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Plasma concentrations and pharmacokinetics of tilmicosin in broiler chicken given through 'in feed' route

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Abstract
The present study has been undertaken to evaluate the various pharmacokinetic parameters of tilmicosin in broiler chicken at the dose rate of 1 kg/tonne of feed through in feed route. Eight birds were used in the study. Tilmicosin was administered by mixing with feed at the recommended dose of 1 g/Kg of feed. Blood samples were collected at different time intervals to separate the plasma and subsequent determination of plasma concentration of tilmicosin. Plasma concentration of tilmicosin was assayed using a validated sensitive HPLC method. Based on time vs concentration curve, various pharmacokinetic parameters were calculated. The desired MIC value of 0.1 µg/ml was maintained up to 12 hours. The results revealed an AUC of 21.51 $\mu g.h^2.ml^{-1}$, half-life of 21.45 h, MRT of 5.74 h. There was significant variability in pk parameters within the 8 birds subjected to trial, among which only 6 birds showed any plausible PK behaviour. It can be concluded that tilmicosin can be given mixed with feed to mainatain therapeutically relevant concentrations.

Keywords: tilmicosin, bioavailability, chicken, in feed, pharmacokinetics

Introduction
Tilmicosin is a broad-spectrum bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary use only (Elkomy and Aboubakr, 2020) [4]. The antibacterial spectrum broadly ranges from *Mycoplasma* spp., *Pasteurella* spp., and various Gram-positive organisms to Gram-positive anaerobic bacteria and Gram-negative respiratory pathogens including *Mannheimia haemolytica* and *Pasteurella multocida* (Prescott, 2000) [10]. Tilmicosin is used for the treatment of respiratory tract infections in poultry caused by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*, and *Pasteurella multocida* (Kempf *et al*., 1997; Jordan *et al.*, 1993) [6, 8]. Tilmicosin has a long elimination half-life and accumulates at high concentrations in lung tissue and is ideal for the treatment of *M. gallisepticum* infections. (Jianzhong *et al*, 2005; Abu Basha *et al*, 2007) [2, 5]. Data on pharmacokinetic studies of tilmicosin in poultry is relatively scarce and most of the pharmacokinetic studies have centred around intramascular route of administration which is not a very common route under field conditions. Often the drug is administered through feed for therapeutic/prophylactic purposes. Studies relating to its bioavailability after in feed administration are scarce. There is a need for dose optimization of tilmicosin by 'in feed' route through adequate pharmacokinetic studies.

Materials and Methods
Chemicals and Reagents
Tilmicosin standard was procured from sigma for the purpose of the study. Tilmicosin required for administration was tilmicosin phosphate which was given as *gratis* from Huve Pharma. Other chemicals for assay such as Ammonium formate, acetonitrile, methanol and trifluroacetic acid, were procured as LC grade.

Pharmacokinetic trial
A total of 8 three-week-old chicken (vencobb strain) of either sex were procured from the local market. After acclimatization for a week they were used for the study. The study was approved by the Institutional Animal Ethics Committee (vide 1803/DFBS/IAEC/2019). The birds were given access to ad libitum feed and water, The birds were tagged properly for the purpose of

identification. On the day of trial, the birds were administered tilmicosin through 'in feed' route. The birds were given medicated feed for 12 h after which unmedicated feed was given ad libitum. The dose used was one gram per Kg of feed as per the manufacturer's recommendations. After exposing the birds to tilmicosin, blood was collected from the tarso-metatarsal vein into heparin coated microcentifuge tubes at 0 min, 15 min, 30 min, 1 hr, 2 h, 3 h, 4 h, 8 h, 12 h and 24 h, calculated from the time of first exposure. After vortexing the blood samples were centrifuged at 2000 g for 7 minutes for extraction of plasma. The plasma collected at different time points were stored at -20 ^{o}C until further use.

HPLC conditions

The HPLC system (Waters, USA) consisted of pump, detector, column oven and powered by *Empower* software. The mobile phase consisted of 0.1 ammonium formate, acetonitrile and methanol in the ratio of 60:30:10 v/v (pH adjusted with trifluoroacetic acid) (Keles *et al.*, 2001) [7]. The flow rate was kept at 1 ml/min. Chromatogram was monitored at a wavelength of 287 nm.

Sample extraction

To 450 µl of plasma sample, equal volume of acetonitrile was added. The tube was vortexed, centrifuged at 2000 x g and then the supernatant was filtered using 0.2 µm filter, in case of standards, spiked blank plasma was used which was processed as mentioned for plasma samples. The method was validated and found to be sensitive for the estimation of tilmicosin in chicken plasma (Sakthivel *et al*, 2021).

Pharmacokinetic analysis

The pharmacokinetic analysis of tilmicosin after administration were calculated based on time versus plasma concentration curve using non-compartmental analysis. The parameters calculated were:

- AUC_{0-t}- Area under plasma concentration- time curve based on trapezoidal method and $AUC_{0-\infty} = AUC_{0-t} + (C_{last}/\beta)$.
- $AUMC_{0-t}$ = Area under the first moment curve using linear trapezoid method and $AUMC_{0-\infty} = AUMC_{0-t} + (C_{last}*t_{last}/\beta) + (C_{last}/\beta*\beta)$.
- MRT- Mean Residence time, where MRT = AUMC/AUC
- V_d area-Volume of distribution, where Vd_{area}/F = (dose/AUC) x β.
- $t_{1/2}\beta$- Elimination half-life, where $t_{1/2}\beta = 0.639/\beta$.
- Cl_B- Total body clearance, where Cl_B/F= dose/AUC.
- C_{max}- The maximum concentration and the corresponding peak time (t_{max}) were taken as observed.

Results

Calibration curve

Calibration curves were constructed based on values for tilmicosin standard solutions prepared in methanol or plasma. Graphs using concentration (µg/ml) in X axis and the corresponding peaks in the Y axis were plotted. The calibration curves for methanolic and plasma standards returned with R^2 values of 0.999 and 0.97 respectively, which is indicative of very high reliability over a broad range from 0.1 µg to 5 µg/ml.

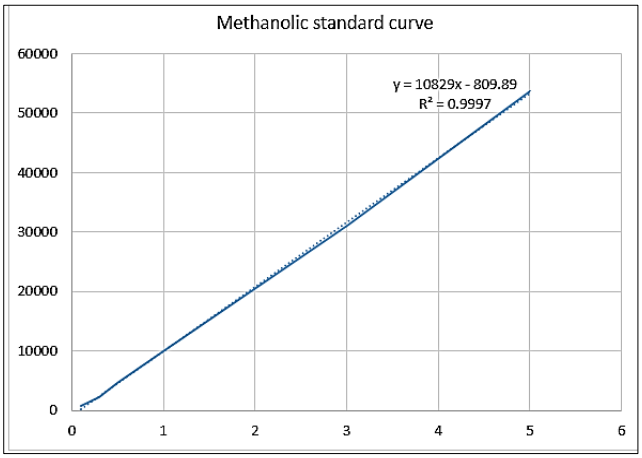

Fig 1: Standard curve of tilmicosin spiked in methanol.

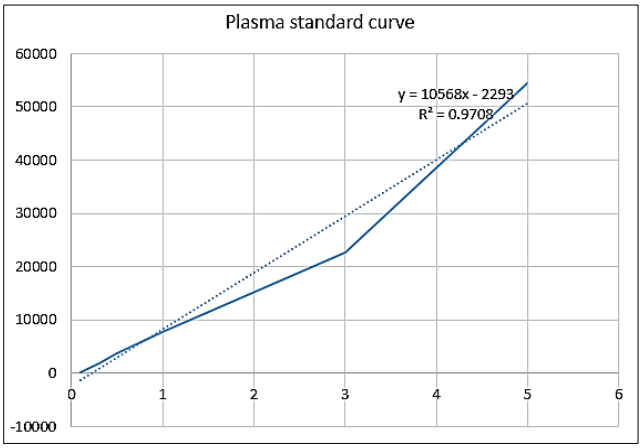

Fig 2: Standard curve of tilmicosin spiked in plasma.

Plasma concentrations of tilmicosin

Plasma concentrations of tilmicosin following "in feed" administration in broiler chicken are shown in Table 1, Fig. 3. The plasma concentrations on average has peaked at 30 minutes and 1 hour meaning that the absorption of the drug has been rapid. The plasma concentrations have remained above the MIC values even up to 12 hrs post administration of the drug indicating that drug is present in sufficient enough quantity to exert its anti-bacterial effect.

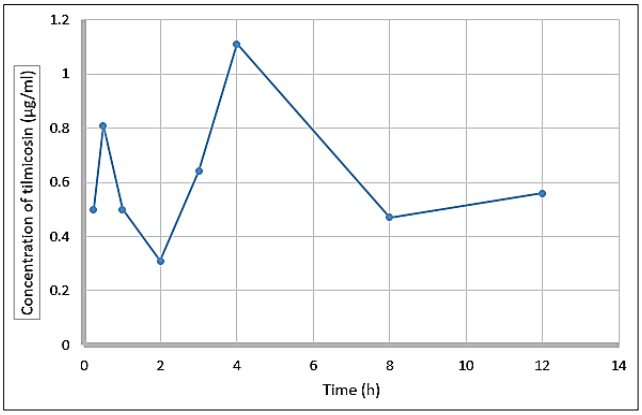

Fig 3: Plasma concentrations of tilmicosin in broiler chicken (mean values of six birds).

Table 1: Concentration of tilmicosin in the plasma (n = 6).

Time (hr)	Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6	Mean ± SD
	Drug concentration (µg/ml)						
0.25 (15 min)					0.36	0.64	0.5 ± 0.19
0.50 (30 min)	0.36	0.53	2.19	1.04	0.37	0.41	0.81 ± 0.71
1	0.47	1.48	0.65	0.41	0	0	0.50 ± 0.54
2	0.28	0.91	0.25	0.46	0	0	0.31 ± 0.34
3	0.83	0.44	0.58	0.29	1.39	0.35	0.64 ± 0.41
4	0.75	0.36	0.54	0.48	4.18	0.40	1.11 ± 1.50
8	0.84	0.66	0.56	0.46	0.35	0	0.47 ± 0.28
12	0.48	0.45	0.46	0.35	1.32	0.34	0.56 ± 0.37

Pharmacokinetic parameters

The mean pharmacokinetic variables for 6 birds subjected to treatment through the 'in feed' route is shown in Table 2. The remaining two birds did not have adequate concentrations at various time points, making it impossible to include them in pharmacokinetic studies. The important pharmacokinetic parameters were as follows: half-life 21.45 ± 17.21; Volume of distribution 417.96 ± 228.24 $L.kg^{-1}$; Clearance 18.70 ± 10.86 $ml.min^{-1}.Kg^{-1}$; $AUC_{0-\infty}$ 21.51 ± 7.42µg.h.ml-1 and MRT 5.7 ± 0.43h.

Table 2: Pharmacokinetic parameters.

Parameters	Mean ± SD	Units
Half life	21.45 ± 17.21	hr
Volume of distribution (Vd_{area})/F	417.96 ± 228.24	$L.kg^{-1}$
Clearance (Cl_B)/F	18.70 ± 10.86	$ml.min^{-1}.kg^{-1}$
C_{max}	1.72 ± 1.32	$\mu g.ml^{-1}$
T_{max}	2.37 ± 3.08	hr
AUC $_{0-\infty}$	21.51 ± 7.42	$\mu g.h.ml^{-1}$
AUMC $_{0-\infty}$	112.84 ± 94.92	$\mu g.h^2.ml^{-1}$
MRT	5.7 ± 0.43	h

Discussion

Tilmicosin is one of the most effective antibiotics against mycoplasma infection and has found rapid acceptance and witnessed widespread usage among the poultry farmers across India given the efficacy and easy availability of the drug. There is an abundance of literature surrounding pharmacokinetic studies of Tilmicosin on a variety of species giving us an insight into the pharmacokinetic behaviour of the drug. Unfortunately, most of the pharmacokinetic studies have been carried out using intramuscular route, which is not a preferred route of administration in poultry, in poultry the most widely used routes are 'in feed' and 'in water' modes across various farms in India. There is also a lack of uniformity in the dose recommendations prescribed by drug companies the current study attempts facilitate the finalization of an appropriate dosage after in feed administration in broiler, and to see the clinical utility of the dose and the route.

The plasma concentrations of tilmicosin have remained above MIC levels (0.05 µg/ml) (Abd-El Ghany, 2009) [1] up to 12 h in six birds that were treated with tilmicosin mixed with feed. However, there is wide variability in the plasma concentrations and also the time points at which it attained Cmax, which could be attributed to a variety of factors such as rate of feed consumption, health of individual birds, physiological idiosyncrasies among birds etc, the fact that adequate plasma levels have been attained at different time points reinforces the utility of the drug in the given dose range.

AUC is the total area under plasma concentration from time zero to infinity. It provides a measure of the extent of drug exposure and it depends directly on dose, modified by absorption and plasma clearance. In the present study $AUC_{0-\infty}$ ranged between 14.18 $\mu g.h.ml^{-1}$ and 29.33 $\mu g.h.ml^{-1}$ with a mean value of 21.51 $\mu g.h.ml^{-1}$. Perusal of literature illustrates huge variability in AUC values with some authors reporting mean AUCs of 67.96 ± 1.56 $\mu g.h.ml^{-1}$ (Keles *et al*., 2001) [7], while others reporting much lower AUCs in the range of 24.2 ± 3.9 and 23.7 ± 4.15 $\mu g.h.ml^{-1}$ (Abu-Basha *et al*., 2007; Elbadawy and Aboubakr, 2017) [2, 3]. The differences in AUC in various studies could be attributed to varying rates of drug intake which depends on factors such as bird strain, dosing pattern, feed and water intake of the birds.

Volume of distribution is the hypothetical volume of fluid, which would be required to contain the amount of drug in the body if it was uniformly distributed at a concentration equal to that in plasma. The V_d area obtained in the present study was recorded to be 417.96 ± 228.24 $L.Kg^{-1}$. Previous studies have indicated huge variability in the volume of distribution between the range of 20.4 ± 2.0 $L.Kg^{-1}$ and 1024.8 ± 87.5 $L.Kg^{-1}$ (Abu-Basha *et al*., 2007; Rassouli *et al*., 2016) [2, 11]. The high standard deviation in the V_d value points to the wide variability that exists between birds when given through the unconventional 'in feed' route. However, it can be surmised that tilmicosin has wide variability as also reported by higher levels of tilmicosin in the lungs (Sakthivel, 2017) [12].

Clearance is the volume of blood cleared of the drug by the various elimination processes per unit time. The clearance in the present study was 18.70 ± 10.86 $ml.min^{-1}.kg^{-1}$. Studies indicate that clearance of tilmicosin is slower in birds as compared to cattle and sheep (Mordic *et al*., 1998) [9]. Slower clearance means longer stasis in the body as clearly seen by presence of the drug up to 12 h.

Elimination half-life is the most frequently reported pharmacokinetic parameter. It is the time required for 50% of the drug to be eliminated from the body after the distribution equilibrium has been attained. It is an index of drug persistence in the body and the main clinical use of this parameter is to select an appropriate length for the dosing interval in circumstances of multiple dose administration. It also aids in the prediction of drug accumulation and the time to reach steady state equilibrium (Toutain and Bousquet-Melou, 2004) [13]. The elimination half-life was recorded to be 21.45 ± 17.21 h.

MRT is the mean time required for a drug molecule to traverse through the body and the $t_{1/2.}$

reflects the time associated with absorption, distribution and elimination. In the present study, the MRT of tilmicosin in chicken was found to be 5.70 ± 0.43.

It is important to note that of the eight birds subjected to trial, only six birds showed plausible pharmacokinetic behaviour. The concentration of tilmicosin remained higher than 0.1 µg/ml at various time points meaning that the drug attains a

concentration that is much higher than the MIC recommended by several studies (Abu Basha *et al*, 2001) [2]. Tilmicosin is a time dependent drug and the concentrations attained in the blood are sufficient to produce antibacterial effect. Based on the integration of pharmacokinetic and pharmacodynamic data, for a time dependent like tilmicosin, maintenance of plasma levels above MIC for 50-60% of the treatment period is important for successful clinical outcome (Toutain *et al*. 2002) [14].

Despite the wide variability in the pharmacokinetics, this study proves that tilmicosin given through feed can produce adequate concentration to be clinically relevant. However, it is to be noted two of the eight birds have not shown desired concentrations. It will be worthwhile to further study the same in infection models in chicken to prove the utility of this route. It is thus concluded that tilmicosin when administered through feed at the dose of 1 g/Kg. of feed in broiler chicken can be clinically useful against susceptible infections.

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