected broiler chickens after a single oral dose of 25mg per kg b.wt. also to assess the effect of amprolium and toltrazuril on the kinetics of tylvalosin disposition in *Mycoplasma gallisepticum* infected broiler chickens.

MATERIALS AND METHODS

Birds:

At the beginning, a total number of eighteen apparently healthy chickens were chosen randomly from a private poultry farm, Qalubia Governorate, Egypt, housed in hygienic floor system chambers, fed on commercial balanced antimicrobial free ration and water ad-libitum and examined to assure that they are free from mycoplasma infection through taking nasal and larynx swabs (Kempf et al. 1992), the chickens were negative for mycoplasma as evident culturally by failure to isolate the organism from the birds, and serologically when tested by serum plate agglutination test. Chickens were left for 15 days as an acclimatization period and to insure the complete excretion of any drug from their bodies.

Drugs:

Avilosin® 62.5% (Each gram contains 625 mg tylvalosin tartrate) is made by ECO Animal Health in London, United Kingdom. It comes in the form of a water-soluble powder. It is administered in the form of an oral dose of twenty five milligrams per kilogram of body weight.

Amprolium was obtained from Adwia company as a water-soluble powder 20 percent under the commercial name Amprolium 20 % ®. Toltrazuril was obtained from Pharma-Swede company, Egypt as a 2.5 percent oral solution under the brand name (Tolacox) ®.

Mycoplasma gallisepticum:

The reference strain was obtained from Mycoplasma Department in Animal Health Research Institute, Agricultural Research Center, Giza, Egypt. The experimental infection was

performed by intranasal administration of 0.2 ml (10^7 CFU) *Mycoplasma gallisepticum* S 6 strain. (Kempf et al.1988)

Experimental design

The experimental infection with *Mycoplasma gallisepticum* was performed on all chicken through administration of 0.2 ml infective dose in a concentration of 10^7 CFU/ml to each chicken intra-nasally (Kempf et al. 1988), then after 7 days of mycoplasma infection (appearance of clinical signs as depression, off food, loss of weight, ruffled feathers, gasping and hard respiration) re-isolation of microorganisms from nasal and larynx swaps was performed to assure that the experimental infection has been established.

Each chicken was individually weighed to estimate the dose of tylvalosin, amprolium, and toltrazuril before their administration then the 35 days old and 1750 grams in average weight chickens were divided into 3 groups to start the current study.

Group (1): This group included six experimentally infected broiler chickens with *Mycoplasma gallisepticum*. Each chicken was given tylvalosin once orally in a dose of twenty five milligrams/kilogram of the body weight. (EMA 2009 and EMA 2010)

Group (2): This group included six experimentally infected broiler chickens with *Mycoplasma gallisepticum*. Each chicken was given amprolium orally in a daily dose of 25 mg/kg b. wt. for a week (EMEA 2001). Tylvalosin was given once orally in a dose of 25 mg/kg b. wt 2 hours after the last dose of amprolium (to ensure that amprolium reached its maximal blood concentration).

Group (3): This group included six experimentally infected broiler chickens with *Mycoplasma gallisepticum*. Each chicken was given toltrazuril orally in a dose of 7 mg/kg b. wt for a couple of days (EMA 1998), after the last dose of toltrazuril by 2 hours (to ensure that toltrazuril reaches its maximal blood concentration), tylvalosin was given once orally in a dose of 25 mg/kg b. wt.

About one milliliter of blood was taken from the right-wing vein of chickens, following the oral administration of tylvalosin at

0.083, 0.167, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours, blood samples were taken. The collected blood samples were allowed to clot and the serum was separated by centrifugation at 3000 r. p.m. for 15 minutes. All serum samples were stored at -20°C until assay.

Analytical Procedures

Tylvalosin was measured in blood samples using a microbiological antibiotic approach (Arret et al. 1971) with *Bacillus subtilis* as the test organism (Elkomy et al. 2019). The serum (from Antibacterial-free chickens) was used to create the standard curves. In standard Petri dishes containing 25 ml agar, six wells, each 8mm in diameter were cut at equal distances. 100 μl of either test samples or tylvalosin standards were poured into the wells. After 2 hours at room temperature, the plates were incubated at 37°C for 18 hours. Micrometers were used to determine inhibition zones, and the standard curve was used to estimate tylvalosin concentrations in the test samples.

Determination of minimum inhibitory concentration (MIC) (Broth micro dilution method):

The MIC of tylvalosin was determined against *Mycoplasma gallisepticum* by broth micro-dilution assay (Abd El-Hamid et al. 2019) in compliance with the aforementioned principles (Hannan, 2000), briefly, tylvalosin was two fold serially diluted from a concentration from 128 to $0.063\mu\text{g/ml}$. *Mycoplasma* isolates were diluted to contain 10^3 to 10^4 color changing unit / 0.2 ml. Consequently, in the 96-well micro-titer plates, 0.1 ml of each dilution was combined with 0.1 ml of each diluted mycoplasma isolate. each plate containing growth controls (tested field mycoplasma strains grown into broth medium with out any tested substances), sterility controls (broth medium without neither tested substances nor mycoplasma inoculums) and pH control (broth medium adjusted to pH 6.8). The plates were sealed and incubated at 37°C with the color indicator being checked at regular intervals. Each experiment was carried out in triplicate and repeated twice to ensure that the results were accurate. The lowest antimicrobial concentration that caused no color change when the color of the mycoplasma growth control

changed was defined as the initial minimum inhibitory concentration (MIC). When there was no more color change in the growth control wells, the final MIC was determined in the broth containing the antimicrobials.

Standard curve of tylvalosin

For the establishment of a standard curve of tylvalosin, a stock solution of 100 µg/ml of tylvalosin standard (obtained from pharma-chem international) in distilled water was prepared. Standard concentrations were obtained by further dilution in drug-free chicken serum to obtain concentrations from 0.098-100 µg/ml. The standard curve was used to calculate the tylvalosin concentrations in the test samples. (EL. Sayed et al. 2018).

Pharmacokinetic and statistical analysis

Data were expressed as mean ± S.E. and the pharmacokinetic parameter calculated by PK Solver: a Microsoft Excel add-in application,

version 2 (Zhang et al. 2010). The data generated were subjected to statistical analysis employing the Student's t-test with $P < 0.05$, $P < 0.01$, and $P < 0.001$ as the levels of significance.

RESULTS

In our study, the concentrations of tylvalosin after single oral administration in a dose of twenty five milligrams/kilogram of the body weight was estimated along twenty four hours, the concentration of tylvalosin alone (group 1), with amprolium (group 2), and with toltrazuril (group 3) was equal to 0.26 µg / ml, 0.18 µg / ml and 0.21 µg / ml respectively at 24 hours. The semi logarithmic serum concentration-time curves of tylvalosin in *Mycoplasma gallisepticum* infected broilers after a single oral administration of 25 mg /kg.b.wt. in group 1, group 2, and group 3 are depicted in Fig. (1). the best model for describing tylvalosin serum curves was found to be a two-compartment open model.

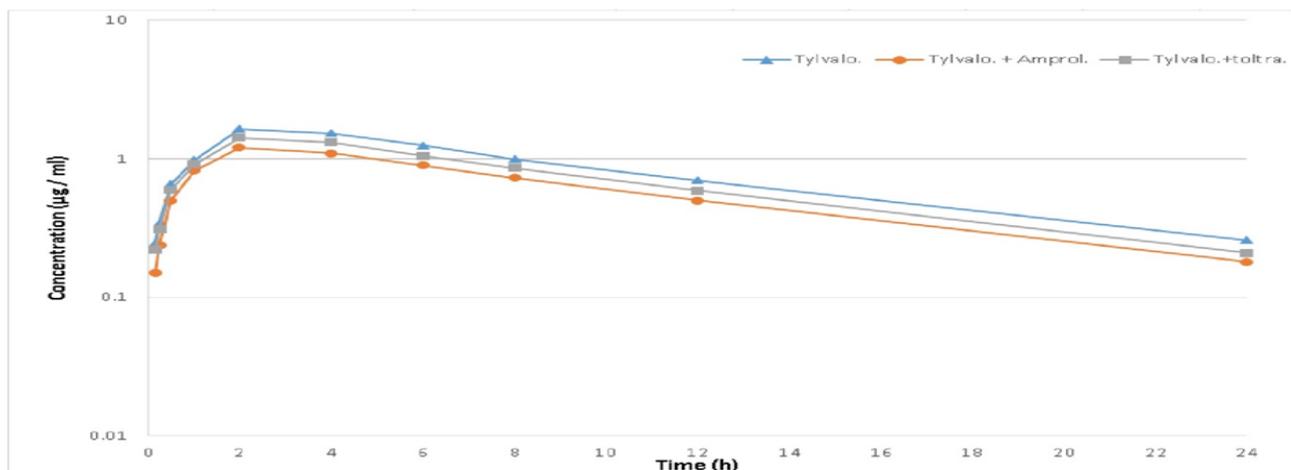


Fig. 1: Graph depicting the time course of tylvalosin in serum of broilers after a single oral administration of 25 mg/kg.b.wt. alone, with amprolium and with toltrazuril.

The minimum **inhibitory concentrations** of tylvalosin against *Mycoplasma gallisepticum* is shown in table 1, where MIC of tylvalosin against *Mycoplasma gallisepticum* was=(0.25 µg / ml)

Table (1): MIC ($\mu\text{g} / \text{ml}$) of tylvalosin against *Mycoplasma gallisepticum*

Parameters	<i>Mycoplasma gallisepticum</i>
	0.25 $\mu\text{g} / \text{ml}$

The pharmacokinetic parameters of tylvalosin in group1, group2 and group3 are shown in table 2, where a significant differences were found in **A, α , k_a , k_{21} , $t_{1/2\alpha}$, $t_{1/2k_a}$, V/F, CL/F, CL_2/F , T_{max} , C_{max} , $AUC_{0-\text{inf}}$, **AUMC and****

MRT between group 2 and group3 when compared with group one.

Table (2): Mean pharmacokinetic parameters of tylvalosin in broilers after a single oral administration of 25mg/kg.b.wt. alone (tylvalo.) (group one), with amprolium (tylvalo. +Ampro.) orally at a dose rate of 25 mg/kg.b.wt (group two) and with toltrazuril (tylvalo.+Toltra.) orally at a dose rate of 7 mg/kg.b.wt (group three)

parameter	unit	Group 1	Group 2	Group 3
A	$\mu\text{g}/\text{ml}$	21.26 \pm 0.830	11.99 \pm 0.75***	15.97 \pm 1.89*
α	1/h	0.41 \pm 0.006	0.44 \pm 0.004***	0.44 \pm 0.01*
B	$\mu\text{g}/\text{ml}$	1.31 \pm 0.032	1.07 \pm 0.04***	1.25 \pm 0.03
β	1/h	0.065 \pm 0.003	0.072 \pm 0.004	0.072 \pm 0.003
k_a	1/h	0.46 \pm 0.006	0.51 \pm 0.005***	0.50 \pm 0.01**
k_{10}	1/h	0.15 \pm 0.002	0.15 \pm 0.003	0.153 \pm 0.003
k_{12}	1/h	0.147 \pm 0.003	0.15 \pm 0.003	0.152 \pm 0.01
k_{21}	1/h	0.177 \pm 0.007	0.21 \pm 0.01*	0.207 \pm 0.01*
$t_{1/2\alpha}$	h	1.69 \pm 0.016	1.56 \pm 0.01***	1.58 \pm 0.02**
$t_{1/2\beta}$	h	10.63 \pm 0.330	9.63 \pm 0.41	9.64 \pm 0.35
$t_{1/2k_a}$	h	1.51 \pm 0.016	1.36 \pm 0.01***	1.4 \pm 0.02**
V/F	(mg)/($\mu\text{g}/\text{ml}$)	7.24 \pm 0.060	10.19 \pm 0.11***	8.56 \pm 0.07***
CL/F	(mg)/($\mu\text{g}/\text{ml}$)/h	1.09 \pm 0.022	1.54 \pm 0.04***	1.31 \pm 0.03***
V_2/F	(mg)/($\mu\text{g}/\text{ml}$)	6.0 \pm 0.302	7.39 \pm 0.46*	6.29 \pm 0.45
CL_2/F	(mg)/($\mu\text{g}/\text{ml}$)/h	1.06 \pm 0.024	1.56 \pm 0.05***	1.3 \pm 0.04***
T_{max}	h	2.95 \pm 0.014	2.8 \pm 0.01***	2.83 \pm 0.02***
C_{max}	$\mu\text{g}/\text{ml}$	1.62 \pm 0.03	1.2 \pm 0.02***	1.41 \pm 0.01***
AUC_{0-t}	$\mu\text{g}/\text{ml} \cdot \text{h}$	18.7 \pm 0.093	13.6 \pm 0.06***	15.98 \pm 0.14***
$AUC_{0-\text{inf}}$	$\mu\text{g}/\text{ml} \cdot \text{h}$	22.91 \pm 0.203	16.24 \pm 0.15***	19.08 \pm 0.26***
AUMC	$\mu\text{g}/\text{ml} \cdot \text{h}^2$	327.88 \pm 6.496	217.4 \pm 5.15***	254.62 \pm 7.35***
MRT	h	14.3 \pm 0.279	13.35 \pm 0.30*	13.39 \pm 0.31*

*Results are expressed as means \pm SE (n=6), student t test

* P <0.05

**P<0.01

***P<0.001

DISCUSSION

The pharmacokinetic drug interaction between agents used as part of a multidrug regimen is important because the interaction may influence drug efficacy. Multi-drug regimens are often administered to birds and animals for the duration of their lives.

Current studies revealed that co-

administration of many anthelmintics (ivermectin, albendazole, and rafoxanide) with florfenicol in goats (Atef et al. 2010) and supplementation of some polyether ionophore anticoccidial drugs (salinomycin, monensin, and maduramycin) as feed additives in broilers (Wang et al. 2013) can change the disposition kinetics of florfenicol. So far, little is recognized about whether the use of anticoccidial

drugs as amprolium and toltrazuril can affect the kinetic profile of tylvalosin in broilers. This situation forced us to study the effect of these coccidiostats on the pharmacokinetics of the administered antibiotic.

In current work, oral administration of tylvalosin alone (group 1), with amprolium (group 2) or with toltrazuril (group 3) in broiler chickens, pharmacokinetics parameter was studied to explain the disposition kinetics of tylvalosin in *Mycoplasma gallisepticum* infected broiler chickens and also to Assess the effect of amprolium and toltrazuril on the kinetics of tylvalosin disposition in *Mycoplasma gallisepticum*-infected broiler chickens.

The present results revealed a significant decrease in distribution half-life $t_{1/2\alpha}$ in group two (1.56h) and group three (1.58h) in comparison with group one (1.69h) in association with a significant increase in volume of distribution V/F in group two [10.19 (mg) / ($\mu\text{g/ml}$)] and group three [8.56 (mg)/($\mu\text{g/ml}$)] in comparison with group one [7.24 (mg)/($\mu\text{g/ml}$)] which indicated that tylvalosin was rapidly absorbed and distributed in broilers which were given amprolium and Toltrazuril, such result agreed with what has mentioned by (El-Sayed et al. 2015) who discovered that the dosing of broilers with amprolium significantly decrease the distribution half-life of lincomycin in comparison with control chickens.

Following PO administration, TVN showed rapid absorption from the alimentary tract of chickens as indicated by small k_a 0.46/h. Similarly, rapid absorption of TVN was recorded where the k_a was 0.799/h (Salman et al. 2016) and 0.69/h (Abo El-Ela et al. 2015) in broiler chickens while a significant increase in k_a and decrease in $t_{1/2ka}$ were found between group two 0.51/h, 1.36h and group three 0.50/h, 1.4h when compared with group one 0.46/h, 1.51h in the present work.

After a single dosage of (25 mg Kg/b.wt.), tylvalosin blood concentration data were best matched to a two compartment open model. Similar findings were observed in healthy chickens by (Salman et al. 2016; Abo El-Ela et al. 2015 and Abeer, 2016), tylvalosin was

detected in serum 10 min post-administration. It was gradually increased after that to achieve its maximal concentration. (C_{max}) equal 1.62 microgram/ml at T_{max} equal to 2.95h post-administration and it could be detected in plasma at 24 hours. Our results were near to what has been recorded by (Elkomy et al. 2019) for Tylvalosin in normal chickens after oral administration (C_{max}) equal 1.36 $\mu\text{g/ml}$ at T_{max} equal to 2.37 h, while they were higher than what has been recorded by (Elbadawy et al. 2017) for Tylvalosin in healthy turkey after oral administration (C_{max}) equal 1.08 $\mu\text{g/ml}$ at T_{max} equal to 2h and lower than what has been recorded by (Abo El-Ela et al. 2015) for Tylvalosin in healthy chickens after oral administration (C_{max}) equal 2.11 $\mu\text{g/ml}$ at T_{max} equal to 2.03 h, These variations might be attributed to anatomical differences between species, healthy status, age variations which could be affecting the degree of protein binding of the drug.

The mean serum concentrations of tylvalosin were lowered at different time intervals in group two and group three broilers compared to group one. Similar findings were previously reported for amprolium with enrofloxacin in broilers (Rania, 2007), who reported that coccidian infected birds which were given amprolium exhibit lower serum enrofloxacin concentrations than broilers which were given enrofloxacin alone, and for toltrazuril with thiamphenicol in broilers (Attia et al. 2021), who reported that broilers which were given toltrazuril exhibit a lower serum thiamphenicol concentration in comparison with broilers which were given thiamphenicol alone.

In our study, there was a significant decrease in C_{max} = 1.2 $\mu\text{g/ml}$ of tylvalosin in group two and C_{max} = 1.41 $\mu\text{g/ml}$ in group three when compared with group one C_{max} = 1.62 $\mu\text{g/ml}$, This difference might be agreed with pharmacological interaction previously recorded by (El Banna et al. 2013) who found that serum concentration of levofloxacin (C_{max}) was significantly lower in chickens which were given amprolium and (Attia et al. 2021) who found that serum concentration of thiamphenicol (C_{max}) was significantly lower in chickens

which were given toltrazuril if compared with control chickens.

This result may be attributed to thiamin deficiency produced by amprolium (a non metabolizable thiamine analog) as it inhibits thiamin absorption from the small intestine and compete with thiamin in its uptake, this amprolium related thiamine deficiency induces cytochrome P450||E1 (CYP2E1) which consequently increases hepatic microsomal monooxygenase activities and results in rapid metabolism of tylvalosin in our study. (EMEA 2001), (Polin et al. 1962) and (Yoo et al. 1990)

Also, the decreased C_{max} of tylvalosin in group three may be attributed to the oxidative oxidative stress of toltrazuril on liver and intestine because toltrazuril induced a significant increase in the level of nitric oxide in the liver and blood which may decrease the absorption of tylvalosin from intestine (Hassan 2014), in addition to the affinity of toltrazuril to form toltrazuril sulfone by CYP3A in the liver which in turn induces CYP 450 enzymes in animals and birds (Benoît et al. 1994) and (Abdel-Maged et al. 2013) which consequently resulted in the rapid metabolism and lower C_{max} of tylvalosin in this work.

In our present work, there was a significant decrease in $AUC_{0-t} = 13.6 \mu\text{g/ml}\cdot\text{h}$, $AUC_{0-inf} = 16.24 \mu\text{g/ml}\cdot\text{h}$ and $AUMC = 217.4 \mu\text{g/ml}\cdot\text{h}^2$ of tylvalosin in group two which was associated with shorter $t_{1/2\beta} = 9.63\text{h}$ which also supported by a significant decrease in $MRT = 13.35\text{h}$, also There was a significant decrease in $AUC_{0-t} = 15.98 \mu\text{g/ml}\cdot\text{h}$, $AUC_{0-inf} = 19.08 \mu\text{g/ml}\cdot\text{h}$ and $AUMC = 254.62 \mu\text{g/ml}\cdot\text{h}^2$ of tylvalosin in group three which Was associated with shorter $t_{1/2\beta} = 9.64\text{h}$ which supported by a significant decrease in $MRT = 13.39\text{h}$ when compared with group one $AUC_{0-t} = 18.7 \mu\text{g/ml}\cdot\text{h}$, $AUC_{0-inf} = 22.91 \mu\text{g/ml}\cdot\text{h}$ and $AUMC = 327.88 \mu\text{g/ml}\cdot\text{h}^2$, with longer $t_{1/2\beta} = 10.63\text{h}$ and which supported by $MRT = 14.3\text{h}$, other studies on amprolium and toltrazuril with tilmicosin in broilers have yielded similar results (El-Hewaity 2016) and with levofloxacin in broilers (El-Banna et al. 2013) another research disagreed with our result and demonstrated that Amprolium brought about a

significance elevation while toltrazuril brought about a significance diminish in AUC_{0-inf} contrasted with enrofloxacin in Eimeria infected chickens (Atef et al. 2020), also our results demonstrated a significant decrease in mean residence time in group two **13.35h** and group three **13.39h** in comparison with group one **14.3h**, the interaction of amprolium and toltrazuril with levofloxacin in broilers (El Banna et al. 2013) and interaction of amprolium and toltrazuril with enrofloxacin in Eimeria infected chickens (Atef et al. 2020) have yielded similar results, while it disagreed with (El-Hewaity 2016) who found no significant differences of Amprolium and toltrazuril contrasted with tilmicosin in broilers, these variation may be attributed to the usage of different antibiotic.

Also, a significant increase in apparent total clearance of the drug (CL/F) and apparent total clearance of the drug from peripheral compartment after oral administration $CL_2/F = 1.54, 1.56 \text{ (mg)/(\mu g/ml)/h}$ of tylvalosin in group two and group three = 1.31, 1.3 (mg)/(\mu g/ml)/h if compared with group one = 1.09, 1.06 (mg)/(\mu g/ml)/h which come consistent with a non-significant shorter half-life time of elimination $t_{1/2\beta} = 9.63\text{h}$ in group two and $t_{1/2\beta} = 9.64\text{h}$ in group three in comparison with group one $t_{1/2\beta} = 10.63\text{h}$ which indicating that amprolium and toltrazuril can rapidly remove the tylvalosin from the blood compared to control chickens (group one). these results agreed with what has mentioned by (El-Hewaity 2016) who recorded a significant decrease in half-life time of elimination $t_{1/2\beta}$ of tilmicosin chickens who were given amprolium and toltrazuril when compared with those who were given tilmicosin only.

The determined minimum inhibitory concentration MIC of tylvalosin for *M. gallisepticum* is 0.25 $\mu\text{g/ml}$, in our study tylvalosin concentration in the second, third groups was lower than the mentioned MIC and in the first group was higher than the mentioned MIC at 24h while tylvalosin concentration in all groups exceeds the mentioned MIC at 12h and such result may be attributed to the effect of anticoccidial drugs as amprolium and toltrazuril on the kinetic profile of tylvalosin in broilers.

Conclusion

The serum concentration following one dose of tylvalosin (25 mg/Kg b. wt./day) was suitable to maintain its therapeutic regimen for treatment of mycoplasma infection in broiler chickens if it is given alone while the administration of amprolium or toltrazuril with oral tylvalosin would alter its kinetic profiles in broiler chickens. Therefore, under this condition, the dose of tylvalosin administration by oral route needs to be carefully adjusted, so it is recommended to give tylvalosin every twenty-four hours if it is given alone while when it is given with amprolium and toltrazuril it should be given every twelve hours.

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Conflict of Interest

The authors declare no conflict of interests

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