

Enrofloxacin against *Escherichia coli* in turkeys: Which treatment scheme is effective?

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ABSTRACT The efficacy of enrofloxacin (ENRO) was evaluated against multidrug-resistant avian pathogenic *Escherichia coli* correlating the minimum inhibitory concentrations (MIC) of 235 *E. coli* field strains with its pharmacokinetics (PK) in 50 healthy turkeys (5 groups) with a PK/pharmacodynamic approach. The treatments were as follows: a) single oral gavage and b) single subcutaneous (SC) treatment at the recommended dose of 10 mg/kg; c) single oral gavage, d) 5 d of 10-h pulsed water medication, and e) 5 d of 24-h continuous water medication at the doubled dose of 20 mg/kg. Blood samples were collected at established times over 24 h. Plasma was analyzed using a liquid chromatography tandem mass spectrometry method that was validated in house. A monocompartmental and a noncompartmental model were applied to the data to obtain the PK results. After gavage administration, the mean maximum concentration C_{\max}/MIC_{50} and area under the curve AUC_{0-24}/MIC_{50} ratios were, respectively, 3.07 ± 0.62 and 7.01 ± 1.03 and 25.48

± 3.04 and 57.2 ± 3.73 for the 10 and 20 mg/kg doses, respectively. After SC administration of 10 mg/kg, C_{\max}/MIC_{50} and AUC_{0-24}/MIC_{50} ratios were 3.45 ± 0.75 and 33.96 ± 7.46 , respectively. After the administration of 10-h pulsed or 24-h continuous medicated water at 20 mg/kg, lower values of C_{\max}/MIC_{50} (10-h pulsed: 3.45 ± 0.7 ; 24-h continuous: 3.05 ± 0.48) and AUC_{0-24}/MIC_{50} (10-h pulsed: 42.42 ± 6.17 ; 24-h continuous: 53.32 ± 5.55) were obtained. Based on these results, the European Union-recommended dosage of 10 mg/kg seems ineffective to achieve adequate drug plasma concentrations and even the 20 mg/kg by 10 h pulsed or continuous medicated water administration did not reach completely efficacious concentrations in plasma against colibacillosis. Although the results obtained were not completely encouraging, the medicated water should preferably be provided continuously. To conclude about the efficacy of ENRO treatment against colibacillosis, target tissue concentration should be extensively considered.

Key words: enrofloxacin, pharmacokinetics/pharmacodynamics, turkey, colibacillosis

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INTRODUCTION

Enrofloxacin (ENRO) is an antimicrobial agent of the fluoroquinolone group approved only in veterinary medicine, with a broad antimicrobial spectrum and high bactericidal activity (Walker et al., 1992). In European Countries, ENRO was approved in the 1990s and is still extensively used in poultry for colibacillosis treatment, due to its unique effectiveness against multidrug-resistant avian pathogenic *Escherichia coli* (Bass et al., 1999; Lutful Kabir, 2010).

In avian species, the low economic value of individual birds makes single therapy cost-prohibitive and drinking water is the most common route of administering mass medication because sick birds continue to drink. For practical reasons, individual therapy by oral or parenteral route is reserved for high value breeders or small flocks.

In commercial turkeys, colibacillosis requires a prompt and efficacious antimicrobial treatment, preferably via the drinking water. The treatments can be conducted following 2 schemes: continuous administration during the entire light period or pulse administration for a limited period between a minimum of 4 and a maximum of 12 h (Charleston et al., 1998). In Europe, turkeys are considered a minor species and the cost of the therapy influences strongly the choice of the drug.

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In recent years, notwithstanding the scarce information on pharmacokinetics (PK) of antimicrobial drugs in turkeys, the ENRO use was increased in this species as a result of its effectiveness and the availability of generic products. However, an indiscriminate use of these drugs may both select for a resistant bacterial population and reduces their clinical efficacy.

Mass therapy, frequently adopted to cure large numbers of animals, is one of the main causes for the development of microbial resistance in veterinary food-producing species (EMA, 2007; Löhren et al., 2008). In poultry, an increase in the number of fluoroquinolone-resistant strains of *E. coli*, *Campylobacter* spp., and *Salmonella* spp. has been frequently reported in recent years (Walsh and Fanning, 2008; EFSA, 2010). Several scientific and health institutions have serious concern over the emergence of fluoroquinolone-resistance, manifesting the need for risk management intervention regarding the use of fluoroquinolones in humans and animals (EFSA, 2010).

During the last 10 yr, particular attention has been devoted to a correct evaluation of efficacious dosages for a more prudent and targeted use of antimicrobials in animal species. The correlation between minimum inhibitory concentrations (MIC) in field isolates and the PK behavior of antimicrobials in target species, known as PK/pharmacodynamic (PD) model is the best tool for a prudent and targeted use of antimicrobials (Martinez et al., 2006).

The aims of the present study were to evaluate 3 different oral treatments (a single oral gavage, 5 d of 10-h pulsed water medication, and 5 d of 24-h continuous water medication) and single parenteral (subcutaneous; SC) treatment using 2 different doses of ENRO (i.e., the EU authorized dose, 10 mg/kg, and double the EU recommended dose, 20 mg/kg). The effectiveness of different treatment schemes against *E. coli* was evaluated by a PK/PD approach, correlating the PK results with the MIC determined for 235 *E. coli* strains isolated from poultry in Italy reported by Vanni et al. (2014).

MATERIALS AND METHODS

Birds

Fifty female turkeys (commercial breed, British United Turkeys, B.U.T.6, Aviagen Turkeys, Cremona, Italy) 62 to 83 d old, weighed between 3.4 and 6.9 kg and determined to be healthy by a thorough physical examination, were selected from a commercial farm. The turkeys were housed according to the requirements of the European Union (Council of Europe, 2007) and were divided into 5 groups of 10 individuals kept into 5 pens of 5 m² on wood shavings. The birds were housed at 20°C and 65% RH and received 16 h of light/day. Standard commercial diet and water were supplied ad libitum in feeders and drinkers. After an acclimatization period of 8 d, the turkeys were weighed and individually marked for identification.

The study was conducted by the Animal Production Research and Teaching Centre of the University of Milan (Lodi, Italy) according to Italian law (D.L. 116/1992) and was ethically approved by the Ethical Committee of University of Milan (Opinion n. 31/11).

Experimental Design

Enrofloxacin was orally administered to turkeys via gavage as a single bolus at the dose of 10 mg/kg of BW or at the doubled dose of 20 mg/kg of BW, or via 10-h pulsed medicated water, or via continuous administration for 5 consecutive days at the doubled dose of 20 mg/kg of BW. Parenteral administration was a single SC injection at 10 mg/kg of BW.

Food and water were withdrawn 8 h before administrations to reduce any variability in the absorption due to drug-feed interaction and overdilution of the drug and treatments were carried out at the beginning of the light period. One hour after single treatments (oral and SC), fresh water and feed were supplied, whereas for repeated water medications only feed was supplied after 1 h.

The turkeys were randomly assigned to 5 groups of 10 birds each, indicated as groups 1, 2, 3, 4, and 5.

Groups 1 and 2 received ENRO (Baytril oral solution 10%, Bayer, Milano, Italy) as single oral dosage via gavage at the doses of 10 and 20 mg/kg of BW, respectively. Groups 3 and 4 were repeatedly treated via drinking water at the dose of 20 mg/kg of BW for 5 d with ENRO (Baytril oral solution 10%, Bayer). The water intake over a period of 10 or 16 h was measured for 3 d before the treatment. Enrofloxacin was added to the water based on the birds' mean weight and mean daily water intake. In group 3 the medicated water (ENRO mean concentration for 5 d: 179.7 ± 48.7 mg/mL) was provided in a pulsed scheme for 10 h/d from 0700 to 1700 h for 5 d and was then replaced with fresh water; in group 4 the medication (ENRO mean concentration for 5 d: 147 ± 1.5 mg/mL) was provided for 24 h and renewed every morning at 0700 h. The daily water consumption was measured at the end of the pulsed period (group 3) or before the each day renewal (group 4) to calculate the mean antibiotic intake.

Birds in group 5 were administered SC at the base of neck with ENRO (Baytril injectable solution 5%, Bayer) at the dose of 10 mg/kg.

Blood samples (maximum 1 mL) were collected from ulnar or metatarsal veins in heparinized tubes in all groups as indicated in Table 1. Plasma was separated by centrifugation at 1,500 × *g* for 10 min and stored at -20°C pending analysis.

Liquid Chromatography–Mass Spectrometry Analysis and Method Validation

The plasma samples purification was performed as reported by Lucatello et al. (2013, 2014). Mass spec-

Table 1. Treatments, doses, and sampling times in all groups of turkeys

Group	Treatment	Dose (mg/kg)	Sampling time (h)
1	Single oral gavage	10	0, 0.25, 0.5, 1, 2, 4, 8, 12, 24
2	Single oral gavage	20	0, 0.25, 0.5, 1, 2, 4, 8, 12, 24
3	5 d 10-h pulsed medicated water	5 d actual dose: 15.06 ± 3.33	d 1 and 5: 0, 1, 3, 6, 9, 11, 12, 14, 18, 24
4	5 d 24-h continuous medicated water	5 d actual dose: 21.9 ± 2.31	d 5: 0, 2, 6, 9, 12, 15, 18, 20, 24
5	Single subcutaneous injection	10	0, 0.25, 0.5, 1, 2, 4, 8, 10, 24

trometric (MS) analysis was performed on a LTQ XL ion trap (Thermo Fischer Scientific, San Jose, CA), equipped as indicated by Lucatello et al. (2013, 2014). The collision energies that were necessary for fragmentation of the parent compounds (ENRO and ciprofloxacin, **CIPRO**) into precursor ions (**MS/MS**) and product ions (**MS/MS/MS**) are shown in Table 2. Calibration curves were constructed using pooled turkey plasma obtained from untreated birds. Blank plasma was spiked with 10 µL of internal standard (**IS**) norfloxacin (3 µg/mL) and with ENRO and CIPRO to obtain a concentration range of 2.5 to 200 ng/mL. Quantification was based on the ratios of the peak areas of the analyte to that of IS and a least squares linear regression analysis was performed to calculate calibration curves.

The method was in-house validated using a set of parameters [linearity, within-run and between-run accuracy and precision, limit of quantification (**LOQ**), limit of detection (**LOD**), and selectivity] that were in compliance with the recommendations defined by the European Community (European Commission, 2002) and with the reference guidelines defined in other European Union and FDA documents (VICH GL 49, 2012). The calibration curves were constructed using matrix-matched calibrator samples (concentration range: 2.5–200 ng/mL) and the correlation coefficients was always $r > 0.99$ for 6 replicates. Within-day precision (repeatability) and accuracy were determined by analyzing blank samples that were spiked with both compounds

at 2.5 (n = 6), 10 (n = 6), and 50 (n = 6) ng/mL on the same day. The between-day precision and accuracy were determined by analyzing quality control samples, concentration level: 2.5 (n = 18), 10 (n = 18), and 50 (n = 18) ng/mL, with each batch of analytical samples on 3 different days. The validation results are reported in Table 2 and fell within the accepted ranges for validation. All values below the LOQ were not included in the plasma concentration-time curves and the pharmacokinetic analysis.

PK and Statistical Analysis

The PK parameters were deduced from plasma concentration-time data using the WinNonLin Prof 6.1 software (Pharsight Corporation, Mountain View, CA), which allows both compartmental and noncompartmental analyses of experimental data. Minimum information criterion estimation (Yamaoka et al., 1978) was used to choose the best fitting model for the data. All of the data points were weighted by the inverse square of the fitted value. Plasma concentrations after single oral bolus, SC, and continuous administration were fitted to a standard monocompartmental model and also a noncompartmental analysis was carried out. The kinetics after the 10-h pulsed administrations was determined at d 1 and 5 using a noncompartmental analysis (Gibaldi and Perrier, 1982). The peak concentrations, C_{\max} , and time to peak T_{\max} were obtained from the experimentally observed data. The elimination half-life

Table 2. Characteristics obtained using mass spectrometry analysis and validation results¹

Item	Mass spectrometry characteristic				
	Precursor ion [M-H] ⁺ (m/z)	Collision energy MS/MS (%)	Precursor ion MS/MS (m/z)	Collision energy MS/MS/MS (%)	Product ion MS/MS/MS (m/z)
Compound					
ENRO	360	46	316	23	245
CIPRO	332	22	288	30	245, 268
Norfloxacin (IS)	320	36	276	30	256, 233
			Validation		
Concentration (ng/mL)	Within-run precision (n. 6)		Between-run precision (n. 18)		
2.5	ENRO: 7.2%; CIPRO: 11.9%		ENRO: 10.6%; CIPRO: 12.6%		
10	ENRO: 6.1%; CIPRO: 5%		ENRO: 5.5%; CIPRO: 7.2%		
50	ENRO: 5.5%; CIPRO: 4.7%		ENRO: 7.1%; CIPRO: 5.7%		
	LOQ		LOD		
	ENRO: 2.47 ng/mL; CIPRO: 1.4 ng/mL		ENRO: 0.82 ng/mL; CIPRO: 0.46 ng/mL		

¹MS = mass spectrometry; ENRO = enrofloxacin; CIPRO = ciprofloxacin; IS = internal standard; LOQ = limit of quantification; LOD = limit of detection.

was calculated as $\ln 2/\lambda_n$; mean residence time (MRT) was determined from the following equation: $MRT = AUMC/AUC$, where AUMC is the area under the moment curve and AUC is the area under plasma concentration-time curve.

Pharmacokinetic parameters are reported as the mean values (\pm SD). The harmonic means and pseudo-standard deviations were calculated for half-lives using a jack-knife technique (Lam et al., 1985). The normality of the kinetics data was assessed using the Kolmogorov-Smirnov test. The differences between the 2 gavage doses (group 1 vs. 2), and between the 10 mg/kg gavage and the 10 mg/kg SC (group 1 vs. 5) were compared by a 2-tailed unpaired *t*-test. The water medication by 10-h pulsed scheme at d 1 and 5 were compared by a 2-tailed unpaired *t*-test; the same test was used to compare the d 5 of 10-h pulsed and continuous administration. A *P*-value < 0.05 was considered statistically significant (GraphPad Prism version 4.00, San Diego, CA).

The following PK/PD indices were calculated as predictors of the success or failure of the therapy: C_{max}/MIC and AUC/MIC ratios. MIC_{50} , defined as minimum inhibitory concentration at which 50% of isolates tested are inhibited, is generally used for PK/PD correlation (Toutain et al., 2002; McKellar et al., 2004). The breakpoint values of $C_{max}/MIC_{50} = 8-10$ and $AUC/$

$MIC_{50} = 100$ h were considered representative of the therapeutic efficacy of fluoroquinolones to prevent the development of resistant bacterial strains in poultry.

RESULTS

The CIPRO concentrations were low in all samples from all birds reaching approximately 3 to 4% of the parent compound. Therefore, the mean plasma concentration-time profiles and PK parameters were reported as the sum of ENRO and its metabolite.

Single Gavage Administration (Groups 1 and 2)

Mean plasma concentrations + SD of ENRO of both doses are shown in Figure 1. Following oral gavage at the dose of 10 mg/kg (group 1) or 20 mg/kg (group 2), ENRO reached the maximum concentrations at approximately 2 h; subsequently, drug levels decreased rapidly, but were still detectable at 24 h after administration with a mean concentration of 0.09 ± 0.02 μ g/mL (group 1) and 0.16 ± 0.03 μ g/mL (group 2).

The pharmacokinetic parameters obtained by mono-compartmental and noncompartmental analysis are presented in Table 3. The C_{max} for ENRO after gavage

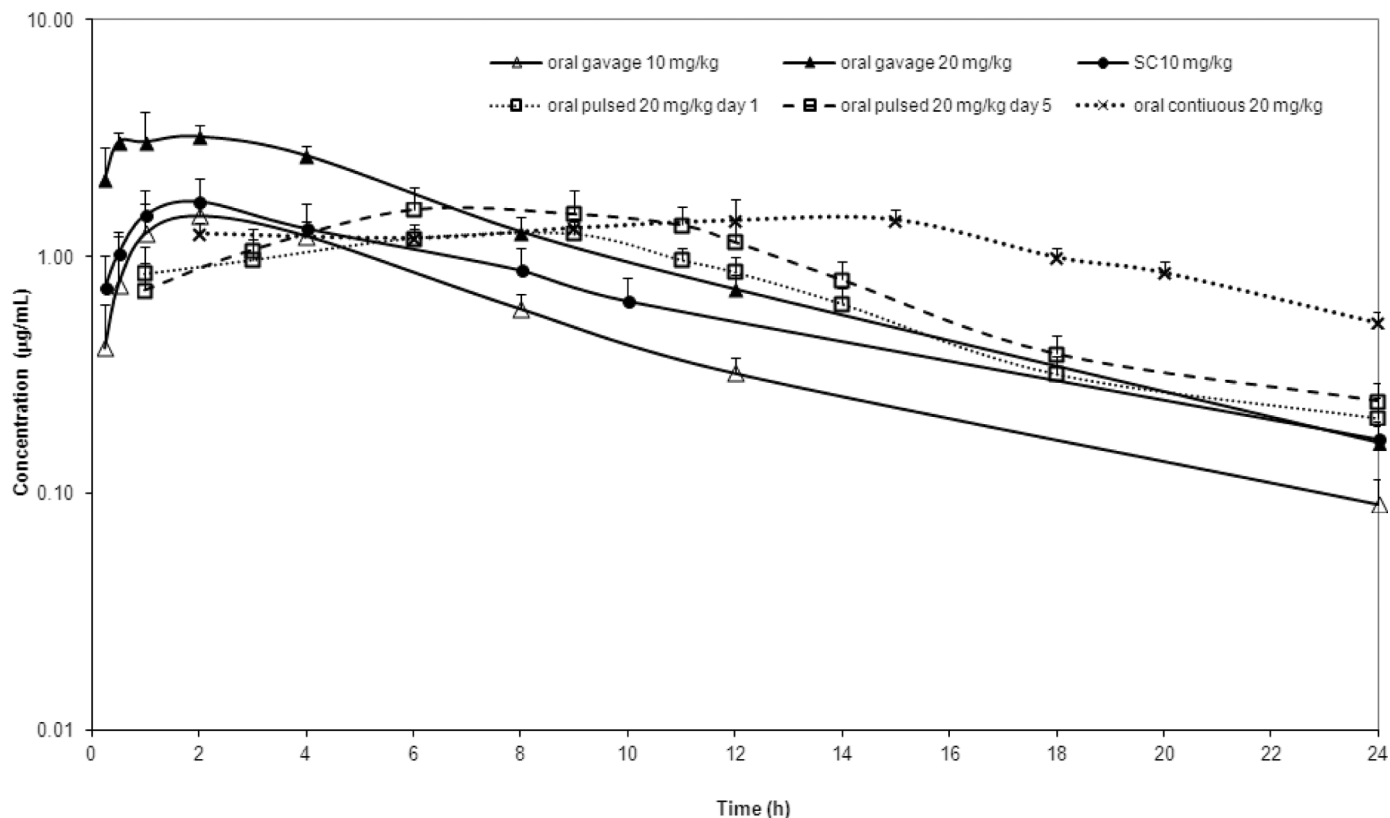


Figure 1. Mean values + SD in the plasma concentration-time profiles of enrofloxacin in all groups of treated birds: group 1 (open triangles, solid line), oral gavage at 10 mg/kg; group 2 (filled triangles, solid line), oral gavage at 20 mg/kg; group 5 (filled circles, solid line), subcutaneous (SC) administration at 10 mg/kg; group 3 at d 1 (open square, dotted line) and 5 (open squares, dashed line) following 10-h oral pulsed administration of an average dosage of 15.06 mg/kg of BW for 5 d and in group 4 (cross, dotted line) at d 5 after continuous water medication of 20 mg/kg for 5 d.

Table 3. Pharmacokinetics parameters in turkeys after oral gavage at 10 (group 1) or 20 mg/kg (group 2) or subcutaneous (SC) administration at 10 mg/kg (group 5)¹

Parameter (unit)	Group 1 Oral gavage 10 mg/kg	Group 2 Oral gavage 20 mg/kg	Group 5 SC 10 mg/kg
T _{max} (h)	1.88 ± 0.33	1.88 ± 0.99	1.87 ± 0.35
C _{max} (µg/mL)	1.53 ± 0.31‡	3.51 ± 0.55†	1.73 ± 0.44
AUC _{0-∞} (h·µg/mL)	13.13 ± 1.40‡§	29.17 ± 1.97†	18.10 ± 3.33†
AUC ₀₋₂₄ (h·µg/mL)	12.74 ± 1.52‡§	28.60 ± 2.00†	16.82 ± 4.05†
AUMC ₀₋₂₄ (h·h·µg/mL)	83.43 ± 9.69‡§	179.25 ± 19.85†	114.88 ± 36.83†
t _{1/2 elim} (h)	5.27 ± 0.67*	4.99 ± 0.32*	6.22 ± 1.36*
MRT ₀₋₂₄ (h)	6.57 ± 0.53	6.26 ± 0.48	6.73 ± 1.32
MIC ₅₀ (µg/mL) for <i>Escherichia coli</i>	0.5		
C _{max} /MIC ₅₀	3.07 ± 0.62‡	7.01 ± 1.03†	3.45 ± 0.75
AUC/MIC ₅₀	25.48 ± 3.04‡§	57.20 ± 3.73†	33.96 ± 7.46†

¹T_{max} = observed time for C_{max}; C_{max} = maximum concentration; AUC_{0-∞} = area under the concentration versus time curve from 0 to infinity; AUC₀₋₂₄ = area under the concentration versus time curve from 0 to 24 h; AUMC₀₋₂₄ = area under moment curve; t_{1/2 elim} = elimination half-life; MRT₀₋₂₄ = mean residence time; MIC₅₀ = minimum inhibitory concentration 50%.

*Harmonic mean ± pseudo SD.

‡Significantly different ($P < 0.05$) from group 2.

§Significantly different ($P < 0.05$) from group 5.

†Significantly different ($P < 0.05$) from group 1.

(1.53 ± 0.31 µg/mL and 3.51 ± 0.55 µg/mL in group 1 and 2, respectively) were attained at 1.88 ± 0.33 (group 1) and 1.88 ± 0.99 h (T_{max}; group 2). The mean AUC₀₋₂₄ and half-lives were 12.74 ± 1.52 h·µg/mL and 5.27 ± 0.67 h in group 1 and 28.60 ± 2.00 h·µg/mL and 4.99 ± 0.32 h in group 2.

10-h Medicated Water Administration (Group 3)

During pulse scheme trials the drug water concentration was adjusted daily based on water intake, the measurement of water at the end of 10-h treatment indicated that the dose received by turkeys was lower than the targeted 20 mg/kg of BW, reaching a value of 14.18 and 16.67 mg/kg at d 1 and 5, respectively; the mean dose received by the group was 15.06 ± 3.33 mg/kg.

The ENRO mean concentration–time profiles following 10-h administration of medicated water are shown in Figure 1, the data refer to d 1 and 5 of therapy. The mean kinetic parameters obtained by noncompartmental analysis are resumed in Table 4; C_{max} were attained at about 8 h and the highest concentrations were achieved on d 5 of treatment (1.28 ± 0.14 µg/mL and 1.72 ± 0.37 µg/mL at d 1 and 5, respectively). The mean AUC₀₋₂₄ at d 1 and 5 were 17.28 ± 2.03 and 21.21 ± 3.30 h·µg/mL ($P < 0.05$), respectively.

24-h Continuous Medicated Water Administration (Group 4)

During continuous treatment the drug water concentration was adjusted daily based on water intake. The measurement of water at the end of 24-h treatment indicated that the dose received by turkeys was between 24.73 and 18.39 mg/kg at d 1 and 5, respectively; the

mean dose received by the group was 21.9 ± 2.31 mg/kg.

The ENRO mean concentration–time profile at d 5 following 24-h administration of medicated water for 5 consecutive days is shown in Figure 1, together with the data from all the other scheme of administration. The mean kinetic parameters obtained by mono- and non-compartmental analysis are resumed in Table 4; C_{max} was attained at about 12 h with a mean value of 1.53 ± 0.24. The mean AUC₀₋₂₄ and elimination half-life were 26.66 ± 2.77 h·µg/mL and 9.78 ± 1.40 h, respectively.

Single SC Administration (Group 5)

Mean plasma concentrations + SD of ENRO after single SC administration at 10 mg/kg are shown in Figure 1. A low interindividual variability was observed in all birds. The ENRO reached the maximum concentrations at approximately 2 h; subsequently, drug levels decreased rapidly, but were still detectable at 24 h after administration with a mean concentration of 0.17 ± 0.03 µg/mL.

The pharmacokinetic parameters obtained by mono- and noncompartmental analysis are presented in Table 3. The C_{max} of 1.73 ± 0.44 µg/mL for ENRO after SC administration was attained at 1.87 ± 0.35 h (T_{max}). The mean AUC₀₋₂₄ and half-life were 16.82 ± 4.05 h·µg/mL and 6.22 ± 1.36 h, respectively.

PK/PD Integration

The PK/PD integrations were calculated for the different trials, based on PK parameters and MIC₅₀ value; the values are presented in Tables 3 and 4. The MIC₅₀, defined by the broth microdilution method for 235 avian *E. coli* strains isolated in Italy and reported by Vanni et al. (2014), resulted in 0.5 µg/mL, and this value was used for PK/PD correlation. Statistical differences (P

Table 4. Pharmacokinetics parameters in turkeys after oral water medication following 10-h (group 3) or continuous administration (group 4) at 20 mg/kg¹

Parameter	Group 3 Oral pulsed 20 mg/kg (d 1)	Group 3 Oral pulsed 20 mg/kg (d 5)	Group 4 Oral continuous 20 mg/kg (d 5)
T _{max} (h)	7.88 ± 1.55	8.50 ± 2.33§	12.02 ± 2.67‡
C _{max} (µg/mL)	1.28 ± 0.14‡	1.72 ± 0.37‡	1.53 ± 0.24
AUC _{0-∞} (h·µg/mL)	—	—	37.37 ± 3
AUC ₀₋₂₄ (h·µg/mL)	17.28 ± 2.03‡	21.21 ± 3.3‡§	26.66 ± 2.77‡
AUMC ₀₋₂₄ (h·h·µg/mL)	160.07 ± 17.87‡	201.72 ± 28.92‡§	309.32 ± 31.33‡
t _{1/2 elim} (h)	—	—	9.78 ± 1.40*
MRT ₀₋₂₄ (h)	9.27 ± 0.22	9.53 ± 0.44§	11.61 ± 0.23‡
MIC ₅₀ (µg/mL) for <i>Escherichia coli</i>	0.5		
C _{max} /MIC ₅₀	3.07 ± 0.62‡	3.45 ± 0.70‡	3.05 ± 0.48‡
AUC/MIC ₅₀	25.48 ± 3.04‡	42.42 ± 6.71‡§	53.32 ± 5.55‡

¹T_{max} = observed time for C_{max}; C_{max} = maximum concentration; AUC_{0-∞} = area under the concentration versus time curve from 0 to infinity; AUC₀₋₂₄ = area under the concentration versus time curve from 0 to 24 h; AUMC₀₋₂₄ = area under moment curve; t_{1/2 elim} = elimination half-life; MRT₀₋₂₄ = mean residence time; MIC₅₀ = minimum inhibitory concentration 50%.

*Harmonic mean ± pseudo SD.

‡Significantly different ($P < 0.05$) from group 3, d 5.

§Significantly different ($P < 0.05$) from group 4.

†Significantly different ($P < 0.05$) from group 3, d 1.

< 0.05) in the C_{max}/MIC₅₀ and AUC/MIC₅₀ ratio were observed when the dose was doubled for the gavage and only in the AUC/MIC₅₀ ratio between oral gavage and SC administration. For the 10-h pulsed administrations, the C_{max}/MIC₅₀ and AUC/MIC₅₀ ratio were significantly different at d 1 and 5, whereas comparisons with continuous administration reported differences for AUC/MIC₅₀ between 10-h pulsed at d 5 and continuous administration (Table 4).

DISCUSSION

Unlike in other animal species, biotransformation of ENRO into its active metabolite CIPRO is low in poultry (Carreras et al., 2004; Dimitrova et al., 2007), as also confirmed by the very low amounts of CIPRO recovered (approximately 3–4% of ENRO) in this study.

After gavage administration of 2 different doses of ENRO, the increase of C_{max} and AUC was related to the dose increase and the concentration-time profiles were similar (Figure 1). Enrofloxacin was rapidly absorbed, T_{max} approximately 2 h, in contrast to what was reported for ENRO by Dimitrova et al. (2007) and for danofloxacin by Haritova et al. (2006), where higher values were recorded (T_{max}: 6.33 ± 2.5 h for enrofloxacin and 6.0 ± 3.29 h for danofloxacin). Conversely, similar results were obtained for flumequine by the same group of authors (T_{max}: 2 h; Ferraresi et al., 2013). The half-life proved to be rather short (t_{1/2 elim}: 5.27 ± 0.67 and 4.99 ± 0.32 h, groups 1 and 2, respectively), and as expected, was not dependent on the dose given.

After SC treatment similar results were obtained, T_{max} approximately 2 h and t_{1/2 elim} 6.22 ± 1.36 h, the maximum concentrations were also comparable, C_{max} 1.73 ± 0.44 µg/mL (group 5) versus 1.53 ± 0.31 µg/mL (group 1).

The C_{max} and AUC₀₋₂₄ obtained, which were higher compared with those with medicated water (Tables 3

and 4), may indicate that individual treatments by oral gavage or SC administration are suitable for efficacious therapy. However, they are not easily practicable in intensive turkey farming due to the high bird density in the sheds, the need for a high number of trained personnel to individually handle the birds, and the stress caused to the birds. This administration route should be preferably adopted in small groups of birds or for breeders because these have an important genetic impact on the progeny, are expected to live longer, and have an high economic value in the flock.

In group 3, the pulsed administration trial showed an AUC₀₋₂₄ and C_{max} increase at d 5 (AUC₀₋₂₄: from 17.28 ± 2.03 to 21.21 ± 3.30 h·µg/mL; C_{max}: from 1.28 ± 0.14 to 1.72 ± 0.37 µg/mL, respectively). These results can be explained by an increase in ENRO concentrations in medicated water due to the low intake of the drug observed at the first day of the trial. In fact, medicated water concentrations were adjusted based on the water intake of previous administrations. The achievement of the targeted dose of 20 mg/kg was never obtained, likely due to the poor palatability of the product and due to the availability of unmedicated water in the remaining 6 h of light period. It is known that drug intake can vary dramatically due to both bird factors (hierarchy, flock size, sex, age, weight, species, breed, health status, and so on) and environmental factors (temperature, humidity, feed and water availability, photoperiod, and so on; Vermeulen et al., 2002).

Comparing these results with those by Russo et al. (2012), who assessed the PK of ENRO at the 10 mg/kg via medicated water in healthy and colisepticemic turkeys, a dose proportional increase of C_{max} and AUC was observed, whereas T_{max} was not affected by dosage.

In group 4, the drug concentration in water was adjusted according to water intake and the dose received by the turkeys (21.90 ± 2.31 mg/kg) was close to the

targeted dose of 20 mg/kg. Notwithstanding the likely poor palatability of the water, the lack of fresh water forced the birds to drink all the available water and take the targeted dose of ENRO. Compared with pulsed administration at d 5, the 24-h continuous administration of ENRO for 5 d resulted in a longer T_{\max} 12.2 ± 2.67 h and MRT of 11.61 ± 0.23 h and higher AUC_{0-24} , whereas C_{\max} was similar.

In agreement with several studies on *E. coli* strains isolated from food-producing animals, a high percentage of strains has proved to be resistant to ENRO (EFSA, 2010; Ozawa et al., 2010). As reported in the co-authored paper by Vanni et al. (2014), 38.7% of *E. coli* tested was resistant, a percentage lower than that observed with old generation fluoroquinolones (70.2% with flumequine), but confirming an increasing trend since the introduction of fluoroquinolones in poultry. The increase in the prevalence of antimicrobial-resistant pathogenic bacteria in farm animals requires re-evaluation of treatment options. As prescribed by EMA (2007) and WHO (2011) in the last sets of guidelines on the prudent use of antimicrobials, fluoroquinolones should be used in turkeys only when a susceptibility test clearly indicates the efficacy of the drug. It was widely accepted that fluoroquinolone dosage regimens that lead to high PK/PD indices as $AUC/MIC >125$ and $C_{\max}/MIC >8$ resulted in less frequent selection of resistant mutants (McKellar et al., 2004). Although specific breakpoints have not been defined for avian colibacillosis, several studies on fluoroquinolones in poultry (Anadón et al., 2001; Dimitrova et al., 2007; Ozawa et al., 2010; Ferraresi et al., 2013) adopted a C_{\max}/MIC ratio of 8 or 10 and an AUC/MIC ratio of 100 as the minimal values required to prevent the selection of resistant bacteria. As reported in Tables 3 and 4, neither type of administration reached the breakpoint values and the PK/PD correlation yielded unsatisfactory results. After gavage administration, the mean C_{\max}/MIC_{50} and AUC_{0-24}/MIC_{50} ratios were, respectively, 3.07 ± 0.62 and 7.01 ± 1.03 , and 25.48 ± 3.04 and 57.2 ± 3.73 for the 10 and 20 mg/kg doses, respectively. After SC administration C_{\max}/MIC_{50} and AUC_{0-24}/MIC_{50} ratios were 3.45 ± 0.75 and 33.96 ± 7.46 , respectively. After the administration of 10-h pulsed (group 3) or 24-h continuous (group 4) medicated water with the dosage regimen of 20 mg/kg lower values of C_{\max}/MIC_{50} (group 3: 3.45 ± 0.70 ; group 4: 3.05 ± 0.48) and AUC_{0-24}/MIC_{50} (group 3: 42.42 ± 6.17 ; group 4: 53.32 ± 5.55) were obtained. These results were similar to those obtained by Russo et al. (2012). Conversely, data published by Dimitrova et al. (2007) supported the efficacy of 10 mg/kg administered via drinking water to turkeys, but PK/PD results were obtained correlating the kinetic parameters with a value of MIC first reported in 1996 and significantly lower than those adopted in the present study (0.06 versus 0.5 $\mu\text{g}/\text{mL}$).

Based on the present results, the EU-recommended dosage of 10 mg/kg might be ineffective to achieve adequate drug plasma concentrations. Also the 20 mg/kg

by 10-h pulsed doses of medicated water did not reach plasma concentrations that were completely efficacious in controlling *E. coli*, the scenario could be even worse when considering the long period with unmedicated water. Indeed, Santos et al. (1997) showed the influence of the photoperiod on the PK of drugs during drinking water administration in turkeys. The eating and drinking patterns can be altered by the light scheme (Classen et al., 1994; Watteyn et al., 2013), which could have a huge influence on the uptake of drinking water medication. Although the results obtained were not completely encouraging, the medicated water should always be provided continuously, as an increase of PK/PD indices was achieved for AUC/MIC .

Escherichia coli is generally located in the intestine and active ENRO concentrations undergo a biliary excretion; thus, plasma concentration does not reflect the same magnitude order of intestinal environment. A similar scenario should be foreseen for pulmonary infections, against which ENRO is frequently used. Indeed, plasma concentrations might not be a good predictor of efficacy, as ENRO concentrations and AUC are reported to be higher in lungs than in plasma (Tang et al., 2007). In addition, interesting results were obtained during the validation process of the LC-MS analytical method by detecting ENRO and CIPRO in lung tissue and intestinal content from turkey treated with ENRO (Lucatello et al., 2014). Both CIPRO and ENRO concentration in lung and intestinal content were much higher than in plasma in turkeys killed after ENRO treatment. Therefore, target tissue concentrations need to be evaluated to define the efficacy of ENRO treatment against colibacillosis.

All the treatment scheme evaluated in this study, based on plasma concentration, were not completely satisfactory against *E. coli*, supporting the unsuitability of the ENRO-recommended dosage scheme. Thus, to improve treatment efficacy and comply with the prudent and responsible use of fluoroquinolones in poultry species, a revision of the ENRO dosage scheme, which includes an extensive distribution study in the target tissues (i.e., intestine and lung), is advisable for a real efficacy evaluation against colibacillosis.

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