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ORIGINAL ARTICLE

Metronidazole pharmacokinetics in geese (*Anser anser domesticus*) after intravenous and oral administrations

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Abstract

Metronidazole (MTZ) is a 5-nitroimidazole anti-bacterial and anti-protozoal drug. In human and companion animal medicine, MTZ remains widely used due to its effectiveness against anaerobic bacteria and protozoa. In farm animals, however, MTZ is currently prohibited in several countries due to insufficient data on nitroimidazoles. The purpose of this study was to assess its pharmacokinetics (PK) in geese after single intravenous (IV) and oral (PO) administrations. Fifteen-month old healthy male geese ($n = 8$) were used. Geese were subjected to a two-phase, single-dose (10 mg/kg IV, 50 mg/kg PO), open, longitudinal study design with a two-week washout period between the IV and PO phases. Blood was drawn from the left wing vein to heparinized tubes at 0, 0.085 (for IV only), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, and 48 h. Plasma MTZ concentrations were measured using HPLC coupled to an UV detector, and the data were pharmacokinetically analyzed using PKanalix™ software with a non-compartmental approach. MTZ was still quantifiable and well above the LLOQ at 24 h after both routes of administration. Following IV administration, terminal elimination half-life, volume of distribution, and total clearance were 5.47 h, 767 mL/kg, and 96 mL/h/kg, respectively. For the PO route, the bioavailability was high (85%), and the mean peak plasma concentration was 60.27 μg/mL at 1 h. When parameters were normalized for the dose, there were no statistically significant differences for any of the PK parameters between the two routes of administration. The study shows that oral administration of MTZ seems to be promising in geese, although comprehensive research on its pharmacodynamics and multiple-dose studies are necessary before its adoption in geese can be further considered.

KEYWORDS
antibacterial, geese, metronidazole, nitroimidazoles, pharmacokinetics

1 | INTRODUCTION

The considerable differences in the biological effects of drugs across various species present a significant hurdle in veterinary pharmacology overall, especially when it comes to comparative pharmacokinetics (PK) as mentioned by Toutain et al. (2000). Such diversity underscores the necessity for conducting PK studies tailored to each species, allowing for the development of species-specific, secure and effective dosage regimens, rather than relying on data extrapolation.

Metronidazole (MTZ) is a 5-nitroimidazole anti-bacterial and anti-protozoal drug. Its notable efficacy lies in its activity against most obligate anaerobes. The antibacterial action of MTZ depends on reduction of its nitro group to form an active intermediary. This reduction product causes DNA strand breakage and organelle damage in the target organism (Jackson et al., 1981). In poultry, MTZ has demonstrated effectiveness in treating trichomoniasis, histomoniasis, giardiasis, and anaerobic bacterial infections (Prescott & Baggot, 1988). It is readily absorbed when taken orally in most species and has the ability to penetrate various tissues, including the central nervous system, bones, placenta, and inflamed tissues like abscesses (Lamp et al., 1999).

The utilization of MTZ in various food-producing animals is presently restricted in multiple countries due to inadequate data on nitroimidazoles, although not universally. For example, in Australia, the treatment of infections caused by microorganisms susceptible to MTZ in ornamental/pet birds and breeder pigeons is approved (ASTAG, 2018). Nevertheless, in the field of human and companion animal medicine, MTZ continues to be highly valuable and extensively employed for a multitude of indications. It is often prescribed either as a standalone treatment or in combination with antibiotics effective against aerobic bacteria to address mixed bacterial infections (USP, 2007). Indeed, the ban of the use of MTZ in poultry in many countries left little alternative for the treatment of histomoniasis, and thus the illicit use of the drug is still reported (Anonymous, 2010, 2014; Granja et al., 2013). Despite the lack of specific data or statistics on practitioners using MTZ in geese by extrapolating the dose, it is probable that such off-label usage takes place in practice. This is particularly likely due to the common occurrence of histomoniasis and coccidiosis, affecting not only chickens but also ducklings, geese, pigeons, and turkeys. Moreover, many online recommendations and local products in various countries promote the use of MTZ in ducks, potentially leading to its use in geese as well (https://gardens.desigusxpro.com/en/utki/metronidazol-dozirovka-v-vodu.html). This is especially true in the USA, where MTZ is commonly used extra-label (Davis & Gookin, 2009).

To ensure safe and effective treatment, it is crucial to rely on species-specific therapy protocols, developed through research and clinical experience for each bird species. To the authors' knowledge, this study represents the first investigation of MTZ's PK in geese, considering both intravenous and oral administration. Due to its physicochemical properties, MTZ exhibits a favorable PK profile in other animal species characterized by rapid and complete absorption following oral administration. The drug's extended volume of distribution and its ability to penetrate various tissues contribute to its broad spectrum of efficacy, making it suitable for a wide range of treatment scenarios. The authors hypothesize that MTZ would display high oral bioavailability and a favorable PK profile in geese.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The pure powders of MTZ and phthalimide, both with a standard purity of 99.0% and the latter used as the internal standard (IS), were obtained from Sigma-Aldrich in Milan, Italy. Methanol (MeOH) was purchased from VWR chemicals (Oud-Heverlee, Belgium) in high-performance liquid chromatography (HPLC) grade. With the aid of a Milli-Q Millipore Water System, deionized water was produced (Millipore, Darmstadt, Germany). The aqueous and organic components of the mobile phase were degassed under pressure and combined in the HPLC system. The mobile phases were filtered through 0.2 μm cellulose acetate membrane filters using a solvent filtration apparatus (Sartorius Stedim Biotech, Goettingen, Germany).

2.2 | Animals and experimental design

This research comprised a selection of eight randomly chosen drug-free male geese, all aged of 15 months, from a larger group. Before the study commenced, these geese were thoroughly assessed for their health through serum chemistry, physical examination, and hematological analyses, and they were found to be in good health. An identity code was applied to the left leg for easier identification. To acclimate them to the study environment, they were kept in a 60 m^2 enclosure with a 9 m^2 indoor shelter, 1 week before the experiment. They were provided with a drug-free pelleted diet, along with access to water ad libitum. Throughout the study, the geese's daily behavior and appetite were carefully observed and recorded. The animal experiment was approved by the Lebanese Ministry of Agriculture ethical committee, verifying that this study complies with appropriate regulations and animal welfare international guidelines (study protocol number 0920234).

A two-phase, two-dose study was conducted with a washout period of 2 weeks. The study design was open and longitudinal in nature. During the first phase, a group of eight geese received intravenous administration of MTZ (Biovis®, 5 mg/mL, Sanofi, France) at a dosage of 10 mg/kg using a sterile 20-gauge 3.75 cm needle in the left-wing vein. In the second phase, the geese were given an oral dose of MTZ at 50 mg/kg via crop gavage, using a rounded tip metal canula. The Flagyl® tablets (Aventis Pharma, France), each containing 250 mg of MTZ, were meticulously grounded, weighed, partitioned and suspended in water to attain a concentration of 100 mg/mL. Each goose was then given a specific volume of this suspension to achieve the desired 50 mg/kg dose, into the crop. Following the

administration, the canula was promptly flushed with 10 mL of water to ensure proper delivery. The geese's body weights ranged from 3 to 4.9 kg.

Approximately 2 mL of blood was obtained from the right-wing vein through direct venipuncture at specific time points: 0, 0.085 (for IV administration only), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 24, and 48 h. The blood was collected in heparinized tubes and then centrifuged at 1500×*g*. Subsequently, the plasma was carefully harvested and stored at a temperature of −20°C. The analysis of the stored plasma was conducted within 25 days from the time of collection.

2.3 | Sample extraction

The sample preparation method was determined using a previously published method (Tabari et al., 2021), slightly modified. To 200 µL of plasma, 100 µL of 200 µg/mL of IS was added. Then, 800 mL of MeOH was added. After vigorous vortex mixing (30 seconds), the samples were shaken at 60 oscillations per min, for 10 min, before being centrifuged at 13,000×*g* for 10 min. The upper layer was transferred to a clean tube and gently steamed with nitrogen while drying at 45°C. The residue was dissolved in 100 µL of mobile phase and vortexed for 1 min. An aliquot of 50 µL of the upper layer was injected onto the HPLC system for analysis.

2.4 | HPLC instrumentation and analytical conditions

An autosampler (AS2055), ternary gradient system (PU 980), in-line degasser (DG-2080-53), and UV multiple wavelength detector (MD-1510) were all part of the LC Jasco HPLC system. The chromatographic separation experiment was carried out using a Luna C18 analytical column (250×4.6 mm inner diameter, 5 µm particle size, Phenomenex, Bologna, Italy) and a Peltier device (CO4062) to keep the column temperature at 30°C. The mobile phase consisted of ammonium acetate 0.05 M adjusted to pH 4.5 and acetonitrile (80:20 v/v). The flow rate of the mobile phase was 0.70 mL/min, and the drug was detected at UV wavelength of 320 nm.

2.5 | Validation of the analytical method

MTZ and IS stock solutions at 1000 µg/mL were diluted in MeOH to 100 µg/mL and stored at −20°C. Dilutions at 50, 10, 5, 2.5, 1, 0.5, 0.1, and 0.05 µg/mL were used for MTZ calibration curve in plasma. Linearity was assessed from 0.05–50 µg/mL using residual plot, fit test, and back calculation. Six plasma samples spiked with IS at high (10 µg/mL), middle (1 µg/mL), and low (0.05 µg/mL) concentration standards were analyzed using the same instrument and operator on the same day and three different days, respectively, to determine the intra-day and inter-day precision. Drug recoveries were evaluated based on detector responses of quality control samples and pure standards. The limit of detection (LOD) and lower limit of quantification (LLOQ) were defined by signal-to-noise ratios (EMA, 2012).

2.6 | Pharmacokinetics and statistical analysis

Using a non-compartmental method, the PK evaluation of the data was performed (PKanalix™ R1; 2023). The concentration vs time curves were used to directly calculate the maximum plasma concentration (C_{max}) and the time required to reach it (T_{max}). By analyzing the concentration-time curve using least squares regression, the elimination half-life (t1/2) was calculated. The area under the curve (AUC) was calculated by linear log trapezoidal rule for the IV administration and by the linear-up log-down rule for the oral administration. The AUC_{rest} for each individual was less than 20% of $AUC_{(0-\infty)}$, and the coefficient of determination (R^2) of the terminal phase regression line was greater than 95%. Values below the LLOQ were excluded from the PK analysis. 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 MTZ have been displayed as geometric means along with their respective ranges (Julious & Debarnot, 2000).

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 MTZ after IV administration was calculated using Cl/CO (Toutain & Bousquet-Mélou, 2004), where CO (mL/kg/min) is the cardiac output calculated according to the allometric equation in birds: 290.7×body weight (in kg)$^{0.69}$ (Grubb, 1983; Waxman et al., 2019).

To determine statistically significant differences in PK variables between the two treatment groups, the paired *t*-test was used. A *p*-value <.05 was considered statistically significant. GraphPad InStat was used for the analyses (GraphPad Software 5.3v).

3 | RESULTS

3.1 | Analytical method validation

The analytical method demonstrated excellent linearity (R^2=0.998; y=0.525x+0.0039) within the range of 0.05–50 µg/mL. The recovery was found to be 81±9.7%. The LOD and LLOQ were 0.01 and 0.05 µg/mL, respectively. Intra-day and inter-day precision showed CV% values lower than 9.8% and 5.3%, respectively. The mean concentrations of the quality control and LLOQ samples were less than 15% and 20% of the nominal values, respectively.

3.2 | Animals

Qualified veterinarians (B L-W; C F) evaluated the health of the geese before, during, and after the study. 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.

3.3 | Pharmacokinetics

MTZ was still quantifiable and well above the LLOQ at 24 h for both routes of administration. Figure 1 depicts the semi-logarithmic plot of the mean (±SD) plasma MTZ concentrations over time after single IV and PO administration. Table 1 displays the mean PK parameters based on a non-compartmental analysis. Following IV administration, the mean Cl value was low at 96 mL/h/kg, and the V_d value was high at 767 mL/kg. The peak plasma concentration (C_{max} = 60.27 μg/mL) occurred at T_{max} = 1 h. The normalized area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) between the two administration routes did not show a significant difference. Likewise, there were no statistically significant distinctions in the mean residence time ($MRT_{0-\infty}$) with values of 6.96 h for IV and 7.43 h for PO, nor in any other parameters when comparing the two routes of administration. The elimination half-life was around 5 h for both the administrations. The oral bioavailability was high, with a mean of 85%. The mean absorption time was 0.57 h, and the body extraction ratio was very low.

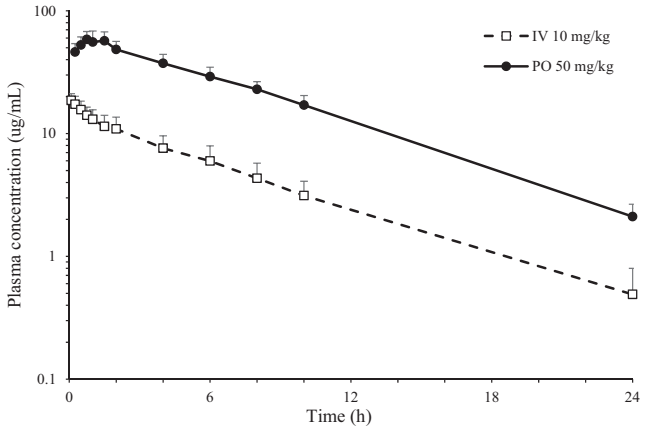

FIGURE 1 Semi-logarithmic mean plasma concentration–time curves and standard deviation (vertical bars) of metronidazole following intravenous and oral administration in geese (n = 8).

4 | DISCUSSION

To the authors' knowledge, there is no prior research on MTZ's PK in geese. Due to technical constraints, choosing a longitudinal rather than a cross-over study design can be viewed as a limitation in this study, as the latter could have minimized the variability. Administered doses of 10 and 50 mg/kg via IV and PO routes, respectively, geese did not exhibit any systemic or local adverse effects. This mirrors the findings in other avian species such as pigeons (Tabari et al., 2021), hens, and quails (Cybulski et al., 1996), as well as turkeys (Świtała et al., 2016), all at comparable dosage levels.

TABLE 1 Mean pharmacokinetic parameters and range in geese (n = 8) after single IV (10 mg/kg) and PO (50 mg/kg) doses of metronidazole.

		IV			PO		
Parameter	Unit	Geo mean	Max	Min	Geo mean	Max	Min
$AUC_{(0-t)}$	h×μg/mL	99.63	142.25	69.46	419.39	563.4	307.84
$AUC_{(0-\infty)}$ D	h×μg/mL	523.3	731.55	358.55	457.99	643.62	315.49
λz	1/h	0.12	0.079	0.15	0.14	0.099	0.16
$t1/2^h$	h	5.47	8.83	4.71	4.79	6.97	4.28
Cl	mL/h/kg	95.54	139.45	68.35	_	_	_
V_d	mL/kg	766.94	1095.41	464.15	_	_	_
$MRT_{(0-t)}$	h	5.75	6.75	5.25	6.07	6.76	4.41
$MRT_{(0-\infty)}$	h	6.96	10.53	6.07	7.43	10.51	6.61
C_{max}	μg/mL	_	_	_	60.27	76.37	41.94
$T_{max}{}^m$	h	_	_	_	1	1.5	0.5
F	%	_	_	_	84.52	96.97	64.28
MAT	h	_	_	_	0.57	1.27	0.23
$E_{(body)}$	_	0.0022	0.0035	0.0019	_	_	_

Abbreviations: $AUC_{(0-\infty)}$ D, area under the curve from 0 h to infinity normalized to the oral dose; $AUC_{(0-t)}$, area under the curve from 0 h to last time collected samples; Cl, plasma clearance; C_{max}, peak plasma concentration; $E_{(body)}$, overall body extraction ratio; F, bioavailability; ^{h}, harmonic mean; ^{m}, median value; MAT, mean absorption time; $MRT_{(0-\infty)}$, mean residence time from 0 h to infinity; $MRT_{(0-t)}$, mean residence time from 0 h to last time point of samples collection; t1/2, terminal half-life; T_{max}, time of peak concentration; V_d, volume of distribution; λz, terminal phase rate constant.

When it comes to birds, ensuring effective oral administration of antibiotics is crucial, with a focus on minimizing stress for the animals. Despite the typical application of individual or flock therapy through drinking water or feed in avian species, these methods were not advised in this study due to limitations like inconsistent drug intake and dosing imprecision (Fadel et al., 2023; Powers, 2006; Turk et al., 2021). Instead, oral gavage was favored for its precise dosing and absence of stability concerns (Flammer, 1994; Vermeulen et al., 2002). While the IV route for MTZ is not recommended in birds, it played in the present study a crucial role for accurately assessing the clearance, volume of distribution, and absolute oral bioavailability. To mitigate the risk of systemic toxicity and adverse effects, the IV dose was intentionally kept lower than the oral dose. Nevertheless, both administration dosages fell within the suggested therapeutic ranges for birds (Fowler & Zalmir, 2001).

The study's findings indicated a substantial oral bioavailability of 85%, which aligns closely with observations in hens (79%) (Cybulski et al., 1996), pigeons (82%) (Tabari et al., 2021), horses (85%) (Sweeney et al., 1986), and falls within the range observed in dogs (60%–100%) (Neff-Davis et al., 1981) and cats (65%) (Sekis et al., 2009). In avian species, specifically, data suggest that MTZ is well absorbed from the gastrointestinal tract (Switala et al., 2009; Świtała et al., 2016; Tabari et al., 2021). MTZ's high bioavailability can be explained by its weakly basic nature, which results in mostly unionized forms at physiological pH. Additionally, it exhibits good solubility in the stomach's acidic environment (proventriculus and gizzard in this case) and efficiently permeates the intestinal biological membranes (Cybulski et al., 1996). Consequently, the comparable $AUC_{(0-\infty)}$ values between the two administration routes (523.3 h μg/mL IV vs. 457.99 h μg/mL PO) when normalized for the dose were a result of the high absorption degree.

It is acknowledged that, in turkeys, up to 25% of MTZ may be hydroxylated in the liver (Świtała et al., 2016). It is not known how efficient the hepatic metabolism of MTZ is in geese, therefore it is difficult to evaluate whether nearly 15% of the dose was bio-transformed after a first-pass effect, or simply remained in the gastrointestinal tract. If unabsorbed, lower bioavailability might aid in targeting digestive system pathogens (Tabari et al., 2021).

MTZ was rapidly absorbed after administration, with a T_{max} of 1 h, which is faster when contrasted with turkeys (3–4 h; Świtała et al., 2016), pigeons (2 h; Tabari et al., 2021), and hens (2 h; Cybulski et al., 1996). Certainly, the absorption rate of MTZ can be influenced by species-specific variances in drug transporters, as well as variations in the gastrointestinal structure and physiology among different bird species (Baert, 2003). The differences might be also attributed to the administration of different pharmaceutical formulations in prior studies, in addition to differences in feeding conditions (Cybulski et al., 1996; Świtała et al., 2016; Tabari et al., 2021). In the investigations carried out by Świtała et al. (2016) and Cybulski et al. (1996), hens and turkeys experienced a fasting period, in contrast to the approach in this study, and were administered an oral solution of MTZ (0.5%) directly into the crop. In the study by Tabari et al. (2021), pigeons were similarly fasted; however, they received an aqueous suspension containing crushed tablets into the crop.

After IV administration, the volume of distribution (V_d) was relatively high, measuring 767 mL/kg, a value comparable to that documented in hens (1100 mL/kg; Cybulski et al., 1996), pigeons (1120 mL/kg; Tabari et al., 2021), horses (900 mL/kg; Britzi et al., 2010), cats (667 mL/kg; Sekis et al., 2009), dogs (940 mL/kg; Neff-Davis et al., 1981), and calves (790 mL/kg; Bhavsar & Malik, 1994). These findings imply that there is a widespread distribution of MTZ in body fluids and tissues, which aligns with expectations for a drug that does not effectively bind to plasma proteins (Neff-Davis et al., 1981). Notably, in all the animal species tested, plasma protein binding was found to be less than 20% (Davis & Gookin, 2009), and in chickens, it was even less than 3% (Cybulski et al., 1996). Furthermore, in quails and hens, a comprehensive whole-body autoradiography of [^{3}H] MTZ revealed substantial absorption of the labeled substance, followed by an extensive and uniform distribution across all body tissues (Cybulski et al., 1996). Additionally, in horses, the significant peak tissue concentrations, with 65% in the peritoneal fluid and 92% in the synovial fluid compared to peak serum concentrations, corroborate these earlier findings (Specht et al., 1992). While plasma protein binding was not specifically assessed in this study, considering the consistency of the values across the animal species and the high distribution volume observed, it is plausible that plasma protein binding is likely very low in geese. Further studies are however needed to confirm this hypothesis.

In this study, the clearance value of MTZ was low (68.35 mL/h/kg), lower than in hens (131 mL/h/kg; Cybulski et al., 1996) and turkeys (73–217 mL/h/kg; Świtała et al., 2016), but substantially higher than in pigeons (0.00021 mL/h/kg; Tabari et al., 2021). Intra- and inter-species clearance variations involve complex factors, such as isoform expression, enzyme activities, genetics, age, body weight, and physiology, among the others (Dantzler, 2016; Świtała et al., 2016). Furthermore, Grabowski et al. (2017) demonstrated the strong correlation between MTZ elimination in turkeys and hemodynamic parameters. These intricate elements make direct clearance comparisons impractical, emphasizing the need to consider body extraction ratios for accurate cross-species assessments (Toutain & Bousquet-Mélou, 2004).

Notably, in geese, the body extraction ratio (E_{body}) was very low (0.0022), confirming a slow clearance and suggesting a minimal contribution of the hepatic and renal systems to the drug's elimination process. This value is indeed below the cut-off thresholds of 0.05 (Toutain & Bousquet-Mélou, 2004). In Cybulski et al. (1996), high labeling in the intestines post-IV administration of MTZ suggests potential biliary excretion predominance, with uncertainty regarding whether MTZ underwent metabolism. This could be the case in geese as well. However, avian species exhibit ubiquitous biotransformation enzymes and unique excretory organ structures, which vary not only from mammals but possibly among avian species as well (Dorrestein, 1991; Toutain et al., 2010; Vermeulen et al., 2002).

Hence, further research in this area is essential for a comprehensive understanding of MTZ metabolism and elimination in geese.

In terms of half-life values, they were deemed relatively moderate, with no statistically significant distinction observed between the IV (5.56 h) and PO route (4.84 h). It can be attributed to both, the slow clearance and the high volume of distribution. The values in the present study found are comparable to those found in hens (4.2–4.7 h; Cybulski et al., 1996), pigeons (5.40–6.16 h; Tabari et al., 2021), and turkeys (3.41–5.94 h; Świtała et al., 2016).

For concentration and time-dependent antimicrobials, such as MTZ, the pharmacokinetic/pharmacodynamic (PK/PD) AUC/MIC surrogate is vital for predicting drug efficacy (Toutain et al., 2017). In vitro susceptibility studies for MTZ have indicated MIC_{90} values below 2 μg/mL for gram-negative anaerobes and under 4 μg/mL for Clostridium species (Prescott & Baggot, 1988). Additionally, an $AUC_{(0-24h)}$/MIC ratio of ≥70 has been recommended for MTZ for optimal efficacy against various anaerobic pathogens (Child et al., 2019; Morales-León et al., 2015). Considering that the plasma protein binding is assumed to be negligible in geese, the $AUC_{(0-24h)}$/MIC ratio surpassed 70 for the oral dose of 50 mg/kg, but fell below 70 for the IV dose of 10 mg/kg, for a MIC value of 4 μg/mL. Consequently, from a PK/PD perspective, achieving a therapeutic goal of $AUC_{(0-24h)}$/MIC ratio above 70 is feasible with the administration of an oral dose of 50 mg/kg of MTZ. Additional research is needed to explore the effectiveness of MTZ, beyond evaluating its plasma protein binding.

In summary, geese administered doses of 10 mg/kg intravenously and 50 mg/kg orally showed no adverse effects, in line with similar findings in various avian species. MTZ demonstrated high oral bioavailability in geese, comparable to other avian species and certain mammals. Extensive absorption, early peak concentration, and wide distribution were noted, emphasizing its potential efficacy. Its slow clearance and high volume of distribution contributed to its moderate half-life, which is essential for sustained therapeutic levels and convenient dosage schedules. The results of the study suggest that the oral administration of MTZ at 50 mg/kg might be suitable for geese in terms of PK and PD. Before considering its adoption for use in geese, further pharmacodynamic and multiple-dose studies are essential, along with the determination of withdrawal periods for edible tissues.

AUTHOR CONTRIBUTION

CF, MG conceived the presented idea, CF, BL, KB carried out the experiment, MG, AP, GH supervised the study, contributing to the interpretation of the results. All authors discussed the results, contributed to the manuscript, and accepted the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest in publishing this work.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal experiment was held in Lebanon and was approved by the Lebanese ministry of Agriculture ethical committee, verifying that this study complies with international standards for animal welfare guidelines (study protocol number 0920234).

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