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Pharmacokinetics of Tylvalosin in Broiler Turkeys (*Meleagris Gallopavo*) After Single Intravenous and Oral Administration

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Pharmacokinetics of tylvalosin (TVN) were determined in eight broiler turkeys following a single intravenous (IV) and peroral (PO) administration of 25 mg/kg b.w using a crossover design with a 3 weeks washout period. Blood samples were taken between 0.083 and 24 h following TVN administration, plasma was separated and assayed for TVN concentrations by HPLC. The non-compartmental analysis was used to analyze plasma concentration-time curves. After IV administration, the pharmacokinetic profile was best described by a two-compartment model. The mean distribution and elimination half-lives were 0.382 and 5.71 h, respectively. The distribution volume at steady state, total body clearance and mean residence time were 8.30 L/kg, 1.17 L/h, and 7.16 h, respectively. After administering orally, the mean absorption half-life and absorption time of TVN was 0.955 and 2.31 h, respectively. The peak plasma concentration was 1.08 μg/mL and achieved at 2.0 h post-administration and the bioavailability was 53.3%. The plasma protein binding percent was 13%. For a successful clinical efficacy of TVN in broiler turkeys, a dosage regimen of 25 mg/kg b.w, given orally each day is recommended to keep efficient plasma levels above the MIC for most susceptible microorganisms.

Keywords: bioavailability, macrolides, pharmacokinetics, tylvalosin, broilers, turkeys

INTRODUCTION

Bacterial infections can endanger the lives of human beings and livestock or cause serious economic losses; therefore, antibacterial intervention is a critical issue. However, due to the frequent use of classical antibiotics, developing resistant bacterial strains continues to be a constant medical problem. New antibacterial agents can solve such an issue.

Tylvalosin is a new, broad-spectrum, third-generation veterinary macrolide antibiotic with 16-membered lactone ring and obtained from tylosin by the change of 3-acetyl-40-isovaleryl group to be acetylisovaleryltylosin tartrate (1, 2). As a macrolide, TVN inhibits the synthesis of bacterial protein by irreversible binding to 50S ribosome subunit of susceptible bacteria. Tylvalosin possesses a wide range of biological activities and significant therapeutic uses (3, 4). Against *Mycoplasma* species, TVN is extremely effective *in vitro* (5), and also against some isolates of *Brachyspira pilosicoli*, *Brachyspira hyodysenteriae,* and some anaerobes like *Clostridium* and *Bacteroides* species (6). Tylvalosin is used in swine for treating porcine proliferative enteritis, swine enzootic pneumonia and swine dysentery (1), and in poultry to control respiratory (*Mycoplasma* Species and *Ornithobacterium rhinotracheale*) and enteric (*Clostridium perfringens*) bacterial infections (6, 7). Moreover, TVN

is better than tylosin in the higher intracellular penetration and accumulation inside respiratory and gut epithelial cells as well as phagocytic cells (3). Furthermore, TVN was shown to exhibit anti-inflammatory like characteristics and alleviates acute lung damage (2). Such data could suggest a substantially improved effect of TVN vs. tylosin. These features make TVN an attractive and prospective alternative against violent susceptible bacteria in the veterinary field. The safety and efficacy of macrolide antibiotics could be interpreted using their pharmacokinetic and pharmacodynamic data especially the cumulative time that the concentration exceeds the MIC (%T > MIC) for the time-dependent macrolides and AUC_{24h}/MIC for the concentration-dependent ones, as azithromycin. However, there are few available data on avian pharmacotherapy and the shortage of pharmacokinetic data impedes the rational use of TVN in targeted avian species. Therefore, the current study was performed to characterize the disposition profile of TVN in broiler turkeys after single oral and intravenous administration.

MATERIALS AND METHODS

Drug and Chemicals

Tylvalosin (Aivlosin®) was obtained as 62.5% water-soluble white granules (ECO Animal Health, London, UK). Each gram powder contains 625 mg of TVN as TVN tartrate. The internal standard, roxithromycin was obtained from Sigma-Aldrich Corp. (St. Louis, U.S.A.). Other chemicals and reagents consumed in the current study were acquired commercially and of HPLC grade.

Experimental Turkeys

Eight clinically healthy broiler black turkeys (*Meleagris gallopavo*, 4 males and 4 females), weighing between 5.2 and 6.5 kg and of 11 weeks age were obtained from a local commercial turkeys farm and utilized to determine the pharmacokinetics of TVN. Turkeys were fed on a balanced ration free from drugs and water was supplemented *ad libitum*. Turkeys were housed in a hygienic room at 22 ± 1°C and 60 ± 10% humidity with a light cycle of 12 h/day for 2 weeks before being used to acclimatize the environment and for ensuring complete clearance of any antibacterial agents. All turkeys were clinically healthy before drug administration. The experimental protocol was approved ethically by the local Ethical Committee of the Faculty of Veterinary Medicine, Benha University, Egypt.

Drug Administration and Blood Sampling

Before TVN administration, each bird was weighed to determine its dose. A Crossover design with a 3 weeks washout interval between the two routes of administration was used. Turkeys were divided into two groups ($n = 4$) and TVN was given as a single dose at 25 mg/kg b.w. (according to the manufacturer instructions) orally (intra-crop using oral gavages) and intravenously into the right brachial vein. Using the left brachial vein, blood samples (1 mL each) were obtained before and 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-drug administration using Venflon IV cannula and centrifuged at 1,600 *g* for 10 min. Plasma was aspirated and stored at −20°C until analyzed.

Analytical Method

Tylvalosin concentrations in turkey's plasma were assayed by HPLC as described before (8) with some modifications. Briefly, roxithromycin (as an internal standard) was mixed with every standard, quality control sample and plasma sample at a level of 1 μg/mL. The plasma samples were mixed with 400 μL of acetonitrile including formic acid (0.1%), vortexed for 10 s and centrifuged at 20,000 *g* for 10 min at 4°C. Subsequently, the supernatant was gathered and evaporated to dryness in a thermostatically controlled water-bath maintained at 35°C (Rotavapor® R-114, Shibata Company, Tokyo, Japan). The residue was reconstituted in 150 μL mobile phase and defatted with 400 μL hexane, and the aqueous layer was collected and filtered by a 0.45 μm HPLC filter (Chromatodisc®, Kurabo Biomedical Company, Osaka, Japan) and 50 μL of the filtrate were injected into the HPLC column.

The HPLC system (Shimadzu Corporation, Kyoto, Japan) composed of a UV detector (SPD-6A), an integrator (Chromatopac C-R7A plus), a pump (LC-10AD) and a loop injector (Model 7125). The mobile phase consists of acetonitrile and (0.15 M) ammonium acetate buffer (49:51, v/v) solution. The analytical separation was accomplished by using Agilent TC-C18 column (5 μm, 4.6 × 250 mm, Agilent Technologies, USA) at 25°C. The flow rate was adjusted at 1 mL per min and the wavelength of the detector was 289 nm.

The calibration was carried out by spiking of 20 μL of diluted TVN standard solutions ranging between 0.019 and 20 μg/mL into 500 μL of blank turkey's plasma and assayed as mentioned above. The result showed that standard calibration curves of TVN were linear ($r = 0.995$). The limit of detection (LOD) was 0.039 μg/mL while limit of quantitation (LOQ) was 0.1 μg/mL. The average plasma recovery rate of TVN was 87.2%. The intra- and inter-day CV values ranged from 4.28 to 4.92% and 4.86 to 5.42%, respectively ($n = 5$, 3 times, 3 days).

The Extent of Plasma Protein Binding of TVN

The plasma protein binding capacity of TVN was estimated *in vitro* by the ultrafiltration method as described previously (9). Different standards of TVN concentrations (as in standard calibration curves of TVN) were prepared, spiked to blank turkey's blank plasma samples in a triplicate manner for each concentration and vortexed for 20 s. Subsequently, samples were kept for 30 min at 37°C to allow binding between plasma protein and TVN. Thereafter, 1 mL of the sample was loaded into the sample reservoir of Ultrafree® centrifugal filter (UFC30LH00, a low-binding hydrophilic PTFE membrane, Millipore Corporation, Japan) with a pore diameter of 0.45 μm and subjected to ultrafiltration by centrifugation at 2,500 *g* at 37°C for up to 30 min or until the required volume of ultrafiltrate was obtained. The ultrafiltrate was assayed for TVN concentration as mentioned before. The extent of plasma protein

binding was estimated with reference to the initial sample concentration according to following Equation;

$$\text{Plasma protein binding (\%)} = \left[\frac{\text{spiked concentration} - \text{ultrafiltrate concentration}}{\text{spiked concentration}}\right] \times 100 \quad (1)$$

Assay of TVN Pharmacokinetic Profile

Following IV injection, the plasma concentration vs. time curves of TVN fit well with the two-compartment model, while after PO administration it fit well with the one-compartment model. Thus, the curves recorded post IV [CP_{IV} (t)] and PO administration [CP_{PO} (t)] were characterized by Equations (2) and (3), respectively.

$$CP_{IV}\ (t) = \frac{\text{Dose}}{V}\left\{\frac{\propto -k_{21}}{\alpha-\beta}\cdot e^{-\alpha\cdot t} + \frac{k_{21-\beta}}{\alpha-\beta}\cdot e^{-\beta\cdot t}\right\} \quad (2)$$

$$CP_{PO}\ (t) = \frac{\text{Dose}\cdot F\cdot ka}{V}\left\{\begin{array}{l}\frac{k_{21-\alpha}}{(ka-\propto)(\beta-\alpha)}\cdot e^{-\alpha\cdot t} \\ +\frac{k_{21-\beta}}{(ka-\beta)(\alpha-\beta)}\cdot e^{-\beta\cdot t} \\ +\frac{k_{21-ka}}{(\alpha-ka)(\beta-ka)}\cdot e^{-ka\cdot t}\end{array}\right\} \quad (3)$$

Equations (2) and (3) were simultaneously fit (10–12) to the plasma concentration vs. time curve of TVN after IV and PO administration into the same turkey, respectively, to determine pharmacokinetic variables by the non-linear least square way using MULTI, a curve fitting program (13).

Several parameters have been calculated using a non-compartmental method of analysis (14). The AUC and AUMC were calculated by the trapezoidal method. The terminal elimination rate constant was estimated using four data points in the terminal part of the concentration vs. time curve by using the non-linear least-square iterative approach. The elimination half-life ($t_{1/2\beta}$) was calculated as $t_{1/2\beta} = 0.693/\beta$, where β is the elimination rate constant. MRT = AUMC/AUC and Cl_{tot} = Dose/$AUC_{0-\infty}$. The absolute oral bioavailability (F) = $AUC_{PO}/AUC_{IV} \times 100$ and MAT = MRT_{PO}-MRT_{IV}. Distribution volume at a steady state (V_{dss}) = Cl_t/MRT_{IV}.

RESULTS

The mean plasma concentration vs. time profile of TVN following a single IV and PO administration of 25 mg/kg b.w. to broiler turkeys were graphed in **Figure 1** and there was a good fitting between the observed points and theoretical curves. The pharmacokinetics data (Mean ± SE) estimated from the curve fitting and non-compartmental analysis were shown in **Table 1**. Following the IV injection, TVN concentration was sloped in a biphasic manner with a rapid and wide distribution and a long elimination half-life. After PO giving, TVN was quickly absorbed followed by slow elimination. The C_{max} was 1.08 µg/mL, reached (Tmax) at 2.0 h. The oral bioavailability of TVN was 53.3%. The *in vitro* plasma protein binding tendency of TVN was low (13 ± 0.785%).

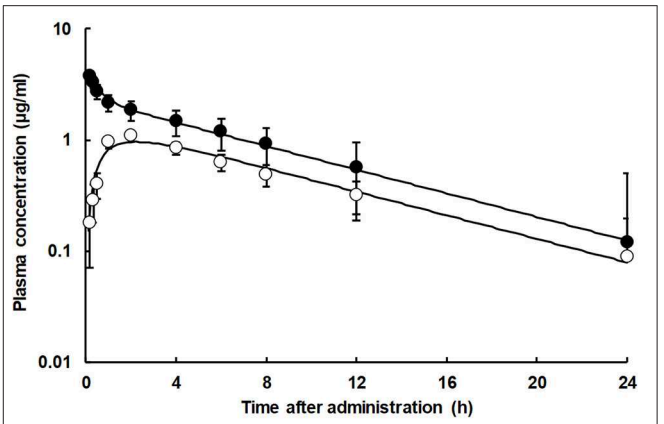

FIGURE 1 | Semi-Logarithmic plot showing the plasma concentration vs. time curve of TVN in broiler turkeys following a single dose of 25 mg/kg b.w. administered intravenously (•) and orally (○). Each mean ± SE ($n = 8$) are represented by each point and vertical bar, respectively. The IV and PO theoretical lines were depicted by Equations (2) or (3), respectively, using pharmacokinetic parameters in **Table 1**.

DISCUSSION

Disposition of 16-membered lactone ring macrolides has been studied in a lot of avian species, and inter-species variations have been demonstrated as for tylosin in pigeons, quail, emus, and cranes (15) and chickens (16–18) as well as for tilmicosin in chicken (19) and turkeys (20). Tylvalosin itself in chickens during a pilot study showed different absorption profiles in between and within individuals when used on separate occasions (5). These differences in the disposition of macrolides among avian species require thorough pre-clinical assessment.

After IV injection of TVN in turkeys, the plasma concentration vs. time curve was tilted in a biphasic pattern, indicating that the disposition profile of TVN obeyed a two-compartment model. Similar data were recently recorded for TVN in turkey (21) and broiler chickens (22–24) and also for tylosin (18, 25, 26) and clarithromycin (27) in broiler chickens. Tylvalosin has good distribution profile in turkey as evidenced by a short (0.382 h) distribution half-life, probably due to extensive tissues distribution. Similarly, a short (0.076 h) $t_{1/2\alpha}$ of TVN in turkeys was recorded (21). Tylvalosin also showed a shorter $t_{1/2\alpha}$ (0.153 h) in broiler chickens at the same dose level (24) and after 10 mg/kg b.w. in laying hens [0.12 h (22)]. Similarly to TVN, the $t_{1/2\alpha}$ of tylosin tartrate (a chemically similar macrolide with time-dependent property) in broiler chickens after IV injection of 50 mg/kg b.w. was 0.385 h (25). Contrarily, shorter distribution half-lives were recorded for tylosin phosphate (0.07 h) and tylosin tartrate (0.09 h) in chickens but after IV administration of 10 mg/kg b.w. (18). In the present study, V_{dss} for TVN was 8.30 L/kg proposing a wide distribution of the drug in tissues of turkeys following IV injection. Similarly, the V_{dss} of TVN in broiler chickens was large (8.74 L/kg) after IV administration of the same dose (24). These are greater than those for tylosin reported earlier in broiler chickens as it was 0.69

TABLE 1 | Mean (±SD, $n = 8$) plasma pharmacokinetic variables of tylvalosin in broiler turkeys determined following a single dose of 25 mg/kg b.w. administered intravenously (IV) and orally (PO).

Parameter	IV	PO
k_a (h^{-1})	—	0.745 ± 0.146
C_{max} (μg/mL)	—	1.08 ± 0.117
T_{max} (h)	—	2.00 ± 0.00
CP_0 (μg/mL)	4.161 ± 0.688	—
α (h^{-1})	1.87 ± 0.393	—
β (h^{-1})	0.122 ± 0.011	—
$t_{1/2ka}$ (h)	—	0.955 ± 0.148
$t_{1/2\alpha}$ (h)	0.382 ± 0.0826	—
$t_{1/2\beta}$ (h)	5.71 ± 0.530	—
AUC (μg·h/mL)	22.1 ± 4.19	11.7 ± 2.95
AUMC (μg·h/mL)	159.4 ± 36.9	112.8 ± 38.9
Cl_t (L/h/kg)	1.17 ± 0.232	—
F (%)	—	53.3 ± 10.8
MRT (h)	7.16 ± 0.398	9.47 ± 1.06
V_{dss} (L/kg)	8.30 ± 1.23	—
MAT (h)	—	2.31 ± 0.83

k_a, absorption rate constant; C_{max}, maximum plasma level; T_{max}, time to achieve maximum plasma level; CP_0, plasma concentration at time zero after IV injection calculated from dose/Vd; α and β, rate constants representing the slope of distribution phase and elimination phase, respectively; $t_{1/2}$ka, $t_{1/2\alpha}$, and $t_{1/2\beta}$, half-lives of absorption phase, distribution phase and elimination phase, respectively; AUC, area under the plasma concentration vs. time curve; AUMC, area under moment curve; Cl_t, total clearance; F, fraction of drug absorbed systemically after oral administration; MRT, mean residence time; V_{dss}, steady-state volume of distribution; MAT, meantime of absorption.

L/kg (16), 0.94–1.09 L/kg (18), 5.30 L/kg (25), and 6.73 L/kg (26) after IV administration. This pharmacokinetic behavior is not surprising since macrolides are weak basic and highly lipophilic drugs with pKa values from 7.1 to 8.9 (pKa of TVN is 7.6) and low plasma protein binding tendency, thus these antibiotics move easily by non-ionic passive diffusion into tissues especially with a lower pH than blood (20, 21, 28). In the current study, the *in vitro* plasma protein binding tendency of TVN was low (13%) as is the case with other macrolides (18–30%) in most species (29). Similarly, tylosin (pKa 7.1) has a low ionization degree and a low binding to serum proteins (40%) is distributed widely in the body and attains greater tissue concentration than plasma levels (30). Our results showed a long (5.71 h) elimination half-life ($t_{1/2\beta}$) of TVN in broiler turkeys after IV administration which is nearly similar to that of broiler chickens [6.67 h, (24)]. Contrarily, TVN showed short $t_{1/2\beta}$ (0.788 and 0.61 h) in turkeys and laying hens after IV administration (21) and (22), respectively. The $t_{1/2\beta}$ of tylosin in broiler chickens was also long [5.62 h (26) and 7.29 h (27)]. Contrarily, a short $t_{1/2\beta}$ of tylosin [0.52 h (16) and 1.04–1.16 h (18)] was recorded in broiler chickens following IV injection of 10 mg/kg b.w. The total clearance of TVN was (1.17 L/h/kg) indicating a relatively quicker clearance in broiler turkeys. Nearly equal clearance values (1.498 and 0.953 L/h/kg) has been detected for TVN in turkeys and broiler chickens after IV injection (21) and (24), respectively. A larger value of TVN clearance (4.37 L/h/kg) was recorded in laying hens following IV injection of 10 mg/kg b.w. (22). In broiler chickens, higher clearance values after IV injection of 10 mg/kg b.w. of tylosin were 1.71 and 1.61 L/h/kg (18) and 5.3 L/h/kg (16).

Following PO administration, TVN showed rapid absorption from the alimentary tract of turkeys as indicated by short $t_{1/2ka}$ (0.955 h) and MAT (2.31 h) as well as small ka (0.745/h). Similarly, rapid absorption of TVN was recorded where the $t_{1/2ka}$ and ka were 0.875 h, 0.799/h (24), 0.94 h, and 0.69/h (23) in broiler chickens and the $t_{1/2ka}$ was 0.74 h in laying hens (22). Moreover, shorter $t_{1/2ka}$ values of TVN after PO dosing of 20 mg/kg b.w. using a rigid (0.471 h) or a flexible (0.175 h) catheter were reported also in broiler chickens (31). Also, the rapid absorption of TVN tartrate after PO giving to broiler chicken was recorded (6). Shorter $t_{1/2ka}$ of tylosin tartrate [0.48 h, (18)], [0.19 h (25) and 0.3 h (26)] were also recorded in broiler chickens. The maximum plasma level (C_{max}) of TVN after PO dosing to turkeys were 1.53 μg/mL and attained shortly (2 h) after administration. Additionally, the plasma concentration of TVN in the current study remained for 24 h higher than the MICs for TVN against several isolates of *Mycoplasma gallisepticum* (0.015–0.03 μg/mL) and *Mycoplasma synoviae* (0.015 μg/mL) isolated recently (Jan., 2017–Dec., 2018) from turkeys in Egypt (32) and TVN was found to be more effective than tilmicosin and tylosin in this study. Also, in another recent study (2014–2016), the MICs of TVN against 17 strains of *Mycoplasma synoviae* isolated from broiler turkeys originating from Central and Eastern Europe were ≤0.25 ug/mL (33). Nearly similar and different values of C_{max} and T_{max} of TVN in turkey and broiler chickens were recorded. In turkeys, the C_{max} and T_{max} of TVN were 0.637 μg/mL and 1.293 h (21). The C_{max} of TVN in broiler chickens were 2.11 and 1.23 μg/mL, attained at 2.03 and 1.72 h (23) and (24), respectively, and that of laying hens (20 mg/kg b.w.) was 22 μg/mL achieved at 0.86 h, respectively (22). Moreover, Cerda' et al. found in broiler chickens that the C_{max} of TVN (20 mg/kg b.w.) using a rigid or a flexible catheter were 6.104 and 1.641 μg/mL and achieved at 1.202 and 0.571 h, respectively (31). The C_{max} of tylosin in broiler chickens was 1.2 (0.18 and 0.44), 3.4 and 4.85 μg/mL attained at 1.5 (1.31 and 1.33), 1.08 and 1.32 h (16, 18, 25, 26), respectively. Our data showed that, the oral bioavailability of TVN was moderate (53.3%), higher than that of TVN in turkey [33.84% (21)] and nearly similar to that of TVN in chickens which was 60.26, 48.39, and 63.83% (22, 24, 34), respectively. However, in broiler chickens, the oral bioavailability of tylosin was from 35.4 to 40.6% (17), 13.74 to 27.0% (18), and 90.3% (25) and 89.2% (26). The differences in kinetic parameters between TVN and tylosin might be due to the differences in the chemical structure of both drugs (3). For anticipating the clinical efficacy of time-dependent antibacterial drugs, using the surrogate marker of the time free drug concentration in plasma is above the minimum inhibitory concentration fT ≥ /MIC (35), TVN would be a successful agent in turkeys for microorganisms with MIC ≤ 0.015–0.03 μg/mL after PO administration. Oral administration of 25 mg/kg b.w. of TVN every 24 h in broiler turkeys would be effective against several bacterial infections as chronic

respiratory diseases caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

In conclusion, administration of TVN (25 mg/kg b.w. each 24 h) might be highly effective for susceptible bacterial diseases in turkeys. However, further studies on tissue residues are necessary.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the manuscript/supplementary files.

ETHICS STATEMENT

The experimental protocol was approved ethically by the local Ethical Committee of the Faculty of Veterinary Medicine, Benha University, Egypt.

AUTHOR CONTRIBUTIONS

ME contributed to the idea, design, performing the experiment, and writing the manuscript. MA contributed to pharmacokinetic analysis and revising the manuscript. AA performed the calculations, English check and grammars, and validation.

REFERENCES

1. Guedes RMC, Franca SA, Machado GS, Blumer MA, Cruz ECD. Use of tylvalosin-medicated feed to control porcine proliferative enteropathy. *Vet Rec.* (2009) 165:342–5. doi: 10.1136/vr.165.12.342
2. Zhao Z, Tang X, Zhao X, Zhang M, Zhang W, Hou S, et al. Tylvalosin exhibits anti-inflammatory property and attenuates acute lung injury in different models possibly through suppression of NF-κB activation. *Biochem Pharmacol.* (2014) 90:73–87. doi: 10.1016/j.bcp.2014.04.015
3. Stuart AD, Brown TDK, Imrie G, Tasker JB, Mockett AA. Intra-cellular accumulation and trans-epithelial transport of aivlosin, tylosin and tilmicosin. *Pig J.* (2007) 60:26–35.
4. Stuart AD, Brown TDK, Mockett APA. Tylvalosin, a macrolide antibiotic, inhibits the *in vitro* replication of European and American porcine reproductive and respiratory syndrome virus (PRRS) viruses. *Pig J.* (2008) 61:42–8.
5. Cerda' RO, Giacoboni GI, Xavier JA, Sansalone PL, Landoni MF. *In vitro* antibiotic susceptibility of field isolates of *Mycoplasma synoviae* in Argentina. *Avian Dis.* (2002) 46:215–8. doi: 10.1637/0005-2086(2002)046[0215:IVASOF]2.0.CO;2
6. Giguère S. Macrolides, azalides, and ketolides. In: Giguère S, Prescott JF, Dowling PM, editors, *Antimicrobial Therapy in Veterinary Medicine*, 5th ed. Wiley-Blackwell (2013). p. 211–31.
7. Forrester CA, Bradbury JM, Dare CM, Domangue RJ, Windsor H, Tasker JB, et al. *Mycoplasma gallisepticum* in pheasants and the efficacy of tylvalosin to treat the disease. *Avian Pathol.* (2011) 40:581–6. doi: 10.1080/03079457.2011.618822
8. Canning P, Bates J, Hammen K, Coetzee J, Wulf L, Rajewski S, et al. Concentrations of tylvalosin and 3-O-acetyltylosin attained in the synovial fluid of swine after administration by oral gavage at 50 and 5 mg/kg. *J Vet Pharmacol Ther.* (2016) 39:621–4. doi: 10.1111/jvp.12309
9. Craig W, Suh B. Protein binding and the antimicrobial effects: methods for the determination of protein binding. In: Lorian V, editor. *Antibiotics in Laboratory Medicine*, 3rd ed. Baltimore, MD: Williams & Wilkins (1991). p. 367–402.
10. Elbadawy M, Sakiyama T, Abohatab R, Sasaki K, Shimoda M. Oral pharmacokinetics of the acidic drugs, diclofenac and sulfamonomethoxine in male Shiba goats. *J Vet Med Sci.* (2015) 77:21–6. doi: 10.1292/jvms.14-0261
11. Elbadawy M, Sasaki K, Miyazaki Y, Aboubakr M, Khalil WF, Shimoda M. Oral pharmacokinetics of acetaminophen to evaluate gastric emptying profiles of Shiba goats. *J Vet Med Sci.* (2015) 77:1331–4. doi: 10.1292/jvms.15-0104
12. Elbadawy M, Ishihara Y, Aboubakr M, Sasaki K, Shimoda M. Oral absorption profiles of sulfonamides in Shiba goats: a comparison among sulfadimidine, sulfadiazine and sulfanilamide. *J Vet Med Sci.* (2016) 78:1025–9. doi: 10.1292/jvms.15-0601
13. Yamaoka K, Tanigawara Y, Nakagawa T, Uno T. A. Pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn.* (1981) 4:879–85. doi: 10.1248/bpb1978.4.879
14. Gibaldi M, Perrier D. Noncompartmental analysis based on statistical moment theory. In: Gibaldi M, Perrier D, editors. *Pharmacokinetics*, 2nd ed. New York, NY: Informa Healthcare (1982). p. 409–17.
15. Locke D, Bush M, Carpenter JW. Pharmacokinetics and tissue concentrations of tylosin in selected avian species. *Am J Vet Res.* (1982) 43:1807–10.
16. Kowalski C, Rolinski Z, Zan R, Wawron W. Pharmacokinetics of tylosin in broiler chickens. *Pol J Vet Sci.* (2002) 5:127–30.
17. Abu-Basha EA, Al-Shunnaq AF, Gehring R. Comparative pharmacokinetics and bioavailability of two tylosin formulations in chickens after oral administration. *J Hell Vet Med Soc.* (2012) 63:159–66. doi: 10.12681/jhvms.15431
18. Ji LW, Dong LL, Ji H, Feng XW, Li D, Ding RL, et al. Comparative pharmacokinetics and bioavailability of tylosin tartrate and tylosin phosphate after a single oral and i.v. administration in chickens. *J Vet Pharmacol Ther.* (2014) 37:312–5. doi: 10.1111/jvp.12092
19. Elbadawy M, Aboubakr M. Pharmacokinetics, tissue residues of tilmicosin phosphate (tilmicor-al®) and it's *in vitro* and *in vivo* evaluation for the control of *Mycoplasma gallisepticum* infection in broiler chickens. *Int J Pharmacol Toxicol.* (2017) 5:11–6. doi: 10.14419/ijpt.v5i1.7084
20. Fricke JA, Clark CR, Boison JO, Chirino-Trejo M, Inglis TE, Dowling PM. Pharmacokinetics and tissue depletion of tilmicosin in turkeys. *J Vet Pharmacol Ther.* (2008) 31:591–4. doi: 10.1111/j.1365-2885.2008.00985.x
21. Radi AM. Pharmacokinetic and bioavailability of tylvalosin after oral, intramuscular and intravenous administration in turkeys. *Int J Pharm Pharm Sci.* (2016) 8:140–4.
22. Liu LN. *Pharmacokinetics of acetylisovaleryltylosin tartrate in laying hens* [Dissertation/master's thesis]. Department of Basic Veterinary Sciences, College of Veterinary Medicine, Huazhong Agricultural University, Huazhong, China.
23. Abo El-Ela FI, El-Banna HA, El-Deen MB, El-Gendy AA, Tohamy MA. Pharmacokinetics of tylvalosin alone or in combination with Vitamin E in broiler chickens. *Asian J Anim Vet Adv.* (2015) 10:556–66. doi: 10.3923/ajava.2015.556.566
24. Salman AH, Youssef SAH, Ramadan A, Soliman AM. Pharmacokinetics of tylvalosin in healthy and experimentally *Mycoplasma gallisepticum* infected broiler chickens. *Int J PharmTech Res.* (2016) 9:72–80.
25. Soliman AM, Sedeik M. Pharmacokinetics and tissue residues of tylosin in broiler chickens. *Pharmacol Pharm.* (2016) 7:36–42. doi: 10.4236/pp.2016.71006
26. Aboubakr M, Elbadawy M. Pharmacokinetics, tissue residues and efficacy of D-Tylo50/25® (tylosin-doxycycline combination) in broiler chickens. *Int J Basic Clin Pharmacol.* (2017) 6:383–8. doi: 10.18203/2319-2003.ijbcp20170334
27. AwadAllah H, Awidat S, El-Mahmoudy A. Pharmacokinetics of clarithromycin after single intravenous and intracrop bolus administrations to broiler chickens. *Int J Pharmacol Toxicol.* (2016) 4:12–8. doi: 10.14419/ijpt.v4i1.5846
28. Goudah A, Abo El Sooud K, Abd El-Aty AM. Pharmacokinetics and tissue residue profiles of erythromycin in broiler chickens after

different routes of administration. *Dtsch Tierarztl Wochenschr.* (2004) 111:162–5.
29. Papich MG. Chloramphenicol and derivatives, macrolides, lincosamides, and miscellaneous antimicrobials. In: Riviere JE, Papich MJ, editors. *Veterinary Pharmacology and Therapeutics*, 10th ed. Hoboken, NJ: John Wiley & Sons, Inc. (2018). p. 912–24.
30. Baggot JD, Gingerich DA. Pharmacokinetic interpretation of erythromycin and tylosin activity in serum after intravenous administration of a single dose to cows. *Res Vet Sci*. (1976) 21:318–23. doi: 10.1016/S0034-5288(18)33344-7
31. Cerda' RO, Petruccelli M, Piscopo M, Origlia J, Landoni M. Impact of the type of catheter on the absorption of tylvalosin (acetylvaleryltylosin) administered orally to broiler chickens. *J Vet Pharmacol Ther*. (2010) 33:202–3. doi: 10.1111/j.1365-2885.2009.01103.x
32. El-Hamid MIA, Awad NFS, Abo-Shama UH, Yousreya MH, Abdel-Rahman MA, Hetta HF, et al. *In vitro* evaluation of various antimicrobials against field *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates in Egypt. *bioRxiv*. (2019). doi: 10.1101/726000
33. Kreizinger Z, Grózner D, Sulyok KM, Nilsson K, Hrivnák V, Benčina D, et al. Antibiotic susceptibility profiles of *Mycoplasma synoviae* strains originating from Central and Eastern Europe. *BMC Vet Res.* (2017) 13:342. doi: 10.1186/s12917-017-1266-2
34. Cerda' RO, Petruccelli MA, Piscopo M, Herrero M, Landoni MF. Effect of the grapefruit juice on the oral absorption of acetylisovaleryltylosin tartrate (aivlosin) in 2 weeks old chickens. Session I: pharmacokinetics. *J Vet Pharmacol Ther*. (2006) 29:239–79. doi: 10.1111/j.1365-2885.2006.00767.x
35. Barbour A, Scaglione F, Derendorf H. Class-dependent relevance of tissue distribution in the interpretation of anti-infective pharmacokinetic/pharmacodynamic indices. *Int J Antimicrob Agents.* (2010) 35:431–8. doi: 10.1016/j.ijantimicag.2010.01.023

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