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Pharmacokinetics of Meloxicam Following Intramuscular, Subcutaneous, and Oral Administration in White Storks (Ciconia ciconia)

Yigit Gunes

yigit.gunes@iuc.edu.tr

Istanbul University-Cerrahpaşa

Ebuderda Gunay

Istanbul University-Cerrahpaşa

Nurcin Yilmaz

Istanbul University-Cerrahpaşa

Emine Irem Deveci

Istanbul University-Cerrahpaşa

Dilek Ozturk Civelek

Istanbul University-Cerrahpaşa

Ceren Anlas

Istanbul University-Cerrahpaşa

Tulay Bakirel

Istanbul University-Cerrahpaşa

Oya Ustuner

Istanbul University-Cerrahpaşa

Ali Serhan Coskun

Istanbul University-Cerrahpaşa

Duygu Sultan Oran

Dokuz Eylül University

Alper Okyar

Istanbul University

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Abstract

Background

Meloxicam (MLX) is a preferential cyclooxygenase-2 inhibitor widely used in avian medicine as an anti-inflammatory agent. Despite marked interspecific variability in avian drug disposition, no pharmacokinetic data are available for MLX in the white stork (*Ciconia ciconia*). This study aimed to characterise and compare the plasma pharmacokinetics of MLX in this species.

Methods

Six clinically stable, non-releasable white storks (three of each sex) received a single 1 mg/kg dose of MLX by the IM, SC and PO routes in a randomised three-way crossover design with a 2-week washout period between treatments. Plasma MLX concentrations were quantified up to 24 h using HPLC and analysed using non-compartmental methods.

Results

Peak plasma concentrations (C_{max}) differed significantly among all three routes (IM 11.49 ± 2.34, SC 6.43 ± 1.62, PO 1.53 ± 0.89 μg/mL; $p < 0.05$) and were reached most rapidly after IM administration. Systemic exposure was comparable between the IM and SC routes (AUC_{0-24h} 80.7 ± 40.2 and 81.1 ± 22.7 μg·h/mL, respectively) and approximately 7-fold greater than after PO administration (11.9 ± 4.8 μg·h/mL; $p < 0.05$), corresponding to relative bioavailabilities versus IM of 100.4% for SC and 14.8% for PO. Terminal half-life progressively increased from IM (9.81 ± 2.30 h) through SC (15.13 ± 4.41 h) to PO (18.85 ± 2.16 h).

Conclusion

SC administration may represent a practical parenteral alternative to IM, whereas PO administration displayed low and variable bioavailability in white storks.

1. Introduction

Pain and inflammation are important clinical conditions that can compromise animal welfare and recovery, particularly in wildlife rehabilitation settings [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutic agents for the management of pain and inflammatory disorders in both domestic and wild animals. Meloxicam (MLX), an oxicam derivative and preferential cyclooxygenase-2 (COX-2) inhibitor, has gained widespread acceptance in avian medicine because of its favourable safety profile and effective anti-inflammatory and analgesic properties [2, 3].

Consequently, MLX is widely used in avian medicine, particularly in wildlife rehabilitation and exotic animal practise [1, 4].

The importance of NSAID safety in birds became particularly evident following diclofenac-associated declines in *Gyps* vulture populations in the Indian subcontinent [5, 6]. The recognition of diclofenac-induced toxicity stimulated extensive research into the safety and pharmacokinetics of alternative NSAIDs in avian species, with particular emphasis on MLX. Subsequent studies demonstrated that MLX was comparatively well tolerated in several vulture species, establishing it as a safer alternative to diclofenac in avian medicine [7–9].

Despite its widespread use, MLX pharmacokinetics exhibit substantial interspecific variability among avian taxa, limiting the reliability of empirical dose extrapolation between species. Previous pharmacokinetic studies have demonstrated marked differences in drug disposition among raptors, psittacines, ratites and domestic poultry [10–14]. Reported elimination half-lives vary from less than 1 h in *Gyps* vultures and red-tailed hawks to up to approximately 31 h in African grey parrots (*Psittacus erithacus*) [8, 13, 14]. In addition, adverse renal effects and visceral gout have been reported following supratherapeutic NSAID exposure in domestic chickens, further emphasising the importance of species-specific pharmacokinetic and safety evaluation prior to clinical application [15]. Collectively, these findings indicate that pharmacokinetic data obtained from phylogenetically distant avian species may not reliably predict drug disposition in unstudied taxa.

The white stork (*Ciconia ciconia*) is a large migratory wading bird of the family Ciconiidae, widely distributed throughout Europe, western Asia and parts of Africa [16]. Owing to its close association with anthropogenic environments, it is frequently admitted to wildlife rehabilitation centres with traumatic injuries, including collision- and electrocution-related lesions, as well as infectious and parasitic conditions that often require analgesic and anti-inflammatory therapy [17–19]. Yet, to our knowledge, no peer-reviewed pharmacokinetic study has evaluated MLX in white storks or any other ciconiid, forcing clinicians to extrapolate dosing regimens from unrelated avian taxa and thereby increasing the risk of therapeutic failure or adverse drug reactions. Because MLX bioavailability and systemic exposure can vary markedly among administration routes and avian species [4, 10, 14], a within-species comparison of the oral, intramuscular and subcutaneous routes commonly used in avian rehabilitation [20, 21] would directly inform rational route selection and the optimisation of analgesic protocols in rehabilitated birds.

The aim of the present study was to characterise and compare the plasma pharmacokinetics of MLX in clinically stable, non-releasable white storks (*Ciconia ciconia*) following a single 1 mg/kg dose administered orally, intramuscularly, and subcutaneously. Establishing such species-specific pharmacokinetic data is essential to guide the rational use of MLX in this species, including the selection of an appropriate administration route and dosing regimen for clinical and rehabilitation practice.

2. Materials and methods

2.1. Reagents and drugs

Meloxicam (MLX) reference standard was kindly donated by Sanovel (Istanbul, Türkiye). Piroxicam was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Potassium dihydrogen phosphate, methanol, acetonitrile, and formic acid (HPLC grade) were obtained from Sigma-Aldrich (USA). Ultrapure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade.

2.2. Animals

The study was conducted on 6 clinically stable, non-releasable white storks (*Ciconia ciconia*) housed at the Department of Wild Animal Diseases and Ecology, Istanbul University-Cerrahpasa Veterinary Faculty Rehabilitation Center. Birds were 1–3 years old and weighed 3.03 ± 0.30 kg, with an equal sex distribution, i.e., three females, three males. Each bird (n = 6) received all three administration routes (oral, intramuscular, and subcutaneous) in a randomised sequence.

All birds were deemed clinically stable prior to inclusion based on physical examination of general appearance, posture, hydration status, mucous membrane colour, respiratory pattern and appetite, with no evidence of acute trauma, active infection or systemic disease. Birds were acclimatised to these enclosures for 7 days before drug administration and no pre-administration fasting was applied in order to minimise management-related stress.

Birds were identified using numbered leg bands and distinctive morphological features. Body weight, used for individual dose calculation, was determined as the mean of two consecutive measurements obtained on a digital platform scale under short-term manual restraint. Throughout the study, birds were monitored for general condition, behaviour, feed and water intake, venipuncture-site reactions and potential adverse effects.

All birds were rehabilitated wild individuals deemed unsuitable for release because of chronic disabilities that had remained clinically stable for at least 6 months prior to the study. They were maintained permanently at the centre in outdoor species-appropriate aviaries under natural photoperiod and prevailing ambient temperature and humidity (Istanbul), fed a meat-based carnivorous diet, with food and municipal tap water provided *ad libitum*.

2.3. Experimental Design

A randomised three-way within-subject (crossover) design was used. Each of the six birds (three females, three males) received a single 1 mg/kg dose of MLX by each of the three administration routes - oral (PO), intramuscular (IM), and subcutaneous (SC) - in a randomised treatment sequence, with a two-week washout period between successive treatments. The dose of 1 mg/kg was selected based on previously reported pharmacokinetic studies of MLX in avian species and its clinical use as a non-steroidal anti-inflammatory drug in birds [8, 22–24]. This dose falls within the reported safe range for avian species [8, 9]. The two-week washout period was selected on the basis of MLX elimination half-

lives previously reported in birds, which range from less than 1 h in *Gyps* vultures and red-tailed hawks to approximately 31 h in African grey parrots [8, 13, 14]. Even for the longest of these reported half-lives, this interval corresponds to more than 10 elimination half-lives, ensuring that negligible drug (< 0.1% of the preceding dose) remained at the time of each subsequent administration. The treatment sequence for each bird was generated using a computer-generated random number sequence (Microsoft Excel RANDBETWEEN function). A within-subject crossover design was selected to eliminate between-animal variability as a confounder in route-of-administration comparisons and to reduce the total number of animals required, in accordance with the 3Rs principle for this protected species. Commercial formulations of MLX were used: Metacam® (1.5 mg/mL oral solution) for oral administration and Metacam (5 mg/mL injectable solution) for subcutaneous and intramuscular administration (both Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany). MLX was administered at a single dose of 1 mg/kg via the assigned route at each treatment period.

Oral MLX administration was performed using a 10F red rubber tube, followed by flushing of the tube with 5 mL of water [22]. SC injections were administered over the keel, whereas IM injections were administered into either the right or left pectoral muscle group [22, 23].

Blood samples were collected immediately before drug administration (0 min) and at 5, 15 and 30 min and 1, 2, 4, 6, 8, 10, 12 and 24 hours post-administration. Approximately 1 mL of blood was withdrawn at each time point using a 1 mL syringe with a 25-gauge needle [22] from the medial metatarsal vein, with venipuncture sites alternated between the right and left legs at consecutive sampling time points to minimise local tissue trauma [23, 25]. All sampling procedures were performed under manual restraint without sedation or general anaesthesia, in order to avoid pharmacological interference with MLX disposition. Although handling stress was not directly quantified, restraint duration was standardised across animals and sampling time points, with each blood sample collected within 30–60 s of initiating restraint. The total volume of blood withdrawn per bird over the 24 h sampling period (approximately 12 mL) corresponded to less than 1% of body weight and to approximately 6% of the estimated total blood volume, remaining well below the 10% threshold considered safe for blood sampling in birds [25]. Blood samples were transferred into lithium heparin-containing tubes and centrifuged at 4000 × g for 10 min (M 4812 P, Elektro-mag, Istanbul, Türkiye). The separated plasma was transferred into clean microcentrifuge tubes and stored at -80°C until analysis.

Blinding of investigators administering the drug was not implemented as the primary outcome (plasma MLX concentration) was determined by an objective HPLC assay performed by an analyst blinded to group allocation.

2.4. Meloxicam Analysis

Plasma concentrations of MLX were determined using an HPLC method adapted with slight modifications from previously reported procedures [26, 27]. Piroxicam was used as the internal standard (IS) and was added to all plasma samples prior to extraction at a final concentration of 1 µg/mL. A total of 0.2 mL of plasma was placed into a microcentrifuge tube, and 0.4 mL of methanol containing 0.1%

formic acid was added for protein precipitation. The samples were vortex-mixed for 45 s and then centrifuged at 12,000 × g for 10 min (MicroCL 21R, Thermo Scientific, Waltham, MA, USA). After centrifugation, 200 µL of the clear supernatant was transferred into autosampler vials, and 20 µL was injected into the HPLC system.

Chromatographic analyses were performed using an Agilent 1260 HPLC system (Agilent, Waldbronn, Germany) consisting of a binary high-pressure gradient system. Separation of MLX was achieved on an ACE® 5 C18 analytical column (5 µm, 250 mm × 4.6 mm; Advanced Chromatography Technologies, Aberdeen, UK), which was maintained at 40°C in a column oven (G1316A, Agilent, Waldbronn, Germany) during analysis. The mobile phase consisted of acetonitrile and 20 mM potassium dihydrogen phosphate (40:60, v/v, pH: 3.5) at a flow rate of 1.2 mL/min. Detection was performed at 355 nm using a diode array detector (G4212B Agilent, Waldbronn, Germany).

The HPLC method was validated in accordance with the principles of the ICH M10 Bioanalytical Method Validation guideline [28] with respect to selectivity, linearity, lower limit of quantification (LLOQ), accuracy, and precision. Calibration curves were prepared in duplicate on three separate days by spiking blank white stork plasma with MLX at eight concentration levels over the range of 0.05-50 µg/mL. Calibration curves were constructed by plotting the peak area ratio of MLX to the IS against nominal concentrations using least-squares linear regression analysis (acceptance criterion: $R^2 \geq 0.99$). The LLOQ was defined as the lowest concentration of the calibration curve at which the analyte could be quantified with accuracy within 80–120% and precision (CV) ≤ 20%; the limit of detection (LOD) was defined as the concentration giving a signal-to-noise ratio of 3:1. Accuracy and precision were determined using quality-control (QC) samples prepared at three concentration levels covering the calibration range (low QC at 3 × LLOQ, medium QC and high QC), with six replicates per level on each of three validation days. Acceptance criteria were ≤ 15% for both accuracy bias and CV at all QC levels, and ≤ 20% at the LLOQ. Absolute recovery was calculated by comparing the peak area of MLX in extracted plasma QC samples with that of post-extraction spiked samples at the same nominal concentrations. Selectivity was evaluated by analysing blank plasma samples from six different white storks for potential interference at the retention times of MLX and the IS.

2.5. Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis in MS Excel (Microsoft Corporation, Redmond, WA, USA) and were independently verified using the PKNCA package (v0.11.0) in R. Plasma concentration–time curves were plotted using GraphPad Prism v.11.0.0 (GraphPad Software, San Diego, CA, USA). The area under the plasma concentration–time curves from 0 to 24 hours (AUC_{0-24h}) were calculated using the linear trapezoidal rule [29, 30], and the curves were obtained using the GraphPad Prism v.11.0.0. $AUC_{0-\infty}$ was obtained by adding the extrapolated area (C_{last}/k_{el}, where C_{last} is the last quantifiable concentration and k_{el} the terminal elimination rate constant) to AUC_{0-24h}. The non-compartmental method was used to calculate the pharmacokinetic parameters of MLX. The values of peak concentration (C_{max}) and time to peak concentration (t_{max}) were recorded as the observed

values from each individual concentration–time profile. k_{el} was derived from the slope of the terminal log-linear phase using at least the last three concentration–time points. Individuals whose terminal log-linear phase was poorly defined (adjusted $R^2 < 0.85$) were excluded a priori from non-compartmental analysis. Elimination half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = \ln 2/k_{el}$. The apparent total body clearance scaled by bioavailability (CL/F) was determined using $CL/F = Dose/AUC_{0-\infty}$, and the apparent volume of distribution scaled by bioavailability (V_d/F) was determined using $(CL/F)/k_{el}$. Because no intravenous reference group was included, CL and V_d are reported throughout as the apparent parameters CL/F and V_d/F, where F denotes the (unknown) absolute bioavailability of the corresponding extravascular route.

2.6. Statistical Analysis

Pharmacokinetic parameters are reported as arithmetic mean ± standard deviation, except t_{max} which is reported as median (range). The sample size (n = 6) was determined by the 3Rs principle for this protected species and by the within-subject crossover design, in which each animal serves as its own control across all three administration routes, thereby maximising statistical efficiency while minimising the number of animals used, consistent with group sizes reported in previous avian pharmacokinetic studies. The normality of the distribution of each pharmacokinetic parameter was assessed with the Shapiro–Wilk test and sphericity with the Greenhouse–Geisser correction. Continuous pharmacokinetic parameters (C_{max}, AUC_{0-24h}, $AUC_{0-\infty}$, $t_{1/2}$, k_{el}, CL/F, V_d/F and MRT) were compared between administration routes using a one-way repeated-measures analysis of variance (RM-ANOVA) followed by Tukey's multiple comparisons test. The t_{max} was compared using the Friedman test followed by Dunn's post-hoc test. The significance threshold was set at $p < 0.05$. Statistical analyses and graphics were performed using GraphPad Prism v.11.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Analytical method validation

The HPLC-DAD assay was linear over the concentration range of 0.05–50 µg/mL ($R^2 = 0.9971$; LLOQ 0.05 µg/mL, LOD 0.015 µg/mL). Intra- and inter-day precision (CV ≤ 7.13%) and accuracy (95.12–100.78%) across all QC levels met ICH M10 acceptance criteria, and no endogenous interfering peaks were detected at the retention times of MLX or piroxicam (Fig. 1; Table 1). All study samples were quantified within the validated calibration range.

Table 1

Validation parameters of the high-performance liquid chromatography method developed for the quantification of meloxicam in white stork (*Ciconia ciconia*) plasma.

Parameter	Value
Linearity	
Calibration range (μg/mL)	0.05–50
Coefficient of determination (R^2)	0.9971
Calibration equation (meloxicam/piroxicam peak area ratio versus concentration)	y = 4.2547x + 3.5989
Sensitivity	
LOD (μg/mL)	0.015
LLOQ (μg/mL)	0.05
Selectivity	No endogenous interfering peaks were observed at the retention times of meloxicam or the internal standard in blank white stork plasma.
Precision (intra-day, %CV; n = 6)	
Low QC (0.15 μg/mL)	6.46
Medium QC (1.0 μg/mL)	2.54
High QC (20 μg/mL)	0.28
Precision (inter-day, %CV; three consecutive days, n = 6)	
Low QC (0.15 μg/mL)	7.13
Medium QC (1.0 μg/mL)	6.21
High QC (20 μg/mL)	4.83
Accuracy (%, n = 6)	
Low QC (0.15 μg/mL)	95.12
Medium QC (1.0 μg/mL)	95.20
High QC (20 μg/mL)	100.78
Validation parameters met the acceptance criteria of the ICH M10 guideline on bioanalytical method validation (precision ≤ 15% CV at all QC levels and ≤ 20% at LOQ; accuracy 85–115% at QC levels and 80–120% at LOQ).	
LOD: limit of detection; LLOQ: lower limit of quantification; CV: coefficient of variation; QC: quality control; IS: internal standard.	

Parameter	Value
Chromatographic conditions	
Column	C18 (5 μm, 250 × 4.6 mm)
Column temperature (°C)	40
Flow rate (mL/min)	1.2
Mobile phase	Isocratic; acetonitrile : 20 mM KH_2PO_4, pH 3.5 (40:60, v/v)
Detection wavelength (nm)	355
Injection volume (μL)	20
Retention time of meloxicam (min)	9.178
Retention time of piroxicam (IS; min)	7.685
Validation parameters met the acceptance criteria of the ICH M10 guideline on bioanalytical method validation (precision ≤ 15% CV at all QC levels and ≤ 20% at LOQ; accuracy 85–115% at QC levels and 80–120% at LOQ).	
LOD: limit of detection; LLOQ: lower limit of quantification; CV: coefficient of variation; QC: quality control; IS: internal standard.	

3.2. Pharmacokinetic parameters of meloxicam

Shapiro-Wilk testing indicated that all pharmacokinetic parameters except t_{max} were normally distributed ($p > 0.05$); accordingly, normally distributed parameters were compared using repeated-measures ANOVA, whereas the non-normally distributed t_{max} was analysed using the Friedman test. All administration routes were well tolerated, with no observable adverse effects. Mean plasma meloxicam concentration-time profiles following IM, SC, and PO administration at 1 mg/kg are shown in Fig. 2, and the corresponding non-compartmental parameters are summarised in Table 2. The most robustly estimated parameters were C_{max}, t_{max} and AUC_{0-24h} (together with the AUC_{0-24h}-based relative bioavailability), whereas $t_{1/2}$, CL/F, V_d/F and MRT depend on terminal-phase extrapolation and are reported as apparent values to be interpreted with corresponding caution.

Table 2
Pharmacokinetic parameters of meloxicam in white storks after intramuscular (IM; n = 6), subcutaneous (SC; n = 6), and oral (PO; n = 6) administration at a single dose of 1 mg/kg.

Parameters	IM Meloxicam	SC Meloxicam	PO Meloxicam
$t_{1/2}$ (h)	9.81 ± 2.30[c]	15.13 ± 4.41	18.85 ± 2.16[a]
k_{el} (h^{-1})	0.0707 ± 0.019[c]	0.0458 ± 0.014	0.0367 ± 0.004[a]
AUC_{0-24h} (μg·h/mL)	80.70 ± 40.22[c]	81.06 ± 22.66[c]	11.94 ± 4.75[a,b]
$AUC_{0-\infty}$ (μg·h/mL)	94.25 ± 30.56[c]	98.04 ± 35.22[c]	13.13 ± 2.42[a,b]
CL/F (mL/h/kg)	10.61 ± 2.78[c]	10.19 ± 2.15[c]	76.20 ± 2.84[a,b]
V_d/F (L/kg)	0.15 ± 0.02[c]	0.22 ± 0.02[c]	2.07 ± 0.03[a,b]
MRT (h)	14.16 ± 3.32[c]	21.84 ± 6.36	27.20 ± 3.11[a]
C_{max} (μg/mL)	11.49 ± 2.34[b,c]	6.43 ± 1.62[a,c]	1.53 ± 0.89[a,b]
t_{max} (h)	0.67 (0.33–1.00)[c]	2.00 (1.00–4.00)	4.00 (2.00–6.00)[a]
$F_{relative}$ (%)	Reference	100.4	14.8

Note: Values are presented as arithmetic mean ± standard deviation (n = 6, randomised three-way crossover design), except t_{max} which is presented as median (min, max). Different superscript letters within the same row denote statistically significant differences between routes ($p < 0.05$): a vs. IM, b vs. SC, c vs. PO. Statistical comparisons were performed using one-way repeated-measures ANOVA, followed by Tukey's multiple comparisons test for continuous parameters, and the Friedman test followed by Dunn's post-hoc test for t_{max}. $F_{relative}$ was calculated as ($AUC_{0-24h,\ test}$ / $AUC_{0-24h,\ IM}$) × 100 [8].

Abbreviations: $t_{1/2}$: Elimination half-life; k_{el}: Elimination rate constant; AUC_{0-24h}: Area under the plasma concentration–time curve from 0 to 24 h; $AUC_{0-\infty}$: Area under the plasma concentration–time curve from time of dosing extrapolated to infinity; CL/F: Apparent total body clearance; V_d/F: Apparent volume of distribution; MRT: Mean residence time; C_{max}: Peak plasma concentration; t_{max}: Time of peak plasma concentration; $F_{relative}$ (%): Relative bioavailability.

Following IM administration, plasma meloxicam concentrations increased rapidly, reaching a C_{max} of 11.49 ± 2.34 μg/mL at 0.67 h. SC administration produced a lower peak concentration (6.43 ± 1.62 μg/mL) at approximately 2 h, with concentrations becoming similar to those observed after IM administration from 6 h onward. In contrast, the PO profile was characterised by lower plasma concentrations throughout the sampling period, with peak concentrations (1.53 ± 0.89 μg/mL) occurring between 2 and 6 h. Peak plasma concentrations differed significantly between all three administration routes (RM-ANOVA, $p < 0.05$ for all pairwise comparisons), with C_{max} following IM administration (11.49 ± 2.34 μg/mL) being approximately 1.8-fold higher than after SC (6.43 ± 1.62 μg/mL; $p = 0.029$) and 7.5-

fold higher than after PO administration (1.53 ± 0.89 µg/mL; p = 0.0007). The time to maximum concentration (t_{max}) differed significantly only between the PO and IM routes (Friedman test, p = 0.012), with t_{max} progressively delayed from IM (median 0.67 h) through SC (2.00 h) to PO (4.00 h).

Total systemic exposure measured as AUC_{0-24h} was comparable between IM and SC administration (80.70 ± 40.22 and 81.06 ± 22.66 µg·h/mL respectively; p = 0.99), whereas PO administration produced approximately 7-fold lower exposure (11.94 ± 4.75 µg·h/mL; PO vs SC p = 0.0019; PO vs IM p = 0.0182) (Fig. 3). Relative bioavailability compared with IM administration (based on AUC_{0-24h}) was 100.4% for SC and 14.8% for PO. Apparent clearance (CL/F) and apparent volume of distribution (V_d/F) were higher after PO administration ($p < 0.05$ compared with both parenteral routes). Terminal half-life ($t_{1/2}$) and mean residence time (MRT) were greater after PO administration than after IM administration ($p < 0.05$). The terminal half-lives observed here (≤ 18.85 h) corresponded to more than 16 elimination half-lives over the two-week washout, confirming that the selected washout period was adequate and that no meaningful carry-over occurred between treatment periods.

Terminal half-life progressively increased from IM (9.81 ± 2.30 h) through SC (15.13 ± 4.41 h) to PO (18.85 ± 2.16 h), with a statistically significant difference between IM and PO (p = 0.011) and an intermediate, non-significantly different SC value.

4. Discussion

The present study provides, to the best of our knowledge, the first species-specific comparison of intramuscular, subcutaneous, and oral MLX pharmacokinetics in white storks (*Ciconia ciconia*). Following a single 1 mg/kg dose, the route of administration markedly influenced the absorption profile of MLX. Intramuscular administration produced the most rapid absorption and was therefore used as the reference for relative bioavailability, subcutaneous administration showed an intermediate, slower profile, and oral administration resulted in the slowest absorption and the lowest systemic exposure. Systemic exposure was comparable between the intramuscular and subcutaneous routes, whereas oral administration yielded a substantially lower relative bioavailability, consistent with absorption-limited kinetics and/or a first-pass effect. Following intramuscular administration, MLX disposition in white storks was characterised by low apparent clearance, a low apparent volume of distribution, and a relatively prolonged elimination half-life ($t_{1/2}$ = 9.81 h) compared with many previously studied avian species [10, 13].

Intramuscular and subcutaneous administration produced comparable systemic exposure, with AUC_{0-24h} values of 80.70 and 81.06 µg·h/mL respectively and a relative subcutaneous bioavailability of approximately 100.4% based on AUC_{0-24h}. This is the first direct comparison suggesting comparable intramuscular and subcutaneous exposure of MLX in any avian species; previous studies have evaluated either the intramuscular route alone or the subcutaneous route alone, often using sustained-release formulations [23, 31, 32]. Comparable or even higher subcutaneous relative to intramuscular

bioavailability is not unusual in birds and has been reported for several other drug classes, including danofloxacin in chukar partridges and swan geese [33, 34], florfenicol in turkeys [35], and levamisole in chukar partridges [36]. The slightly higher subcutaneous $AUC_{0-\infty}$ observed in the present study most likely reflects sustained absorption from the subcutaneous depot together with a larger contribution of the extrapolated portion of the AUC associated with the longer apparent subcutaneous half-life. These findings support the subcutaneous route as a reliable parenteral alternative to intramuscular administration in this species.

In contrast to the comparable parenteral exposure, oral administration produced markedly reduced systemic availability, with a relative bioavailability of 13.9% based on $AUC_{0-\infty}$ and 14.8% based on AUC_{0-24h}. The relative oral bioavailability observed here was substantially lower than values previously reported in other avian species, ranging from approximately 47–60% in Pekin ducks and pigeons through 50–75% in parrots and flamingos to essentially complete absorption in *Gyps* vultures [4, 8, 14, 22, 23, 37–39]. White storks therefore appear to represent not only a slow-eliminator species but also a species with comparatively low oral bioavailability of MLX.

When considered within the broader avian literature, MLX pharmacokinetics in white storks appear most consistent with slow-eliminator species such as emus, Pekin ducks, African grey parrots, and laying hens [4, 11, 14, 38, 39], and contrast with the substantially shorter elimination half-lives reported in *Gyps* vultures, domestic pigeons, red-tailed hawks, and great horned owls [8, 13, 37]. These findings support previous reports that avian MLX pharmacokinetics may vary markedly among species and may not be reliably predicted from phylogeny, feeding ecology, or body size alone [14, 22, 23]. The present findings therefore support previous recommendations that NSAID dosing in birds should be informed by species-specific pharmacokinetic data rather than empirical interspecies extrapolation [1, 15].

A notable feature of the present study was the progressive prolongation of the apparent terminal half-life from intramuscular (9.81 ± 2.30 h) through subcutaneous (15.13 ± 4.41 h) to oral (18.85 ± 2.16 h) administration, although a statistically significant difference was observed only between the intramuscular and oral routes ($p < 0.05$). This pattern may be suggestive of flip-flop kinetics, a phenomenon thought to occur when absorption from the extravascular administration site is slower than systemic elimination, such that the terminal slope of the concentration–time curve is considered to reflect absorption rather than elimination processes [40]. Consistent with this interpretation, t_{max} was progressively delayed in the same rank order (IM < SC < PO), providing absorption-based support for this pattern that is independent of the terminal half-life estimates.

The markedly higher apparent clearance and volume of distribution after oral administration, while these parameters remained similar between the two parenteral routes, is consistent with this interpretation. Similar patterns have been reported, although less formally demonstrated, in Pekin ducks and domestic pigeons [37, 38]. Although the absence of an intravenous reference precludes formal confirmation of flip-flop disposition, the observed progressive prolongation of apparent $t_{1/2}$ across the three extravascular routes provides internally consistent support for this interpretation. The clinical implication is that dosing

intervals may be more appropriately guided by the intramuscular half-life, which may be closer to the true elimination half-life, rather than by the apparently longer terminal half-lives obtained after subcutaneous or oral administration. In the absence of species-specific pharmacodynamic data for white storks, the plasma concentration of 3.5 µg/mL associated with analgesic efficacy in Hispaniolan Amazon parrots [4, 12] can only be used as a provisional and unvalidated reference threshold. At the single 1 mg/kg dose administered in the present study, intramuscular plasma concentrations remained above this threshold for a period broadly compatible with once-daily dosing; this should, however, be regarded as a preliminary estimate of dosing interval rather than a validated dose recommendation. Establishing an optimal dose and dosing interval would require species-specific PK/PD studies incorporating efficacy endpoints, such as the integrated PK/PD modelling approach applied in emus [11].

A further consideration is the fed state of the birds, as no pre-administration fasting was applied and food was available *ad libitum*. In principle, the presence of food in the gastrointestinal tract could influence oral absorption. However, in mammals the extent of MLX absorption is essentially unaffected by food, with co-administration altering only the time to peak concentration rather than overall bioavailability [41]. The pronounced reduction in oral bioavailability observed here is therefore unlikely to be explained by the fed state alone, and more plausibly reflects species-specific differences in pre-systemic handling, including intestinal efflux and biliary elimination [42].

The markedly reduced oral bioavailability is likely multifactorial, reflecting pre-systemic processes that have not been investigated in storks. MLX is a substrate of the efflux transporter BCRP/ABCG2, intestinal efflux of which is a major determinant of its oral bioavailability in mammals [43]; critically, chickens express functional intestinal BCRP under transcriptional control of the chicken xenobiotic receptor, and its induction lowers the oral C_{max} and AUC of substrate drugs such as florfenicol [44]. A heightened constitutive intestinal BCRP activity in white storks could therefore contribute to the low oral exposure observed here. Avian first-pass metabolism may also differ from that of mammals, as birds possess unique CYP3A isoforms and avian-specific CYP2C variants with no clear mammalian orthologues [45], and MLX phase II conjugation in *Gyps* vultures diverges substantially from the predominant mammalian pathway [46]. Because these transporter and enzyme systems have been characterised almost exclusively in mammals, the present mechanistic interpretation remains necessarily speculative.

The slow apparent clearance and long apparent half-life observed here may also reflect enterohepatic recirculation and biliary–faecal elimination of MLX, processes that contribute substantially to its disposition in mammals [47] and that, in *Gyps* vultures, result in excretion of substantial amounts of unmetabolised parent drug in faeces [46]. None of these pathways was directly evaluated in the present study, and each warrants targeted investigation in white storks.

The high inter-individual variability observed after oral administration (C_{max}) further suggests that gastrointestinal absorption is influenced by individual factors including gastrointestinal content, intestinal transit, and possibly regurgitation [4, 10]. Such variability may also be compatible with inter-individual differences in intestinal efflux transporter activity and first-pass metabolism, although these

mechanisms were not directly evaluated in the present study. From a clinical perspective, the markedly reduced oral bioavailability indicates that oral administration may not reliably achieve systemic exposure comparable to parenteral administration at the same dose. Although higher oral doses could theoretically be required to approach parenteral exposure, such dose escalation should not be empirically applied, as meloxicam toxicity in domestic chickens has been reported at oral doses substantially below the level required to compensate for 14.8% bioavailability [15].

The pharmacokinetic profile reported here supports several practical considerations for the clinical use of MLX in white storks. The prolonged elimination half-life and sustained plasma concentrations observed after intramuscular and subcutaneous administration may support consideration of once-daily dosing, although clinical efficacy and repeated-dose safety require further evaluation. Because subcutaneous administration produced systemic exposure comparable to intramuscular administration while potentially reducing pectoral muscle trauma and injection-site muscle injury [48], the subcutaneous route may represent a practical parenteral alternative for repeated administration in rehabilitated birds. Intramuscular administration remains useful when rapid systemic absorption is desired, whereas oral administration should be interpreted cautiously because of the low and variable bioavailability observed here.

Several limitations should be acknowledged. First, the absence of an intravenous reference group precluded the calculation of absolute bioavailability and the direct determination of true systemic clearance and volume of distribution; the reported parameters should accordingly be interpreted as apparent values (CL/F and Vd/F). Second, the 24-hour sampling window did not fully capture the terminal elimination phase in all individuals. For the oral route the extrapolated fraction of $AUC_{0-\infty}$ was small (mean ≈ 9%), supporting reliable estimation of total oral exposure, whereas for the intramuscular and subcutaneous routes it was larger (means ≈ 14% and ≈ 17%, respectively), and all three values remained below the 20% threshold generally considered acceptable for reliable extrapolation, although the corresponding $AUC_{0-\infty}$ values should therefore be interpreted with some caution. Importantly, the relative bioavailability estimates were based on AUC_{0-24h} rather than $AUC_{0-\infty}$ and are thus unaffected by this extrapolation. Future studies would benefit from extended sampling to characterise the terminal elimination phase more completely. It should also be acknowledged that the progressive prolongation of apparent terminal half-life across routes could be influenced in part by this truncated sampling window rather than reflecting flip-flop absorption alone. Third, the present study evaluated only single-dose pharmacokinetics in clinically stable, non-releasable white storks; repeated-dose pharmacokinetics, safety, and disposition in actively diseased, juvenile, or free-ranging individuals remain unknown. Although the study included an equal number of males and females, the small sample size (three of each sex) was not designed to detect sex-related differences in MLX disposition; no apparent sex-related difference in plasma exposure was observed, although this should be confirmed in adequately powered studies. Finally, the mechanistic explanations advanced here are hypothetical: transporter expression, metabolite profiling, CYP activity, and faecal/urinary excretion were not directly evaluated in white storks, and targeted *in vitro* and *in vivo* studies will be required to confirm or refute these interpretations.

5. CONCLUSION

This study provides the first pharmacokinetic evaluation of MLX in white storks and demonstrates marked route-dependent differences in systemic exposure. Intramuscular and subcutaneous administration produced comparable exposure profiles, supporting subcutaneous administration as a practical parenteral alternative when repeated administration is required, whereas oral administration resulted in substantially reduced bioavailability and high inter-individual variability, which may be partly associated with intestinal efflux transport, avian-specific first-pass metabolism, and biliary-faecal elimination; however, these mechanisms require direct experimental verification. These findings highlight the importance of species-specific pharmacokinetic evaluation in avian medicine and support cautious interpretation of empirical dose extrapolation across bird species.

Abbreviations

ABCG2
ATP-binding cassette subfamily G member 2
ANOVA
Analysis of variance
ARRIVE
Animal Research:Reporting of In Vivo Experiments
AUC
Area under the plasma concentration–time curve
$AUC_{0\text{-}24h}$
Area under the plasma concentration–time curve from 0 to 24 h
$AUC_{0\text{-}\infty}$
Area under the plasma concentration–time curve from time zero to infinity
BCRP
Breast cancer resistance protein
CL/F
Apparent total body clearance
C_{max}
Maximum plasma concentration
COX-2
Cyclooxygenase-2
CV
Coefficient of variation
CYP
Cytochrome P450
DAD
Diode-array detection

F

Bioavailability (fraction of the dose reaching the systemic circulation)

$F_{relative}$

Relative bioavailability

HPLC

High-performance liquid chromatography

ICH

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IM

Intramuscular

IS

Internal standard

k_{el}

Elimination rate constant

LLOQ

Lower limit of quantification

LOD

Limit of detection

MLX

Meloxicam

MRT

Mean residence time

NSAID

Non-steroidal anti-inflammatory drug

PD

Pharmacodynamic

PK

Pharmacokinetic

PK/PD

Pharmacokinetic/pharmacodynamic

PO

Oral administration (per os)

QC

Quality control

R^2

Coefficient of determination

RM-ANOVA

Repeated-measures analysis of variance

SC

Subcutaneous

SD
Standard deviation
$t_{1/2}$
Elimination (terminal) half-life
t_{max}
Time to maximum plasma concentration
V_d/F
Apparent volume of distribution

Declarations

Ethics approval and consent to participate

All experimental procedures involving animals were approved by the Istanbul University-Cerrahpasa Local Animal Ethics Committee of Animal Experiments (Approval no: 2025/106).

The white stork (*Ciconia ciconia*) is a protected wild species in Türkiye. No animals were captured or collected from the wild for the purposes of this study; all birds were non-releasable individuals already held under permanent care at the Istanbul University-Cerrahpasa Veterinary Faculty Rehabilitation Center. In addition to the institutional animal ethics approval, and in accordance with national legislation governing protected wildlife species, scientific research permission to conduct this study was obtained from the General Directorate of Nature Conservation and National Parks (Doğa Koruma ve Milli Parklar Genel Müdürlüğü) of the Republic of Türkiye Ministry of Agriculture and Forestry (permit no. E-72784983-288.04-22432043, dated 15 December 2025). All procedures were carried out in compliance with the conditions specified in this permit and with the relevant institutional and national guidelines for the use of protected species.

The study is reported in accordance with the ARRIVE 2.0 Essential 10 guidelines [49]; a completed checklist is provided as Supplementary Material S1.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting this study’s findings are available from the corresponding author upon reasonable request.

Competing interests

All authors declare that they have no conflicts of interest.

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Authors' Contributions

Conceptualization: YG and EG. Data curation: YG, DOC, and AO. Investigation: YG, EG, NY, EID, ASC and DSO. Methodology: YG, EG, NY, DOC, and AO. Writing – original draft: YG. Validation: YG, DOC, and AO. Supervision: OU and TB. Project administration: YG, EG, and CA. Writing – review & editing: YG, CA, DOC, OU, TB, and AO. All authors reviewed and approved the final manuscript.

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ORCID

Yigit Gunes https://orcid.org/0000-0002-9403-3545

Ebuderda Gunay https://orcid.org/0000-0002-6029-4157

Nurcin Yilmaz https://orcid.org/0009-0001-1946-3502

Emine Irem Deveci https://orcid.org/0009-0006-2411-6894

Dilek Ozturk Civelek https://orcid.org/0000-0003-2485-891X

Ceren Anlas https://orcid.org/0000-0002-7997-016X

Tulay Bakirel https://orcid.org/0000-0001-5805-2178

Oya Ustuner https://orcid.org/0000-0002-4354-8207

Ali Serhan Coskun https://orcid.org/0009-0006-0192-0476

Duygu Sultan Oran https://orcid.org/0000-0002-1453-3751

Alper Okyar https://orcid.org/0000-0002-9891-5588

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Figures

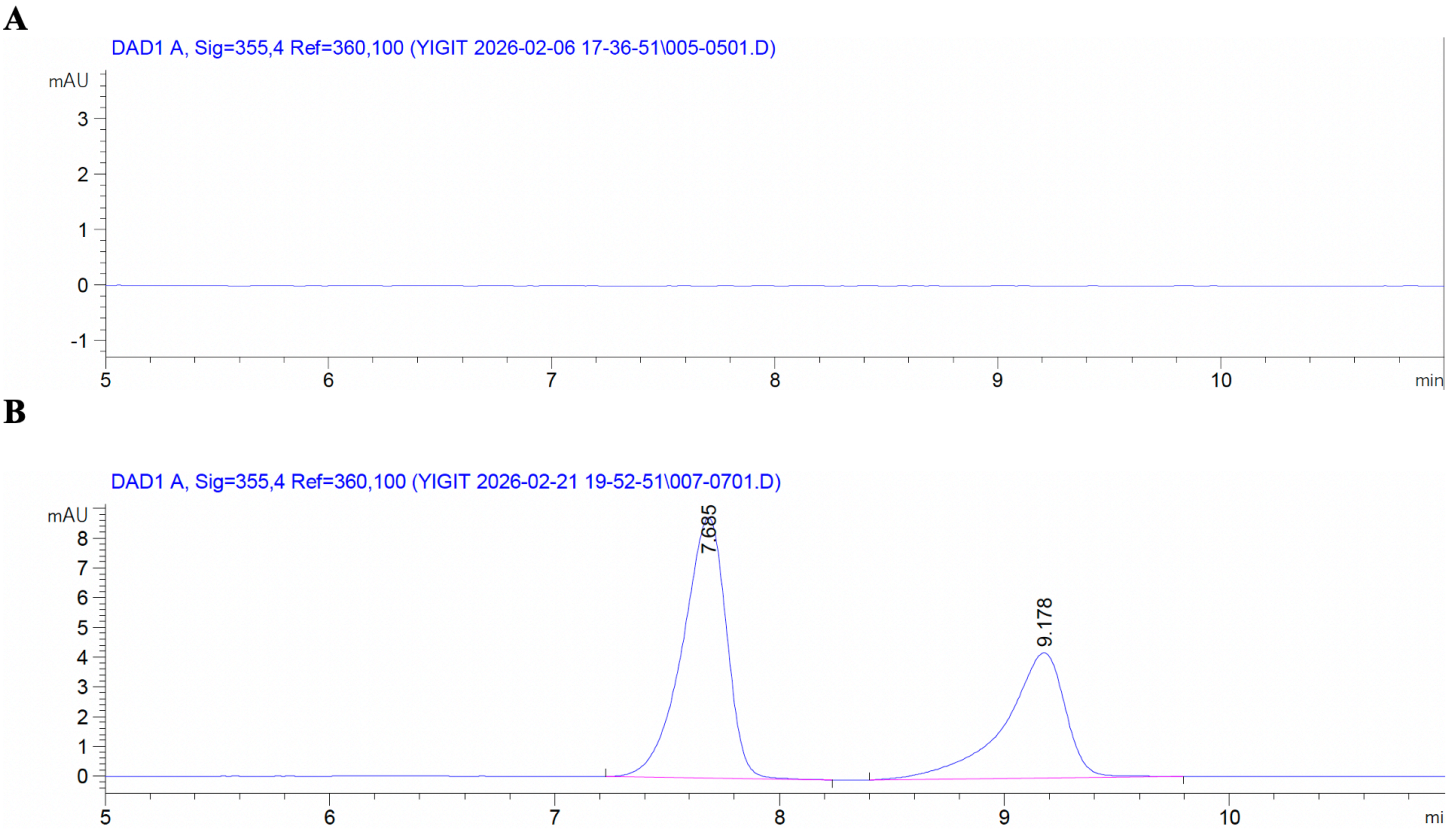


Figure 1

Representative HPLC–DAD chromatograms (355 nm) of (A) blank white stork (*Ciconia ciconia*) plasma and (B) plasma spiked with the internal standard piroxicam (7.685 min) and meloxicam (9.178 min), demonstrating baseline separation and the absence of interfering peaks in blank plasma.

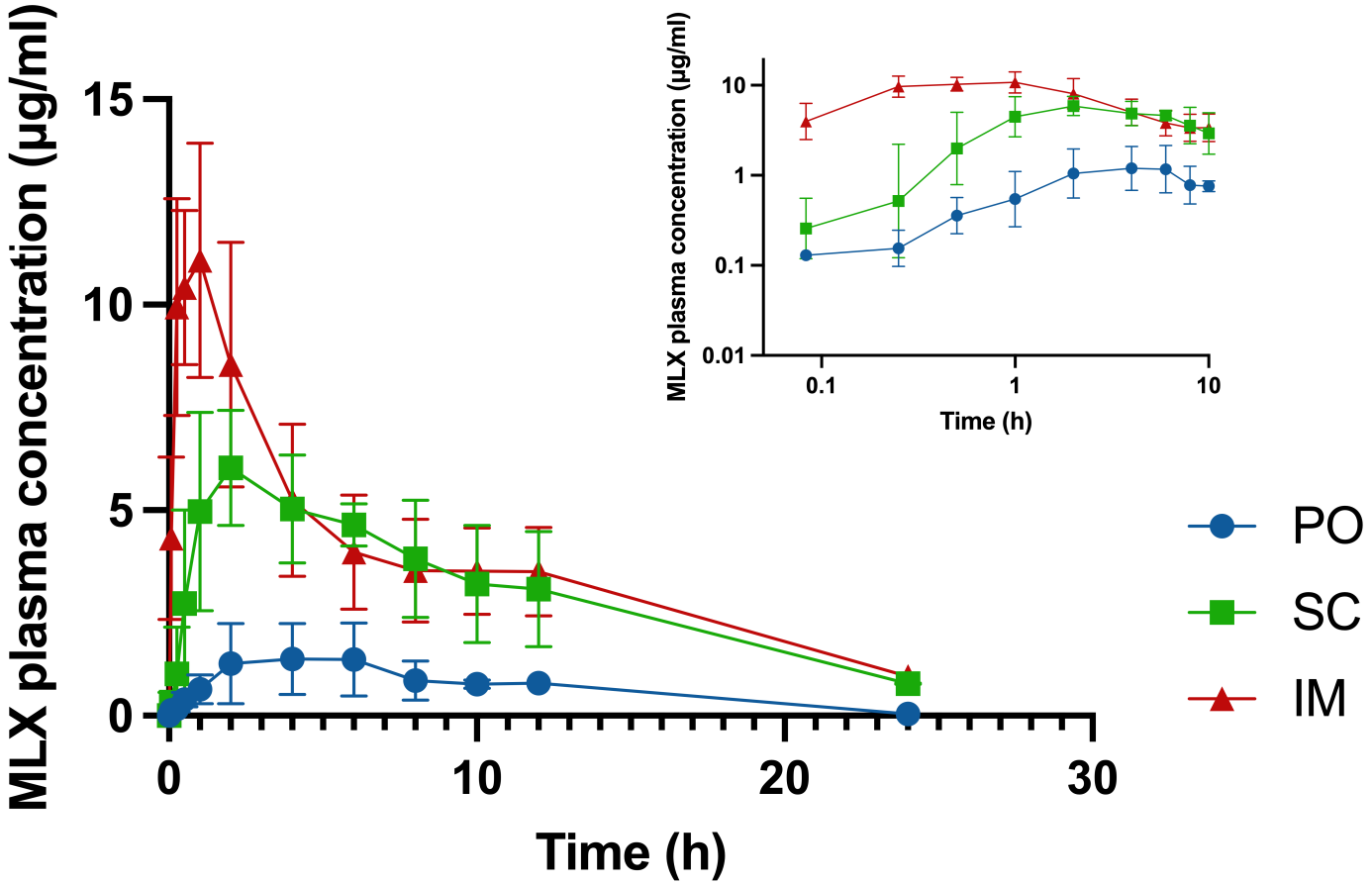


Figure 2

Plasma meloxicam (MLX) concentration–time profiles in adult white storks (*Ciconia ciconia*; n = 6). MLX was administered a single 1 mg/kg dose by the oral (PO; blue circles), subcutaneous (SC; green squares), or intramuscular (IM; red triangles) route, in a randomised three-way crossover design with a two-week washout between treatments. Data are presented as mean ± SD. The inset shows the same data on a log–log scale, highlighting the early absorption phase and the flip-flop pattern in terminal slopes.

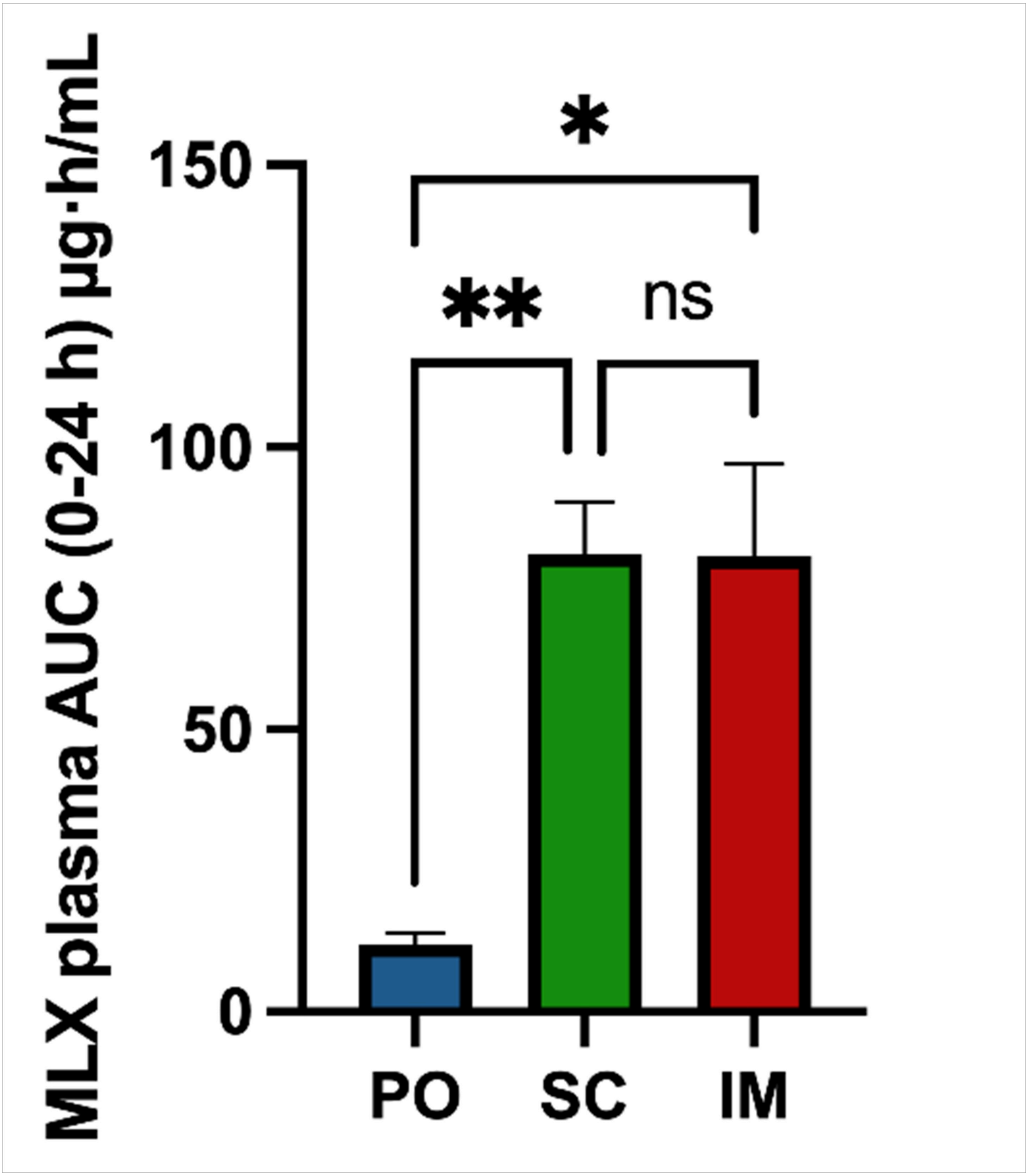


Figure 3

Mean plasma $AUC_{0\text{-}24h}$ of meloxicam in white storks (*Ciconia ciconia*, n = 6) following a single 1 mg/kg dose administered by the oral (PO), intramuscular (IM) and subcutaneous (SC) routes. Bars represent the mean and error bars the standard deviation. Groups were compared by repeated-measures ANOVA followed by Tukey's multiple comparisons test. $AUC_{0\text{-}24h}$ was significantly lower after oral administration

than after both parenteral routes (PO vs SC, p = 0.0019; PO vs IM, p = 0.0182), whereas the two parenteral routes did not differ (SC vs IM, p = 0.9999). ** p < 0.01; * p < 0.05; ns, not significant.

Supplementary Files

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