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Pharmacokinetics and tissue residues of colistin following intravenous, and single and repeated oral dosing in domestic geese (*Anser anser domesticus*)

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ABSTRACT

Colistin, also known as polymyxin E, is a member of the polymyxin group of antibiotics. It is approved in Europe to treat enteric infections caused by Gram-negative bacteria, such as *Escherichia coli,* in poultry, although the similarity of infections between species make it likely used off-label in geese as well.This study investigated the pharmacokinetics and tissue residues of colistin in geese through *in vivo* experiments. The study involved longitudinal open studies on 16 healthy adult male geese, divided into three phases separated by one-month washout period. Geese were administered colistin via intravenous (IV, 1 mg/kg), single oral (PO, 30 mg/kg), and multiple oral (SID, 2.5 mg/kg for five consecutive days) routes, with blood samples drawn at specific intervals. Tissue samples were also collected at pre-assigned times for subsequent analysis. Colistin levels in geese plasma were quantified using a fully validated UHPLC-MS/MS method.

Plasma concentrations could be quantified up to 24 h for the single PO (n= 2) and IV (n= 4) routes, and up to 10 h (n= 6) from the last dose administered for the multiple PO route (n=6). The bioavailability was significantly low, averaging 3 %. The terminal half-life in geese was 2.18 h following IV administration, similar to values found in other avian species. Following IV administration, clearance and volume of distribution values were 0.11 $mL{\cdot}h^{-1}{\cdot}g^{-1}$ and 0.41 $mL{\cdot}g^{-1}$, respectively. The body extraction ratio was low at 0.2 %, indicating minimal hepatic and renal elimination of colistin. Multiple oral doses showed no plasma accumulation, and tissue levels consistently remained below the maximum residue limit (MRL) set for food-producing animals. This study highlights the minimal systemic bioavailability and tissue penetration of colistin in geese, consistent with findings in other poultry and mammals. Future research should focus on intestinal colistin content in geese to optimize dosing strategies and minimize anti-microbial resistance.

Introduction

Colistin, also known as polymyxin E, is part of the polymyxin group of antibiotics. Similar to other polypeptide antibiotics, colistin is a complex compound made up of over 13 cyclic polypeptides, varying primarily in the length of their fatty acyl segments (Decolin et al., 1997). The two main components, colistin A and B (polymyxin E1 and E2), can constitute up to 95 % of its total mass. Colistin has a narrow antimicrobial spectrum. It is effective against fermentative Gram-negative bacteria such as *Escherichia coli*, *Klebsiella spp.*, and *Salmonella spp.*, as well as non-fermentative Gram-negative bacteria like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Poirel et al., 2017).

In veterinary medicine, colistin has been widely used, particularly in swine, bovine, and poultry, for prevention, treatment, and metaphylaxis (Catry et al., 2015; Rhouma et al., 2016; Elbadawy and Aboubakr, 2017). It has also been employed as a growth promoter in many countries, including China, India, and Vietnam (Kempf et al., 2016; Guetiya Wadoum et al., 2016). However, following the discovery of mobilized

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colistin resistance (mcr) genes (Liu et al., 2016), many countries have restricted its use as a feed additive and growth promoter. In 2015, the European Union limited the use of colistin in veterinary medicine to therapy or metaphylaxis, eliminating its use for prophylactic purposes. Its indications were specifically restricted to the treatment of gastrointestinal (GI) infections caused by non-invasive, susceptible Enterobacteriaceae in pigs, cattle, small ruminants, and poultry (EMA/CVMP, 2015; Catry et al., 2015).

Specifically, colistin is mainly employed in pig and cattle production to control enteric infections caused by *E. coli* and *Salmonella* (e.g. post-weaning diarrhoea in piglets) (Catry et al., 2015; Rhouma et al., 2016). For poultry, there are no relevant indications given other than the prohibition of its use against *Salmonella* infections (Löhren et al., 2008). Indeed, while not restricted for use against *Salmonella* in all countries, the World Health Organization (WHO) advocates for the reduction of colistin use in food animals, particularly in poultry, as a measure to prevent the spread of resistant bacteria, including *Salmonella*. Nonetheless, several publications mention that colistin is frequently used in poultry to treat mild colibacillosis (Kempf et al., 2016; Catry et al., 2015; Lima Barbieri et al., 2017), and in combination with amoxicillin for treating necrotic enteritis in broiler chickens (Elbadawy and Aboubakr, 2017).

When used in farm animals, including poultry, colistin is typically administered orally, through drinking water (Stefaniuk, Tyski, 2019). While there is no specific data on the use of colistin in geese, it is plausible that such off-label practices are occurring. This assumption is based on the widespread incidence of colibacillosis, which affects not only chickens but also geese, ducklings, turkeys, and pigeons (Lutful Kabir, 2010). While the pharmacokinetics (PK) of colistin have been studied in various animal species, research specific to geese is notably missing. This absence of comprehensive data on antibacterial use in geese poses a significant challenge for the industry. Understanding the efficacy and appropriate usage of antibacterials is crucial to address public health issues and ensure the well-being of the birds. Moreover, the growing problem of antimicrobial resistance to colistin highlights the need for targeted research each species to avoid suboptimal non-effective concentrations by relying on dosage extrapolations from other species. Therefore, the primary objective of this study was to conduct a detailed analysis of the PK characteristics and tissue residue patterns of colistin in healthy geese. This investigation included single intravenous (IV) administration, and both single and repeated oral (PO) administrations.

Materials and methods

Animals

This study used 16 healthy adult male Bilgorajska geese (*Anser anser domesticus*), aged between 22 and 26 months, with weights ranging from 2.8 to 4.5 kg. These geese were sourced from a local farm (Majątek Rutka, Puchaczow, Poland). They have not received any prior antibiotic treatment and were individually distinguished by rings on their left legs. Prior to the study, a thorough evaluation by the supervising veterinarians (K B.; B. L-W) confirmed their healthy status. The geese underwent a week-long acclimatization period in the new environment, which included daily monitoring of their behavior and appetite. They were housed in a 60 m^2 enclosed area with an indoor shelter measuring 10 m^2 and had unrestricted access to feed and water. The animal experiment was carried out in accordance with European law (2010/63/UE) and approved (nr. 59/2022; 76/2023) by the Institutional Animal Care and Use Committee of the University of Lublin (Poland).

Experimental applications

The study design involved a series of longitudinal, open studies conducted on the previously described geese. Each phase was separated by a one-month washout period. The multiple PO doses used were based on the recommended doses for poultry (FAO, 2006; Mead et al., 2021). For IV administration, a lower dose was deliberately chosen to minimize potential toxic effects. In contrast, a higher dose was selected for the single PO administration to address the possibility of very low systemic concentrations, as observed with lower doses in previous poultry studies (FAO, 2006).

In the initial phase, geese received an IV dose of colistin at 1 mg/kg (Colistin TZF 1000000 IU), in 5 mL of saline solution, administered through a sterile 20-gauge 3.75 cm needle in the left-wing vein. In the second phase, a single oral administration of 30 mg/kg b.w. colistin solution (Colimed, Biofactor, Poland, 1200,000 IU/g) was administered via crop gavage, with the cannula being flushed promptly afterwards with 5 mL of water to ensure proper delivery. The third phase (multiple PO) involved administering a colistin solution (Colimed, Biofactor, Poland, 1200,000 IU/g) to geese via crop gavage at a dose of 2.5 mg/kg once a day for 5 consecutive days at 08:00 am.

Blood samples, approximately 1 mL each, were drawn from the right-wing vein at specific time intervals during all phases: 0, 5 (for IV administration only), 15, 30, 45 min, and 1, 1.5, 2, 4, 6, 8, 10, and 24 h. For the multiple PO, blood samples were taken every 24 h, just before the administration of the daily dose. On the fifth day, blood sampling followed the same pattern as the single PO, with an additional sample taken at 36 h. The blood was collected in heparinized tubes and then centrifuged at 1500 *x g*. Subsequently, the plasma was carefully harvested and stored at a temperature of −20° C. The analysis of the stored plasma was conducted within 40 days from the time of collection, after each phase. Regarding tissue sampling, sets of four geese were euthanized, via stunning and bleeding, at specific time points: 6, 10, 24 and 36 h after the last oral dose of the multiple administration. Samples of approximately 20 g of tissue were collected from muscle, liver, heart, lungs, and kidney, in each animal and stored at −20° C for subsequent analysis.

Colistin quantification in plasma and tissues

The method determination for colistin in plasma was developed and validated by the authors. To extract colistin from plasma samples, 200 µl of plasma was aliquoted into polypropylene Eppendorf tubes. Then, 50 µl of the internal standard (IS) working solution (Polymyxin B; 2 µg/mL) was added and briefly vortex-mixed. Extraction was carried out using 800 µl of acetonitrile (ACN) containing 0.1 % formic acid (HCOOH) and 0.2 % trifluoroacetic acid (TFA). The samples were vortexed, stirred for 10 min, and then centrifuged at 15,000 *x g* for 10 min. Subsequently, 1 mL of the supernatant was transferred to a clean Eppendorf tube and evaporated to dryness using a nitrogen stream at 40° C. The dry residue was reconstituted in 0.2 mL of a solution containing 1 % HCOOH in water and 1 % HCOOH in ACN (95/5; *v/v*). This reconstituted mixture was then transferred to Eppendorf tubes with filters and centrifuged at 15,000 *x g* for 5 min. Finally, the supernatant was transferred to an HPLC vial with a glass insert and analyzed using UHPLC-MS/MS.

For tissue samples, the determination method was based on previous studies (Boison et al., 2015; Kumar et al., 2021). All tissues were homogenized using a mechanical mixer designed for laboratory use before extraction. 1 g of each tissue was weighed and placed into a polypropylene centrifuge tube. Then, 100 µl of the IS working solution (2 µg/mL) was added and briefly vortexed. Extraction was carried out using 7 mL of ACN containing 0.1 % HCOOH and 0.2 % TFA. The samples were stirred for 10 min and then centrifuged at 4500 *x g* for 10 minutes. Following centrifugation, 6 mL of the supernatant was transferred to a glass tube and evaporated to dryness using a nitrogen stream at 40° C. The dry residue was reconstituted in 0.5 mL of a solution containing 1 % HCOOH in water and 1 % HCOOH in ACN (95/5; *v/v*). The reconstituted solution was filtered through a 0.22 µm PVDF filter, transferred to an HPLC vial with a glass insert, and analyzed using

UHPLC-MS/MS.

UHPLC-MS/MS conditions

The analysis utilized ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). LC separation was conducted on a Shimadzu Nexera X2 UHPLC system (Shimadzu, Japan), employing a Luna Omega Polar C18 column (100×2.1 mm × 1.6 µm) coupled with a Security Guard ULTRA holder and cartridge for UHPLC C_{18} columns (Phenomenex, USA). The mobile phase consisted of 1 % formic acid in water (solvent A) and 1 % formic acid in acetonitrile (solvent B). The gradient elution program was set as follows: starting with 5 % solvent B at 0.00 min, increasing to 30 % solvent B from 0.01 to 3.0 min, further increasing to 95 % solvent B from 3.01 to 4.50 min, and then decreasing back to 5 % solvent B from 4.51 to 6.0 min. The column oven temperature was maintained at 45° C, with a flow rate of 0.4 mL/min and an injection volume of 10 µl.

The mass spectrometry analysis was conducted using a SCIEX 5500 triple quadrupole mass spectrometer (Sciex, USA), controlled by Analyst 1.6.3 software (SCIEX, Framingham, MA, USA). The mass spectrometer operated in electrospray ionization (ESI+) mode with MS data acquisition performed in multiple reaction monitoring (MRM). The analysis monitored specific precursor ions for colistin at *m/z* 386 and corresponding product ions at *m/z* 380 and *m/z* 374, and for polymyxin B at *m/z* 402 with a product ion at *m/z* 396. Key mass spectrometer parameters included curtain gas at 20 psi, nebulizer gas at 50 psi, auxiliary gas at 60 psi, ion spray voltage at 5500 V, and a temperature of 400° C.

For colistin, the MS/MS parameters included declustering potential (DP) of 70 V, cell exit potential (CXP) of 15 V, entrance potential (EP) of 10 V, and collision energies (CE) of 16 V and 18 V for ion 1 and ion 2, respectively. For polymyxin B, the parameters were DP = 75 V, CE = 16 V, CXP = 16 V, and EP = 5 V.

Validation of the analytical method for plasma and tissues

The UHPLC-MS/MS method was validated for plasma and each tissue type in terms of several parameters including linearity, precision (repeatability and within-laboratory reproducibility), recovery, limit of detection (LOD), and limit of quantification (LOQ).

Linearity was evaluated using blank plasma or tissue samples to prepare two calibration curves spanning concentrations from 5 to 10,000 ng/mL for plasma and 5–200 ng/g for tissues. Repeatability was assessed by analyzing samples (n = 6) spiked at three levels (50, 5000, and 10000 ng/mL for plasma; 5, 50, and 200 ng/g for tissues) on the same day using the same instrument and operator.

Within-laboratory reproducibility was determined by analyzing two additional sets of fortified samples, similar to those used for repeatability, over three different days with different operators. Precision was expressed as the coefficient of variation (CV, %) calculated from these measurements.

Concentrations were calculated relative to internal standards using matrix-matched calibration curves. Recovery (%) was evaluated by comparing the mean measured concentration to the spiked concentration of the samples in the same experiment. The LOD was defined as the drug concentration producing a signal-to-noise ratio of 3, and the LOQ was determined as the lowest concentration point on the matrix calibration curve, following the guidance document on the estimation of LOQ and LOD for measurements in the field of contaminants in feed and food (Wenzl et al., 2016).

Pharmacokinetic and statistical analysis

PK analyses were performed using PKanalix™ software (R1, 2023) employing a non-compartmental approach. Concentration-time curves were utilized to directly determine the maximum plasma concentration (C_{max}) and the corresponding time (T_{max}). The elimination half-life (t1/2) was calculated by analyzing the concentration-time curve using least squares regression. The area under the curve (AUC) was computed using the linear log trapezoidal rule for the IV administration and the linear-up log-down rule for the PO administration. With the exception of T_{max}, which is presented as a categorical variable with its median value and range, and the terminal half-life expressed as the harmonic mean, the PK parameters of colistin have been displayed as geometric means along with their respective ranges (Julious and Debarnot, 2000).

The accumulation index was calculated as the ratio of $AUC_{(0\text{-}\infty)\ D}$ after the final dose in the multiple dose regimen to $AUC_{(0\text{-}\infty)\ D}$ after the single dose (Toutain and Bosquet-Mélou, 2004a). A value approaching 1 suggests minimal accumulation occurred.

The PO bioavailability (F) was calculated using the following equation:

$$F\% = 100 \times \frac{AUC(PO) \times Dose(IV)}{AUC(IV) \times Dose(PO)}$$

The mean absorption time (MAT) was calculated using the following equation:

$$MAT_{(PO)} = MRT_{(PO)} - MRT_{(IV)}$$

The body extraction ratio (E_{body}) for colistin after IV administration was calculated using Cl/CO (Toutain and Bousquet-Mélou, 2004a), where CO (mL/kg/min) is the cardiac output calculated according to the allometric equation in birds: 290.7 × body weight (in kg)$^{0.69}$ (Waxman et al., 2019).

Statistical analysis of PK variables among the treatment groups was conducted using repeated-measures ANOVA, followed by Bonferroni's multiple comparison test as a post-hoc analysis to assess significant variations. Normality was assessed for each parameter using the Shapiro test. The data were non-normally distributed, and min-max values were used to represent values instead of the standard deviation. A p-value < 0.05 was considered statistically significant. GraphPad InStat was used for the analyses (GraphPad Software 5.3 v).

Residue analysis

Due to the limited number of time points available on the elimination curve, it was not feasible to use a naïve pooled-data approach with a non-compartmental analysis to calculate the PK parameters for colistin in the selected tissues. In addition, determining withdrawal times using EMA's software was impractical under the study conditions. Therefore, the present study focused on comparing tissue concentrations exclusively against the maximal residual limits (MRL) set by European commission for food-producing animals: 150 ng/g for muscle and liver, and 200 ng/g for kidneys (Commission Regulation EU No 37/2010, 2009).

Results

Analytical method validation

The UHPLC-MS/MS method was validated for plasma and each tissue type in terms of linearity, precision and recovery. Table 1 displays the validation outcomes of the analytical method, detailing the parameters evaluated in plasma and all analyzed tissues. The method exhibited satisfactory recovery rates and demonstrated good linearity across all matrices studied, along with low LOD and LOQ values.

Animals

Following IV and single PO administrations, throughout the entire study period, the geese did not exhibit any noticeable immediate or delayed (up to 7 days) adverse effects, either locally or systemically. Furthermore, during tissue collection, no post-mortem macroscopic lesions or pathological changes were found.

Table 1
Results of the UHPLC-MS/MS method validation for colistin quantification in plasma and tissues in geese.

Parameter	Unit	Matrix					
		Plasma	Muscle	Kidney	Liver	Lung	Heart
Equation		y = 1.049x – 74.24	y= 0.0942x + 3.106	y= 0.995x + 0.2723	y= 1.039x – 1.243	y= 1.049x – 1.590	y= 1.156x – 2.434
R^2		0.992	0.994	0.995	0.993	0.992	0.993
Repeatability, CV	%	8.5	9.4	6.9	6.7	9.0	6.8
Within-lab reproducibility, CV	%	8.3	7.4	6.2	6.3	6.9	6.3
Recovery	%	93.9	105	96.1	101	96.9	94.8
LOD	ng/mL; ng/g	2	2	2	2	2	2
LOQ	ng/mL; ng/g	5	5	5	5	5	5

Pharmacokinetics

Plasma concentrations could be quantified up to 24 h for the single PO (n= 2) and IV (n= 4) routes, and up to 10 h (n= 6) from the last dose administered for the multiple PO route. Fig. 1 depicts the semi-logarithmic plot of the mean (± SD) plasma colistin concentrations over time following IV, single and multiple PO administrations. Table 2 displays the mean PK parameters based on non-compartmental analysis. AUC_{rest} values for each individual were less than 20 % of $AUC_{(0-\infty)}$, and the coefficient of determination (R^2) for the terminal phase regression line exceeded 95 %. Samples below the LOQ were excluded from the PK analysis.

A relatively short terminal half-life, slow clearance and low volume of distribution, were observed following IV administration. The body extraction rate was low at 0.2 %. The absolute bioavailability was very low after oral administration, and ranged between 0.5 % and 12 %. In the multiple PO administrations, there was no observed accumulation (< 1) of colistin in plasma by the fifth day of administration. Initially, plasma concentrations were undetectable in most geese during the first four 24-h samples. However, by the fifth day, plasma concentrations were detectable in 12 geese, with a mean concentration of 12 ng/mL.

Tissue residues

Fig. 2 shows the histogram of the mean (± SD) tissue concentrations of colistin over time, following the fifth day of multiple PO administrations, in regard to the MRL set in poultry tissues (excluding lungs and heart). Colistin was detected in all tissues at each sampled time point. For the liver, muscles, and kidneys, colistin did not exceed the MRL at any of the time points set for the corresponding tissues in poultry.

Discussion

Understanding the disposition kinetics of a drug is essential for developing an effective dosage regimen. In antimicrobial chemotherapy, it is equally important to consider the susceptibility of the infecting microorganisms to determine the effective and non-toxic drug concentrations in both plasma and tissues. Additionally, assessing tissue residues is crucial for safety considerations and ensuring consumer well-being. To the authors' knowledge, this study represents the first PK investigation of colistin in geese. At the employed doses in the present study, and with a single PO dose up to 30 mg/kg, none of the animals showed any visible systemic or local effects.

The bioavailability of colistin in geese was found to be very low, averaging between 1.5 % and 5 %, and consistent with the 4 % bioavailability observed in broiler chickens (Soliman et al., 2016). Similarly, in mammals such as pigs, calves, dairy cows, and humans, colistin absorption from the gastrointestinal tract is minimal or absent (Guyonnet et al., 2010; Grégoire et al., 2017; Kumar et al., 2020). In pigs, colistin was found to be rapidly degraded in simulated gastric fluid due to pepsin-mediated cleavage of peptide bonds in the tail tripeptide moiety, retaining antimicrobial activity in the GI tract however (Grégoire et al., 2017).

Despite the low bioavailability in poultry, colistin is purposefully

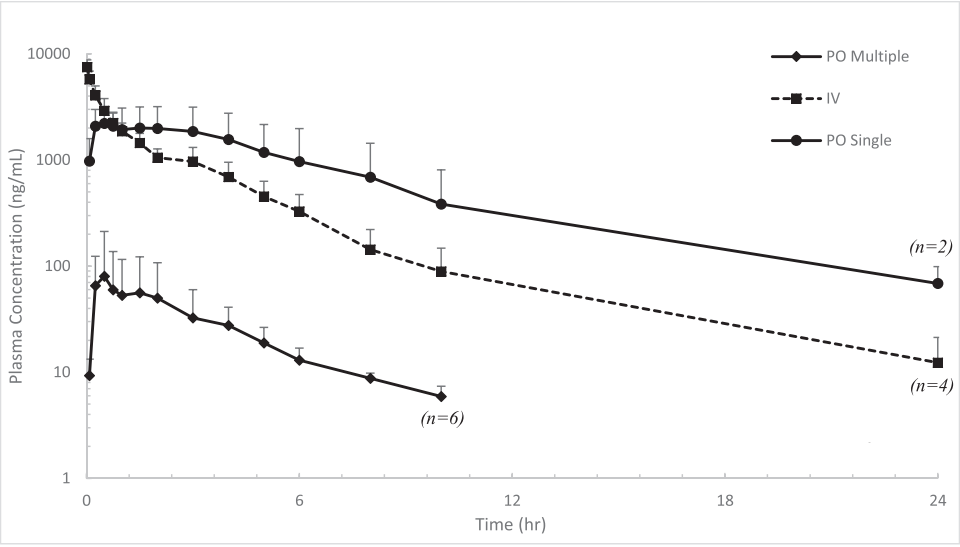

Fig. 1. Semi logarithmic mean plasma concentration–time curves of colistin following intravenous (1 mg/kg), single (30 mg/kg) and multiple (SID, 2.5 mg/kg) oral administrations in geese (n = 16).

Table 2
Pharmacokinetic parameters of colistin in geese (n = 16) after intravenous (1 mg/kg), single (30 mg/kg) and multiple (SID, 2.5 mg/kg for 5 days) oral administrations.

		IV			Single PO			Multi PO		
Parameter	Unit	Geo mean	max	min	Geo mean	max	min	Geo mean	max	min
$AUC_{(0-\infty)\ D}$	$h{\cdot}ng{\cdot}mL^{-1}$	8774.05	11414.91	6496.81	274.70[a]	839.23	132.4	132.94[a]	380.50	65.915
λz	h^{-1}	0.30	0.49	0,15	0.26	0.35	0.20	0.26	0.54	0.12
t1/2°	h	2.18	4.70	1.49	2.59	3.49	1.96	2.88	5.40	1.47
Cl	$mL{\cdot}h^{-1}{\cdot}g^{-1}$	0.11	0.12	0.09	-	-	-	-	-	-
Vd	$mL{\cdot}g^{-1}$	0.41	0.84	0.20	-	-	-	-	-	-
$MRT_{(0-\infty)}$	h	2.66	4.89	1.72	4.18	6.49	3.26	4.56	2.73	8.14
$C_{max\ D}$	$ng{\cdot}mL^{-1}$	-	-	-	84.63	188.33	39.00	30.73[b]	209.5	6.65
$T^{\S}_{max}$	h	-	-	-	0.50	3.00	0.50	0,50	3.00	0.25
F	%	-	-	-	5.00	12.00	1.00	1.50	5.00	0.50
MAT	h	-	-	-	1.52	4.80	1.10	1.88	6.19	0.51

Note: $AUC_{(0-\infty)}$ D, area under the curve from 0 h to infinity normalized to 1 mg/kg of the administered dose; λz, terminal phase rate constant; t1/2, terminal half-life; Cl, plasma clearance; Vd, volume of distribution; $MRT_{(0-\infty)}$, mean residence time from 0 h to infinity; $C_{max\ D}$, peak plasma concentration normalized to 1 mg/kg; T_{max}, time of peak concentration; F, bioavailability; MAT, mean absorption time.
[a], statistically different from IV; [b], statistically different from single PO; [§], median value; [°], harmonic mean.

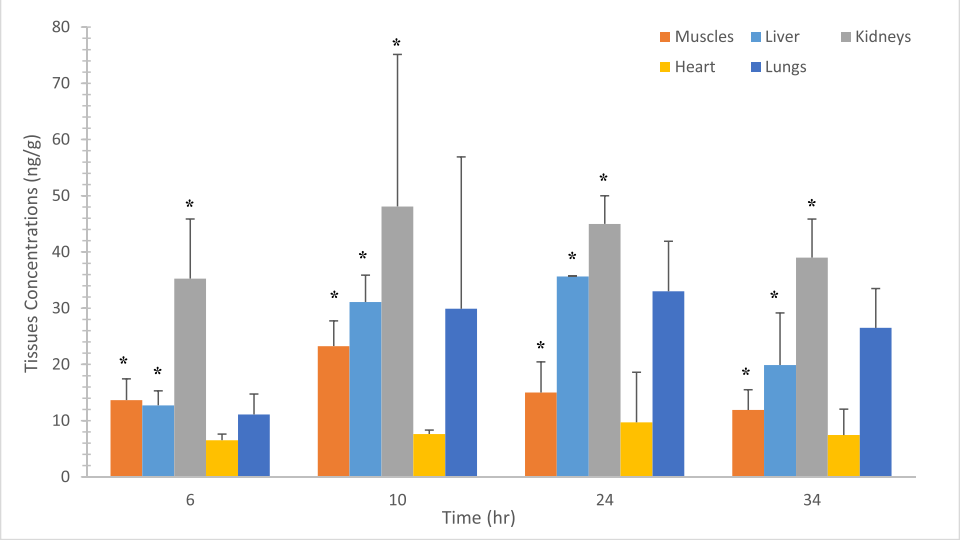

Fig. 2. Histogram of mean (± SD) tissue concentrations of colistin over time following the fifth day of multiple oral administrations (2.5 mg/kg) in geese (n = 16; 4 per time point). * Indicates all the concentrations are below the MRL set for the corresponding tissues in food-producing animals.

administered to treat enteric infections, as it achieves high concentrations at the site of infection in the GI tract with minimal risk of antimicrobial residues in the meat, permitting a short or even no withdrawal period (Mead et al., 2021). This rationale might apply to geese as well, where colistin's primary use is for local treatment of GI infections without the need for systemic absorption. Although there are anecdotal reports of colistin being used for systemic indications as well, it is generally not recommended for treating systemic infections in poultry (Lin et al., 2005; Apostolakos and Piccirillo, 2018). The blood and tissue levels attained may be inadequate to address common infections (Apostolakos and Piccirillo, 2018; Kumar et al., 2020). Additionally, as seen in Table 2, the bioavailability and systemic exposure $AUC_{(0-\infty)}$ of colistin varied widely and may be minimal in some geese, compromising treatment effectiveness on a population/flock level. Such variability should be anticipated with oral administration, and its impact on treatment outcomes is especially significant when the F% is low (Toutain and Bousquet-Mélou, 2004b).Other drugs, such as sulfonamides, tetracyclines, and penicillins, are certainly more appropriate choices (Löhren et al., 2008).

The Cl of colistin following IV administration was relatively slow at 0.11 $mL{\cdot}h^{-1}{\cdot}g^{-1}$, and comparable to that found in poultry (0.13 $mL{\cdot}h^{-1}{\cdot}g^{-1}$; Lashev and Haritova, 2003). Such comparable clearance rates suggest that the metabolic and excretory mechanisms involved in colistin elimination are likely similar across these avian species (Fadel et al., 2023; Bourdo et al., 2024). The extraction ratio in geese, reported at a low 0.2 %, indicates minimal ability of clearing organs to eliminate colistin. Despite this, the combination of a short half-life and low plasma and tissue levels ensures effective drug elimination, supporting the safety of colistin use in geese without concern for drug accumulation, as explained later on.

The V_d in geese was low to moderate at 0.41 $mL{\cdot}g^{-1}$, matching that found in broiler chickens (0.41 $mL{\cdot}g^{-1}$; Lashev and Haritova, 2003). In cattle, values were slightly higher at 1.3 $mL{\cdot}g^{-1}$ (FAO, 2006), yet still indicating an extracellular distribution of the drug. Plasma protein binding was not evaluated in this study, but in other species, it varies widely: 40 % in cattle (FAO, 2006), 55 % in rats and dogs (Li et al., 2003a,b), 70 % in sheep (FAO, 2006), and 91 % in mice (Cheah et al., 2015). Notably, colistin is thought to predominantly bind to alpha-1-acid glycoprotein, levels of which can markedly rise during bacterial infections, potentially altering colistin kinetics in diseased states (Grégoire et al., 2017).

Regarding the terminal half-life, values found in geese (2.5 h) were consistent with those in broiler chickens (2.19 h; Lashev and Haritova, 2003) and ducks (2.33 h; Zeng et al., 2010). This suggests similar PK behaviors among these avian species, despite their physiological differences. This similarity may be associated to comparable metabolic rates, V_d, body size and composition, plasma protein binding, and clearance efficiencies (Fadel et al., 2023).

Regarding tissue residues, in the liver, muscles, and kidneys, colistin did not exceed the MRL set for food-producing animals, at any of the time points. This is a positive indicator, suggesting that the recommended treatment protocol for local enteric infections in geese is unlikely to compromise withdrawal times or pose public health risks. This was in consistence with data found in other species (Archimbault et al., 1980; Roudaut, 1989; Zeng et al., 2010). Colistin was undetectable in both serum and milk, following oral administration in cows (Archimbault et al., 1980). In ducks 20 mg per kg of feed of colistin for 10 consecutive days, colistin was undetectable in plasma and tissues, except in the intestines (Zeng et al., 2010). In laying hens, given the same oral dosage regimen as in the present study, no detectable residues of colistin were found in eggs (Roudaut, 1989). While this trend may extend to laying geese, further studies specifically examining colistin residues in geese eggs are necessary to validate this assumption. It is important to note that caution should be exercised when comparing the present results with older studies, as modern analytical methods offer significantly improved sensitivity, allowing for quantification at lower limits. Nonetheless, the overall findings highlight colistin's minimal systemic exposure and tissue penetration when administered orally, thereby reducing the risk of residues in consumable products. This characteristic might make colistin a practical choice for farmers, enabling effective treatment of GI infections without requiring prolonged withdrawal periods. However, it should only be used after sensitivity testing, as resistance may require alternative treatments despite its favorable PK profile.

Regarding the consumption of heart and lung tissues in some countries (Seong et al., 2015), the low residue levels found suggest minimal risk. Similar residue profiles to the commonly consumed tissues (see Fig. 2) may support using existing MRLs for liver, kidney, and muscle as preliminary limits for heart and lungs. In addition, daily intake and dietary exposure data indicate very low consumption of these organs, further minimizing exposure risks and supporting the safety of these preliminary MRLs.

Due to technical constraints, the intestinal content of colistin was not assessed in the present study, representing a limitation. Indeed, assessing the intestinal concentrations alongside the PK of colistin is pivotal for optimizing its therapeutic use and mitigating the risk of antimicrobial resistance. In poultry, the half-life of disappearance of colistin from the luminal intestinal content was 2.5 h after cessation of dosing via drinking water (75,000 IU/kg/day; Mead et al., 2021). Other studies in chickens reported transit times ranging from 3.82 to 5.65 h (Rougière and Carré, 2010; Ravindran, 2013). A further investigation of these parameters in geese is particularly important due to potential differences in intestinal transit times compared to chickens. Variations in GI physiology among avian species could influence colistin's elimination, and consequently affecting its therapeutic outcomes. In fact, considering colistin's primary activity in the intestines, it may be a practical and effective to base the frequency of administration and dosing regimen on its intestinal transit time and local antimicrobial activity, rather than solely on its plasma parameters (Davis et al., 1986). While the similar Cl, V_d, and t1/2 between geese and chickens suggest that the same dosing regimen could be applicable, optimizing it should ideally focus on evaluating intestinal content and transit time.

The present study confirms that a substantial amount of colistin is retained in the GI tract of geese, given the minimal F %. Furthermore, while oral gavage was employed in this study, on-farm administration typically involves colistin via drinking water, which should result in even lower systemic exposure (Mead et al., 2021). Future steps should involve assessing intestinal colistin content following multiple administrations via drinking water to further refine therapeutic strategies in geese.

In conclusion, this study demonstrates that colistin has a short half-life and low systemic bioavailability in geese, with minimal tissue penetration and drug concentrations remaining below MRLs for food-producing animals. These findings support the use of colistin for local GI treatment in geese, emphasizing its safety and practicality for farmers. Future research should focus on intestinal colistin content to optimize dosing regimens and minimize antimicrobial resistance. It is also important to consider colistin's position in the antibiotic armamentarium as a last-resort treatment. The careful use of colistin is crucial to preserve its effectiveness in combating multidrug-resistant bacterial infections.

Conflict of Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

CRediT authorship contribution statement

Anna Gajda: Validation, Resources, Methodology. **Mario Giorgi:** Writing – review & editing, Validation, Supervision, Conceptualization. **Charbel Fadel:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Krzysztof Bourdo:** Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Amnart Poapolathep:** Writing – review & editing, Data curation. **Magdalena Bilecka:** Resources, Methodology. **BEATA ŁEBKOWSKA-WIERUSZEWSKA:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author, upon reasonable request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2024.106245.

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