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# INTERACTION KINETICS OF ENROFLOXACIN WITH HYDRATED SODIUM CALCIUM ALUMINOSILICATE – A TOXIN BINDER IN BROILER CHICKEN AUTHORS

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## ABSTRACT

The study was conducted to explore the influence of hydrated sodium calcium aluminosilicate (HSCAS), a toxin binder commonly used in poultry feed, on the pharmacokinetic behavior of enrofloxacin in broiler chicken. Control group received normal feed free of toxin binder whereas HSCAS group was supplemented with HSCAS @ 0.5% in feed. Enrofloxacin was administered as single pulse dose (@10mg.kg$^{-1}$) through drinking water to both the groups. Blood samples were collected at predetermined time intervals after drug administration and plasma was separated and analyzed for enrofloxacin concentrations using HPLC. Highly significant ($p<0.01$) decrease in mean plasma concentration of enrofloxacin was noticed in HSCAS group at 4 and 6h which was preceded (2h) by significant ($p<0.05$) decrease when compared to control group. The $C_{max}$ of HSCAS group was significantly ($p<0.01$) lower than the control group (0.97±0.06 *vs* 1.38±0.04µg.mL$^{-1}$). $T_{max}$ was significantly ($p<0.05$) delayed in HSCAS group (6.67±0.67 *vs* 4.33±0.67h) when compared to control group. Highly significant increase in $V_{d\ ss}/F$ (9.34±0.48 *vs* 7.21±0.20L.kg$^{-1}$) was noticed in HSCAS group when

compared to control group. Relative bioavailability of HSCAS group was calculated to be 88.88±15.03% by treating the bioavailability of control group as 100%. The other pharmacokinetic parameters did not differ significantly though there were numerical differences. Integration of PK/PD parameters revealed that the dose of enrofloxacin (10mg.kg$^{-1}$) was sufficient to treat only moderately sensitive organisms (MIC≤0.125µg.mL$^{-1}$) both in the presence and absence of toxin binder and increase in dosage is needed to treat less sensitive organisms. Hence it is suggested to either increase the dosage of enrofloxacin or withdraw the feed containing HSCAS during enrofloxacin treatment in broiler chicken in order to prevent the emergence of resistance as well as to obtain clinical cure.

**KEYWORDS:** Pharmacokinetics, Enrofloxacin, HSCAS, Toxin binder, Pulse dosing, HPLC.

## INTRODUCTION

Intensive rearing of poultry with high stocking density and selection for fast growing strains necessitates the use of antimicrobial agents to combat bacterial infections. Among the various classes of antimicrobials used in poultry, fluoroquinolones are one of the most important categories. Enrofloxacin was the first fluoroquinolone developed and introduced exclusively for veterinary use. The drug is widely used in birds due to its broad spectrum of activity, bactericidal effect at low concentration, large volume of distribution and high bioavailability.[1,2] Since the introduction of enrofloxacin numerous pharmacokinetic studies have been conducted in various species. But very limited work has been carried out on the interaction kinetics of enrofloxacin with co-administered drugs and feed additives. Among the feed additives, toxin binders or adsorbents form an important inclusion in poultry diet to combat the problem of mycotoxicosis.[3]

Toxin binders available in the market are composite mixtures of many ingredients of which hydrated sodium calcium aluminosilicate (HSCAS) is an important and major ingredient. HSCAS was developed by Phillips *et al.*[4] and later reported to be aflatoxin selective binder. But Huwig *et al.*[5] reported the need for high inclusion rates of vitamins and minerals when HSCAS was added in the feed. Hence the specificity of HSCAS with respect to aflatoxin is speculative and the present study was undertaken to explore the influence of toxin binder hydrated sodium calcium aluminosilicate on the pharmacokinetics and bioavailability of widely used antimicrobial, enrofloxacin administered through drinking water in broiler chicken.

## MATERIALS AND METHODS

### Experimental birds

The study was conducted in twenty four six weeks old apparently healthy broiler chicken of Cobb strain of either sex weighing between 2.0 and 2.2kg. The birds were procured from a commercial broiler farm at the age of 4 weeks and were acclimatized for two weeks prior to commencement of the experimental trial. The birds were reared in individual cages under standard and uniform conditions with natural day-night cycle and fed *ad libitum* feed and water free of antibacterials. The experimental trial on birds was approved by Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India.

### Drugs and Chemicals

Pure compound of enrofloxacin, ciprofloxacin hydrochloride and analytical grade heparin sodium salt were procured from M/s. Himedia Laboratories Private Limited, India.

HSCAS was obtained as *gratis* from M/s. Nutricon Ltd., Chennai. Acetonitrile, methanol and triethylamine of HPLC grade and orthophosphoric acid (analytical grade) were procured from M/s Merck Specialities Limited, India. All solvents and solutions used for HPLC analysis were filtered through 0.2µ HNN nylon membrane filter (MDI Advanced Microdevices Pvt. Ltd, India) and degassed using sonicator.

### Administration of drug and collection of blood sample

The birds were divided into two groups of twelve each and control group was provided with normal feed free of drugs and toxin binder whereas HSCAS group received normal feed supplemented with 0.5 per cent HSCAS starting from the acclimatization period to the end of the trial. Birds in both the groups were administered enrofloxacin as single pulse dose through drinking water at the rate of 10mg.kg$^{-1}$ body weight. During the acclimatization period of the trial, the mean daily water consumption of each bird was recorded. During the experimental trial the total dose of enrofloxacin was dissolved in one fourth volume of the total daily water intake of the bird and assured that it was consumed within 4h. After consumption of medicated water, the birds were provided drug free water *ad libitum* for the rest of the day. The birds were deprived of feed and water for 2h before administration of medicated water.

Blood samples (0.75 to 1mL) were collected from medial metatarsal vein in heparinised tubes (10U.mL$^{-1}$) immediately before and at 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 30.0, 36.0 and 48.0h after drug administration. Plasma was separated by centrifuging at 1000g for 10 minutes using micro centrifuge and stored at -80°C until assay.

**Drug assay**

Concentrations of enrofloxacin and its metabolite ciprofloxacin in plasma were assayed using HPLC as per the method of Kung *et al*.[6] The HPLC system consisted of LC-20AD double plunger pump, PDA detector and LC Solution software for data analysis. A reverse phase $C_{18}$ column (Hibar® 250-4, 6 RP-18 endcapped, particle size 5µm, 4.6 x 250mm, Merck, Darmstadt, Germany) served as a stationary phase. The column was protected with a 2 to 8mm Phenomenax® guard column (KJO-4282). The mobile phase consisted of a mixture of acetonitrile: methanol: water in the ratio of 17:3:80 containing 0.4 per cent orthophosphoric acid and 0.4 per cent triethylamine (pH adjusted to 3.0 with triethylamine). The scan range of photodiode array UV-Vis detector was 220 to 400nm, and the detection wavelength was 278 nm. The flow rate of mobile phase was 1.0mL.min$^{-1}$ and samples were analyzed for 10 minutes at 40°C. There were no interfering peaks in the plasma at the retention time of ciprofloxacin (5.85 min) and enrofloxacin (7.45 min). The data collected were analyzed with chromatopak software taking into account the peak area of the drug.

The plasma samples were subjected to liquid-liquid extraction according to the method of Nielsen and Hansen.[7] To 500µL of plasma, 750µL of HPLC grade acetonitrile was added in the ratio of 1:1.5 and vortexed thoroughly for 15 seconds and centrifuged at 900g for 10 min at 4°C. The clear supernatant thus obtained was mixed with twice the volume of water in a separate microcentrifuge tube and mixed by vortexing. The aliquot was then filtered through 0.2µ HNN nylon membrane filter and 20µL of this filtrate was injected into the HPLC system.

Working plasma standards of enrofloxacin and ciprofloxacin (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5, and 10.0µg.mL$^{-1}$) were prepared from respective stock solutions after diluting with pooled drug free chicken plasma. The plasma standards were subjected to liquid-liquid extraction and analyzed using HPLC as described above. Standard calibration curves for spiked plasma samples were prepared separately for enrofloxacin and ciprofloxacin by plotting peak area against concentration of the drug. The standard curves of enrofloxacin and ciprofloxacin

were linear in the range of 0.025 to 10.0µg.mL$^{-1}$ and 0.05 to 10µg.mL$^{-1}$, respectively. The equation for enrofloxacin obtained from the calibration plot was y= 27618x + 2779.7 with $r^2$ value > 0.999. The equation for ciprofloxacin from the calibration plot was y= 18495x + 177.1 with $r^2$ value > 0.997. The overlay report for various concentrations of enrofloxacin and ciprofloxacin is shown in Fig. 1.

The mean absolute recovery was within the range of 95.12 to 99.67 per cent for plasma and the percentage of CV was 1.76 to 5.80 per cent suggesting the suitability of the method for analysis of enrofloxacin and ciprofloxacin in chicken plasma.[8] The intra-day and inter-day CVs were within the limits (<15 %) specified and hence the method was suitable for assay of both enrofloxacin and ciprofloxacin in chicken plasma. The limit of detection and quantification were 0.01 and 0.025 µg.mL$^{-1}$ for enrofloxacin and 0.025 and 0.05µg.mL$^{-1}$ for ciprofloxacin, respectively.

**==== Shimadzu LC solution Analysis Report ====**

Data1 : C:\Documents and Settings\micro\Desktop\Mekala standard\0.025microgram.lcd

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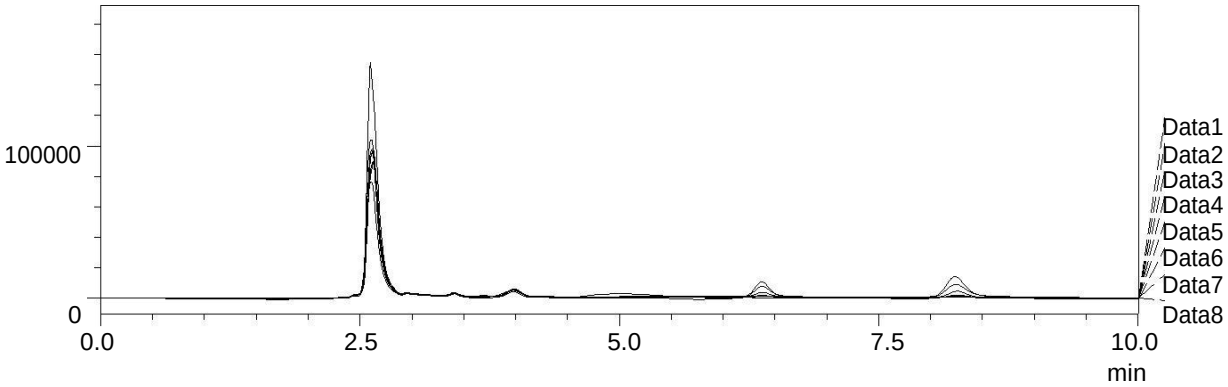
Data4 : C:\Documents and Settings\micro\Desktop\Mekala standard\0.25 microgram.lcd

Data5 : C:\Documents and Settings\micro\Desktop\Mekala standard\0.5 microgram.lcd

Data6 : C:\Documents and Settings\micro\Desktop\Mekala standard\2.5 microgram.lcd

Data7 : C:\Documents and Settings\micro\Desktop\Mekala standard\5 microgram.lcd

Data8 : C:\Documents and Settings\micro\Desktop\Mekala standard\10 microgram.lcd uV



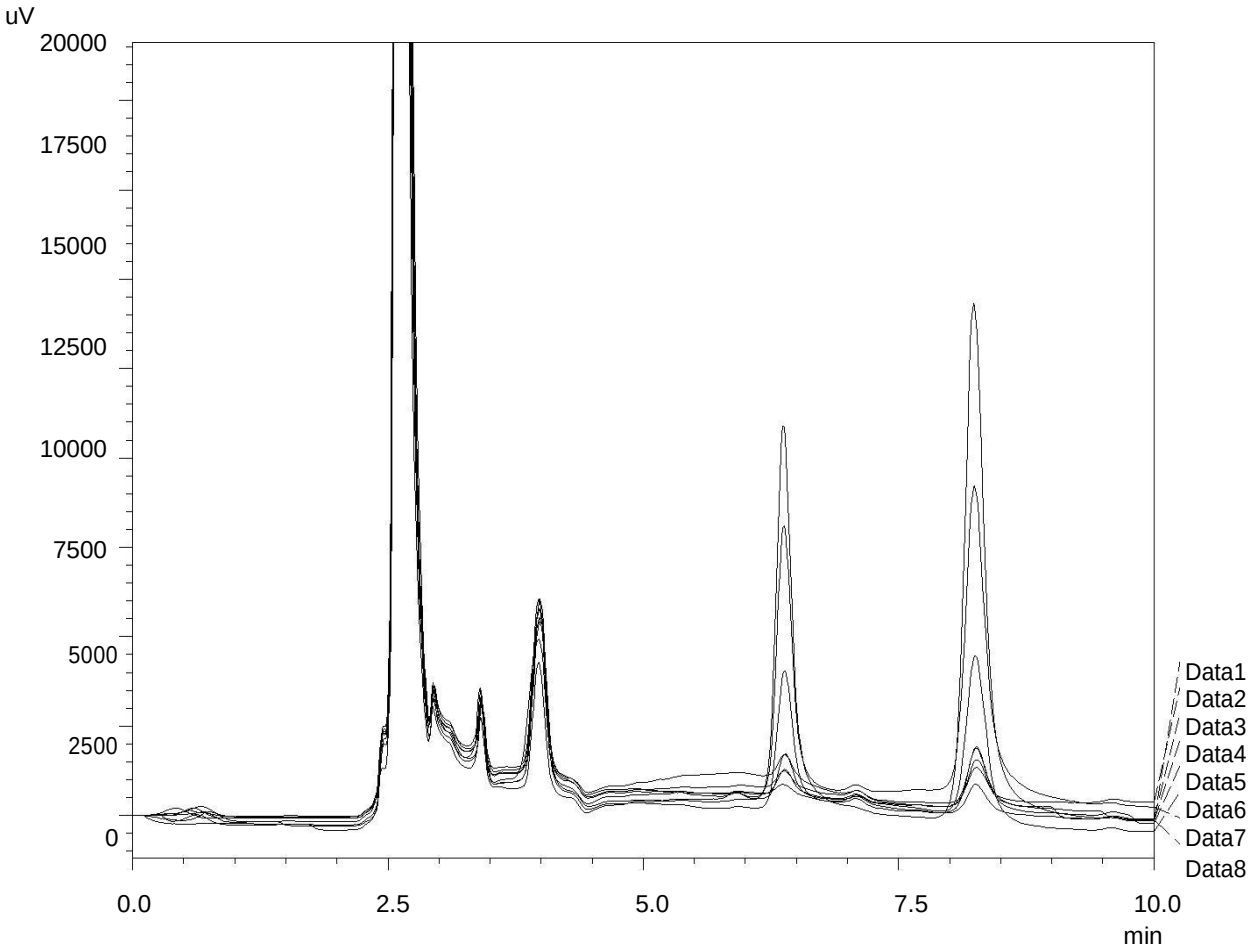



**Fig. 1. Overlay report of ciprofloxacin and enrofloxacin in broiler chicken plasma**

**Pharmacokinetic analysis**

The plasma drug concentration-time data of enrofloxacin were analyzed by non-compartmental techniques based on statistical moments theory[9] using the pharmacokinetic software PK function.[10] The terminal elimination rate constant (β) was calculated from semi-logarithmic plot of the concentration-time curve using linear regression analysis.

The elimination half-life ($t_{1/2\beta}$) was calculated according to $t_{1/2\beta} = \ln 2/\beta$, where ln 2 = 0.693. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated using the trapezoidal rule and extrapolated to infinity by means of the elimination rate constant. The mean residence time (MRT = AUMC/AUC), total body clearance ($CL_B/F$ = Dose/AUC), volume of distribution at steady state ($Vd_{ss}/F = CL_B$ / MRT) and apparent volume of distribution ($Vd_{area}/F$ = Dose/β x AUC) were calculated. Relative bioavailability is calculated by comparing the AUC of both the groups. The maximum plasma concentration ($C_{max}$) and time to reach maximum concentration ($t_{max}$) were taken from the observed values.

**Pharmacokinetic/pharmacodynamic (PK/PD) integration**

The hypothetical $MIC_{90}$ values (0.05, 0.125, 0.25 and 0.5μg.mL$^{-1}$) and hypothetical MPC values (0.2, 0.5, 1.0 and 2μg.mL$^{-1}$) which are four times the MIC were taken into consideration for calculation of AUC/MIC, $C_{max}$/MIC, T>MIC and AUC/MPC, $C_{max}$/MPC, T>MPC, respectively.

**Statistical analysis**

Statistical analysis of the data was performed by using SPSS 17.0 software. The results were expressed as mean ± SE. Harmonic mean was used with data not distributed normally. Student's t-test was applied to find out the significance between the groups.[11]

**RESULTS**

The plasma concentrations of enrofloxacin at different time intervals were analyzed (Table 1) and the pharmacokinetic parameters (Table 2) were calculated for all the samples and expressed as mean (±SE). Ciprofloxacin, the metabolite of enrofloxacin could not be determined consistently in many samples and hence not reported. Inconsistency in detection of ciprofloxacin was also reported in chicken[12] and Muscovy ducks.[13] Ovando *et al.*[14] and Jakubowski *et al.*[15] also reported that the metabolic conversion was less than 10 and 7 per cent, respectively and hence did not calculate the pharmacokinetic parameters for ciprofloxacin. In this study also meaningful pharmacokinetics could not be established for ciprofloxacin as a metabolite of enrofloxacin and hence the pharmacokinetics of enrofloxacin alone is reported.

After administration of enrofloxacin through drinking water as pulse dosing, enrofloxacin was detected in plasma as early as 0.5h in all the samples. In control group the mean (±SE) initial concentration was 0.41±0.04μg.mL$^{-1}$ which gradually increased to 1.24±0.06μg.mL$^{-1}$ at 6h and then declined to 0.03±0.01μg.mL$^{-1}$ at 48h. The plasma concentrations in samples could be detected up to 36h in all samples and up to 48h in three samples. Similarly in HSCAS group enrofloxacin was quantified in all post dosing samples as early as 0.5h with mean (±SE) value of 0.35±0.04μg.mL$^{-1}$. Enrofloxacin was detected in all samples up to 36h and two samples up to 48h. In both the groups mean enrofloxacin concentration at 12h was 0.5μg.mL$^{-1}$ and above. Highly significant ($p<0.01$) increase in mean plasma concentration was noticed in control group at 4 and 6h which was preceded (2h) by significant ($p<0.05$)

increase when compared to HSCAS group. In the remaining period there was no significant difference between the two groups.

**Table 1. Comparison of mean plasma concentration (μg.mL$^{-1}$) after single pulse dosing of enrofloxacin alone and in the presence of HSCAS**

| Time (h) | Mean±SE | |
|---|---|---|
| | Control Group | HSCAS Group |
| 0.5 | 0.41±0.04 | 0.35±0.04 |
| 1 | 0.53±0.11 | 0.43±0.04 |
| 1.5 | 0.72±0.12 | 0.50±0.03 |
| 2 | 0.96*±0.15 | 0.56±0.03 |
| 4 | 1.22**±0.09 | 0.82±0.06 |
| 6 | 1.24**±0.06 | 0.90±0.06 |
| 8 | 0.96±0.05 | 0.87±0.07 |
| 10 | 0.73±0.06 | 0.68±0.05 |
| 12 | 0.53±0.07 | 0.50±0.06 |
| 24 | 0.34±0.06 | 0.30±0.03 |
| 30 | 0.16±0.03 | 0.17±0.02 |
| 36 | 0.10±0.02 | 0.08±0.01 |
| 48 | 0.03±0.01 | 0.02±0.01 |

* p<0.05 ** p<0.01

**Table 2: Comparison of mean pharmacokinetic parameters after single pulse dosing of enrofloxacin alone and in the presence of HSCAS**

| Parameters | Units | Mean±SE | |
|---|---|---|---|
| | | Control Group | HSCAS Group |
| β | $h^{-1}$ | 0.080±0.008 | 0.076±0.004 |
| $AUC_{0-\infty}$ | $\mu g.h.mL^{-1}$ | 19.67±1.68 | 16.42±1.24 |
| $AUMC_{0-\infty}$ | $\mu g.h^2.mL^{-1}$ | 285.83±44.07 | 251.57±30.62 |
| MRT | h | 14.09±1.10 | 15.12±0.78 |
| MAT | h | 5.23±1.10 | 6.26±0.78 |
| $V_{d\ area}/F$ | $L.kg^{-1}$ | 6.39±0.13 | 8.13±0.45 |
| $V_{d\ ss}/F$ | $L.kg^{-1}$ | 7.21±0.20 | 9.34**±0.48 |
| $Cl_B/F$ | $L.h^{-1}.kg^{-1}$ | 0.53±0.05 | 0.63±0.04 |
| $t_{1/2\beta}$ | h | 8.70±0.74 | 9.12±0.48 |
| $C_{max}$ | $\mu g.mL^{-1}$ | 1.38**±0.04 | 0.97±0.06 |
| $t_{max}$ | h | 4.33±0.67 | 6.67*±0.67 |
| $F_{rel}$ | % | 100 | 88.88±15.03 |

* p<0.05 ** p<0.01

$AUC_{0-\infty}$ and $AUMC_{0-\infty}$ of control group was 1.2 and 1.14 fold higher than HSCAS group, respectively. The MRT, MAT, $V_{d\ area}/F$, $Cl_B/F$ and $t_{1/2\beta}$ were 1.07, 1.20, 1.27, 1.19 and 1.05 folds higher in HSCAS group when compared to control group, respectively. The $V_{d\ ss}/F$ of HSCAS group was 1.30 fold higher than control group and was highly significant ($p<0.01$). The $C_{max}$ of control group was significantly ($p<0.01$) higher by 1.41 fold than HSCAS group. $T_{max}$ was delayed in HSCAS group (6.67±0.67h) which was significant ($p<0.05$) when compared to control group (4.33±0.67h). Relative bioavailability for HSCAS group was calculated to be 88.88% by treating the bioavailability of control group as 100%. Except $V_{d\ ss}/F$, $C_{max}$ and $t_{max}$ there was no significant difference in rest of the pharmacokinetic parameters.

**PK/PD integration**

The PK/PD integration parameters such as AUC/MIC, $C_{max}$/MIC, T>MIC, AUC/MPC, $C_{max}$/MPC and T>MPC were calculated from the obtained PK parameters and presented in Table 3a and 3b.

**Table 3a. PK/PD integration**

| MIC ($\mu g.mL^{-1}$) | $AUC_{0-24}$/MIC | | $C_{max}$/MIC | | T>MIC | |
|---|---|---|---|---|---|---|
| | Control Group | HSCAS Group | Control Group | HSCAS Group | Control Group | HSCAS Group |
| 0.05 | 319.25±19.07 | 261.30±16.34 | 27.50±0.77 | 19.47±1.27 | 36 | 36 |
| 0.125 | 127.70±7.63 | 104.52±6.53 | 11.00±0.31 | 7.79±0.51 | 30 | 30 |
| 0.25 | 63.85±3.82 | 52.26±3.27 | 5.50±0.15 | 3.89±0.25 | 24 | 24 |
| 0.5 | 31.93±1.91 | 26.13±1.63 | 2.75±0.08 | 1.95±0.13 | 12 | 12 |

**Table 3b. PK/PD integration**

| MPC ($\mu g.mL^{-1}$) | $AUC_{0-24}$/MPC | | $C_{max}$/MPC | | T>MPC | |
|---|---|---|---|---|---|---|
| | Control Group | HSCAS Group | Control Group | HSCAS Group | Control Group | HSCAS Group |
| 0.2 | 79.81±4.77 | 65.33±4.08 | 6.88±0.19 | 4.87±0.32 | 24 | 24 |
| 0.5 | 31.93±1.91 | 26.13±1.63 | 2.75±0.08 | 1.95±0.13 | 12 | 12 |
| 1.0 | 15.96±0.95 | 13.07±0.82 | 1.38±0.04 | 0.97±0.06 | 6 | - |
| 2.0 | 7.98±0.48 | 6.53±0.41 | 0.69±0.02 | 0.49±0.03 | - | - |

**DISCUSSION**

Enrofloxacin administered as single pulse dosing through drinking water to control group attained maximum plasma concentration ($C_{max}$) of 1.38±0.04$\mu g.mL^{-1}$ which was higher than the reported $C_{max}$ of 1.1$\mu g.mL^{-1}$[16] and 1.17$\mu g.mL^{-1}$[17] for the same dose rate after pulse

dosing. But Sumano *et al.*[18] obtained a higher $C_{max}$ of 2.43±0.59µg.mL$^{-1}$ probably due to shorter period (2 to 2.5h) of consumption of medicated water when compared to the present study (4h). The $C_{max}$ attained in this study was also higher than previous reports of 0.38[16] and 0.6µg.mL$^{-1}$[12] reached during continuous medication program in chicken. The findings confirm the advantage of pulse dosing over continuous medication. Since fluoroquinolones are concentration dependent antibacterials higher $C_{max}$ is required for better effect. In HSCAS group significantly ($p<0.01$) lower $C_{max}$ of 0.97±0.06µg.mL$^{-1}$ was detected at significantly ($p<0.05$) delayed $t_{max}$ of 6.67±0.67h when compared to control group which might be due to adsorption of drug to HSCAS.

The $AUC_{0-24}$ (15.96±1.04µg.h.mL$^{-1}$) obtained for control group concurs with Sumano *et al.*[18] wherein the $AUC_{0-24}$ was 15.80±3.47µg.h.mL$^{-1}$ but was higher than the reported value of 9.6µg.h.mL$^{-1}$[17] for pulse dosing and 12.48µg.h.mL$^{-1}$[19] for continuous dosing in chicken. The higher value of AUC obtained in this study could be attributed to longer absorption phase. This was further supported by higher MRT (14.09±1.10h) and MAT (5.23±1.10h) observed in the present study. However, comparatively lower MRT of 9.2[17] and 4.83h[18] were reported for pulse dosing in chicken. This variation might be due to the difference in the duration of intake of medicated water as well as quality of drinking water which further affects the water consumption. In HSCAS group $AUC_{0-\infty}$ was 16.42±1.24µg.h.mL$^{-1}$ which was lower than control group (19.67±1.68µg.h.mL$^{-1}$) and relative bioavailability was 88.88±15.03%. Sumano *et al.*[20] reported that as water hardness increased, $C_{max}$, AUC and F of enrofloxacin administered directly into the proventriculus decreased. The authors reported that the formation of $Ca^{++}$ and $Mg^{++}$ coordination compounds might have reduced the absorption of enrofloxacin through gastrointestinal epithelium and resulted in reduced concentration. Aguilera *et al.*[21] also reported that enrofloxacin absorption was lowered when coadministered with calcium in broiler chicken. The interaction of fluoroquinolones with multivalent cations present in antacids was already proven.[22] HSCAS also contains calcium and aluminium ions which might have chelated enrofloxacin leading to reduced bioavailability.

The $V_{d\ area}$/F and $V_{d\ ss}$/F were 6.39±0.13 and 7.21±0.20L.kg$^{-1}$, respectively for control group which confirms wider distribution of enrofloxacin. The $Cl_B$/F was 0.53±0.05L.h$^{-1}$.kg$^{-1}$ and $t_{1/2\beta}$ was 8.70±0.74h after pulse dosing. At the same dose level a lower $t_{1/2\beta}$ of 6.47h for pulse

dosing but very high value of 27.05h for continuous dosing in chicken were reported.[17] Sumano *et al.*[18] also reported very low $t_{1/2\beta}$ of 1.12±0.054h which might be due to variation in strain, age and body weight of the birds. In HSCAS group numerically higher $V_{d\ area}/F$ and $Cl_B/F$, and highly significant increase in $V_{d\ ss}/F$ were noticed when compared to control group. Increase in volume of distribution can occur either due to binding or sequestration of the drug in other tissues.[23] In this case HSCAS might have adsorbed the drug in the GI tract resulting in increased $V_d$ and reduced $C_{max}$. The MRT value of 15.12±0.78h was higher than control group which might be because of delayed absorption due to binding of drug to HSCAS as evidenced by delayed $t_{max}$ (6.67±0.67h) and MAT (6.26±0.78h). Perusal of literature revealed lack of information on *in vivo* interaction of enrofloxacin with toxin binders. Yan *et al.*[24] reported that cation exchange was a major contributor to sorption of cationic enrofloxacin species on clay such as smectite. Other *in vitro* studies have demonstrated the ability of enrofloxacin to interact with various types of clay wherein the interaction was limited to external surfaces in non swelling clays but extended to interlayer spaces in swelling clays.[25] Since HSCAS is a swelling type of clay, the drug would have interacted with both external and interlayer spaces. In addition, enrofloxacin would have interacted with aluminium ion present on the surface of HSCAS similar to ciprofloxacin[26] and inturn resulted in reduced bioavailability.

Further the interaction of HSCAS with enrofloxacin would be similar to chelation of aflatoxin by HSCAS wherein it was proposed to interact with various edge site metals such as aluminum or interlayer cations especially calcium. HSCAS was also postulated to bind to dicarbonyl system of aflatoxin which was found essential for chemisorption.[27] The structure of enrofloxacin also contains dicarbonyl group, hence it might have bound to HSCAS similar to that of aflatoxin and thus reduced the bioavailability.

One of the main objectives of conducting pharmacokinetic studies of antimicrobial agents is to suggest appropriate dosage regimen for treating microbial infections in the target species. Dosage schedule for antimicrobials were rationalized based on the integration of pharmacokinetic parameters such as AUC and $C_{max}$ with pharmacodynamic parameters such as MIC and MPC. For concentration dependent antimicrobials like fluoroquinolone, the pharmacological indices AUC/MIC and $C_{max}$/MIC are the best indicators of clinical outcome. Of late, to prevent the emergence of mutant population AUC/MPC and $C_{max}$/MPC are taken

into consideration. The MPC is defined as the minimum inhibitory concentration of the least susceptible first step resistant mutant present in bacterial population.[28]

In order to maximize clinical efficacy and minimize the development of resistance AUC/MIC>100-125 and $C_{max}$/MIC>8-12 should be achieved.[29] From the results of the study it is clear that single administration of enrofloxacin @ 10mg.kg$^{-1}$ by pulse dosing to control group is likely to produce clinical success as reflected by AUC/MIC>125 and $C_{max}$/MIC>10 for microorganisms having MIC of 0.125µg.mL$^{-1}$. In agreement with this finding, El-Aziz *et al.*[30] suggested that a dosage of 10mg.kg$^{-1}$ orally every 24h was appropriate for treatment of infections in chickens involving pathogens that exhibit MIC of 0.12µg.mL$^{-1}$. Sumano *et al.*[20] reported that a dosage of 10mg.kg$^{-1}$ once daily through properly handled drinking water was sufficient to protect chicken against most common pathogens.

In the presence of toxin binder the administered dose would be effective if the microorganism has an MIC below 0.125µg.mL$^{-1}$ because even though AUC/MIC is more than 100 for HSCAS group, $C_{max}$/MIC is less than 8 which is a better predictor of clinical efficacy. Randall *et al.*[17] also reported that 2.5 times the recommended dose by pulse dosing was better than 5 day treatment with recommended dose in chicken because of increased antibiotic concentration.

Mutant prevention concentration appears to be useful for determining dosing strategies for enrofloxacin[31] in order to overcome the problem of emergence of antimicrobial resistance. AUC/MPC above 13.41 and $C_{max}$/MPC above 1.2 for complete eradication of *S. pneumonia* by moxifloxacin and levofloxacin[32], AUC/MPC>14 for restricting selection of double mutant and 35 for preventing selection of single step mutant[33] in *E. coli* were previously reported. In the present study AUC/MPC above 14 and $C_{max}$/MPC above 1.2 were maintained by single pulse dose administration of enrofloxacin for MPC $\leq$ 1.0µg.mL$^{-1}$. Addition of binder in feed reduced both the values and higher dose may be needed to prevent mutation for organisms with MPC $\geq$ 1.0µg.mL$^{-1}$.

The mean plasma concentration was maintained above MIC/MPC of 0.5µg.mL$^{-1}$ up to 12h in both the groups. For fluoroquinolones which is a concentration dependent antimicrobial, integration with AUC and $C_{max}$ is more appropriate than time above MIC or MPC, since they possess prolonged post antibiotic effect.[34] However based on T>MIC and T>MPC and for antimicrobials like enrofloxacin with PAE of 4 to 8h the dose may be sufficient only for

moderately sensitive organisms and increase in dosage may be needed to treat less sensitive organisms.

## CONCLUSION

The results of single pulse dosing of enrofloxacin alone and in the presence of HSCAS revealed that maximum plasma concentration was significantly lowered, attained later and volume of distribution was increased due to binding interaction with HSCAS added in the feed. PK/PD integration also revealed that the dose of enrofloxacin (10mg.kg$^{-1}$) was capable of treating only moderately sensitive organisms (MIC≤0.125µg.mL$^{-1}$) both in the presence and absence of toxin binder and higher dosage is needed for less sensitive organism. It can be concluded that continuous administration of enrofloxacin to HSCAS supplemented broilers would lead to decrease in clinical efficacy and promote development of antimicrobial resistance. Hence careful adjustment of dosage or withdrawal of the usage of toxin binder containing HSCAS in feed during enrofloxacin treatment is recommended.

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