The influence of immune stress induced by *Escherichia coli* lipopolysaccharide on the pharmacokinetics of danofloxacin in broilers

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ABSTRACT This study aimed to determine whether the challenge from *Escherichia coli* (***E. coli***) lipopolysaccharide (**LPS**) affects the pharmacokinetics of danofloxacin in broilers. Twenty 1-day-old Arbor Acres (**AA**) broilers were equally and randomly divided into 2 groups. When the chickens were 23, 25, 27, and 29 days old, *E. coli* LPS (1 mL; 0.5 mg/kg body weight [**BW**]) and sterile saline (1 mL) were intraperitoneally injected into the two groups. After the last injection, danofloxacin was given to all chickens by gavage at the dose of 5 mg/kg BW. Then serum and plasma samples at each time point were collected through the wing vein. Danofloxacin concentrations in plasma were detected through the high-performance liquid chromatography (**HPLC**) method and subjected to noncompartmental analysis using Phoenix software. The levels of chicken interleukin-1β (**IL-1β**) and corticosterone (**CORT**) in serum were measured by the Enzyme-linked immunosorbent assay (**ELISA**) kit. In addition, after the collection of plasma or serum samples, 7 chickens (31 days of age) in each group were killed to calculate the organ indices. Compared with the control group, the challenge of LPS significantly decreased the parameters of $AUC_{0\text{-}\infty}$, C_{max}, and $t_{1/2\lambda z}$ and increased the parameters of T_{max} and λz. Additionally, in the LPS group, the absorption time of danofloxacin was prolonged; however, the elimination was accelerated, which resulted in reduced internal exposure.

Key words: broiler, danofloxacin, pharmacokinetics, *Escherichia coli* lipopolysaccharide

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INTRODUCTION

With the improvement of intensive poultry breeding in China, most poultry is suffering from the increased risk of microbial infection due to the poor sanitary environment in production and the high density of chickens (Chanie et al., 2009; Zhuang et al., 2014). Exposure of animals to infectious agents can induce adaptive immunological response (Webel et al., 1998). The activated immune system can further resist the damage of exogenous microbes to the animal body, but excessive activation of the immune system will produce immune stress (Li et al., 2017).

Lipopolysaccharide (**LPS**) is a pathogenic component of Gram-negative bacteria and can stimulate the secretion of immune elements (Yousefi et al., 2021). Multiple intraperitoneal injections of LPS were commonly used to induce immune stress in broilers (Webel et al., 1998; Li et al., 2015; Wu et al., 2017). It was shown that the immune stress induced by LPS would reduce the early growth performance of broilers (Li et al., 2015). Additionally, multiple intraperitoneal injections of LPS would cause intestinal mucosal damage in broilers (Jiang et al., 2019). These changes may further affect the pharmacokinetics of the drug. The LPS intramuscularly given to broilers upregulated the P-glycoprotein expression in the small intestine, which further altered the pharmacokinetics of enrofloxacin in LPS-treated chickens. Its absorption of enrofloxacin was prohibited after oral administration (Bughio et al., 2017). In another study in broilers, intravenous injection of LPS caused changes in hepatic blood flow and reduced the concentration of an active metabolite of tepoxalin (De Boever et al., 2010). There are few studies on the potential effect of intraperitoneal injection of LPS on the pharmacokinetics in broilers.

Danofloxacin is a third-generation fluoroquinolone developed especially for veterinary applications. It has the advantages of the broad antibacterial spectrum, high bactericidal activity, and wide distribution in the body (Fan et al., 2015; Vardali et al., 2017). The antibacterial spectrum of danofloxacin includes a few Gram-positive (**G+**) and most Gram-negative (**G-**) bacteria

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(Haritova et al., 2013). It mainly works by inhibiting bacterial DNA gyrase (EMA, 2002). It has been commonly used to treat respiratory and gastrointestinal infections in chickens, pigs, and cattle (Real et al., 2011; Perez et al., 2013; Yu et al., 2013). In broilers, danofloxacin is usually given through drinking water, providing 5 mg/kg body weight (**BW**) daily for 3 d. The potential effects of LPS on the pharmacokinetics of danofloxacin have been observed in pigs and goats (Ismail, 2006; Yao et al., 2017). Additionally, similar effects were also observed for enrofloxacin in broilers (Bughio et al., 2017). Therefore, this study aimed to determine the potential impact of the LPS challenge on the pharmacokinetics of danofloxacin in broilers.

MATERIALS AND METHODS

Drugs and Reagents

The LPS from *E. coli* O55:B5 (L2880) was bought from Sigma Aldrich Chemical Co. (St. Louis, MO). The analytical standard for danofloxacin mesylate (Lot No. h0201210) with a purity of 94.2% was supplied by the China Institute of Veterinary Drugs Control (Beijing, China). The raw material of danofloxacin mesylate (Lot No. 201217-1) with a purity of 95.37% was donated by Zhejiang Guobang Pharmaceutical Co., Ltd. (Hangzhou, China). The Enzyme-linked immunosorbent assay (**ELISA**) kit of chicken corticosterone (**CORT**) and interleukin-1β (**IL-1β**) were obtained from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China). Acetonitrile and methanol were of chromatographic grade and purchased from the Shanghai Linen Technology Development Co., Ltd (Shanghai, China). Phosphoric acid and triethylamine were domestic analytical reagents purchased from Tianjin Deen Chemical Reagent Co., Ltd. (Tianjin, China) and Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China), respectively.

Experimental Animals

Thirty healthy 1-day-old Arbor Acres (**AA**) broilers of both sexes were purchased from a hatchery near Luoyang City. Under the experimental conditions, the animals were strictly raised following the Modern Broiler Production Manual. The diet was free of any antibiotics and coccidiostats. All broilers were equally arranged in 5 cages, each with an automatic water dispenser and trough. All broilers had free access to feed and water. The temperature of the animal room was gradually reduced from 34 to 24°C (d 1−21) and was constant at 21°C from 22 d of age. The humidity was maintained at 60% ± 5%, and illumination was maintained for 14 h every day. Natural ventilation was maintained. All procedures used in this study were approved by the Institutional Animal Care and Use Committee (**IACUC**) of Henan University of Science and Technology (approved # 20200904).

Experimental Design and Sample Collection

All broilers were subjected to an acclimatization period of 3 wk and were cultured under the experimental conditions. On the 22nd day of age, 20 broilers with a mean BW of 1.11 ± 0.12 kg were randomly selected and equally divided into 2 groups. At 23, 25, 27, and 29 d of age, the LPS group was injected intraperitoneally with LPS of *E. coli* at the dose of 0.5 mg/kg. And the activity dose was 2.5×10^5 EU/kg. While in the control group, chickens were intraperitoneally injected with the same volume (1 mL) of sterile saline at the same time points. The raw material of danofloxacin mesylate was dissolved in sterile water to get a final concentration of 5 mg/mL (calculated as danofloxacin base). Broilers in both groups were given danofloxacin by gavage at a dose of 5 mg/kg BW (calculated as danofloxacin base) immediately after the last injection. Then approximately 1 mL of blood was collected from the wing vein of each bird at 0 (before drug administration), 10, 30 min, 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 h into heparinized tubes. The plasma was obtained by centrifugation at 3,000 × *g* for 10 min at 4°C. At 2, 8, and 24 h after drug administration, another 1 mL blood sample was collected into a procoagulant tube, and the serum was obtained by centrifugation at 4,000 × *g* for 15 min at 4°C. At the end of the blood sample collection, 7 chickens (31 d of age) in each group were euthanized by CO_2 asphyxiation. The samples of the heart, liver, spleen, thymus, glandular stomach, muscular stomach, and bursa were obtained by dissection. The weight of each organ was immediately measured, and the organ indices were calculated using the following formula: organ indices = organ weight (g)/body weight (kg).

Determination of Cytokine Levels

The levels of CORT and IL-1β in chicken serum were detected strictly according to the instructions of the ELISA kit (Nanjing Jiancheng Institute of Bioengineering). An automated microtiter plate reader measured absorbances at 450 nm (Bio-Rad, Hercules, CA). The logistic curve (4-parameter) model in the ELISA Calc v 0.2 software was used to fit the standard curve, and then all concentration values were calculated using this standard curve. The detection limits were 2 to 600 ng/mL and 0.5 to 200 ng/L for CORT and IL-1β, respectively.

Determination of Danofloxacin Concentrations in Plasma

The detection method referred to Tian (Tian et al., 2019) with some adjustments. Waters 2695 high-performance liquid chromatography (**HPLC**) system (Waters) was used in this study, and the separation was accomplished on a reversed-phase chromatographic column (Hypersil BDS C18; 4.6 mm × 250 mm, 5 μm; Dalian Elite Analytical Instruments Co., Ltd.) at 30°C. The mobile phase was a mixed solution of phosphoric

acid buffer (0.05%; adjusting the pH to 2.8 with triethylamine) and acetonitrile (85:15, v/v), and the flow rate was configured to 1.0 mL/min. Waters 2475 fluorescence detector was performed at 280 nm (excitation) and 450 nm (emission) to detect danofloxacin. Control of the HPLC system, data acquisition, and peak integration were wholly performed by the EMPOWER software (Waters Corporation, Shanghai, China).

The extraction process was as follows: a total of 200 μL of plasma sample was accurately collected into a 1.5-mL Eppendorf tube and mixed with 400 μL of acetonitrile following vortexing for 30 s and centrifugation at 15,000 $\times$ g for 10 min at 4°C. The supernatant was moved into a 15-mL glass tube, and the sediment was re-extracted. All supernatants were collected and evaporated to dryness with a stream of nitrogen at 60°C. The residue was redissolved in 1 mL of the mobile phase. After vortexing for 1 min and centrifugation at 15,000 $\times$ g for 10 min at 4°C, the supernatant was filtered through a 0.22-μm filter into the autosampler glass vial. The supernatant (20 μL) was injected onto the C-18 column.

This method had good specificity. And no interference of endogenous compounds was observed at the retention time of danofloxacin in plasma samples. The analytical standard of danofloxacin mesylate was dissolved in an appropriate amount of pure water and then diluted with methanol to obtain a stock solution with a concentration of 1 mg/mL (calculated as danofloxacin base) and stored at −20°C until use. Calibration standards and quality control samples were prepared by diluting the stock solution with blank plasma samples. The concentration range of the calibration curve is between 0.005 and 2 μg/mL. The limits of detection (**LOD**) and quantitation (**LOQ**) for danofloxacin based on a signal-to-noise ratio >3 and >10 were 0.001 and 0.005 μg/mL, respectively. The recovery rate was 96.69 to 103.12%, and the coefficients of variation of intraday and interday precision were lower than 4.81%.

Pharmacokinetic Analysis

The pharmacokinetic parameters of danofloxacin in both groups were calculated using a noncompartmental method in the program of Phoenix WinNonlin (version 8.1.0; Pharsight Corporation, Mountain View, CA). The linear trapezoidal method was used to calculate the areas under the concentration vs. time curve from time 0 to infinity ($\mathbf{AUC_{0\text{-}\infty}}$) and area under the first moment from 0 to the infinity ($\mathbf{AUMC_{0\text{-}\infty}}$). And the mean residence time (**MRT**) was then obtained as the ratio of $AUMC_{0\text{-}\infty}$ to $AUC_{0\text{-}\infty}$. In addition to $AUC_{0\text{-}\infty}$, the areas under the concentration vs. time curve from time 0 to T_{max} ($AUC_{0\text{-}Tmax}$) and from time 0 to 24 h ($\mathbf{AUC_{0\text{-}24h}}$) were also calculated. The peak concentration ($\mathbf{C_{max}}$) and time to reach it ($\mathbf{T_{max}}$) were directly read from the individual concentration vs. time curve. The first-order rate constant associated with the terminal phase (λz) was derived from the slope of the apparent terminal phase. The terminal half-life ($t_{1/2\lambda z}$) was calculated as 0.693/λz.

Statistical Analysis

The Kolmogorov-Smirnov test in SPSS 20.0 software was used to determine the data normality of the organ indices, cytokine levels, and pharmacokinetic parameters. Except for $AUMC_{0\text{-}\infty}$, all other data were determined to be normally distributed. Then the $AUMC_{0\text{-}\infty}$ between the two groups was subjected to the Mann-Whitney U test, and the others were subjected to the independent sample t test in SPSS 20.0. All data were represented by the mean ± standard deviation (Mean ± SD). A P value lower than 0.05 indicated that the difference was significant.

RESULTS

Effects of Immune Stress Induced by E. coli LPS on the Organ Indices

The effects of immune stress induced by *E. coli* LPS on the organ indices are shown in Table 1. It can be seen that immune stress induced by LPS in broilers significantly increased the organ indices of liver ($P = 0.010$), spleen ($P = 0.009$), and glandular stomach ($P = 0.003$). However, there were no significant difference in the organ indices of heart ($P = 0.051$), thymus ($P = 0.593$), bursa ($P = 0.125$), and muscular stomach ($P = 0.289$).

Effects of Immune Stress Induced by E. coli LPS on the Serum Cytokine Levels in Broilers

The effects of immune stress induced by *E. coli* LPS on the serum levels of CORT and IL-1β are shown in Table 2. It was demonstrated that both cytokine levels in the LPS group at 2 h were significantly higher than those in the control group ($P < 0.05$). However, the concentrations of CORT and IL-1β at 8 and 24 h in the LPS group were higher than those in the control group without significant difference.

Table 1. Comparison of mean organ indices (g/kg of body weight) (±SD) of broilers in the LPS and control group.

Items	Control	LPS	*P*-value
Heart	5.85 ± 1.03	6.84 ± 0.63	0.051
Liver	29.21 ± 5.64	36.60 ± 2.93*	0.010
Spleen	1.85 ± 0.59	2.96 ± 0.72*	0.009
Thymus	2.90 ± 1.05	3.20 ± 1.01	0.593
Bursa	1.05 ± 0.21	1.30 ± 0.33	0.125
Muscular stomach	12.10 ± 2.18	13.31 ± 1.89	0.289
Glandular stomach	3.53 ± 0.60	4.54 ± 0.39*	0.003

Control: sterile saline control group; LPS: *E. coli* LPS challenge group.
Abbreviations: LPS, lipopolysaccharide; SD, standard deviation.
*There are significant differences between the two groups of parameters ($P < 0.05$).

Table 2. Comparison of mean serum cytokine levels (±SD) of broilers between the LPS and control groups.

Items	Control	LPS	*P*-value
2 h after the last injection of LPS			
IL-1β (ng·L^{-1})	160.85 ± 43.62	261.93 ± 66.79*	0.022
CORT (ng·mL^{-1})	70.16 ± 39.95	149.91 ± 28.32*	0.026
8 h after the last injection of LPS			
IL-1β (ng·L^{-1})	127.53 ± 53.48	163.83 ± 67.59	0.374
CORT (ng·mL^{-1})	95.33 ± 42.07	139.50 ± 54.33	0.298
24 h after the last injection of LPS			
IL-1β (ng·L^{-1})	66.65 ± 41.97	102.54 ± 44.26	0.225
CORT (ng·mL^{-1})	111.93 ± 55.11	132.59 ± 57.73	0.653

Control: sterile saline control group; LPS: *E. coli* LPS challenge group.
Abbreviations: CORT, corticosterone; IL-1β, interleukin-1β; LPS, lipopolysaccharide; SD, standard deviation.
*There are significant differences between the two groups of parameters ($P < 0.05$).

Effects of Immune Stress Induced by E. coli *LPS on the Pharmacokinetic Characteristics of Danofloxacin in Broilers*

Semi-logarithmic plots of the average concentration of danofloxacin in both groups after a single dose of danofloxacin (5 mg/kg BW) are presented in Figure 1. The comparison of the pharmacokinetic parameters between the two groups are shown in Table 3. It can be seen that immune stress induced by LPS in broilers significantly increased the parameters of T_{max} ($P = 0.006$), λz ($P = 0.001$); however, the parameters of $t_{1/2\lambda z}$, C_{max}, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}\infty}$, MRT of broilers in the LPS group were significantly lower than those in the control group ($P < 0.05$). No significant difference was observed for the parameter of $AUC_{0\text{-}Tmax}$ ($P = 0.594$).

DISCUSSION

All broilers developed typical clinical manifestations during the LPS challenge, including anorexia, susceptibility to startle, and diarrhea. However, all chickens survived. And after receiving each exposure to LPS, the clinical manifestations were similar.

Table 3. Comparison of mean pharmacokinetic parameters (±SD) between the LPS and control groups after a single gavage of danofloxacin (5 mg/kg BW) in broilers (n = 10) (based on the results of noncompartmental analysis).

Parameters	Unit	Control	LPS	*P*-value
λz	1/h	0.07 ± 0.02	0.12 ± 0.04*	0.001
$t_{1/2\lambda z}$	h	11.10 ± 3.12	6.21 ± 2.18*	0.001
T_{max}	h	2.92 ± 1.54	4.99 ± 1.42*	0.006
C_{max}	μg/mL	0.53 ± 0.17	0.36 ± 0.09*	0.015
$AUC_{0\text{-}Tmax}$	h•μg/mL	0.92 ± 0.39	1.02 ± 0.45	0.597
$AUC_{0\text{-}24h}$	h•μg/mL	5.36 ± 1.50	4.19 ± 1.05	0.058
$AUC_{0\text{-}\infty}$	h•μg/mL	6.94 ± 2.43	4.71 ± 1.57*	0.028
Vz/F	mL/kg	12.31 ± 4.20	9.82 ± 3.26	0.157
Cl/F	mL/h/kg	0.82 ± 0.31	1.13 ± 0.25*	0.023
$AUMC_{0\text{-}\infty}$	h^2•μg/mL	109.11 ± 58.86	54.26 ± 34.34*	0.002
MRT	h	14.84 ± 3.45	11.01 ± 2.56*	0.011

Control: sterile saline control group; LPS: *E. coli* LPS challenge group.
Abbreviations: $AUMC_{0\text{-}\infty}$: area under the first moment curve from the time of dosing to infinity; $AUC_{0\text{-}Tmax}$, area under the concentration versus time curve from 0 to T_{max}; $AUC_{0\text{-}24h}$, area under the concentration versus time curve from 0 to 24 h; $AUC_{0\text{-}\infty}$, area under the concentration versus time curve from the time of dosing to infinity; BW, body weight; λz, first-order rate constant associated with the terminal phase; C_{max}, peak concentration; Cl/F, ratio of body clearance rate and bioavailability; MRT, mean residence time extrapolated to infinity; LPS, lipopolysaccharide; $t_{1/2\lambda z}$, terminal half-life; T_{max}, time to reach peak concentration; SD, standard deviation; Vz/F, ratio of apparent distribution volume and bioavailability based on terminal phase.
*There are significant differences between the two groups of parameters ($P < 0.05$).

In this experiment, multiple intraperitoneal injections of LPS significantly increased the indices in the spleen, liver, and glandular stomach (Table 1). These increases might be because that LPS, as a nonspecific immunogen, could induce immune stress in animals and damage immune organs. The current results in the spleen indices were consistent with the previous ones (Li et al., 2015; Liu et al., 2015; Zhang et al., 2020). As an essential lymph organ, the spleen can activate lymphocytes after LPS injections, further increasing the number of lymph nodes in the white pulp, the inflammatory cytokines, and the activity of the organ. The liver indices in the LPS group was also increased significantly, which might be due to the inflammatory response induced by the LPS. After injection, LPS would activate the nuclear

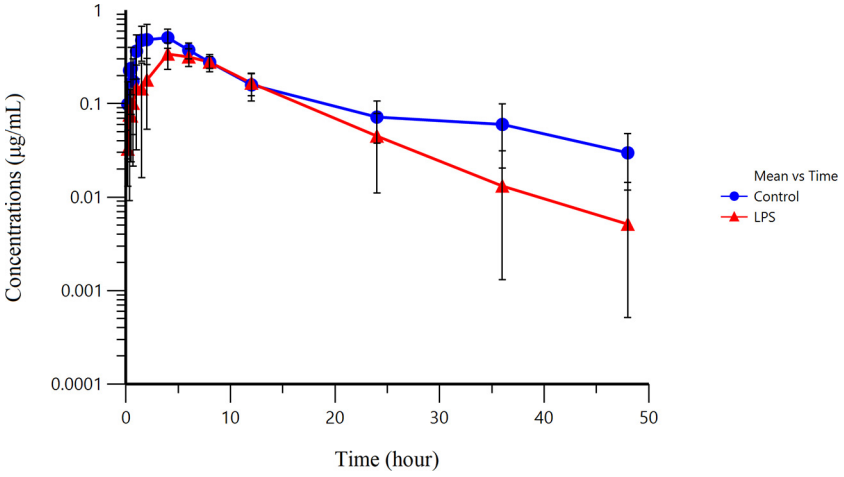

Figure 1. Mean ± SD plasma concentrations (μg/mL) of danofloxacin in the LPS and control groups after a single gavage of danofloxacin (5 mg/kg BW) in broilers. Control: sterile saline control group; LPS: *E. coli* LPS challenge group. Abbreviations: BW, body weight; LPS, lipopolysaccharide; SD, standard deviation.

factor kappa-B (**NF-κB**) signaling pathway, produce downstream proinflammatory cytokines and chemokines, and enhance liver tissue inflammation and significant expansion of hepatic sinusoids (Chen et al., 2021; Xu et al., 2021). There were no reports similar to the substantial increase in the organ indices of the glandular stomach caused by multiple injections of LPS. Therefore, further research is still needed to explore the mechanism.

It was shown that at 2 h after the last injection of LPS, the serum CORT and IL-1β levels in the LPS group were significantly higher than those in the control group (Table 2). The LPS challenge induced an inflammatory response in broilers. The current results were consistent with the previous ones (Baert et al., 2005; Zheng et al., 2020). LPS could be recognized by the toll-like receptor 4 (**TLR4**), which would result in the activation of myeloid differentiation factor 88 (**MyD88**) (Triantafilou and Triantafilou, 2002; Jerala, 2007). The triggering of the MyD88 pathway ultimately would lead to the activation of the transcription factor NF-κB, and then produce a large number of proinflammatory cytokines, such as tumor necrosis factor-alpha (**TNF-α**), interferon-γ (**IFN-γ**), interleukin-6 (**IL-6**), and interleukin-1β (**IL-1β**) (Verstrepen et al., 2008). The increased secretion of IL-1β and other inflammatory cytokines will lead to a corresponding increase in corticosterone levels. As the essential adrenocortical hormone in chickens, the CORT has been widely used as a critical indicator to measure the degree of stress in broilers (Curtis et al., 1980). However, in the present study, its increase at 8 and 24 h was not statistically significant, which was inconsistent with some previous results (Han et al., 2020; Zheng et al., 2020). In those studies (Han et al., 2020; Zheng et al., 2020), taurine and pyrroloquinoline quinone disodium were supplemented to broiler feed to reduce the inflammatory responses caused by LPS. Compared with taurine and pyrroloquinoline quinone disodium, danofloxacin might have a stronger ability to alleviate the secretion of these proinflammatory cytokines in chickens. In chicks, enrofloxacin treatment might change the proportions of lymphocyte subsets in lymphoid organs, thereby affecting the immune response of chicks to bacterial endotoxins (Klaudia and Alina, 2015). In addition, fluoroquinolones could reduce serum IL-6 and TNF-α levels in LPS-treated mice (Khan et al., 2000). Danofloxacin has also been reported to significantly inhibit the increase of IL-1β, IL-6, and other proinflammatory cytokines in LPS-induced piglets (Yao et al., 2017). We speculate that danofloxacin could alleviate proinflammatory cytokine secretion in LPS challenged broilers by regulating the immune response. However, further experiments are still needed to explore the mechanism by which this occurs.

The LPS challenge significantly reduced the peak concentration of danofloxacin, and the absorption time was also significantly prolonged (Table 3). However, the $AUC_{0\text{-}Tmax}$ changes in the LPS group were not significantly different. Thus, although the LPS challenge leads to immune organ damage and inflammatory responses in broilers, it may not affect the absorption extent of danofloxacin. Other researchers also reported similar results. In turkeys, the LPS challenge also had no effects on the pharmacokinetic properties of amoxicillin (Poźniak et al., 2017).

Interestingly, the parameter values of $t_{1/2\lambda z}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-}\infty}$, and MRT in the LPS group were significantly lower than those in the control group, and λz was significantly increased. These results indicated that the LPS challenge would substantially accelerate its elimination and shorten the average residence time, ultimately decreasing internal exposure. Similar results were observed for enrofloxacin in broilers (Bughio et al., 2017), and LPS decreased the $AUC_{0\text{-}\infty}$ after oral dosing. It was proved that LPS could upregulate the expression of P-glycoprotein (**P-gp**) in the small intestine of broilers, thereby affecting the absorption of enrofloxacin (Bughio et al., 2017). However, a slower elimination was observed for enrofloxacin (Bughio et al., 2017), which was inconsistent with the present result. Another study reported that intraperitoneal administration of *E. coli* lipopolysaccharide rapidly increased expression of P-gp in the liver, spleen, and kidney of broilers, but not in the duodenum (Barnes, 2001). Danofloxacin has been reported to be the substrate of the efflux transporter P-gp and MRP2 (Schrickx and Fink-Gremmels, 2007). Therefore, the LPS challenge in the present study would increase the expression of P-gp in the liver and kidney, which further accelerated the elimination of danofloxacin. On the other hand, the changes in the pharmacokinetics also might be caused by the hemodynamic changes and diarrhea caused by LPS because both of them could lead to the alert of distribution volume.

In the present study, the LPS challenge decreased C_{max} and $AUC_{0\text{-}24h}$ values of danofloxacin in broiler plasma. As a concentration-dependent drug, the AUC_{24h}: MIC ratio was the PK/PD parameter associated with the clinical efficacy of danofloxacin (Craig, 1998). A previous study has proved that an AUC_{24h}/MIC ratio above 6.73 h is required for the complete killing of *E. coli* in turkeys (Haritova et al., 2006). Another study reported that the MIC_{50} of danofloxacin to poultry *E. coli* was 0.25 μg/mL (Ozawa et al., 2010). In the present study, the $AUC_{0\text{-}24h}$: MIC ratio in the LPS group (16.76 h) was lower than that in the control group (21.44 h); however, both were higher than 6.73 h. Therefore, the challenge from LPS might have no effects on the clinical efficacy of danofloxacin in broilers; however, further studies are needed to verify this.

In conclusion, the challenge from LPS would prolong the absorption time of danofloxacin and accelerate the elimination, which could further result in a reduced internal exposure. However, the challenge from LPS might have no effects on the clinical efficacy of danofloxacin in broilers to treat *E. coli* infection.

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DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in the manuscript entitled "The influence of immune stress induced by *Escherichia coli* lipopolysaccharide on the pharmacokinetics of danofloxacin in broilers".

REFERENCES

Baert, K., L. Duchateau, S. De Boever, M. Cherlet, and P. De Backer. 2005. Antipyretic effect of oral sodium salicylate after an intravenous *E. coli* LPS injection in broiler chickens. Br. Poult. Sci. 46:137–143.

Barnes, D. M. 2001. Expression of P-glycoprotein in the chicken. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 130:301–310.

Bughio, S., T. Guo, F. He, Y. Liu, Y. Song, and L. Wang. 2017. Lipopolysaccharide upregulating P-glycoprotein in small intestine alters pharmacokinetics of orally administered enrofloxacin in broilers. Pak. Vet. J. 37:399–404.

Chanie, M., T. Negash, and S. B. Tilahun. 2009. Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in central Ethiopia. Trop. Anim. Health. Prod. 41:1309–1317.

Chen, S., Y. Lin, L. Miao, W. Pan, W. Jiang, L. Qian, J. Hao, B. Xi, B. Liu, and X. Ge. 2021. Ferulic acid alleviates lipopolysaccharide-induced acute liver injury in *Megalobrama amblycephala*. Aquaculture 532:735972.

Craig, W. A. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. 26:1–12.

Curtis, M. J., I. H. Flack, and S. Harvey. 1980. The effect of *Escherichia coli* endotoxins on the concentrations of corticosterone and growth hormone in the plasma of the domestic fowl. Res. Vet. Sci. 28:123–127.

De Boever, S., E. A. Neirinckx, E. Meyer, S. de Baere, R. Beyaert, P. de Backer, and S. Croubels. 2010. Pharmacodynamics of tepoxalin, sodium-salicylate and ketoprofen in an intravenous lipopolysaccharide inflammation model in broiler chickens. J. Vet. Pharmacol. Ther. 33:564–572.

EMA. 2002. Danofloxacin (extension to all food producing species) summary report (6). Committee for Veterinary Medical Products. EMA/MRL/818/02-FINAL. European Medicines Agency: London, UK.

Fan, Y. C., S. Y. Sheu, H. T. Lai, M. H. Chang, P. H. Chen, Y. C. Lei, T. F. Kuo, and C. Y. Wang. 2015. Residue depletion study of danofloxacin in cultured tilapia (*Oreochromis mossambicus*). J. AOAC. Int. 98:575–579.

Han, H., J. Zhang, Y. Chen, M. Shen, E. Yan, C. Wei, C. Yu, L. Zhang, and T. Wang. 2020. Dietary taurine supplementation attenuates lipopolysaccharide-induced inflammatory responses and oxidative stress of broiler chickens at an early age. J. Anim. Sci. 98:skaa311.

Haritova, A., D. Dimitrova, T. Dinev, R. Moutafchieva, and L. Lashev. 2013. Comparative pharmacokinetics of enrofloxacin, danofloxacin, and marbofloxacin after intravenous and oral administration in Japanese quail (*Coturnix coturnix japonica*). J. Avian Med. Surg. 27:23–31.

Haritova, A. M., N. V. Rusenova, P. R. Parvanov, L. D. Lashev, and J. Fink-Gremmels. 2006. Pharmacokinetic-pharmacodynamic modelling of danofloxacin in turkeys. Vet. Res. Commun. 30:775–789.

Ismail, M. 2006. A pharmacokinetic study of danofloxacin in febrile goats following repeated administration of endotoxin. J. Vet. Pharmacol. Ther. 29:313–316.

Jerala, R. 2007. Structural biology of the LPS recognition. Int. J. Med. Microbiol. 297:353–363.

Jiang, J., L. Qi, Z. Lv, S. Jin, X. Wei, and F. Shi. 2019. Dietary stevioside supplementation alleviates lipopolysaccharide-induced intestinal mucosal damage through anti-inflammatory and antioxidant effects in broiler chickens. Antioxidants 8:575.

Khan, A. A., T. R. Slifer, F. G. Araujo, Y. Suzuki, and J. S. Remington. 2000. Protection against lipopolysaccharide-induced death by fluoroquinolones. Antimicrob. Agents. Chemother. 44:3169–3173.

Klaudia, C., and W. Alina. 2015. The influence of enrofloxacin, florfenicol, ceftiofur and *E. coli* LPS interaction on T and B cells subset in chicks. Vet. Res. Commun. 39:53–60.

Li, Y., H. Zhang, Y. P. Chen, M. X. Yang, L. L. Zhang, Z. X. Lu, Y. M. Zhou, and T. Wang. 2015. *Bacillus amyloliquefaciens* supplementation alleviates immunological stress in lipopolysaccharide-challenged broilers at early age. Poult. Sci. 94:1504–1511.

Li, K., P. Zhang, B. Shi, J. Su, Y. Yue, M. Tong, and S. Yan. 2017. Dietary *Artemisia ordosica* extract alleviating immune stress in broilers exposed to lipopolysaccharide. Ital. J. Anim. Sci. 16:301–307.

Liu, L., J. Shen, C. Zhao, X. Wang, J. Yao, Y. Gong, and X. Yang. 2015. Dietary *Astragalus* polysaccharide alleviated immunological stress in broilers exposed to lipopolysaccharide. Int. J. Biol. Macromol. 72:624–632.

Ozawa, M., K. Baba, Y. Shimizu, and T. Asai. 2010. Comparison of in vitro activities and pharmacokinetics/pharmacodynamics estimations of veterinary fluoroquinolones against avian pathogenic *Escherichia coli* isolates. Microb. Drug. Resist. 16:327–332.

Perez, M., J. A. Otero, B. Barrera, J. G. Prieto, G. Merino, and A. I. Alvarez. 2013. Inhibition of ABCG2/BCRP transporter by soy isoflavones genistein and daidzein: effect on plasma and milk levels of danofloxacin in sheep. Vet. J. 196:203–208.

Poźniak, B., U. Pasławska, K. Motykiewicz-Pers, and M. Świtała. 2017. The influence of growth and *E. coli* endotoxaemia on amoxicillin pharmacokinetics in turkeys. Br. Poult. Sci. 58:462–468.

Real, R., E. Egido, M. Pérez, L. González-Lobato, B. Barrera, J. G. Prieto, A. I. Alvarez, and G. Merino. 2011. Involvement of breast cancer resistance protein (BCRP/ABCG2) in the secretion of danofloxacin into milk: interaction with ivermectin. J. Vet. Pharmacol. Ther. 34:313–321.

Schrickx, J. A., and J. Fink-Gremmels. 2007. Danofloxacin-mesylate is a substrate for ATP-dependent efflux transporters. Br. J. Pharmacol. 150:463–469.

Tian, E., C. Chen, W. Hu, Y. Miao, I. Muhammad, Q. Zhang, Y. Liu, L. Xu, J. Bao, L. Ding, and J. Li. 2019. Population pharmacokinetics for danofloxacin in the intestinal contents of healthy and infected chickens. J. Vet. Pharmacol. Ther. 42:556–563.

Triantafilou, M., and K. Triantafilou. 2002. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends. Immunol. 23:301–304.

Vardali, S. C., Y. P. Kotzamanis, A. E. Tyrpenou, and V. F. Samanidou. 2017. Danofloxacin depletion from muscle plus skin tissue of European sea bass (*Dicentrarchus labrax*) fed danofloxacin mesylate medicated feed in seawater at 16 degrees C and 27 degrees C. Aquaculture 479:538–543.

Verstrepen, L., T. Bekaert, T. L. Chau, J. Tavernier, A. Chariot, and R. Beyaert. 2008. TLR-4, IL-1R and TNF-R signaling to NF- kappaB: variations on a common theme. Cell. Mol. Life. Sci. 65:2964–2978.

Webel, D. M., R. W. Johnson, and D. H. Baker. 1998. Lipopolysaccharide-induced reductions in food intake do not decrease the efficiency of lysine and threonine utilization for protein accretion in chickens. J. Nutr. 128:1760–1766.

Wu, Q. J., Y. Q. Wang, and Y. X. Qi. 2017. Influence of procyanidin supplementation on the immune responses of broilers challenged with lipopolysaccharide. Anim. Sci. J. 88:983–990.

Xu, Q., J. Xu, K. Zhang, M. Zhong, H. Cao, R. Wei, L. Jin, and Y. Gao. 2021. Study on the protective effect and mechanism of *Dicliptera chinensis* (L.) Juss (Acanthaceae) polysaccharide on immune liver injury induced by LPS. Biomed. Pharmacother. 134:111159.

Yao, M., W. Gao, H. Tao, J. Yang, and T. Huang. 2017. The regulation effects of danofloxacin on pig immune stress induced by LPS. Res. Vet. Sci. 110:65–71.

Yousefi, M., H. Jonaidi, and B. Sadeghi. 2021. Influence of peripheral lipopolysaccharide (LPS) on feed intake, body temperature and hypothalamic expression of neuropeptides involved in appetite regulation in broilers and layer chicks. Br. Poult. Sci. 62:110–117.

Yu, C. H., Z. Y. Liu, L. S. Sun, Y. J. Li, D. S. Zhang, R. T. Pan, and Z. L. Sun. 2013. Effect of danofloxacin on reactive oxygen species production, lipid peroxidation and antioxidant enzyme activities in kidney tubular epithelial cell line, LLC-PK1. Basic. Clin. Pharmacol. Toxicol. 113:377–384.

Zhang, P., D. Sun, B. Shi, L. Faucitano, X. Guo, T. Li, Y. Xu, and S. Yan. 2020. Dietary supplementation with *Artemisia argyi* extract on inflammatory mediators and antioxidant capacity in broilers challenged with lipopolysaccharide. Ital. J. Anim. Sci. 19:1091–1098.

Zheng, Y. W., J. Y. Zhang, H. B. Zhou, Y. P. Guo, Q. G. Ma, C. Ji, and L. H. Zhao. 2020. Effects of dietary pyrroloquinoline quinone disodium supplementation on inflammatory responses, oxidative stress, and intestinal morphology in broiler chickens challenged with lipopolysaccharide. Poult. Sci. 99:5389–5398.

Zhuang, Q. Y., S. C. Wang, J. P. Li, D. Liu, S. Liu, W. M. Jiang, and J. M. Chen. 2014. A clinical survey of common avian infectious diseases in China. Avian. Dis. 58:297–302.