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Pharmacokinetic enhancement of marbofloxacin by alpha-1-monolaurin pre-treatment in broiler chickens

Sheen Tukra, Ratn Deep Singh*, Hiteshkumar Patel, Vaidehi Sarvaiya, Sanjaykumar Vaghela, Ankitkumar Patel, Shaileshkumar Mody

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, India.

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Abstract

The present study investigated the prospect of improvement in pharmacokinetic (PK) parameters of marbofloxacin due to alpha-1-monolaurin pre-treatment in broiler chickens. Two groups of broilers were administered a single oral dose of marbofloxacin (5.00 mg kg^{-1} body weight): Group-I without pre-treatment and Group-II with alpha-1-monolaurin pre-treatment (4.00 g kg^{-1} feed for 10 days). Blood sampling was done periodically for both groups and plasma marbofloxacin concentrations were determined using ultra-high performance liquid chromatography. Pharmacokinetic parameters using non-compartmental modelling approach were calculated with the PKSolver software. Statistical analysis revealed significant differences in plasma marbofloxacin concentrations between the two groups at 1, 2, and 24 hr. Group-II birds exhibited a higher mean maximum plasma concentration (2.43 µg mL^{-1}) at an earlier time (T_{max}: 1.38 hr) compared to Group-I. The plasma concentrations of marbofloxacin were maintained above 0.10 and 0.18 µg mL^{-1} up to 24 hr in Group-I and Group-II broilers, respectively. Significant differences were observed in PK parameters such as the area under the curve and total body clearance. The mean relative oral bioavailability of Group-II birds compared to Group-I was 119.61%. The findings of the study provided evidence of PK parameters enhancement of marbofloxacin in the alpha-1-monolaurin pre-treated group. The calculated PK-pharmacodynamic indices for marbofloxacin predicted clinical efficaciousness in the broiler chickens.

Introduction

The global poultry sector is witnessing a consistent and significant rise in demand for meat consumption. This trend is predominantly driven by a growing preference for white meats and their relatively lower price compared to other meat sources.[1] The restrictions in the use of antibiotics in poultry production have led to an increased focus on exploring feed additives with effective properties to enhance the gut health of broiler chickens. Medium-chain fatty acids (MCFAs) are commonly utilized as feed additives in broiler diets as natural alternatives to antibiotic growth promoters. These MCFAs prevent the colonization of enteric pathogens, maintain gut health and promote optimal performance in broilers.[2] Alpha-1-monolaurin, known as monolaurin, is a widely employed MCFA in the modern broiler industry. This compound, an alpha-monoglyceride, has demonstrated to have the ability to bolster the bird immunity against bacterial and viral infections.[3] Monolaurin displays inhibitory properties against pathogenic microbes being present in the gut and maintains stability even under high-heat and acidic conditions. Additionally, it contributes to a pleasant taste and flavour; thereby, promoting feed intake and improving digestibility.[4,5] Monolaurin, at the recommended dose of 4.00 g kg^{-1} of diet, demonstrated a significant enhancement in chick performance, particularly in terms of body weight (BW) gain and food conversion ratio. Additionally, it also exhibited positive effects on immunological and biochemical parameters such as pro-inflammatory cytokines (tumour necrosis factor alpha and interleukin 12) and total anti-oxidant capacity.[6] Monolaurin facilitates nutrient absorption

***Correspondence:**

Ratn Deep Singh. MVSc, PhD
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, India
E-mail: rdsingh@kamdhenuuni.edu.in

through the positive effects on intestinal villi potentially impacting nutritional and drug bioavailability.[6,7] Marbofloxacin, a third-generation fluoroquinolone exclusively employed in veterinary medicine, functions as a concentration-dependent, broad-spectrum anti-microbial drug and exhibits a significant post-antibiotic effect. Additionally, its short withdrawal period makes it a preferred choice to treat bacterial diseases in broiler production.[8] Marbofloxacin, an off-label drug in poultry, is employed to manage systemic colibacillosis. Its efficacy in combating this condition has been evaluated and proven effective under field conditions.[9] Similar to enrofloxacin, marbofloxacin, a drug exclusively meant for veterinary use, demonstrates potential in addressing challenging bacterial infections in poultry. In light of the increasing resistance of pathogens to enrofloxacin and ciprofloxacin resulting due to their extensive use in the poultry industry, use of marbofloxacin is promising.[10,11] Considering the rising trend of alpha-1-monolaurin utilization as a growth promoter in poultry flocks, it is likely that the gut-related effects could impact the pharmacokinetic (PK) characteristics of marbofloxacin, if used to treat susceptible bacterial infections in these flocks. Hence, it is crucial to quantify the magnitude of alpha-1-monolaurin pre-treatment effect on PK parameters of marbofloxacin in poultry aiming to predict an effective dosage regimen tailored for broiler chickens. Thus, due to lack of literature regarding such information, this study was planned to assess the influence of alpha-1-monolaurin pre-treatment on the PK parameters of marbofloxacin in broiler chickens.

Materials and Methods

Experimental birds. Sixteen healthy male broiler chickens of the Cobb strain belonging to the same hatch batch were procured at the age of 3 - 4 weeks. At the time of PK trial, birds were weighing above 1.50 kg. The Institutional Animal Ethics Committee of the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, India, approved the experiment under the protocol number of VETCOLL/ IAEC/22/19/PROTOCOL-14/09-01-2022. The study was conducted in accordance with ethical guidelines and animal welfare regulations to ensure the compassionate treatment of the birds. Before commencing the experiment, a 10-day acclimatization period was observed.

Experimental design. The sixteen chickens were randomly allocated to two groups, namely Group I and Group II, each comprising eight birds. Group I was designated as a control group, receiving no pre-treatment of alpha-1-monolaurin and only receiving a single oral dose of marbofloxacin at a rate of 5.00 mg kg^{-1} BW. Conversely, Group II underwent a 10-day pre-treatment period with alpha-1-monolaurin (Feed grade; Symbio Nutrients, Mumbai, India), incorporated into the feed at a dose rate of 4.00 g kg^{-1} of feed, as recommended by Mustafa *et al*.[6] Following the pre-treatment phase, Group II birds were administered a single oral dose of marbofloxacin at the same dose rate used for Group I. The selection of the oral dose of marbofloxacin for the present study was based on a literature review and the results of a previous experiment that was focused on deriving the appropriate dosage of marbofloxacin in broiler chickens and it was found to be 5.00 mg kg^{-1} BW.[8,12] A 12-hr fasting period was implemented before the oral dosing of marbofloxacin during which the birds were deprived of feed to exclude the possibility of food-drug interaction.

Ultra high-performance liquid chromatography (UHPLC) analysis. For standard preparation, a certified sample of pure marbofloxacin powder (99.00% w per w) was procured from Nexia Enterprise, Mumbai, India. All the HPLC-grade chemicals and reagents were procured from the S.D. Fine Chemicals Limited, Mumbai, India. For the drug quantification, an UHPLC system from Dionex (Ultimate 3000; Thermofisher, Karlsruhe, Germany) was employed. The UHPLC apparatus consisted of a gradient solvent delivery pump, a manual injector and an ultra-violet (UV) detector. Chromatographic separation was conducted using a reverse-phase C-18 column (ODS-3V; GL Science Inc., Tokyo, Japan) with particle size of 5.00 µm, 25.00 cm length and 4.60 mm internal diameter, at room temperature. The sample was loaded using a 50.00 µL capacity loop. Data integration was performed using Chromeleon Chromatography Data System Software (version 6.8; Thermofisher, Karlsruhe, Germany). The chromatographic conditions employed for the quantification of marbofloxacin using UHPLC were adapted from previously published methods.[8,13] In brief, liquid-liquid extraction of drug from plasma was done using equal volume of 20.00% perchloric acid. The mobile phase used for the UHPLC analysis was consisted of a mixture of 0.01 M formic acid and acetonitrile in a ratio of 82:18. The aqueous portion of the mobile phase was prepared by combining formic acid with HPLC grade water resulting in a 0.01 M formic acid buffer with a pH of 3.10. The pH of the buffer was then adjusted to 3.70 by adding approximately 500 µL of triethylamine. Under isocratic conditions, the mobile phase flowed through the system at a rate of 1.00 mL per min. The effluents were monitored using UV detection at a wavelength of 297 nm. For sample analysis, 50.00 µL of the extracted supernatant sample was manually injected into the UHPLC system. In the resultant chromatogram with a run time of 12 min, the peak corresponding to marbofloxacin was detected at an average retention time of 5.60 min (Fig. 1). The UHPLC assay utilized in the study was accurate and reproducible with precision. The calibration

curve constructed using nine different concentrations of standards ranging from 0.039 to 10.00 µg mL^{-1} showed acceptable linearity, as indicated by a mean correlation coefficient value of 0.9996. To assess accuracy, three different concentrations of 0.10, 1.00, and 10.00 µg mL^{-1} (low, mid and high, respectively) were studied. The mean accuracy values for these concentrations were calculated to be 94.31%, 94.01% and 98.78%, respectively. The mean extraction recovery values for the same concentrations were determined to be 91.74%, 91.18% and 96.39%, respectively. The assay precision was evaluated in terms of intra-day and inter-day variability and expressed as the percent coefficient of variance (%CV). Both intra-day and inter-day %CV were found to be less than 2.00%, indicating high precision of the assay.

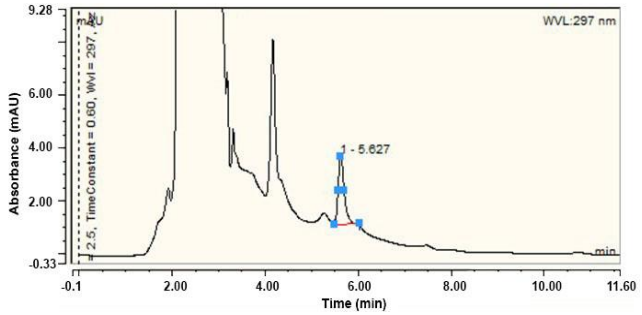

Fig. 1. High-performance liquid chromatography peak of marbofloxacin at 5.627 min for plasma sample collected at 8 hr after oral administration in a pre-treated bird.

Pharmacokinetic and statistical analysis. To determine the PK parameters of marbofloxacin, the plasma concentration-time curves of individual birds were subjected to non-compartmental analysis. The analysis was conducted using PKSolver version 2.0 (China Pharmaceutical University, Nanjing, China) which is a user-friendly add-in program for Microsoft Excel (version 12.0; Microsoft Corporation, Redmond, USA) specifically designed for PK analysis.[14] To measure the extent and rate at which a drug is absorbed and becomes available at the site of action in test group compared to a reference or control group, relative bioavailability was calculated using the following formula:[15]

$$Relative\ Bioavailability\ (\%) = (AUC_{Group\ II} / Dose_{Group\ II}) \times (AUC_{Group\ I} / Dose_{Group\ I})^{-1} \times 100$$

where, *AUC* is the area under the curve.

The data on plasma drug concentrations and values of PK parameters were showcased as mean values along with their corresponding standard errors. To compare the plasma concentrations and oral PK parameters of marbofloxacin between Group I and Group II broiler chickens, the *t*-test was employed at the significance levels of $p \leq 0.05$ and $p \leq 0.01$. The analysis was performed using SPSS Software (version 20.0; IBM Corp., Armonk, USA).

Results

Figure 2 represents a comparison between Group I (broiler chickens without pre-treatment) and Group II (broiler chickens pre-treated with feed grade alpha-1-monolaurin at a concentration of 4.00 g kg^{-1} in feed for 10 days) in terms of mean plasma marbofloxacin concentrations after a single oral dose (5.00 mg kg^{-1} BW) administration.

The plasma drug levels observed in both Group I and Group II broiler chickens indicated the absence of detectable drug concentrations in plasma samples collected beyond 24 hr. No drug was detected in samples from either group at 36 or 48 hr. The marbofloxacin concentrations in both groups exhibited significant differences at 1, 2, and 24 hr ($p \leq 0.05$; Table 1).

Apart from these time points, no statistically significant differences were observed in the concentrations. A comparison of mean values for various PK parameters between Group I and Group II broiler chickens (Table 2) revealed significant differences in parameters such as maximum plasma concentration (C_{max}) and the area under the first moment of the plasma drug concentration ($p \leq 0.05$). Furthermore, parameters *viz*. the AUC and total body clearance (Cl_B) demonstrated highly significant differences ($p \leq 0.01$). Throughout the 24-hr period following oral drug administration (Fig. 2), Group I and Group II birds maintained mean plasma concentrations of marbofloxacin above 0.10 and 0.18 µg mL^{-1}, respectively. The relative oral bioavailability of marbofloxacin in Group I birds, compared to Group II birds was 119.61%.

Table 3 displays the PK-pharmacodynamic indices computed for predicting the efficacy of marbofloxacin in Group I and Group II birds. The average AUC/minimum inhibitory concentration (MIC) ratios were determined as 115.81 and 138.39; while, the mean C_{max}/MIC ratios were 15.62 and 19.47 for Group I and Group II birds, respectively.

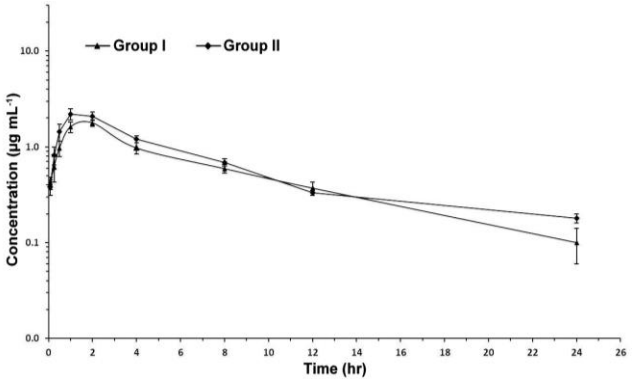

Fig. 2. Semi-logarithmic plot illustrating the plasma marbofloxacin concentration versus time in broiler chickens. Group I and Group II are compared (n = 8).

Table 1. Comparison of plasma marbofloxacin concentrations following single-dose oral administration (5.00 mg kg^{-1} body weight) in Group I (non-pre-treated; n = 8) and Group II (pre-treated with alpha-1-monolaurin; n = 8).

Time after administration (hr)	Concentrations (μg mL^{-1})	
	Group I	Group II
0.083	0.42 ± 0.06	0.38 ± 0.07
0.25	0.61 ± 0.18	0.82 ± 0.18
0.5	0.97 ± 0.18	1.44 ± 0.29
1	1.61 ± 0.21	2.19 ± 0.29*
2	1.77 ± 0.14	2.09 ± 0.21*
4	0.97 ± 0.13	1.20 ± 0.10
8	0.59 ± 0.06	0.69 ± 0.06
12	0.37 ± 0.06	0.33 ± 0.02
24	0.10 ± 0.04	0.18 ± 0.02*
36	ND	ND
48	ND	ND

ND: Not detected.
* indicates statistically significant difference at $p \leq 0.05$.

Table 2. Comparison of pharmacokinetic (PK) parameters of marbofloxacin following single-dose oral administration (5.00 mg kg^{-1} body weight) in Group I (non-pre-treated; n = 8) and Group II (pre-treated with alpha-1-monolaurin; n = 8).

Parameters	Values of PK parameters	
	Group I	Group II
C_{max} (μg mL^{-1})	1.95 ± 0.13	2.43 ± 0.22*
T_{max} (hr)	1.63 ± 0.19	1.38 ± 0.183
β (per hr)	0.12 ± 0.01	0.11 ± 0.004
$t_{1/2\beta}$ (hr)	6.22 ± 0.48	6.47 ± 0.25
AUC (μg hr mL^{-1})	14.48 ± 1.29	17.32 ± 1.51†
AUMC (μg hr^2 mL^{-1})	119.83 ± 12.56	158.16 ± 18.87*
MRT (hr)	8.28 ± 0.53	9.03 ± 0.48
$V_{d(area)}$ (L kg^{-1})	3.26 ± 0.35	2.83 ± 0.24
Cl_B (L kg^{-1} per hr)	0.36 ± 0.02	0.30 ± 0.02†

C_{max}: Maximum plasma concentration; T_{max}: Time at which C_{max} was observed; β: Elimination rate constant; $t_{1/2\beta}$: Terminal half-life; AUC: Area under the curve; AUMC: Area under first moment of the plasma drug concentration curve; MRT: Mean resident time; $V_{d(area)}$: Apparent volume of distribution (scaled by bio-availability); Cl_B: Total body clearance (scaled by bioavailability).
*, † indicate statistically significant difference at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Discussion

Previous studies have extensively investigated the PK profile of marbofloxacin in broiler chickens.[8,12,16-18] However, the present investigation specifically addresses a significant gap in the scientific literature regarding the influence of alpha-1-monolaurin pre-treatment on the PK parameters of marbofloxacin in broiler chickens. The results of this study offered invaluable guidance to poultry veterinarians in devising efficacious treatment approaches for combating bacterial diseases in flocks where alpha-1-monolaurin is employed as a growth promoter. For the present study, the oral route was chosen as the extra-vascular administration method as it was the most suitable and widely employed approach for mass medication in poultry flocks. No adverse reactions were observed in broiler chickens following oral administration of marbofloxacin as they exhibited normal behaviour without any signs of pain or suffering. Similarly, no negative impact of marbofloxacin in broiler chickens was also reported in other study.[17]

Following oral administration of marbofloxacin, the plasma drug concentrations at 1, 2, and 24 hr were significantly higher ($p \leq 0.05$) in Group II birds which received alpha-1-monolaurin pre-treatment, compared to Group I birds. This increased value also caused highly significant differences in important PK parameters such as AUC and total Cl_B when analyzed between the two groups. The major reasons that can contribute to an increase in plasma drug concentration after oral administration are absorption enhancement and food effect.[19,20] In this study, we eliminated the influence of food by observing a fasting period prior to the administration of marbofloxacin. Consequently, the observed increase in plasma concentrations can be attributed to the alpha-1-monolaurin's ability to enhance absorption or permeation. This enhancement is primarily attributed to the positive impact of alpha-1- monolaurin on the gut of broiler chickens, as it improves the permeability and functionality of the intestinal barrier, enhances drugs and nutrients

Table 3. Calculated values of pharmacokinetic-pharmacodynamic indices calculated for oral dose of marbofloxacin (5.00 mg kg^{-1} BW) in Group I and Group II broiler chickens (n = 8).

No.	Group I				Group II			
	AUC (μg hr mL^{-1})	AUC per MIC	C_{max} (μg mL^{-1})	C_{max} per MIC	AUC (μg hr mL^{-1})	AUC per MIC	C_{max} (μg mL^{-1})	C_{max} per MIC
1	11.55	92.40	1.59	12.72	13.19	105.52	1.80	14.40
2	20.35	162.80	2.79	22.32	23.13	185.04	3.23	25.84
3	13.14	105.12	1.97	15.76	14.73	117.84	2.03	16.24
4	10.12	80.96	1.96	15.68	13.08	104.64	2.16	17.28
5	17.66	141.28	1.98	15.84	22.12	176.96	3.49	27.92
6	13.16	105.28	1.77	14.16	14.07	112.56	1.95	15.60
7	12.87	102.96	1.88	15.04	16.33	130.64	2.53	20.24
8	16.96	135.68	1.68	13.44	21.74	173.92	2.28	18.24
Mean	14.48	115.81	1.95	15.62	17.30	138.39	2.43	19.47

C_{max}: Maximum plasma concentration; AUC: Area under the curve; MIC: Minimum inhibitory concentration.
Reference MIC: 0.125 μg mL^{-1}.[9]

absorption by improving the villi height to crypt depth (VH:CD) ratio in the duodenum, jejunum, and ileum, and modulates the composition of the gut microbiota.[5-7,21] An increase in the ratio of VH:CD leads to an expansion of the absorptive surface area, facilitating improved absorption of drugs resulting in higher C_{max}. This C_{max} value is particularly significant for concentration-dependent anti-microbial drugs like marbofloxacin. The objective was to attain higher and quick C_{max} levels for such drugs in order to maximize therapeutic effectiveness while minimizing the risk of resistance development. As shown in Table 2, Group II broiler chickens displayed a higher average C_{max} (2.43 μg mL^{-1}) compared to Group I (1.95 μg mL^{-1}). Also, Group II broiler chickens achieved peak levels of marbofloxacin in a shorter time (1.38 hr) compared to Group I (1.63 hr). Therefore, Group II birds reached their peak levels approximately 0.25 hr earlier than Group I. An attainment of early C_{max} holds significance for anti-bacterial drugs, particularly when treating an acute bacterial infection. In contrast to the current investigation, a previous study observed a reduction in the C_{max} and AUC of marbofloxacin in broiler chickens following lactic acid pre-treatment. Lactic acid possibly modulates marbofloxacin elimination kinetics by augmenting biotransformation activity within the hepato-pancreatic system and stimulating microsomal enzyme activity.[22]

The mean terminal half-lives for Group I and Group II birds were determined as 6.22 and 6.47 hr, respectively, with no statistically significant difference between the two groups. These values closely were in agreement with the reported mean half-life of 6.30 hr in broiler chickens[16] and 6.19 hr in Japanese quail.[23] The average mean resident time following single-dose oral administration of marbofloxacin was calculated as 8.28 and 9.03 hr in Group I and Group II birds, respectively. A longer resident time leads to sustained exposure to therapeutic concentrations, enhancing bacterial killing and increasing the likelihood of complete eradication of bacteria while minimizing the survival and proliferation of resistant strains.[24]

High average apparent volumes of distribution ($V_{d(area)}$; scaled by bioavailability) of 3.26 and 2.83 L kg^{-1} were observed in Group I and Group II birds, respectively, showing no significant difference. Since the total body water volume in broiler chickens was approximately 70.00% or 0.70 L kg^{-1}, the high $V_{d(area)}$ values obtained in this study suggested extensive tissue distribution of marbofloxacin in this species. Such extensive distribution is crucial for the effective treatment of infections in physiologically remote organs such as pulmonary or integumentary organs.

In our study, value of mean total Cl_B observed for Group II was significantly higher than that for Group I (0.36 versus 0.30 L kg^{-1} per hr). These values were similar or higher than the previously reported range of 0.18 - 0.30 L kg^{-1} per hr in broiler chickens.[8,12,16] A moderate to high clearance rate is beneficial for drugs used in livestock raised for food, as it indicates effective elimination from the body and thus, requiring a shorter withdrawal period.

The significant relative oral bioavailability (119.61%) observed in the present study indicated an improvement in bioavailability following a single oral administration of marbofloxacin after pre-treatment with alpha-1-monolaurin. The observed increase in bioavailability could potentially be attributed to the positive effects of alpha-1-monolaurin on poultry gut health such as an increase in intestinal VH providing more absorptive area.[7,21] Previous studies have reported lower absolute oral bioavailability of marbofloxacin as 56.82% and 76.22% in broiler and layer chickens, respectively.[16,25] Considering the reported low bioavailability values, the observed improvement in bioavailability in alpha-1-monolaurin pre-treated group is noteworthy.

The clinical efficacy of concentration-dependent anti-microbial drugs, including marbofloxacin, is predicted using two important PK-pharmacodynamic indices: AUC per MIC and C_{max} per MIC ratios. An AUC per MIC ratio greater than 100 - 125 hr and a C_{max} per MIC value of ≥ 10 are associated with clinical cure rates exceeding 80.00% and reduced risk of bacterial resistance, particularly in Gram-negative bacteria.[26] In this study, the efficacy prediction for Group I and Group II was based on the PK-pharmacodynamic indices being presented in Table 3, using a MIC value of 0.125 μg mL^{-1} encompassing common Gram-negative bacteria in veterinary species including poultry.[9,27,28] The mean AUC per MIC ratios were calculated as 115.81 and 138.39, and the mean C_{max} per MIC ratios were 15.62 and 19.47 for Group I and Group II, respectively. These values suggested that an oral dose of marbofloxacin at 5.00 mg kg^{-1} BW would effectively treat bacterial infections in alpha-1-monolaurin pre-treated broiler chickens, as the AUC per MIC ratio exceeded 120 and the C_{max} per MIC ratio exceeded 10. Furthermore, from the results it was evident that alpha-1-monolaurin pre-treated birds (Group II) consistently maintained therapeutic plasma concentrations above the MIC of 0.125 μg mL^{-1} for up to 24 hr. Based on the discussion, it could be concluded that pre-treatment with alpha-1-monolaurin in broiler chickens enhanced drug absorption leading to an improved PK profile and increased therapeutic efficacy of the marbofloxacin.

Acknowledgments

None.

Conflict of interest

The authors declare that there are no conflicts of interest to disclose regarding this work.

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