



Pharmacokinetics and Chicken Meat Residue of Tilmicosin after Administration Through Drinking Water in Broiler Chicken

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10.18805/ag.D-6192

ABSTRACT

Background: Tilmicosin is an antimycoplasmic drug of great utility in controlling mycoplasmosis in chicken. The drug despite its efficacy, has a high residue impact leading to a long withdrawal period. In this study, an attempt has been made to rationalize the dosage to reduce the residue burden by adopting a lower dose.

Methods: The dose of tilmicosin was arrived at based on a pilot study wherein different doses were administered, and the levels were checked in plasma and meat. In the main study, tilmicosin was administered to broiler chicken as a bolus oral dose or "in drinking water" at the dose rate of 25 mg. kg⁻¹ body weight. Blood was collected at different time points for the study. A trial to assay drug residues in tissues was also carried out. The assay of tilmicosin in chicken plasma/tissues was done using a sensitive HPLC assay.

Result: In both pharmacokinetics study groups, tilmicosin concentrations could be reasonably detected only in 4 of the eight birds tested. In the birds that responded, the mean plasma concentrations remained above the MIC values of 0.05 µg.ml⁻¹. The residue level in the lung and muscle could be detected up to day 14, and no residue from 18th day, after administration of tilmicosin through drinking water. The lung, the organ of mycoplasma infection revealed high concentrations of tilmicosin up to 14 days after the last dose. The results show that the Tilmicosin administered at a reduced rate of 25 mg.Kg⁻¹ can be useful therapeutically, with a reduced residue burden in broiler chicken.

Key words: Lung, Pharmacokinetics, PK-PD integration, Residue, Tilmicosin.

INTRODUCTION

Poultry industry has witnessed rapid expansion in the last few decades due to the increasing demand for poultry meat. Respiratory diseases in poultry are very common and pose serious risks in terms of economic loss (Rajkumar *et al.*, 2021; Parvin *et al.*, 2022). *Mycoplasma gallisepticum* is responsible for chronic respiratory diseases (CRD) in chickens as well as sinusitis in turkeys (Ley and Yoder, 2008; Nisa *et al.*, 2019). Macrolides are quite effective in treating *Mycoplasma gallisepticum* (Wu *et al.*, 2005; Duraisamy *et al.*, 2022). Macrolide antibiotics inhibit bacterial growth by binding to prokaryotic ribosomes and interfering with protein synthesis (Franceschi *et al.*, 2004). Tilmicosin, which is synthesized from tylosin, is a macrolide antibiotic and has an antibacterial spectrum similar to tylosin, but with enhanced activity against Gram-negative bacteria such as *Pasteurella* spp. and *Actinobacillus* spp. (Ziv *et al.*, 1995). Tilmicosin also tends to accumulate in higher concentrations in the lung, making it a suitable candidate for treating mycoplasmosis (Jianzhong *et al.*, 2005; Abu-Basha *et al.*, 2007; Zhang *et al.*, 2016). Tilmicosin is one of the most effective antibiotics for combating respiratory diseases in poultry. However, PK data when the drug is administered through unconventional routes like 'in water' and 'in feed' routes are relatively scarce and further studies are required to optimise the right dosage and reduce the residue load. In a previous study conducted by Duraisamy *et al.* (2022), the pharmacokinetic parameters

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How to cite this article: Anurag, B., Srinivasan, R., Thangamalai, R., Raghavan, G., Sesh, P.S.L. and Tirumurugaan, K.G. (2025). Pharmacokinetics and Chicken Meat Residue of Tilmicosin after Administration Through Drinking Water in Broiler Chicken. *Agricultural Science Digest*. **45 (Special Issue)**: 208-214. doi: 10.18805/ag.D-6192.

Submitted: 10-09-2024 **Accepted:** 27-11-2024 **Online:** 03-02-2025

of tilmicosin given @ 40 mg /Kg⁻¹ through 'oral' and 'in water' routes were satisfactory, but the residue load with the given dose was high and prolonged. The present study has been undertaken to identify the suitable dosage that will produce adequate therapeutic concentrations of the drug but leaving a lower residue burden in the meat.

MATERIALS AND METHODS

Chemicals and reagents

Tilimicosin standard was procured from Sigma (Cat No. 33864) for the purpose of the study. Tilimicosin required for administration was tilimicosin phosphate which was given *gratis* from Huve Pharma. Other chemicals for assay such as Ammonium formate, acetonitrile, methanol and trifluoroacetic acid, were procured as LC grade.

Pharmacokinetic trial

A pilot study with two birds for every dose was conducted in which tilimicosin was given by feed or through drinking water and the plasma concentrations were studied in the birds. Based on the pilot study, the dose of tilimicosin was fixed at 25 mg.Kg⁻¹.

In the main study, a total of sixteen three-week old chicken (vencobb strain) of either sex weighing 0.5 to 0.8 Kg approx. procured from the local market were used. 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 experiment was conducted in 2021 at Madras Veterinary College. The birds were given access to ad libitum feed and water and they were tagged for the purpose of identification.

On the day of the trial, one group of birds was administered tilimicosin through oral route and the total dose was given as a bolus dose. The dose used was 25 mg.kg⁻¹ body weight. Another group of birds was administered tilimicosin through "in water" route by mixing with drinking water at the dose rate of 25 mg.kg⁻¹ body weight. During acclimatization, the mean water consumption was recorded. On the test day, the birds were deprived of water for a few hours to stimulate water consumption. The drug was mixed with 25% of mean volume of water consumed and kept for drinking. After the birds had consumed all the medicated water, plain water was given up to 24 hrs. In both the groups of birds, blood was collected from the tarsometatarsal vein into heparin coated microcentrifuge tubes at 0 min, 15 min, 30 min, 1 hr, 2 hrs, 3 hrs, 4 hrs, 8 hrs, 12 hrs and 24 hrs post drug administration. The blood samples were subjected to vortexing and centrifugation (2000xg for 10 minutes) to separate the plasma. The plasma collected at different time points was stored at -20°C until further use.

Residue trial

A total of 15 three-week old chicken (vencobb strain) were reared in a deep litter system and given sufficient time for acclimatization. Tilimicosin was administered through the drinking water at the dose rate of 25 mg.kg⁻¹ for 5 days. The drug was mixed in 1/4th of the water consumed by each bird in a day and supplemented with fresh water upon exhaustion of medicated water. After the fifth day, the birds were slaughtered on days 10, 14, 18, 22 and 25 after the last dose @ three birds per time point. Pieces of lung and

muscles were collected to assess the residue status in these organs. The tissues were subjected to HPLC assay. The value was expressed as the mean±SE of three tissues was for every time point.

Assay of tilimicosin using HPLC

HPLC

The HPLC system consisted of pump (515 HPLC) detector (Waters 2489 UV/ Visible), connected to Empower® software.

Sample preparation

To 450 µl of plasma sample, equal volume of acetonitrile was added. The tube was vortexed and centrifuged at 2000 g and then the supernatant was filtered using 0.2 µm filter. The lung or muscle tissue was prepared by homogenization with 1:1 water (5 grams in 5 ml). To 450 µl of tissue homogenate 50 µl of perchloric acid was added, vortexed and centrifuged at 1000xg for 5 min. The supernatant was filtered using 0.2 micorn filter. 10 µl of plasma / tissue supernatant was injected into the HPLC system.

The mobile phase consisted of ammonium formate, acetonitrile and methanol in the ratio of 60:30:10. C₁₈ column was the stationary phase.

Calculation of concentrations of tilimicosin

A standard curve using tilimicosin was constructed separately for lung tissue, muscle tissue and plasma, to quantify the concentration of tilimicosin. The concentrations of tilimicosin in plasma or tissues were calculated from the standard curve constructed using spiked plasma, muscle or lung samples.

Recovery of tilimicosin

Recovery was calculated for plasma/ meat standards as a ratio of the peak areas obtained for tissue-based standards and those obtained for mobile phase based standards. For each concentration, three determinants were made. Per cent recovery was calculated according to the regression formula:

$$\text{Per cent recovery} = \frac{n \in XY - (\in X)(\in Y)}{n \in X^2 - (\in X)^2}$$

Where,

X = Concentration of drug spiked.

Y = Concentration found by assay method.

(recovery × concentration spiked).

n = Number of observations.

Pharmacokinetic analysis

The pharmacokinetic analysis of tilimicosin after administration was calculated based on the 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-inf} = AUC_{0-t} + (C_{last}/β).
- t_{1/2β} - Elimination half-life, where t_{1/2β} = 0.639/β.
- t_{max} - Time at which C_{max} was observed.

- C_{max} - The maximum concentration and the corresponding peak time (t_{max}) were taken as observed.
- Clearance - Cl/F – clearance with the formula Dose / AUC
- Volume of distribution - Vd/F - calculated as Dose / ($\beta \cdot AUC$).
- Relative bioavailability (F) of the drinking water route was determined as the ratio of AUC of drinking water to that of the oral route, calculated based on mean value of the two groups.

RESULTS AND DISCUSSION

Recovery of tilmicosin

The ability of the assay procedure to extract tilmicosin from the plasma matrix is expressed in terms of recovery. The recovery of tilmicosin from chicken plasma is given in Table 1. The acceptable recovery percentage for an assay procedure is 80-120% (Urban *et al.*, 2009). In this method, the recovery was 85.07%, which was within the acceptable range.

Calibration curve

The calibration curves for methanolic and plasma standards returned with R^2 values of 0.99, which is

indicative of very high reliability over a broad range from 0.1 μg to 5 $\mu\text{g}/\text{ml}$. The calibration curve of plasma standard is depicted in Fig 1.

Plasma concentrations of tilmicosin through “in crop” and “in water” route

The plasma concentrations of tilmicosin revealed a highly irregular pattern of absorption. Only four out of 8 birds responded to treatment orally and “in water”. This inconsistency in the water route can be expected since the drug entry into the body largely depends on water consumption patterns, which may vary among birds. However, the birds that had responded, the plasma concentration remained above a MIC value of 0.05 $\mu\text{g}/\text{ml}$ for up to 8 hrs. In the group that was given orally, therapeutic value was maintained up to 12 hours. This satisfies the PK/PD value of T/MIC as the plasma concentrations of tilmicosin when administered through the oral route, have remained above the MIC value of 0.05 $\mu\text{g}/\text{ml}$ (Abd-El Ghany, 2009) for the majority of the treatment duration. The tilmicosin plasma concentrations could be detected only till 8 hrs when administered through “in water” route, which could be attributed to the degradability of the drug when exposed to

Table 1: Analytical recovery of tilmicosin from chicken plasma.

Concentration spiked ($\mu\text{g} \cdot \text{ml}^{-1}$)	Methanol peak area	Plasma spiked peak area	Percentage range	Mean	Recovery percentage
0.1 $\mu\text{g}/\text{ml}$	910	812	89.23	85.42	
0.1 $\mu\text{g}/\text{ml}$	877	688	78.44		
0.1 $\mu\text{g}/\text{ml}$	1001	887	88.61		
0.5 $\mu\text{g}/\text{ml}$	8616	7643	88.70	86.22	85.07%
0.5 $\mu\text{g}/\text{ml}$	8653	7896	91.25		
0.5 $\mu\text{g}/\text{ml}$	9991	7865	78.72		
1 $\mu\text{g}/\text{ml}$	18754	15328	81.73	83.58	
1 $\mu\text{g}/\text{ml}$	20154	15122	75.03		
1 $\mu\text{g}/\text{ml}$	17654	16593	93.99		

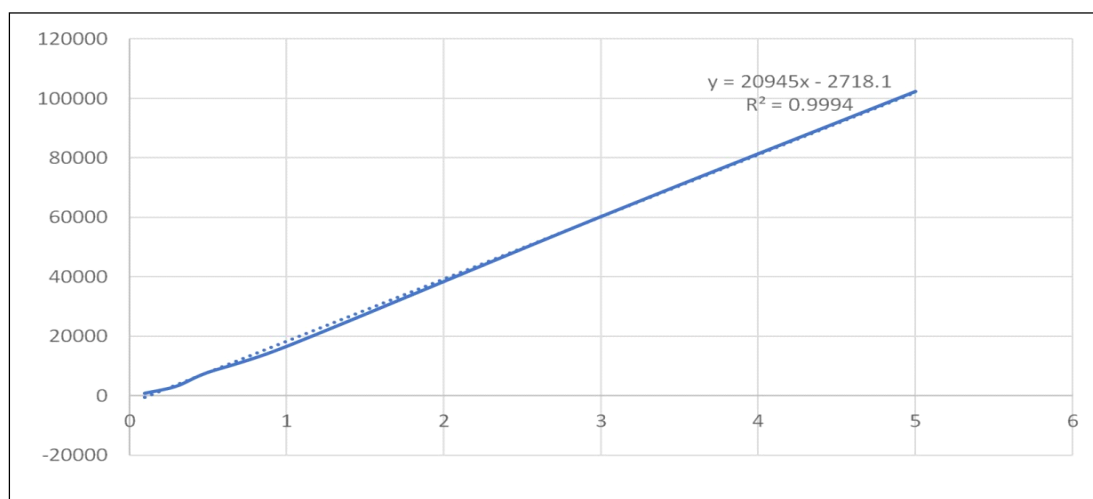


Fig 1: Standard Curve of tilmicosin in plasma detected by HPLC.

water coupled with the heightened scope for contamination (Gbylik-Sikorska *et al.*, 2016). The plasma time concentration graphs of tilmicosin through oral and “in water” routes are depicted in Fig 2. The chromatogram depicting tilmicosin detection is given in Fig 3 (a).

Pharmacokinetic parameters

The important pharmacokinetic parameters of tilmicosin calculated for the two routes are shown in Table 2. In the

present study, when the drug was administered orally, $AUC_{0-\infty}$ ranged between $4.02 \mu\text{g.h.ml}^{-1}$ and $18.23 \mu\text{g.h.ml}^{-1}$, with a mean value of $8.61 \mu\text{g.h.ml}^{-1}$. When administered through “in water” route, $AUC_{0-\infty}$ ranged between $0.99 \mu\text{g.h.ml}^{-1}$ and $8.78 \mu\text{g.h.ml}^{-1}$ with a mean value of $4.73 \mu\text{g.h.ml}^{-1}$. AUC is the total area under plasma concentration from time zero to infinity, thus providing a measure of the extent of drug exposure which depends directly on dose and is modified by absorption and plasma clearance. On perusal of the

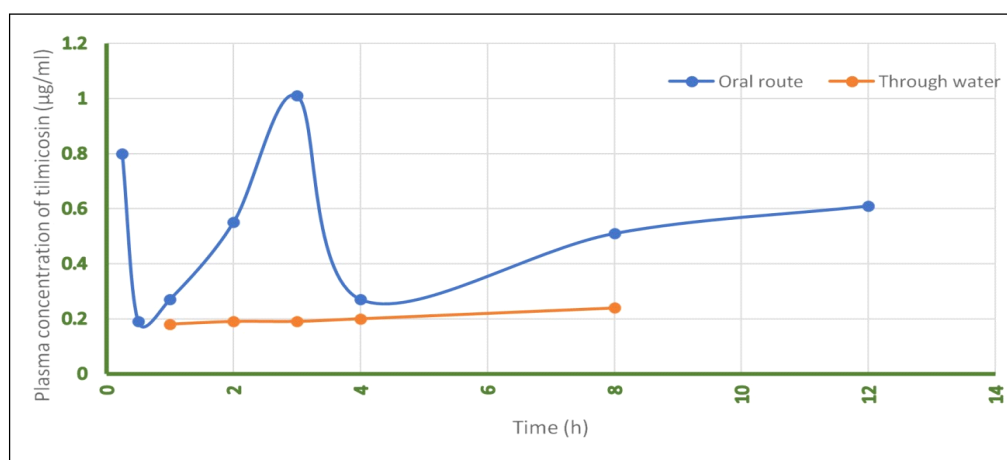


Fig 2: Plasma concentrations of tilmicosin after administration through oral route and drinking water (25 mg.Kg^{-1}).

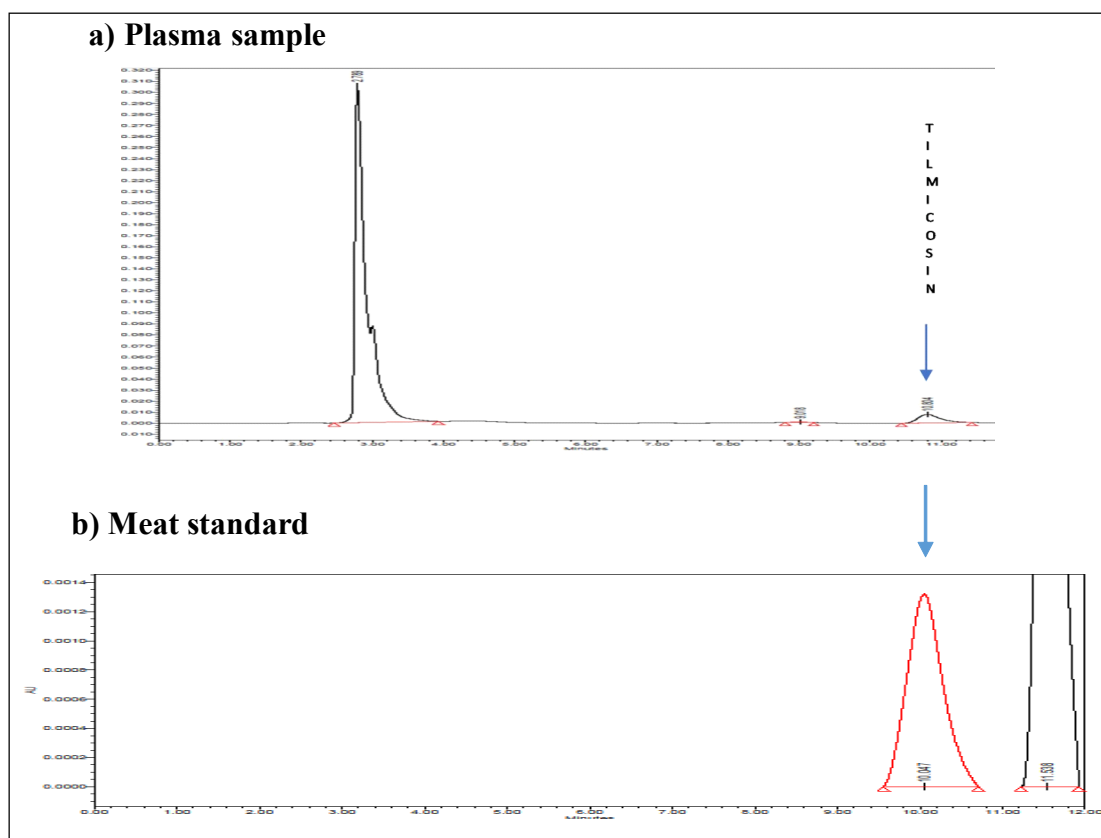


Fig 3: Chromatogram of tilmicosin.

literature indicates wide variability in AUC values, with some authors reporting mean AUCs of $67.96 \pm 1.56 \mu\text{g.h.ml}^{-1}$ (Keles *et al.*, 2001), while others reported much lower AUCs in the range of 24.2 ± 3.9 and $23.7 \pm 4.15 \mu\text{g.h.ml}^{-1}$ (Abu-Basha *et al.*, 2007; Elbadawy and Aboubakr, 2017). The elimination half-life was recorded to be 6.13 ± 5.67 hrs for the 'in crop' group and 13.24 ± 13.01 hrs for the 'in water' group. 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 under the 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). The parameters of AUC and C_{max} are important to indicate the drug absorption pattern and the persistence in the body. However, it is to be borne in mind that some of the birds did not respond with adequate concentration. This could be due to the wide variation in consumption pattern and possible degradation of the drug in water or in the GI tract. The relative bioavailability of tilmicosin after administration through drinking water was reported as 54.94%, which suggests appreciable absorption and suitability of water route.

PK - PD integration

In this study, we have not carried out the MIC of tilmicosin against *Mycoplasma*. However, the MIC of $0.05 \mu\text{g.ml}^{-1}$ (Abd-El Ghany, 2009) has been taken into consideration. Since tilmicosin is a time-dependent antibiotic with the surrogate parameter of $T > \text{MIC}$, satisfactory efficacy can be achieved if the concentration above MIC were maintained for 50-80% of the treatment period (Toutain *et al.*, 2002). In our study, the concentrations above the MIC value are maintained up to 12 hours (oral) and up to 8 hrs (in water). This suggests that the dose used will be clinically relevant.

Tissue concentration of tilmicosin

The concentrations of tilmicosin in lung (from a therapeutic perspective) and muscle (from a residue perspective) were detected and quantified in the present study (Fig 3b and Table 3).

Lung tissue

In the present study, the concentrations of tilmicosin in the lung were assayed. Even if there could be some apprehensions about the utility of tilmicosin, vis a vis its plasma concentrations, the concentration of tilmicosin in the lung is clinically important since the lungs are the seat of infection of *Mycoplasma* in chicken. Studies have shown that the levels of tilmicosin are many fold higher than in plasma. In our previous study too, lung concentration was higher than that of plasma (Duraismy *et al.*, 2022). But that was at a higher dose of 40 mg /Kg. We wanted to test if the reduction in the dose would affect the lung concentration. To our satisfaction, even at the reduced dose used in this study, the concentration in lungs on day 10 ($0.99 \mu\text{g.g}^{-1}$) and day 14 ($0.75 \mu\text{g.g}^{-1}$) were far higher than the MIC for the organism, which suggests a satisfactory dose level used in this study.

Muscle residue

From a food safety point of view, it is important to ensure that the drug concentration remains lower in the edible tissues. In our previous study, (Duraismy *et al.*, 2022), the residue load was found to be $4.61 \mu\text{g/g}$ on the 14th day and $1.37 \mu\text{g/g}$ on the 22nd day when tilmicosin was administered via the 'in water' route at the dose rate of 40 mg/kg body weight. Our idea was to minimize the residue burden. In this study, we found that reducing the dose to 25 mg.Kg⁻¹, the last detected residue was at 14 days and no residue could be detected beyond that. The FSSAI standards (FSSAI, 2011) and Codex (2018) prescribe an MRL of $0.15 \mu\text{g/g}$.

Table 2: Pharmacokinetic parameters of tilmicosin through 'oral' and 'in water' route (25 mg / Kg⁻¹) in broiler chicken.

Parameters	Mean $\pm$ SD (in crop)	Mean $\pm$ SD (in water)	Units
Half life	6.13 ± 5.67	13.24 ± 13.01	h
C_{max}	1.0 ± 0.53	0.25 ± 0.06	$\mu\text{g.ml}^{-1}$
T_{max}	4 ± 2.7	4.25 ± 2.62	h
AUC_{0-t}	4.79 ± 3.43	1.17 ± 0.73	$\mu\text{g.h.ml}^{-1}$
$\text{AUC}_{0-\text{inf}}$	8.61 ± 6.50	4.73 ± 3.42	$\mu\text{g.h.ml}^{-1}$
Relative bioavailability (F)	-	54.94	per cent

Table 3: The residue of tilmicosin in lungs and muscles after administration through drinking water (25 mg / Kg⁻¹) in broiler chicken.

Days	Lungs	Muscles
10 th Day	$0.99 \pm 0.09 \mu\text{g/g}$	$0.80 \pm 0.44 \mu\text{g/g}$
14 th Day	$0.75 \pm 0.04 \mu\text{g/g}$	$0.44 \pm 0.19 \mu\text{g/g}$
18 th , 22 nd and 25 th days	Below detectable limits	

In our study the residue was above MRL (0.80 µg/g) on 10th day but below MRL on 14th day. This is advantageous in the farm practice since the drug can be administered up to 4th week of age since the marketing age is 6 weeks or more.

CONCLUSION

In conclusion, tilmicosin @ 25 mg. kg⁻¹ used in this study, can be considered suitable for administration through water. Since the concentration in the lungs are adequate and are maintained far beyond the period of therapy. The results of residue studies also indicate a desirable reduction in residue status while proving to be effective in the lungs. The experiment has to be further replicated with a larger number of birds to get a clearer picture.

ACKNOWLEDGEMENT

The present study was supported and funded by Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Tamil Nadu.

Declaration

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript

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