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Research Article

INFLUENCE OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE AND ACTIVATED CHARCOAL ON THE PHARMACOKINETICS OF SINGLE PULSE DOSING OF ENROFLOXACIN IN BROILER CHICKEN

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ABSTRACT

Objective: The present study was undertaken to evaluate the interaction kinetics of enrofloxacin, the commonly used antibacterial in poultry with mycotoxin binders namely hydrated sodium calcium aluminosilicate (HSCAS) and activated charcoal (AC), which have become inevitable components of poultry feed.

Methods: Control group received normal feed free of toxin binder, whereas HSCAS and AC group were supplemented with HSCAS and AC at 0.5% in feed, respectively. Enrofloxacin was administered as single pulse dose (at 10 mg/kg) through drinking water to all the groups. Blood samples were collected at predetermined time intervals after drug administration, and plasma was separated and analyzed for enrofloxacin concentrations using high-performance liquid chromatography.

Results: Significant decrease in area under the plasma concentration-time curve ($AUC_{0-\infty}$) was noticed in AC group when compared to control group (13.90±1.15 vs. 19.67±1.68 µg.h/ml), whereas HSCAS group (16.42±1.24 µg.h/ml) neither differed significantly from AC nor control group. The volume of distribution and clearance were significantly high in AC group when compared to control group (8.31±0.89 vs. 6.39±0.13 l/kg; 0.77±0.07 vs. 0.53±0.05 l/h/kg). HSCAS group was intermediate and did not differ significantly from the other two groups (8.13±0.45 l/kg; 0.63±0.04 l/h/kg). However, volume of distribution at steady state was significantly high in both AC (10.42±1.09 l/kg) and HSCAS group (9.45±0.48 l/kg) when compared to control group (7.21±0.20 l/kg). Maximum plasma concentration was significantly low (0.99±0.04, 0.97±0.06, 1.38±0.04 µg/ml) and time to reach maximum plasma concentration was significantly delayed (7.33±0.42, 6.67±0.67, 4.33±0.67 h) in AC and HSCAS group when compared to control group, respectively. The relative bioavailability was significantly low in both AC and HSCAS group (74.95±10.70, 88.88±15.03%) when compared to control group. Pharmacokinetic/pharmacodynamic integration revealed that the dose of enrofloxacin (10 mg/kg) was capable of treating only moderately sensitive organisms (minimum inhibitory concentration ≤0.125 µg/ml) both in the presence and absence of toxin binder and higher dosage is needed for the less sensitive organism.

Conclusion: The study revealed that the administration of enrofloxacin to HSCAS and AC supplemented broilers would lead to decrease in clinical efficacy and promote the development of antimicrobial resistance. AC was found to interact more with enrofloxacin than HSCAS as observed from the PK parameters. Hence, careful adjustment of dosage or withdrawal of the usage of toxin binder containing either HSCAS or AC in feed during enrofloxacin treatment is recommended.

Keywords: Enrofloxacin, Pulse dosing, Hydrated sodium calcium aluminosilicate, Activated charcoal, Interaction kinetics.

INTRODUCTION

Enrofloxacin, a fluoroquinolone antibacterial was developed exclusively for veterinary purpose and is commonly used in the poultry industry for prevention and treatment of bacterial infections owing to its broad spectrum of activity, bactericidal effect at low concentration, large volume of distribution and high bioavailability [1]. Pharmacokinetic (PK) behavior of the drug was widely explored in poultry and various other species, but limited studies were reported on the interaction with co-administered drugs. Poultry diet is formulated with a number of feed additives of which toxin binders are inevitable inclusions to combat the problem of mycotoxicosis [2]. As per the request of the European Commission [3], European Food Safety Authority proposed guidelines for authorization of these feed additives. It emphasized the importance of exploring the possible interaction of mycotoxin binders with veterinary medicinal products [4] since additives that exert their activity mainly by binding, may affect the oral bioavailability of drugs. To the best of our knowledge, perusal of literature revealed a lack of information on the interaction kinetics of enrofloxacin with toxin binders used in poultry.

Toxin binders available in the market are composite mixtures of many ingredients of which either hydrated sodium calcium aluminosilicate (HSCAS) or activated charcoal (AC) or both are major ingredients. HSCAS was initially reported to be aflatoxin selective binder [5]. But, later studies reported the need for high inclusion rates of vitamins and minerals when HSCAS was added in the feed [6]. AC is a non-specific binder capable of adsorbing a wide variety of agents from mycotoxins to nutrients in feed [7]. Hence, the present study was undertaken to explore the influence of HSCAS or AC included in the feed on the PK behavior of enrofloxacin administered through drinking water as pulse dosing in broiler chicken.

METHODS

Experimental birds

The study was conducted in 36 apparently healthy broiler chicken of Cobb strain of 6 weeks old weighing between 2.0 and 2.2 kg. The birds were procured from a commercial broiler farm at the age of 4 weeks and were acclimatized for 2 weeks prior to the 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 high-performance liquid chromatography (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 0.2 µ 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 three groups of 12 each and control group was provided with normal feed free of drugs and toxin binder, whereas HSCAS and AC groups received normal feed supplemented with 0.5% HSCAS and AC, respectively, starting from the acclimatization period to the end of the trial. All the birds were administered enrofloxacin as single pulse dose through drinking water at the rate of 10 mg/kg 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 4 hrs. 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 2 hrs before administration of medicated water.

Blood samples (0.75-1 ml) were collected from medial metatarsal vein in heparinized tubes (10 u/ml) 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 48.0 hrs after drug administration. Plasma was separated by centrifuging at 1000 g for 10 minutes using microcentrifuge 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 Küng *et al*. [8]. The HPLC system consisted of an LC-20AD double plunger pump, photo diode array (PDA) detector, and LC Solution software for data analysis. A reverse phase C_{18} column (Hibar® 250-4, 6 RP-18 end capped, particle size 5 µm, 4.6 × 250 mm, Merck, Darmstadt, Germany) served as a stationary phase. The column was protected with a 2-8 mm 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% orthophosphoric acid and 0.4% triethylamine (pH adjusted to 3.0 with triethylamine). The scan range of PDA detector was 220-400 nm, and the detection wavelength was 278 nm. The flow rate of mobile phase was 1.0 ml/minute, and samples were analyzed for 10 minutes at 40°C. There were no interfering peaks in the plasma at the retention time of ciprofloxacin (6.38 minutes) and enrofloxacin (8.25 minutes). 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 Gyrd-Hansen [9]. To 500 µl of plasma, 750 µl of HPLC grade acetonitrile was added in the ratio of 1:1.5 and vortexed thoroughly for 15 seconds and centrifuged at 900 g for 10 minutes 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 0.2 µ HNN nylon membrane filter and 20 µl of this filtrate was injected into the HPLC system.

Working plasma standards of ciprofloxacin and enrofloxacin (0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5, and 10.0 µg/ml) 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 ciprofloxacin and enrofloxacin by plotting peak area against the concentration of the drug. The standard curves of ciprofloxacin and enrofloxacin were linear in the range of 0.05-10.0 µg/ml and 0.025 to 10 µg/ml, respectively. The equation for ciprofloxacin from the calibration plot was y=18495x+177.1 with r^2>0.997. The equation for enrofloxacin obtained from the calibration plot was y=27618x+2779.7 with r^2>0.999. The overlay report for various concentrations of ciprofloxacin and enrofloxacin is shown in Fig. 1.

The mean absolute recovery was within the range of 95.12-99.67% for plasma, and the percentage of CV was 1.76-5.80% suggesting the suitability of the method for analysis of enrofloxacin and ciprofloxacin in chicken plasma [10]. The intra-day and inter-day CVs were within the limits (<15%) specified; hence, the method was suitable for assay of both ciprofloxacin and enrofloxacin in chicken plasma. The limit of detection and quantification were 0.025 and 0.05 µg/ml for ciprofloxacin and 0.01 and 0.025 µg/ml for enrofloxacin, respectively.

PK analysis

The plasma drug concentration-time data of enrofloxacin were analyzed by non-compartmental techniques based on statistical moments

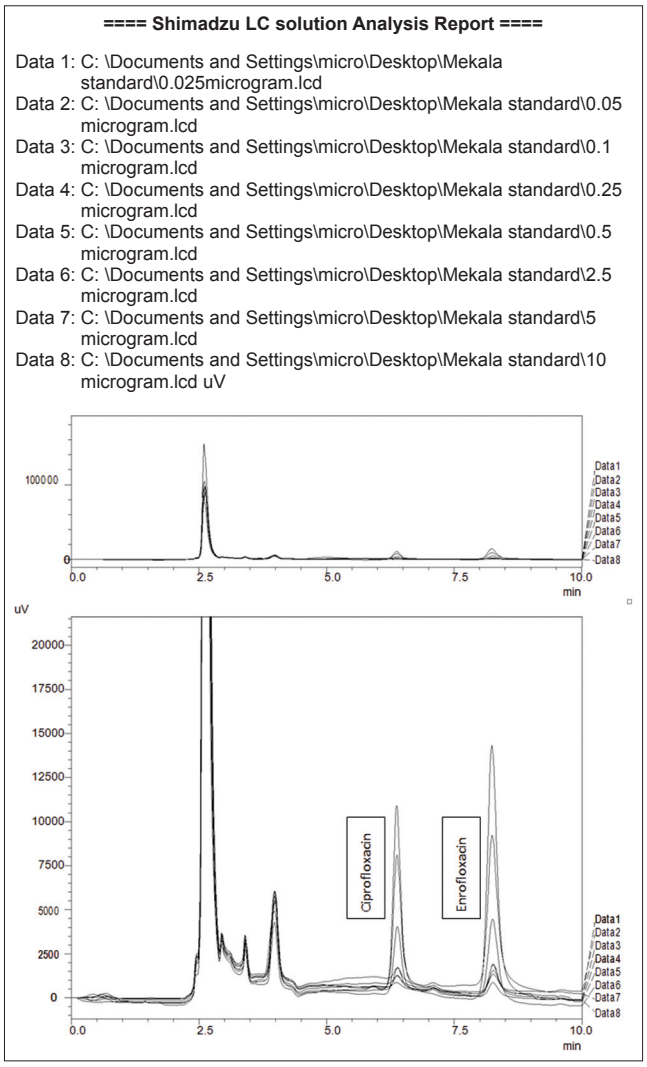

Fig. 1: Overlay report of ciprofloxacin and enrofloxacin in broiler chicken plasma

theory [11] using the PK software PK function [12]. The terminal elimination rate constant (β) was calculated from semi-logarithmic plot of the concentration-time curve using linear regression analysis from which the elimination half-life ($t_{1/2\beta}$) was calculated. 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, total body clearance, the volume of distribution at steady state, and apparent volume of distribution were calculated. Relative bioavailability was calculated by comparing the AUC of binder groups with the control group. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were taken from the observed values.

PK/PD integration

The hypothetical minimum inhibitory concentration (MIC_{90}) values (0.05, 0.125, 0.25, and 0.5 µg/ml) were used for integrating with the PK parameters for calculating AUC/MIC and C_{max}/MIC.

Statistical analysis

Statistical analysis of the data was performed by using SPSS 17.0 software. The results were expressed as mean±standard error. Harmonic mean was used with data not distributed normally. One-way ANOVA was applied to find out the differences among various groups [13], and the means were compared by Duncan's multiple range tests as described by Kramer [14] to find out the significance between the groups.

RESULTS

The plasma concentrations of enrofloxacin at different time intervals were analyzed (Table 1), and the PK parameters (Table 2) were calculated for all the samples and expressed as mean ± standard error of mean.

The mean plasma concentration was above 1 µg/ml (1.22±±0.09 and 1.24±±0.06 µg/ml) from 4 to 6 hrs in the control group, whereas the maximum concentration was 0.9±±0.06 and 0.89±±0.05 µg/ml mat 6 hrs in HSCAS and AC group, respectively. The mean concentration at 12 hrs was 0.5 µg/ml mand above in HSCAS and control group, whereas it was 0.45 µg/ml in AC group. Enrofloxacin concentration was detected up to 48 hrs in control and HSCAS group, whereas as it was detected up to 36 hrs in AC group.

Comparison of mean PK parameters of the three groups is presented in Table 2. $AUC_{0-\infty}$ of the control group was 1.2-fold higher than HSCAS group, but it was not significant, whereas it was higher by 1.42 times than AC group and it differed significantly ($p<0.05$) which indicates that the interaction of the drug with AC was more than HSCAS. $AUMC_{0-\infty}$ of the control group was 1.14 and 1.54 folds higher than HSCAS and AC group, respectively, but it did not differ significantly. Though there were numerical difference in Mean residence time (MRT) and mean absorption time, there was no significant difference between the three groups.

The $V_{d\ area}/F$ and Cl_B/F were significantly higher ($p<0.05$) in AC group when compared to control group. There was no significant difference in $V_{d\ area}/F$ and Cl_B/F between HSCAS and AC group, as well as between control and HSCAS group. $V_{d\ ss}/F$ of HSCAS and AC group were significantly higher ($p<0.05$) than the control group.

The C_{max} of control group was significantly higher ($p<0.05$) by 1.42 and 1.39 folds than HSCAS and AC group, respectively. T_{max} was higher in AC group followed by HSCAS, group but there was no significant difference between them, whereas they differed significantly ($p<0.05$) from control group. The relative bioavailability (F_{rel}) was calculated by comparing the AUC of binder groups with the control group. The binder groups differed significantly ($p<0.05$) among themselves as well as from the control group of which the F_{rel} of HSCAS group was intermediate between the control and AC groups.

Table 1: Comparison of mean plasma concentration (µg/ml) after single pulse dosing of enrofloxacin

Time (h)	Control group	HSCAS group	AC group
0.5	0.41±0.04	0.35±0.04	0.21±0.03
1	0.53±0.11	0.43±0.04	0.24±0.02
1.5	0.72±0.12	0.50±0.03	0.35±0.03
2	0.96±0.15	0.56±0.03	0.48±0.04
4	1.22±0.09	0.82±0.06	0.63±0.06
6	1.24±0.06	0.90±0.06	0.89±0.05
8	0.96±0.05	0.87±0.07	0.87±0.10
10	0.73±0.06	0.68±0.05	0.65±0.07
12	0.53±0.07	0.50±0.06	0.45±0.05
24	0.34±0.06	0.30±0.03	0.26±0.04
30	0.16±0.03	0.17±0.02	0.12±0.02
36	0.10±0.02	0.08±0.01	0.03±0.01
48	0.03±0.01	0.02±0.01	ND

Results are expressed as mean±SEM (n=12), SEM: Standard error of mean, ND: Not detected, HSCAS: Hydrated sodium calcium aluminosilicate, AC: Activated charcoal

Table 2: Comparison of mean pharmacokinetic parameters after single pulse dosing of enrofloxacin

Parameters	Units	Control group	HSCAS group	AC group
β	/h	0.080±0.008	0.076±0.004	0.093±0.005
$AUC_{0-\infty}$	µg.h/ml	19.67^{b}±1.68	16.42^{ab}±1.24	13.90^{a}±1.15
$AUMC_{0-\infty}$	$\mu g.h^2/ml$	285.83±44.07	251.57±30.62	185.73±20.44
MRT	h	14.09±1.10	15.12±0.78	13.60±0.52
MAT	h	5.23±1.10	6.26±0.78	4.74±0.52
$V_{d\ area}/F$	l/kg	6.39^{a}±0.13	8.13^{ab}±0.45	8.31^{b}±0.89
$V_{d\ ss}/F$	l/kg	7.21^{a}±0.20	9.45^{b}±0.48	10.42^{b}±1.09
Cl_B/F	l/h/kg	0.53^{a}±0.05	0.63^{ab}±0.04	0.77^{b}±0.07
$t_{1/2\beta}$	h	8.34±0.74	9.00±0.48	7.54±0.40
C_{max}	µg/ml	1.38^{b}±0.04	0.97^{a}±0.06	0.99^{a}±0.04
t_{max}	h	4.33^{a}±0.67	6.67^{b}±0.67	7.33^{b}±0.42
F_{rel}	%	100^{c}	88.88^{b}±15.03	74.95^{a}±10.70

Results are expressed as mean±SEM (n=12), SEM: Standard error of mean. Mean values bearing different superscripts differ significantly ($p<0.05$). MRT: Mean residence time, MAT: Mean absorption time, AUC: Area under the plasma concentration-time curve, AUMC: Area under the first moment curve, HSCAS: Hydrated sodium calcium aluminosilicate, AC: Activated charcoal

PK/PD integration

The PK/PD integration parameters such as AUC/MIC and C_{max}/MIC were calculated from the obtained PK parameters and presented in Table 3.

DISCUSSION

Ciprofloxacin, the metabolite of enrofloxacin could not be determined consistently in many samples and hence not reported. Inconsistency in the detection of ciprofloxacin was also reported in chicken [15] and Muscovy ducks [16]. Other authors [17,18] also reported that the metabolic conversion was less than 10 and 7%, respectively, and hence did not calculate the PK parameters for ciprofloxacin. In this study also meaningful PKs could not be established for ciprofloxacin as a metabolite of enrofloxacin and hence the PKs of enrofloxacin alone is reported.

The significant increase in $V_{d\ area}/F$ and Cl_B/F would have occurred due to binding of the drug to AC since the high volume of distribution can also occur either due to binding or sequestration in tissues [19]. The absorbed drug was cleared rapidly in AC group since it is capable of binding the drug that undergoes enterohepatic recirculation [20] or effectively favors trans-intestinal elimination or recirculation as in the case of ciprofloxacin [21]. It would have resulted in numerical decrease in MRT and half-life when compared to other groups. Repeated administration of the binders would bind the drug in GI tract and

Table 3: PK/PD integration

MIC (µg/ml)	AUC_{0-24}/MIC			C_{max}/MIC		
	Control group	HSCAS group	AC group	Control group	HSCAS group	AC group
0.05	319.25±19.07	261.30±16.34	233.54±16.61	27.50±0.77	19.47±1.27	19.83±0.83
0.125	127.70±7.63	104.52±6.53	93.42±6.64	11.00±±0.31	7.79±0.51	7.93±0.33
0.25	63.85±3.82	52.26±3.27	46.71±3.32	5.50±0.15	3.89±0.25	3.97±0.17
0.5	31.93±1.91	26.13±1.63	23.35±1.66	2.75±0.08	1.95±0.13	1.98±0.08

MIC: Minimum inhibitory concentration, AUC: Area under the plasma concentration-time curve, HSCAS: Hydrated sodium calcium aluminosilicate, AC: Activated charcoal, PK: Pharmacokinetic, PD: Pharmacodynamic

lead to increase in $V_{d\ ss}$/F. The binding of enrofloxacin to toxin binders administered through feed would have resulted in decrease in C_{max}, increase in t_{max} and decrease in F_{rel} in AC group followed by HSCAS group.

Perusal of literature revealed lack of information on *in vivo* interaction of enrofloxacin with toxin binders. The cation exchange was a major contributor to sorption of cationic enrofloxacin species on clay such as smectite [22]. 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 [23]. Since HSCAS is a swelling type of clay, the drug would have interacted with both external and interlayer spaces. Fluoroquinolones were reported to interact with multivalent cations present in antacids [24], form coordination compounds with calcium and magnesium present in hard water [25] as well lead to reduced bioavailability in calcium primed broilers [26]. Thus, the presence of tri and divalent cations such as aluminum and calcium in HSCAS and dicarbonyl group in enrofloxacin would facilitate their interaction resulting in reduced bioavailability.

AC is a non-specific adsorbent with high surface area; hence, the chance for binding is more than HSCAS. It was reported to adsorb some essential nutrients in feed [6]. Dose-dependent binding activity for AC toward quinolones was also demonstrated by agar dilution method [27]. AC was proven to have high adsorption capacity for fluoroquinolones than bentonite and kaolin in *in vitro* studies [28]. In the present study, AC might have adsorbed enrofloxacin administered through drinking water and thus reduced the C_{max}. Similar to the present study repeated doses of AC lowered the C_{max} and AUC of single-dose intravenous infusion and oral administration of moxifloxacin in human [29]. Between the binders, there was a significant difference in F_{rel} but AC was identified to have bound enrofloxacin more than HSCAS since AC group differed significantly with reference to most of the PK parameters from the control group.

Dosage schedule for antimicrobials was rationalized based on the integration of PK parameters such as AUC and C_{max} with PD parameters such as MIC. For concentration-dependent antimicrobials like fluoroquinolone, the pharmacological indices AUC/MIC and C_{max}/MIC are the best indicators of clinical outcome. 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 [30]. PK PD predictors of clinical efficacy suggested that single pulse dosing of enrofloxacin at 10 mg/kg is likely to produce clinical success for microorganisms having MIC ≤0.125 µg/ml.In the presence of either of the toxin binders, following single administration, the dose would be effective only for those microorganisms with an MIC below 0.125 µg/ml because even though AUC/MIC is more than 100 for HSCAS, C_{max}/MIC is less than 8 which is a better predictor of clinical efficacy and for AC group both the values were lower. In agreement with this finding, a dosage of 10 mg/kg orally every 24 hrs was reported to be appropriate for treatment of infections in chickens involving pathogens that exhibit MIC of 0.12 µg/ml [31]. A dosage of 10 mg/kg once daily through properly handled drinking water was found to be sufficient to protect chicken against most common pathogens [25]. Yet, another report suggested that 2.5 times the recommended dose by pulse dosing was better than 5 days treatment with recommended dose in chicken because of increased antibiotic concentration [32].

CONCLUSION

The results of single pulse dosing of enrofloxacin alone and in the presence of either HSCAS or AC revealed that maximum plasma concentration was significantly lowered, attained later, and volume of distribution was increased due to binding interaction with HSCAS and AC added in the feed. AC was found to interact more with enrofloxacin than HSCAS as observed from the PK parameters. PK/PD integration also revealed that the dose of enrofloxacin (10 mg/kg) was capable of treating only moderately sensitive organisms (MIC≤0.125 µg/ml) both in the presence and absence of toxin binder and higher dosage is needed for less sensitive organism. It can be concluded that AC interacts more with enrofloxacin than HSCAS leading to decrease in clinical efficacy. Hence, careful adjustment of dosage or withdrawal of the usage of toxin binder containing either HSCAS or AC in feed during enrofloxacin treatment is recommended. However, interaction between enrofloxacin and toxin binders in the presence of natural mycotoxin contamination in the feed of broiler chicken need to be explored.

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