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Egg residue and depletion of meloxicam in Jing Hong laying hens following multiple oral doses

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ABSTRACT Meloxicam is a nonsteroidal anti-inflammatory drug (**NSAID**) commonly used in an extra-label manner in commercial laying hens for the treatment of foot lesions, which are a common issue in this species. The present study aimed to determine the depletion profiles of meloxicam in eggs with multiple oral administration under 2 different dosing regimens and to further recommend reasonable withdrawal intervals (**WDIs**). Meloxicam (1 mg/kg) was administered orally to laying hens under 2 dosing schedules: 10 doses at 24-h intervals and 15 doses at 12-h intervals. Eggs were collected daily after the first dosing, and meloxicam concentrations in both yolk and white were determined by a high-performance liquid chromatography (**HPLC**) method. The weight ratio of white to yolk in the whole egg was 1.54 (the mean of 20 eggs with repeated tests), and this value combined with the meloxicam concentrations in white and yolk were used to calculate the drug concentrations in whole eggs. Meloxicam was quickly eliminated from egg white, and its concentrations could only be quantified at 2 time points during the elimination phase. The elimination half-lives in yolk and whole egg were 3.07 ± 1.00 and 2.98 ± 0.88 d, respectively, after 10 repeated doses. And the corresponding elimination half-lives were 2.30 ± 0.83 and 2.18 ± 0.67 d, respectively, after repeated 15 doses. Considering the time when meloxicam was not detectable in eggs with the time of ovum development and maturation, a withdrawal interval (**WDI**) was suggested as 17 d for both dosing schedules. The current results enriched the study on the residue of meloxicam in domestic Jing Hong laying hens and provided WDIs to help ensure animal-derived food safety.

Key words: withdrawal interval, meloxicam, Jing Hong laying hens, HPLC, the weight ratio of white to yolk

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INTRODUCTION

Egg production shows a slow growth trend from a global perspective and is expected to increase further in response to the rising demand for animal protein sources (FAO, 2022). This growth in egg production will correspondingly entail an increase in the number of breeding hens. About 35% of the world's eggs are produced in China (Molnar and Szollosi, 2020).

Laying hens, as a species usually kept in captivity, are particularly susceptible to foot lesions that may indicate the need for analgesia. Nonsteroidal anti-inflammatory drugs (**NSAIDs**) are frequently used to treat inflammation and relieve pain (Paul-Murphy and Ludders, 2001). However, they also have some side effects, including gastrointestinal ulceration and platelet dysfunctions, caused by a suppression of cyclooxygenase-1 (**COX-1**) activity. Meloxicam is an NSAID with preferential cyclooxygenase-2 (**COX-2**) inhibitory activity, which is commonly used in an extra-label manner in commercial laying hens to treat foot lesions. Due to its higher COX-2 selectivity, meloxicam has a reduced incidence of adverse effects (Gates et al., 2005).

There are some studies on meloxicam residue in eggs laid by different hen breeds, including White Leghorn hens (Souza et al., 2017, 2018), Wyandotte hens (Souza et al., 2021), Bantam Cochin Chickens (Stilz et al., 2022), and Hy-line hens (Yuan et al., 2022b). However, few cases have been reported in Jing Hong laying hens, which is an excellent breed known for its strong adaptability, high egg production, and low feed consumption (Wu et al., 2016). The breed is widely cultivated in China. Additionally, the WDI for meloxicam was only calculated in Hy-line hens (Yuan et al., 2022b), without any information on the other breeds.

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The breed differences have been reported for the disposition of meloxicam in different laying hens (Souza et al., 2017, 2018, 2021). Therefore, it is essential to recommend the WDI of meloxicam in each laying hen breed. The present study aimed to determine meloxicam depletion profiles in eggs from Jing Hong hens with multiple oral doses under 2 dosage regimens and recommend the corresponding WDIs.

MATERIALS AND METHODS

Animals and Experiment Design

Twenty-six 6-mo-old Jing Hong hens were obtained from a commercial source and housed in a climate-controlled environment for 2 wk. Animals were housed in individual wire cages (55 × 55 × 45 cm), and all hens could observe each other. The hens were maintained on a 16 h light and 8 h dark cycle. The drug-free powder feed was given to chickens twice daily, which was produced by a domestic enterprise upon our request. And the water was supplied ad libitum. Each hen was considered healthy based on an external physical examination. And their average egg-laying frequency is about 7 to 9 eggs every 10 d.

All chickens were randomly divided into 2 groups. The chickens were weighed before the first dose and then given multiple doses based on their body weights (**BWs**), which were 1.60 to 2.48 kg. Hens in Group 1 ($n = 14$) were given meloxicam by gavage at 1 mg/kg for 10 doses at 24-h intervals. Hens in Group 2 ($n = 12$) were given meloxicam at 1 mg/kg for 15 doses at 12-h intervals. In both groups, the first dosing was conducted at 8:00 pm. During drug treatments, eggs were collected each evening before dosing. Egg collection continued for 10 d after the last dose. Each egg was refrigerated whole at 4°C. Meloxicam in egg samples was shown to be stable for 30 d when stored at 4°C (data not shown here). In the present study, the period from sample collection to determination did not exceed 15 d.

Reagents and Standards

Meloxicam analytical standard (Lot No. G1025591) with purity above 99.66% was provided by LGC Labor Gmbh (Shanghai, China). Meloxicam raw material (purity of 95.66%; Lot No. RH355441) was purchased from Shanghai Yien Chemical Technology Co., Ltd. (Shanghai, China). Dimethyl sulfoxide, methanol, and acetonitrile were of HPLC grade and purchased from Tianjin Komiou Chemical Reagent Co., Ltd. (Tianjin, China). Phosphoric acid (H_3PO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Tianjin Deen Chemical Reagent Co., Ltd. (Tianjin, China). All the other reagents were purchased from commercial sources.

HPLC Analysis of Samples

The e2695 HPLC system equipped with an ultraviolet detector (2487 series; Waters; Milford, MA) was used to quantify the concentrations of meloxicam. The chromatographic separation was performed with a reverse-phase C18 analytical column (250 × 4.6 mm inner diameter, 5 μm particle size; Dalian Elite Analytical Instruments Co., Ltd.; Dalian, China) maintained at 30°C. The mobile phase consisted of 0.05 M potassium phosphate buffer (pH = 3.2) and acetonitrile (v:v = 60:40 for egg yolk; v:v = 55.5:44.5 for egg white), whose flow rate was set as 1.0 mL/min. The injection volume was 20 μL. The detection wavelength was set at 365 nm.

Egg yolks and whites were manually separated after the eggs returned to room temperature. After fully stirring, each was weighed 0.5 g for subsequent extraction. The preparation process of egg yolk samples referred to a published method (Shao et al., 2022) with slight modifications and was briefly described below. The sample was added with 1 mL of acetonitrile, vortexed for 3 min, and centrifuged at 12,000 × g for 10 min. The supernatant was taken into a tube, and 1 mL of n-hexane was added followed by a complete vortex and centrifugation at 8,000 × g for 10 min. The lower layer of liquid was collected and 20 μL was injected into the HPLC system. Based on a previous method (Bae et al., 2007), the egg white sample was prepared as follows. A total of 0.5 g egg white was mixed with 100 μL of 5 M HCl and vortexed for 30 s, then 6 mL of diethyl ether was added and the mixture was vortexed for 30 s. Then the mixture was centrifuged at 2,500 × g for 10 min, followed by collecting the organic layer into a glass tube and drying with a nitrogen flow at 40°C. The residue was redissolved with 1 mL of mobile phase, vortexed for 3 min, and subsequently filtered through a 0.22-μm filter. Finally, 20 μL of the filtrate was injected into the HPLC system.

Method Validation

Meloxicam stock solution with a concentration of 1 mg/mL was prepared in dimethyl sulfoxide, which was stable at room temperature and stored in the dark. The stock solution was diluted with 50% methanol to obtain serial working solutions. To determine the accuracy and precision of the present detection methods, 5 replicate blank samples were spiked with meloxicam at 3 different concentrations (20, 100, and 500 ng/g in yolks and 10, 50, and 500 ng/g in whites). Then the recovery, intraday, and interday coefficient of variation were determined for 3 consecutive days. Calibration standards samples were prepared by diluting the stock solution with blank samples. The limits of quantification (**LOQ**) and detection (**LOD**) were determined based on signal-to-noise ratios of ≥10 and ≥3, respectively.

Data Processing

A total of 20 blank eggs were subjected to manual separation into egg white and yolk, and each white and yolk were weighed. Subsequently, the weight ratio between white and yolk was determined for each egg, and its mean value was determined as 1.54. Based on this

weight ratio combined with meloxicam concentrations in the white and yolk, drug concentrations in the whole egg were further calculated. Pharmacokinetic parameters for meloxicam in egg white, egg yolk, and whole egg were further estimated using Phoenix software (version 8.1; Pharsight; Mountain View, CA) by noncompartmental analysis. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule with extrapolation to time infinity (Song et al., 2023). Other pharmacokinetic parameters included terminal phase rate constant (λz), terminal half-life ($t_{1/2\lambda}$), peak concentration (C_{max}), and the time to reach it (T_{max}).

The Kolmogorov-Smirnov test in SPSS 20.0 software was used to determine the data normality of each pharmacokinetic parameter. The independent sample t test in SPSS 20.0 was used for parameters with normal distribution, and the Mann-Whitney U test was used for other parameters. A P value <0.05 was considered significant.

RESULTS

Validation of Analytical Methods

The current assay methods were selective to egg yolk and egg white, and there was no interference peak at the retention time of meloxicam. The calibration curve of meloxicam had repeatability and linearity (yolk: 20−500 ng/g; egg white: 10−500 ng/g). The recovery of meloxicam in egg yolk was between 88.62 and 107.25%, and the intraday and interday coefficients of variation were lower than 6.65%. The recovery rate of meloxicam in egg white was between 75.15 and 101.22%, and the intraday and interday coefficients of variation were below 7.07%. LODs of meloxicam in egg white and egg yolk were 5 and 10 ng/g respectively, and LOQs were 10 and 20 ng/g respectively.

Residue Depletion

During the entire experimental period, hens remained in good general health, without obvious adverse clinical symptoms, such as inappetence, diarrhea, or regurgitation. The concentration-time curves and data of meloxicam under both dosing regimens are shown in Figure 1 and Tables 1 and 2. The pharmacokinetic parameters of egg white, yolk, and whole egg are provided in Tables 3 and 4. The statistical analysis results of pharmacokinetic parameters between the 2 groups are shown in Table 5.

In the treatment group with 24-h dosing intervals, the C_{max} in egg yolk, egg white, and whole egg were 75.34 ± 23.40, 24.43 ± 3.89, and 44.18 ± 10.77 ng/g, respectively; and the corresponding T_{max} values were 10.14 ± 0.53, 9.64 ± 0.63, and 9.64 ± 0.63 d, respectively. The elimination half-life in egg yolk was 3.07 ± 1.00 d, and that in the whole egg was 2.98 ± 0.88 d. There was no elimination half-life result in the egg white because meloxicam was quickly eliminated from it, and drug concentrations could only be quantified at 2 time points during the elimination phase.

In the treatment group with 12-h dosing intervals, the C_{max} in egg yolk, egg white, and whole egg were 93.17 ± 16.73, 37.23 ± 8.89, and 58.55 ± 11.12 ng/g, respectively. And the T_{max} from fast to slow were egg white (7.67 ± 0.65 d), whole egg (8 d), and yolk (8.33 ± 0.65 d). The elimination half-lives in yolk and whole egg were 2.30 ± 0.83 and 2.18 ± 0.67 d, respectively.

In the statistical analysis of 2 dosing groups, most pharmacokinetic parameters adhered to a normal distribution. However, we found that T_{max} in the yolk, egg white, and whole egg did not comply with this pattern. We observed no significant disparities in both groups for λz, $t_{1/2\lambda}$, and $AUC_{0-\infty}$ in the yolk ($P > 0.05$). Conversely, other pharmacokinetic parameters did diverge significantly between both groups in the yolk, egg white, and whole egg ($P < 0.05$).

Due to the absence of established maximum residue limit (**MRL**) values in any country, we adopted an analysis method similar to Souza et al. (2018), where we combined the time when meloxicam was not detected in eggs with the time of ovum development and maturation. Based on our analysis, we suggest a WDI of 17 d.

DISCUSSION

The present study seeks to evaluate the WDIs of meloxicam in hens' eggs to protect food safety. Two different dosing regimens were applied and the following findings were made: 1) the highest concentrations of meloxicam were found in egg yolk, with the slowest depletion rate; 2) the recommended WDIs in eggs for both dosing regimens were 17 d.

Under both dosing regimens, higher concentrations were observed in the yolk, consistent with the results in Hy-line hens (Yuan et al., 2022b). The difference is thought to be due to several factors including the properties of the drug (lipophilic with better permeability in the lipophilic yolk; Azeem et al., 2009) and the different physio-chemical processes involved in the formation of these 2 compartments (Donoghue et al., 1997; Johnson, 2015). For example, it takes 9 to 10 d for the rapid growth phase of the yolk and the absorption of meloxicam into the preovulatory yolk during administration (Donoghue et al., 1997). Furthermore, the yolk then passes through several segments of the oviduct during which the egg white is formed (Donoghue, 2005): the infundibulum (15−30 min); the magnum (3 h); the isthmus (1.25 h); the uterus (20−21 h). This longer process of yolk formation is likely to result in a higher concentration of meloxicam in it as compared to egg white. Additionally, the drug was found to be quickly eliminated from the egg white and could not be quantified on the third day after administration, a much shorter timeframe than that required in the yolk (eighth day). In general, drug levels in egg white mostly depend on circulating plasma levels (Kolanovic et al., 2019). Previous studies have suggested that the pharmacokinetic parameters of meloxicam, such as $t_{1/2\lambda}$, C_{max}, and T_{max}, are not altered in domestic chickens when administered in

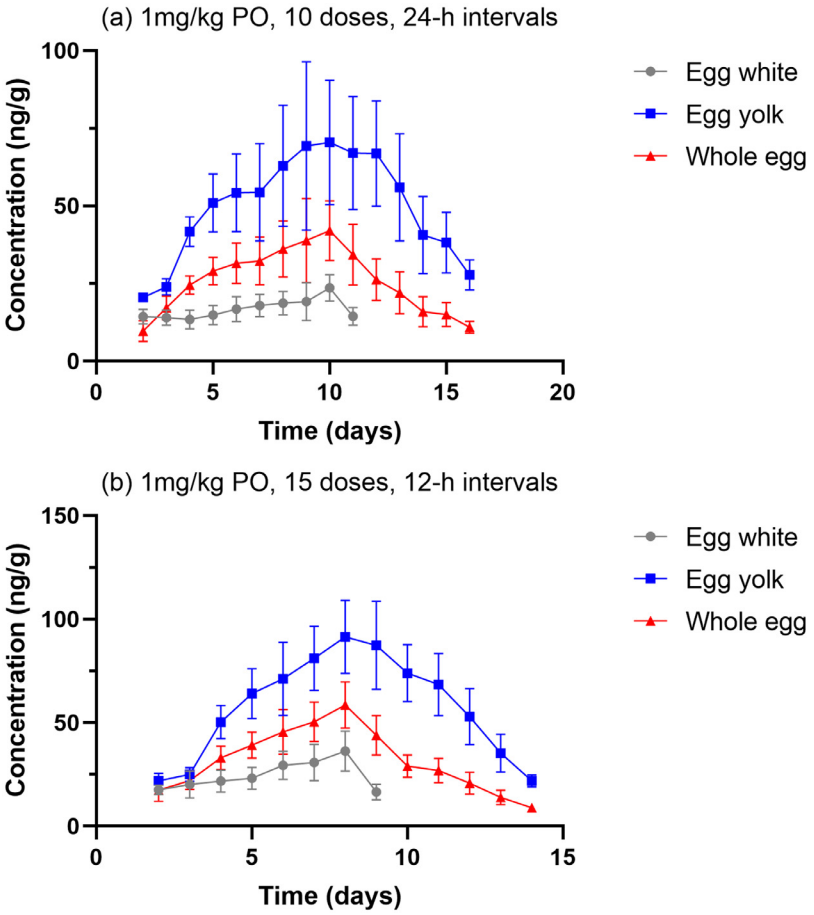

Figure 1. Concentration-time curves of meloxicam (mean ± SD) in the egg white, egg yolk, and whole egg under 2 dosing regimens. (A) 10 PO doses at 1 mg/kg with 24-h intervals; (B) 15 PO doses at 1 mg/kg with 12-h intervals.

either single or multiple doses (Souza et al., 2018). These results indicate that significant plasma accumulation does not occur and that the drug is quickly eliminated from plasma upon drug withdrawal (Kolanovic et al., 2019). Consequently, meloxicam is also rapidly eliminated from egg whites.

Table 1. Concentration data in the egg yolk, egg white, and whole egg after 10 repeated oral doses of meloxicam at 1 mg/kg with 24-h intervals.

		Concentrations of meloxicam (average ± SD, ng/g)		
Dosage #/time	Time (d)	Egg yolk	Egg white	Whole egg
During treatment				
1, 8:00 pm	0	NC	NC	NC
2, 8:00 pm	1	ND	ND	ND
3, 8:00 pm	2	20.56 ($n = 1$)	14.34 ± 2.36 ($n = 8$)	9.71 ± 3.37 ($n = 8$)
4, 8:00 pm	3	23.96 ± 2.62 ($n = 11$)	14.08 ± 2.46 ($n = 12$)	17.18 ± 3.80 ($n = 12$)
5, 8:00 pm	4	41.68 ± 4.76 ($n = 10$)	13.47 ± 3.02 ($n = 10$)	24.58 ± 2.97 ($n = 10$)
6, 8:00 pm	5	50.97 ± 9.39 ($n = 10$)	14.85 ± 3.08 ($n = 10$)	29.07 ± 4.45 ($n = 10$)
7, 8:00 pm	6	54.21 ± 12.48 ($n = 12$)	16.79 ± 3.96 ($n = 12$)	31.53 ± 6.52 ($n = 12$)
8, 8:00 pm	7	54.42 ± 15.67 ($n = 11$)	17.97 ± 3.63 ($n = 11$)	32.32 ± 7.71 ($n = 11$)
9, 8:00 pm	8	62.91 ± 19.53 ($n = 12$)	18.71 ± 3.78 ($n = 12$)	35.54 ± 8.48 ($n = 12$)
10, 8:00 pm	9	69.29 ± 27.11 ($n = 9$)	19.19 ± 6.10 ($n = 9$)	39.68 ± 13.70 ($n = 9$)
After cessation of treatment				
NA	10	70.49 ± 20.03 ($n = 10$)	23.63 ± 4.25 ($n = 10$)	42.08 ± 9.62 ($n = 10$)
NA	11	67.06 ± 18.15 ($n = 10$)	14.45 ± 2.80 ($n = 9$)	34.78 ± 8.98 ($n = 10$)
NA	12	66.84 ± 16.97 ($n = 13$)	ND	26.31 ± 6.68 ($n = 13$)
NA	13	55.94 ± 17.22 ($n = 10$)	ND	22.03 ± 6.78 ($n = 10$)
NA	14	40.59 ± 12.42 ($n = 9$)	ND	15.98 ± 4.89 ($n = 9$)
NA	15	38.24 ± 9.78 ($n = 10$)	ND	15.06 ± 3.85 ($n = 10$)
NA	16	27.81 ± 4.85 ($n = 6$)	ND	10.95 ± 1.91 ($n = 6$)
NA	17	ND	ND	ND
NA	18	ND	ND	ND
NA	19	ND	ND	ND

n, number of samples with quantifiable meloxicam concentrations; NA, not applicable; NC, not collected; ND, not detectable.

Table 2. Concentration data in the egg yolk, egg white, and whole egg after 15 repeated oral doses of meloxicam at 1 mg/kg with 12-h intervals.

Dosage #, dosing time	Time (d)	Concentration of meloxicam (average ± SD, ng/g)		
		Egg yolk	Egg white	Whole egg
During treatment				
1, 8:00 pm	0	NC	NC	NC
2, 8:00 am; 3, 8:00 pm	1	ND	ND	ND
4, 8:00 am; 5, 8:00 pm	2	21.95 ± 3.60 ($n = 8$)	17.52 ± 2.23 ($n = 8$)	17.45 ± 5.38 ($n = 8$)
6, 8:00 am; 7, 8:00 pm	3	25.09 ± 3.28 ($n = 11$)	20.15 ± 6.59 ($n = 11$)	22.09 ± 4.34 ($n = 11$)
8, 8:00 am; 9, 8:00 pm	4	50.26 ± 8.07 ($n = 9$)	21.82 ± 5.31 ($n = 9$)	33.02 ± 5.71 ($n = 9$)
10, 8:00 am; 11, 8:00 pm	5	64.06 ± 11.97 ($n = 12$)	23.13 ± 5.26 ($n = 12$)	39.24 ± 6.27 ($n = 12$)
12, 8:00 am; 13, 8:00 pm	6	71.23 ± 17.69 ($n = 11$)	29.49 ± 6.76 ($n = 11$)	45.56 ± 10.72 ($n = 11$)
14, 8:00 am; 15, 8:00 pm	7	81.04 ± 15.49 ($n = 12$)	30.83 ± 8.76 ($n = 12$)	50.41 ± 9.58 ($n = 12$)
After cessation of treatment				
NA	8	91.40 ± 17.72 ($n = 12$)	36.35 ± 9.65 ($n = 12$)	58.55 ± 11.12 ($n = 12$)
NA	9	87.31 ± 21.19 ($n = 10$)	16.58 ± 3.81 ($n = 10$)	43.90 ± 9.58 ($n = 10$)
NA	10	73.95 ± 13.80 ($n = 8$)	ND	29.12 ± 5.43 ($n = 8$)
NA	11	68.43 ± 14.99 ($n = 8$)	ND	26.94 ± 5.90 ($n = 8$)
NA	12	52.90 ± 13.50 ($n = 10$)	ND	20.83 ± 5.31 ($n = 10$)
NA	13	35.31 ± 9.10 ($n = 11$)	ND	13.90 ± 3.58 ($n = 11$)
NA	14	21.92 ± 2.95 ($n = 5$)	ND	8.88 ± 1.08 ($n = 5$)
NA	15	ND	ND	ND
NA	16	ND	ND	ND
NA	17	ND	ND	ND

n, number of samples with quantifiable meloxicam concentrations; NA, not applicable; NC, not collected; ND, not detectable.

Egg white and egg yolk reached peak concentrations on the first day after multiple doses since the formation of the eggshell would take 25 to 27 h and repeat itself daily (Wang et al., 2021). In particular, when 1 mg/kg of meloxicam was administered orally for 10 doses at 24-h intervals, the C_{max} values in the egg yolk and egg white were 75.34 ± 23.40 and 24.43 ± 3.89 ng/g, respectively. These values were observed to be higher than those in similar conditions involving 8 doses at 24-h intervals (50.3 ng/g in egg yolk and 16.8 ng/g in egg white) (Yuan et al., 2022b). In the group orally given for 15 doses at 12-h intervals, the C_{max} values in egg yolk and white were 93.17 ± 16.73 and 37.23 ± 8.89 ng/g, respectively, which were lower than those reported for the same dosing frequency but with twenty doses (egg yolk: 215 ng/g; egg white: 47.5 ng/g; Yuan et al., 2022b). At the same dosing interval of 12 h, the current C_{max} values in both egg yolk and egg white were higher than those after 9 oral doses of meloxicam at 1 mg/kg (egg yolk: 88 ng/g; egg white: 19.5 ng/g) (Souza et al., 2018). Meloxicam concentrations varied across studies due to differences in dosing numbers and intervals. However, higher drug concentrations in both egg whites and yolks were generally observed as the number of doses increased. In this study, the C_{max} values in the yolk, egg white, and whole egg were significantly higher after 15 doses than after 10 doses ($P < 0.05$). In another breed of laying hens (White Leghorn), previous studies found that multiple doses of meloxicam resulted in significantly higher residue concentrations in both egg yolk and egg white compared to single dosing (Souza et al., 2017, 2018). Rapid elimination was also observed in egg whites (Souza et al., 2018). Due to the lower incidence of adverse effects associated with meloxicam compared to other dosing regimens, we opt for intermediate doses at 12-h intervals instead of higher doses at 24-h intervals to achieve better efficacy. Frequent administration with 12-h dosing intervals may have better therapeutic effects, and treatment cycles should be shorter for 12-h dosing intervals compared to 24-h dosing intervals. Hence, we administered a total of 10 doses at 24-h intervals and 15 doses at 12-h intervals.

Table 3. Pharmacokinetic parameters in the egg yolk, egg white, and whole egg after 10 repeated oral doses of meloxicam at 1 mg/kg with 24-h intervals.

Parameter (unit)	Egg yolk	Egg white	Whole egg
C_{max} (ng/g)	75.34 ± 23.40	24.43 ± 3.89	44.18 ± 10.77
T_{max} (d)	10.14 ± 0.53	9.64 ± 0.63	9.64 ± 0.63
$AUC_{0-\infty}$ (ng·d/g)	848.52 ± 174.36	NC	421.62 ± 79.97
λz (1/d)	0.2509 ± 0.0857	NC	0.2532 ± 0.0793
$t_{1/2\lambda}$ (d)	3.07 ± 1.00	NC	2.98 ± 0.88

NC, not calculatable; T_{max} refers to the peak time after the first dose rather than the last one.

Various methods have been employed to calculate the WDIs of meloxicam in chickens. Previous studies (Souza et al., 2017, 2018, 2021; Stilz et al., 2022) suggest an egg WDI of 2 wk based on the time it takes for meloxicam to be undetectable in eggs and a review of drug residue literature (usually less than 2 wk; Goetting et al., 2011). Their dosing regimens included a single dose of 1 mg/kg and 1 mg/kg q12h for 9 doses. In another study (Yuan

Table 4. Pharmacokinetic parameters in egg yolk, egg white, and whole egg after 15 repeated oral doses of meloxicam at 1 mg/kg with 12-h intervals.

Parameter (unit)	Egg yolk	Egg white	Whole egg
C_{max} (ng/g)	93.17 ± 16.73	37.23 ± 8.89	58.55 ± 11.12
T_{max} (d)	8.33 ± 0.65	7.67 ± 0.65	8
$AUC_{0-\infty}$ (ng·d/g)	844.74 ± 148.59	NC	451.99 ± 79.14
λz (1/d)	0.3437 ± 0.1374	NC	0.3464 ± 0.1111
$t_{1/2\lambda}$ (d)	2.30 ± 0.83	NC	2.18 ± 0.67

NC, not calculatable; T_{max} refers to the peak time after the first dose rather than the last one.

et al., 2022b), the tolerance method recommended by FDA was used to calculate the egg WDIs for meloxicam, which was between 12 and 36 d under different dosing regimens. The physiologically based pharmacokinetic (**PBPK**) modeling combined with Monte Carlo simulations was also applied to determine the WDIs of meloxicam in laying hens, which were 22 and 4 d in yolk and white after 14 repeated oral dosing (1 mg/kg) at 24-h intervals (Yuan et al., 2022a). In another backyard chickens study (Richards et al., 2022), 3 different model-based approaches (nonlinear mixed-effect model, artificial neural network, and support vector machine) were used to determine WDIs of meloxicam in the tissues rather than eggs.

The $t_{1/2\lambda}$ of meloxicam in egg yolk in Hy-line chickens showed variability with different doses and intervals (Yuan et al., 2022b). With multiple oral doses of 1 mg/kg meloxicam at the same dosing frequency (24 h), the $t_{1/2\lambda}$ in egg yolk was faster with 8 doses (54.15 h) than the current result with 10 doses (3.07 ± 1.00 d). At a frequency of 12 h, the $t_{1/2\lambda}$ after 20 doses (112.54 h) in egg yolk was slower than the present results after 15 doses (2.30 ± 0.83 d) (Yuan et al., 2022b). The difference in terminal half-life values may be that meloxicam elimination in eggs varies by breed. In the present study, the $t_{1/2\lambda}$ in the whole egg with 12-h intervals was significantly faster compared to the 24-h intervals ($P < 0.05$). Due to the good safety profile of meloxicam, a 12-h dosing interval may have a better therapeutic effect (1 mg/kg) from the perspective of analgesia. Although the C_{max} values of 15 doses of meloxicam in yolk, egg white, and whole egg were significantly higher than those of 10 doses ($P < 0.05$), the faster $t_{1/2\lambda}$ resulted in the consistent WDI of the drug in eggs.

There were no established MRLs in any country, Codex Committee on Residues of Veterinary Drugs in Foods (**CCRVDF**), or elsewhere for meloxicam in eggs. Therefore, we use the method of Souza et al. (2018) to derive the recommendation of the WDIs according to the time that meloxicam is undetectable in eggs. In both regimens, meloxicam was not detected in egg yolk on the eighth day, and in egg white on the third day. A previous study showed that meloxicam could be detected in both primary and secondary follicles after multiple doses, with the observed concentrations in secondary follicles being higher than those in primary follicles (Yuan et al., 2022b), indicating that meloxicam might be slowly absorbed from the ovary into the yolk before ovulation. However, an ovum took 9 to 10 d to form a mature yolk (Donoghue et al., 1997). Consequently, undetectable meloxicam in the egg yolk could be present in developing ova. After combining the time when meloxicam was undetectable in eggs with the time of ovum development and maturation, a WDI of 17 d is recommended. Further studies should be carried out to determine the MRL values of meloxicam in eggs, and at that time the WDI for meloxicam in eggs can be precisely determined.

Table 5. Statistical analysis results of pharmacokinetic parameters of meloxicam after 10 oral doses at 24-h intervals and 15 oral doses at 12-h intervals.

	P value		
Parameter	Egg yolk	Egg white	Whole egg
C_{max}	0.038	<0.001	0.003
T_{max}	<0.001	<0.001	<0.001
$AUC_{0-\infty}$	0.955	NC	0.350
λz	0.060	NC	0.023
$t_{1/2\lambda}$	0.053	NC	0.018

$P < 0.05$ indicates a significant difference between the 2 groups.

CONCLUSIONS

This study was the first to analyze the depletion of meloxicam in eggs from Jing Hong laying hens after multiple oral doses. Meloxicam concentrations in the whole egg were calculated based on the average weight ratio between the egg white to egg yolk (1.54), and the pharmacokinetic parameters were further assessed in the whole egg and egg yolk. To ensure animal-derived food safety, a 17-d WDI should be sufficient to avoid drug residue in eggs. Based on the WDI and analgesic effect of the 2 dosing regimens, 12-h dosing interval is recommended.

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Ethical Approval: All applicable international, national, and institutional guidelines for the care and use of animals were followed.

DISCLOSURES

All authors declare that they have no conflicts of interest.

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