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Pharmacokinetics of meloxicam following intramuscular, subcutaneous, and oral administration in white storks (*Ciconia ciconia*)

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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 the median time to peak concentration (tmax) was progressively delayed from IM (0.67 h) through SC (2.00 h) to PO (4.00 h). Systemic exposure did not differ significantly between the IM and SC routes ($AUC_{0–24\ h}$ 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.

Keywords Avian · *Ciconia ciconia* · Meloxicam · Pharmacokinetics · White stork

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Introduction

Pain and inflammation are common clinical signs and underlying pathophysiological processes 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]. Although this preferential COX-2 inhibition underlies the anti-inflammatory action of MLX, the cyclooxygenase system of birds is not identical to that of mammals: interspecific differences in the constitutive and inducible expression of avian COX isoenzymes, and in their sensitivity to preferential COX-2 inhibitors, are thought to contribute to the pronounced variation in NSAID efficacy and tolerability among bird species, including the marked susceptibility of some taxa to NSAID-induced renal injury and visceral gout [3, 4]. In mammals, MLX is well absorbed after oral administration and undergoes extensive hepatic biotransformation, predominantly by cytochrome P450 (CYP2C9 and, to a lesser extent, CYP3A)-mediated oxidation of its thiazolyl methyl group to essentially inactive hydroxymethyl and carboxylic-acid metabolites that are excreted in urine and faeces, with only negligible amounts of unchanged drug eliminated [5, 6]. Because these oxidative pathways, and the cyclooxygenase target itself, may differ appreciably between mammals and birds, neither the potency nor the disposition of MLX can be reliably extrapolated to avian species from mammalian data. Consequently, MLX is widely used in avian medicine, particularly in wildlife rehabilitation and exotic animal practise [1, 7].

The importance of NSAID safety in birds became particularly evident following diclofenac-associated declines in *Gyps* vulture populations in the Indian subcontinent [8, 9]. 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 [10–12].

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, with avian pharmacokinetic profiles varying substantially according to species and drug characteristics [13–18]. 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*) [11, 16, 17]. 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 [4]. 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 [19]. 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 [20–22]. 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 [7, 13, 17], a within-species comparison of the oral, intramuscular and subcutaneous routes commonly used in avian rehabilitation [23, 24] 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 may help inform the selection of an appropriate administration route and provide a pharmacokinetic basis for future studies aimed at defining dosing regimens for clinical and rehabilitation practice.

Materials and methods

Reagents and drugs

Meloxicam (MLX) reference standard (Ph. Eur. grade; batch AFMLXNF010; assay 99.97% by potentiometry) was kindly donated by Sanovel (Istanbul, Türkiye). Piroxicam (purity 99.71%; Cat. No. HY-B0253) 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.

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 procedures were approved by the Istanbul University-Cerrahpasa Local Ethics Committee of Animal Experiments (Approval no: 2025/106). Because the white stork (*Ciconia ciconia*) is a legally protected species in Türkiye, scientific research permission was additionally obtained from the General Directorate of Nature Conservation and National Parks of the Republic of Türkiye Ministry of Agriculture and Forestry (permit no. E-72784983-288.04-22432043, dated 15 December 2025). 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 rehabilitation centre. Full details are provided in the ethics approval and consent to participate section.

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*.

Experimental design

A randomised, three-period, three-treatment, within-subject crossover design was used. The same six white storks (three females, three males) participated in all three treatment periods. In each period, each bird was administered a single dose of 1 mg/kg of MLX via one of three routes of administration (oral (PO), intramuscular (IM), and subcutaneous (SC)); thus, each stork received all three treatments over the course of the study. The treatment order was randomised for each bird, and a two-week washout period was allowed between consecutive treatment periods. 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 [11, 25–27]. This dose falls within the reported safe range for avian species [11, 12]. 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 [11, 16, 17]. 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 PO administration and Metacam (5 mg/mL injectable solution) for SC and IM 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 10 F red rubber tube, followed by flushing of the tube with 5 mL of water [25]. SC injections were administered over the keel, whereas IM injections were administered into either the right or left pectoral muscle group [25, 26].

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 h post-administration. Approximately 1 mL of blood was withdrawn at each time point using a 1 mL syringe with a 25-gauge needle [25] 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 [26, 28]. 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 [28]. 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 high-performance liquid chromatography (HPLC) assay performed by an analyst blinded to group allocation.

Meloxicam analysis

Plasma concentrations of MLX were determined using an HPLC method adapted with slight modifications from previously reported procedures [29, 30]. 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 diode-array detector (DAD) 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 DAD (G4212B Agilent, Waldbronn, Germany).

The HPLC method was validated in accordance with the principles of the ICH M10 Bioanalytical Method Validation guideline [31] with respect to selectivity, linearity, lowest limit of quantification (LLOQ), accuracy, and precision. Calibration curves were prepared in duplicate on each of 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% of the nominal concentration and a precision (coefficient of variation, CV) ≤ 20%; performance at the LLOQ was assessed from the back-calculated concentrations of the lowest calibration standard (0.05 µg/mL), analysed in duplicate on each of the three validation days ($n = 6$). 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.

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 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. The area under the plasma concentration–time curves from 0 to 24 h ($AUC_{0–24\,h}$) was calculated using the linear trapezoidal rule [32, 33]. The terminal elimination rate constant (k_{el}) was derived from the slope of the terminal log-linear phase of the individual concentration-time curve, fitted by least-squares linear regression using at least the last three quantifiable 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 as $t_{1/2} = \ln 2/k_{el}$. $AUC_{0–\infty}$ was obtained by adding the area extrapolated beyond the last quantifiable concentration (C_{last}/k_{el}, where C_{last} is the last quantifiable concentration) to $AUC_{0–24\,h}$. For each individual profile, the fraction of $AUC_{0–\infty}$ arising from this extrapolation was calculated as $\%AUC_{extrap} = [(AUC_{0–\infty} - AUC_{0–24\,h})/AUC_{0–\infty}] \times 100$, and $AUC_{0–\infty}$ was regarded as reliable only when $\%AUC_{extrap}$ remained below the 20% threshold conventionally accepted in non-compartmental analysis. Because relative bioavailability was intended to be independent of any extrapolation-related bias, $F_{relative}$ was calculated from $AUC_{0–24\,h}$ rather than from $AUC_{0–\infty}$. Mean residence time (MRT) was calculated as $MRT = AUMC_{0–\infty}/AUC_{0–\infty}$, where $AUMC_{0–\infty}$ is the area under the first moment curve, computed by the linear trapezoidal rule up to the last quantifiable concentration and extrapolated to infinity as $(t_{last} \times C_{last})/k_{el} + C_{last}/k_{el}^2$. The apparent total body clearance scaled by bioavailability (CL/F) was determined as $CL/F = Dose/AUC_{0–\infty}$, and

the apparent volume of distribution scaled by bioavailability (V_d/F) was determined as $(CL/F)/k_{el}$. 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.

Statistical analysis

Pharmacokinetic parameters are reported as arithmetic mean ± standard deviation together with 95% confidence intervals, so that the precision of each estimate can be appraised alongside the corresponding p-values, 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 [7, 17]. The normality of the distribution of each pharmacokinetic parameter was assessed with the Shapiro–Wilk test. Sphericity was not assumed, and the Greenhouse–Geisser correction was applied to all repeated-measures analyses. Continuous pharmacokinetic parameters (C_{max}, $AUC_{0–24\ h}$, $AUC_{0–\infty}$, $t_{1/2}$, 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).

Results

Analytical method validation

The calibration curve was linear over the concentration range of 0.05–50 µg/mL ($R^2 = 0.9971$). The LLOQ and LOD were 0.05 µg/mL and 0.015 µg/mL, respectively. MLX and piroxicam eluted at 9.18 and 7.69 min, respectively, and no endogenous interfering peaks were detected at these retention times in blank plasma obtained from six different white storks, confirming the selectivity of the method (Supplementary Material 1). At the LLOQ (0.05 µg/mL), the observed accuracy was 100.99% of the nominal concentration and the precision was 8.51% CV ($n = 6$), fulfilling the ICH M10 acceptance criteria for the LLOQ. Across the low, medium and high QC levels, intra- and inter-day precision (CV ≤ 7.13%) and accuracy (95.12–100.78%) met the ICH M10 acceptance criteria, and the mean absolute recovery, calculated across the QC levels, was 84.90% (CV 4.40%) for MLX and 82.83% (CV 3.32%) for the internal standard, confirming consistent and reproducible extraction efficiency (Table 1). All study samples were quantified within the validated calibration range.

Pharmacokinetic parameters of MLX

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. Pairwise differences are reported as mean differences with 95% confidence intervals (CI) and adjusted p-values. All administration routes were well tolerated, with no observable adverse effects. Mean plasma MLX concentration–time profiles following IM, SC and PO administrations at 1 mg/kg are shown in Fig. 1, and the corresponding non-compartmental parameters are summarised in Table 2. C_{max}, t_{max} and $AUC_{0–24\ h}$ were obtained directly from the observed concentration–time data and are therefore the most robustly estimated parameters, together with the $AUC_{0–24\ h}$-based relative bioavailability, CL/F and V_d/F are reported as apparent parameters because the absolute bioavailability of each extravascular route is unknown in the absence of an intravenous reference group, whereas $t_{1/2}$ and MRT depend

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
Calibration range (µg/mL)	0.05–50
Coefficient of determination (R^2)	0.9971
Calibration equation (MLX/IS peak area ratio vs. concentration)	y = 4.2547x + 3.5989
LOD / LLOQ (µg/mL)	0.015 / 0.05
Precision, intra-day (%CV; low / medium / high QC)	6.46 / 2.54 / 0.28
Precision, inter-day (%CV; low / medium / high QC)	7.13 / 6.21 / 4.83
Accuracy (%; low / medium / high QC)	95.12 / 95.20 / 100.78
Accuracy / precision at LLOQ (0.05 µg/mL)	100.99% / 8.51%CV
Absolute recovery (%; MLX / IS)	84.90 (CV 4.40) / 82.83 (CV 3.32)
Selectivity	No endogenous interference at the retention times of MLX or IS
Column (temperature)	C18, 5 µm, 250 × 4.6 mm (40 °C)
Mobile phase	Acetonitrile : 20 mM KH_2PO_4, pH 3.5 (40:60, v/v)
Flow rate / injection volume	1.2 mL/min / 20 µL
Detection wavelength	355 nm
Retention time (MLX / piroxicam, IS)	9.178 / 7.685 min

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. Low, medium and high QC samples correspond to 0.15, 1.0 and 20 µg/mL, respectively; MLX: meloxicam

on accurate characterisation of the terminal slope; all four parameters should accordingly be interpreted with caution.

Following IM administration, plasma MLX 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): IM exceeded SC by 5.07 µg/mL (95% CI 0.70 to 9.44; adjusted $p = 0.029$) and PO by 9.96 µg/mL (95% CI 6.29 to 13.64; $p = 0.0007$), while SC exceeded PO by 4.90 µg/mL (95% CI 2.55 to 7.25; $p = 0.0025$). The t_{max} was progressively delayed from IM (median 0.67 h; range 0.33–1.00) through SC (2.00 h; 1.00–4.00) to PO (4.00 h; 2.00–6.00), with a statistically significant difference only between the IM and PO routes (Dunn's test, adjusted $p = 0.012$); the IM–SC and SC–PO comparisons were not significant ($p = 0.182$ and $p = 0.937$, respectively).

Total systemic exposure measured as $AUC_{0–24\ h}$ did not differ significantly between IM and SC administration (80.70 ± 40.22 and 81.06 ± 22.66 µg·h/mL respectively; mean difference (IM − SC) − 0.37 µg·h/mL, 95% CI − 80.65 to 79.92; $p = 0.99$), whereas PO administration produced approximately 7-fold lower exposure (11.94 ± 4.75 µg·h/mL) being lower than SC by 69.12 µg·h/mL (95% CI 37.96 to 100.3; $p = 0.0019$) and lower than IM by 68.76 µg·h/mL (95% CI 16.24 to 121.3; $p = 0.0182$). Relative bioavailability compared with IM administration (based on $AUC_{0–24\ h}$) was 100.4% for SC and 14.8% for PO.

Apparent clearance was markedly higher after PO administration (78.70 ± 14.00 mL/h/kg) than after SC (11.50 ± 3.66 mL/h/kg; mean difference 67.20 mL/h/kg, 95% CI 53.46 to 80.94; $p < 0.0001$) or IM administration (11.70 ± 3.44 mL/h/kg; 67.00 mL/h/kg, 95% CI 52.98 to 81.02; $p < 0.0001$), whereas the two parenteral routes did not differ (− 0.20 mL/h/kg, 95% CI − 0.48 to 0.08; $p = 0.151$). The apparent volume of distribution followed the same pattern, being higher after PO administration (2.17 ± 0.44 L/kg) than after SC (0.27 ± 0.10 L/kg; mean difference 1.900 L/kg, 95% CI 1.506 to 2.294; $p < 0.0001$) or IM administration (0.18 ± 0.06; 1.990 L/kg, 95%

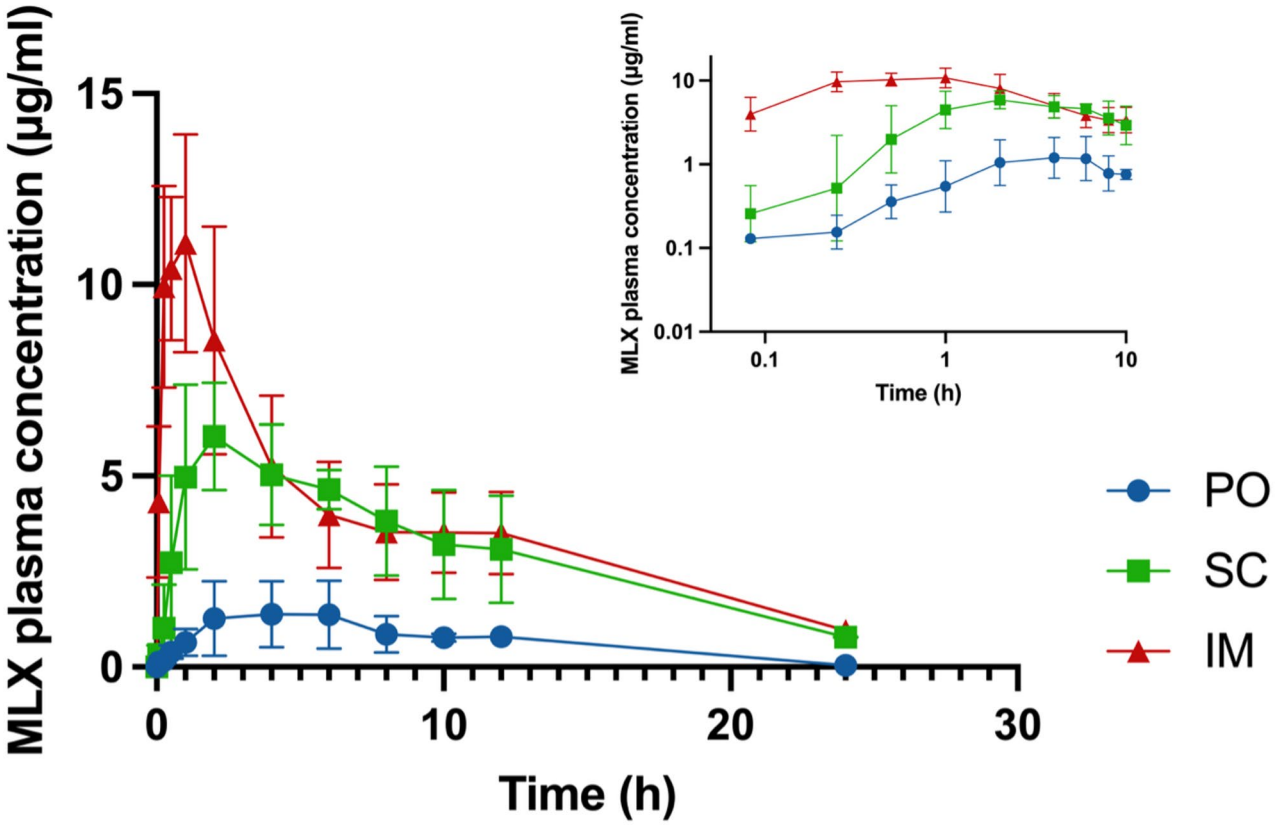


Fig. 1 Plasma meloxicam (MLX) concentration–time profiles in adult white storks (*Ciconia ciconia*; $n=6$). MLX was administered a single 1 mg/kg dose by the oral (PO; blue circles), subcutaneous (SC; green squares), or intramuscular (IM; red triangles) route, in a randomised three-way crossover design with a two-week washout between treatments. Data are presented as mean ± SD. The inset shows the same data on a log–log axes to resolve the early absorption phase (0–10 h) and the apparent differences in terminal slopes among three routes. Individual concentration–time profiles for each bird and route are provided in Supplementary material 2, allowing inter-individual variability to be assessed

CI 1.596 to 2.384; $p<0.0001$), with no significant difference between SC and IM (0.090 L/kg, 95% CI −0.304 to 0.484; $p=0.826$).

MRT was shortest after IM administration (12.43 ± 1.19 h) and progressively longer after SC (17.54 ± 3.72 h) and PO administration (23.25 ± 8.93 h). MRT was significantly longer after SC than after IM administration (mean difference 5.11 h, 95% CI 1.38 to 8.84; adjusted $p=0.015$) and after PO than after IM administration (10.82 h, 95% CI 0.11 to 21.53; $p=0.048$), whereas the PO and SC routes did not differ significantly (5.71 h, 95% CI −1.83 to 13.25; $p=0.121$). Because MRT incorporates the mean absorption time in addition to disposition, this ordering provides an index of progressively slower absorption across the three routes that does not depend solely on the terminal slope estimate.

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 routes (mean difference 9.68 h, 95% CI 4.13 to 15.22; adjusted $p=0.011$) whereas the SC value was intermediate and did not differ significantly from either IM (5.96 h, 95% CI −3.19 to 15.11; $p=0.140$) or PO (3.72 h, 95% CI −3.14 to 10.57; $p=0.205$). 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.

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]
$AUC_{0-24\ h}$ (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)	11.70 ± 3.44[c]	11.50 ± 3.66[c]	78.70 ± 14.00[a, b]
V_d/F (L/kg)	0.18 ± 0.06[c]	0.27 ± 0.10[c]	2.17 ± 0.44[a, b]
MRT (h)	12.43 ± 1.19[b, c]	17.54 ± 3.72[a]	23.25 ± 8.93[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 ($AUC_{0-24\ h,\ test}$ / $AUC_{0-24\ h,\ IM}$) × 100 [11]

Abbreviations: $t_{1/2}$ Elimination half-life, k_{el} Elimination rate constant; $AUC_{0-24\ h}$, 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

Discussion

The present study provides, to the best of our knowledge, the first species-specific comparison of IM, SC and PO 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. IM administration produced the most rapid absorption and was therefore used as the reference for relative bioavailability, SC administration showed an intermediate, slower profile, and PO administration resulted in the slowest absorption and the lowest systemic exposure. Systemic exposure did not differ significantly between the IM and SC routes, whereas PO administration yielded a substantially lower relative bioavailability, consistent with absorption-limited kinetics and/or a first-pass effect. Following IM administration, white storks exhibited a relatively prolonged apparent terminal half-life with relatively low CL/F and Vd/F values compared to those reported in many other bird species [13, 16]. However, interspecies comparisons of CL/F and Vd/F values should be interpreted with caution, as these parameters are influenced by the unknown absolute bioavailability of the relevant extravascular pathway. Consequently, differences between species cannot be attributed solely to differences intrinsic clearence or volume of distribution. A low apparent clearance is therefore equally compatible with efficient absorption as with slow elimination, and the present data do not permit white storks to be characterised as a slowly eliminating species; such a conclusion would require intravenous administration.

IM and SC administration produced similar systemic exposure, with $AUC_{0-24\ h}$ values of 80.70 and 81.06 µg·h/mL respectively and a relative SC bioavailability of approximately 100.4% based on $AUC_{0-24\ h}$. It should be stressed that the absence of a statistically significant difference between these routes does not in itself demonstrate equivalence. Formal demonstration would require an equivalence-testing framework with pre-specified acceptance limits, or confidence intervals for the subcutaneous/intramuscular AUC ratio, neither of which the present sample size ($n = 6$) was designed to support; the two routes are therefore described throughout as producing similar, rather than equivalent, exposure. This is the first direct comparison of the IM and SC routes for MLX in any avian species; previous studies have evaluated either the IM route alone or the SC route alone, often using sustained-release formulations [26, 34, 35]. SC exposure similar to, or even higher than, IM exposure is not unusual in birds and has been reported for several other drug classes, including danofloxacin in chukar partridges and swan geese [36, 37], florfenicol in turkeys [38], and levamisole in chukar partridges [39]. The slightly higher

SC $AUC_{0-\infty}$ observed in the present study most likely reflects sustained absorption from the SC depot together with a larger contribution of the extrapolated portion of the AUC associated with the longer apparent SC half-life. These findings support the SC route as a reliable parenteral alternative to IM administration in this species.

In contrast to the similar parenteral exposure, PO administration produced markedly reduced systemic exposure, with a relative oral bioavailability of only 14.8% (oral $AUC_{0-24\ h}$ 11.94 µg·h/mL). This value is substantially lower than the oral bioavailabilities reported for most other avian species at a comparable 1 mg/kg PO dose, including domestic pigeons (86.3%; oral $AUC_{0-\infty}$ 5.33 µg·h/mL) [40], laying hens (70.1%; 27.09 µg·h/mL) [41], Hispaniolan Amazon parrots (62%; 113 µg·h/mL) [7] and African grey parrots (38.1%) [17], as well as red-tailed hawks (74%) and great horned owls (62%) dosed at 0.5 mg/kg [16]; in Gyps vultures, oral exposure even approached that of IM administration (relative bioavailability ≈ 107%) [11]. Notably, the low oral bioavailability in white storks was not accompanied by a correspondingly low absolute oral AUC, because the prolonged apparent terminal disposition in this species sustains plasma concentrations once the drug has been absorbed; oral bioavailability, rather than absolute AUC, is therefore the more appropriate metric for interspecies comparison of the extent of absorption. White storks thus appear to represent a species with comparatively low oral bioavailability of MLX.

When considered within the broader avian literature, the apparent terminal half-life of MLX in white storks ($t_{1/2}$ = 18.85 h after PO administration) is among the longest so far reported in birds. Because no intravenous data are available, these values represent apparent terminal half-lives that may be influenced by the rate of absorption as well as by elimination, so cross-species comparisons should be regarded as indicative rather than definitive. The value falls within the upper range of apparent terminal half-lives reported in birds, close to those described in African grey parrots (31.4 h) [17], Hispaniolan Amazon parrots (15.8 h) [7] and emus [14], and contrasts markedly with the substantially shorter terminal half-lives reported in *Gyps* vultures (0.32 h) [11], domestic pigeons (2.59 h) [40], red-tailed hawks (3.97 h), and great horned owls (5.07 h) [16]. 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 [17, 25, 26], and reinforce previous recommendations that NSAID dosing in birds should be informed by species-specific pharmacokinetic data rather than by empirical interspecies extrapolation [1, 4].

More broadly, the disposition of MLX in poultry and other birds differs from that in mammals in several respects of direct relevance to dosing. In most mammals MLX exhibits high oral bioavailability, very high (> 97%) plasma protein binding and an elimination half-life of the order of 12–24 h, with clearance governed largely by hepatic CYP-mediated oxidation [5, 6]. Birds, including domestic poultry, instead display a considerably wider range of elimination half-lives and frequently lower and more variable oral bioavailability, together with a greater propensity for renal tubular injury and visceral gout at supratherapeutic doses [4, 13, 17]. These differences are consistent with species variation in both the cyclooxygenase target and the enzyme and transporter systems governing MLX disposition, and they reinforce the conclusion that avian dosing should rest on species-specific pharmacokinetic data rather than on extrapolation from mammals or from phylogenetically distant birds.

A notable feature of the present study was the progressive prolongation of the apparent terminal half-life from IM (9.81 ± 2.30 h) through SC (15.13 ± 4.41 h) to oral (18.85 ± 2.16 h) administration, although a statistically significant difference was observed only between the IM and PO routes ($p < 0.05$). This pattern is consistent with possible 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 [42]. Consistent with this possibility, t_{max} was progressively delayed in the same rank order (IM < SC < PO), which is also compatible with progressively slower absorption across administration routes. We emphasise, however, that these observations constitute indirect evidence only and do not establish flip-flop disposition. Formal confirmation would require intravenous administration to define the true elimination half-life, or compartmental modelling with explicit estimation of the absorption rate constant, neither of which was possible with the present dataset. Flip-flop kinetics is therefore

presented throughout as a hypothesis consistent with the observed data rather than as a demonstrated property of MLX disposition in white storks.

The markedly higher apparent clearance and apparent volume of distribution after PO administration, while these parameters remained similar between the two parenteral routes, is also compatible with this hypothesis, although it is equally explicable by the low oral bioavailability by which both parameters are scaled. Similar patterns have been reported, although less formally demonstrated, in Pekin ducks and domestic pigeons [40, 43]. It is important to distinguish clearly between the pharmacokinetic observations reported here and any therapeutic recommendation. No pharmacodynamic data are available for white storks, and the plasma concentration of 3.5 µg/mL associated with analgesic efficacy in Hispaniolan Amazon parrots [7, 15] derives from a single, distantly related species and is not a validated threshold for white storks. The observation that IM plasma concentrations remained above this concentration for part of the sampling period is therefore a pharmacokinetic observation only, and should not be interpreted as evidence that once-daily dosing is efficacious or safe in this species. 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 [14].

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 [44]. 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 [5].

The markedly reduced oral bioavailability is likely to be multifactorial. Differences in presystemic processing, including intestinal excretion and first-pass metabolism, may contribute to low oral exposure; however, these mechanisms have not been directly evaluated in white storks and therefore remain speculative [45–48].

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 [6] and that, in *Gyps* vultures, result in excretion of substantial amounts of unmetabolised parent drug in faeces [48]. None of these pathways was directly evaluated in the present study, and each warrants targeted investigation in white storks. A further consideration when interpreting the apparent volume of distribution is plasma protein binding. MLX is highly protein-bound in most mammalian species (approximately 97%) [5], whereas protein binding in birds appears to be appreciably lower (below 80%) and to vary among species [26]. Because the Vd/F value is also influenced by the unknown absolute bioavailability of the extravascular compartment, interspecies differences in this apparent parameter should be interpreted by caution. In addition, interspecies differences in protein binding may also contribute to the variability in apparent volume of distribution reported among avian species, and the low Vd/F values observed here should be interpreted with these considerations in mind. Plasma protein binding of MLX has not been determined in white storks and would be a valuable adjunct to future studies.

The high inter-individual variability observed after PO administration (C_{max}) further suggests that gastrointestinal absorption is influenced by individual factors including gastrointestinal content, intestinal transit, and possibly regurgitation [7, 13]. 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. Appreciable variability was likewise observed in $AUC_{0–24\ h}$ after IM administration (coefficient of variation approximately 50%), which exceeded that observed after subcutaneous administration (approximately 28%). Variability of this magnitude is not unexpected for IM injection in birds and may arise from differences in pectoral muscle perfusion at the time of dosing, from variation in injection technique and in the precise placement of the injectate within the muscle, and from differences in body condition and pectoral muscle mass among individuals. All birds in the present study were non-releasable residents of a rehabilitation centre with chronic disabilities, and inter-individual differences in body condition, activity level, and duration of captivity may therefore have contributed further to this variability. From a clinical perspective, the markedly

reduced oral bioavailability indicates that PO administration may not reliably achieve systemic exposure similar to that of 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 [4].

The pharmacokinetic profile reported here supports several practical considerations for the clinical use of MLX in white storks, which should be read as pharmacokinetic observations rather than as validated dosing recommendations. Because SC administration produced systemic exposure similar to that of IM administration while potentially reducing pectoral muscle trauma and injection-site muscle injury [49], the SC route may represent a practical parenteral alternative for repeated administration in rehabilitated birds. From an animal-welfare perspective the PO and SC routes therefore differ in an important respect: SC injection delivered systemic exposure that did not differ significantly from that after IM administration and would be expected to attain putatively analgesic plasma concentrations more consistently than the PO route, whereas oral dosing, although non-invasive, produced low and highly variable exposure and hence less predictable exposure. In debilitated or recently traumatised storks, in which prompt and dependable analgesia is itself a welfare priority, the modest and transient discomfort of a SC injection is likely to be outweighed by the more reliable exposure it provides relative to PO administration. In practical terms, at 1 mg/kg both parenteral routes attained plasma concentrations exceeding the value associated with analgesia in Hispaniolan Amazon parrots, whereas the PO route at the same dose did not approach this concentration at any sampling time (C_{max} 1.53 µg/mL) and should therefore not be regarded as therapeutically interchangeable. Pending species-specific efficacy and repeated-dose safety data, the SC route represents the more dependable provisional option for MLX in rehabilitated white storks, although the appropriate dosing interval cannot be defined from the present pharmacokinetic data alone. IM administration remains useful when rapid systemic absorption is desired, whereas PO 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), and no inference regarding the intrinsic elimination capacity of this species can be drawn from them. Second, the 24-hour sampling window did not fully capture the terminal elimination phase in all individuals. This is particularly relevant for the PO route, for which the estimated terminal half-life (18.85 h) approached the duration of sampling, so that the terminal log-linear phase was defined by relatively few concentration–time points. For the oral route the extrapolated fraction of $AUC_{0-\infty}$ was small (mean≈9%, individual range 5–15%), supporting reliable estimation of total oral exposure, whereas for the IM and SC routes it was larger (means≈14% and ≈17%, individual ranges 10–18% and 12–19%, respectively); all individual 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 $AUC_{0-24\,h}$ rather than $AUC_{0-\infty}$ and are thus unaffected by this extrapolation. The adjusted R^2 values of the individual terminal log-linear regressions ranged from 0.879 to 0.999 confirming adequate definition of the terminal phase, and no individual was excluded on the basis of the pre-specified criterion. 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 being attributable to flip-flop absorption. Third, the bioanalytical method was validated for linearity, sensitivity, selectivity, accuracy, precision, and absolute recovery, but several elements recommended in current bioanalytical validation guidelines were not assessed, namely carry-over, freeze–thaw and autosampler stability, dilution integrity, and matrix effect evaluation; their absence should be taken into account when appraising the analytical data, although all study samples were quantified within the validated calibration range. Fourth, 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. The small sample size also limits the precision of all reported parameter estimates, and the confidence intervals presented in the results section should be consulted alongside the corresponding p-values. 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.

Conclusion

This study provides the first pharmacokinetic evaluation of MLX in white storks and demonstrates marked route-dependent differences in systemic exposure. IM and SC administration produced similar exposure profiles, and although formal equivalance between the two routes was not established, the SC route may represent a practical parenteral alternative when repeated administration is required, whereas PO 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. Because no intravenous reference group was included, all disposition parameters are reported as apparent values, and the present findings should be regarded as pharmacokinetic observations rather than as validated dosing recommendations. 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

ANOVA	Analysis of variance
ARRIVE	Animal Research: Reporting of In Vivo Experiments
AUC	Area under the plasma concentration–time curve
$AUC_{0–24h}$	Area under the plasma concentration–time curve from 0 to 24 h
$AUC_{0-\infty}$	Area under the plasma concentration–time curve from time zero to infinity
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

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

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 whitestork (*Ciconia ciconia*) is a protected wild species in Türkiye. No animals were captured orcollected from the wild for the purposes of this study; all birdswere non-releasable individuals already held under permanent care atthe Istanbul University-Cerrahpasa Veterinary Faculty RehabilitationCenter. In addition to the institutional animal ethics approval, andin accordance with national legislation governing protected wildlifespecies, scientific research permission to conduct this study wasobtained from the General Directorate of Nature Conservation andNational 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). Allprocedures were carried out in compliance with the conditionsspecified in this permit and with the relevant institutional andnational guidelines for the use of protected species.
The study is reported in accordance with the ARRIVE 2.0 Essential 10 guidelines [50]; a completed checklist is provided as Supplementary Material 3.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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