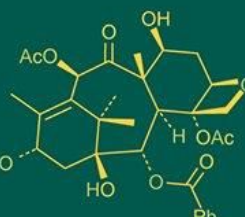
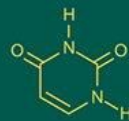
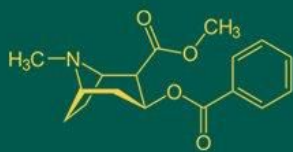


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(6): 498-501
www.biochemjournal.com
Received: 13-03-2025
Accepted: 16-04-2025

Darshana K Asari
Department of Veterinary
Pharmacology & Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

RD Singh
Assistant Professor,
Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

HB Patel
Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

CM Modi
Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

HA Patel
Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

Corresponding Author:
RD Singh
Assistant Professor,
Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Science &
Animals Husbandry,
Kamdhenu University,
Sardarkrushinagar, Gujarat,
India

In vitro binding dynamics of Danofloxacin in broiler chicken plasma

Darshana K Asari, RD Singh, HB Patel, CM Modi and HA Patel

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i6Sg.4666>

Abstract

Danofloxacin is a fluoroquinolone antimicrobial agent used exclusively in veterinary medicine. It exhibits broad-spectrum activity against several Gram-negative bacterial pathogens such as *Escherichia coli*, *Klebsiella*, *Salmonella*, and some Gram-positive organisms like *Staphylococcus* species, and thus has potential therapeutic applications for treating bacterial infections in poultry. Plasma protein binding significantly influences the pharmacokinetics and pharmacodynamics of antimicrobial drugs. Despite its clinical relevance, data on the plasma protein binding of danofloxacin in broiler chickens remain scarce. This study aimed to determine the extent of danofloxacin binding to plasma proteins in healthy broilers using *in vitro* equilibrium dialysis, with subsequent quantification by Ultra High-Performance Liquid Chromatography. Results showed that danofloxacin's plasma protein binding ranged from $24.99 \pm 3.38\%$ to $33.81 \pm 4.68\%$ across different concentrations, with an average binding of $28.84 \pm 1.27\%$. These findings suggest that danofloxacin exhibits low to moderate plasma protein binding in broiler chickens and offer important baseline data to better understand the drug's kinetic behaviour in this species.

Keywords: Broilers chickens, danofloxacin, equilibrium dialysis method, plasma protein binding, UHPLC

Introduction

The intensification of poultry production systems has led to increased incidences of bacterial infections, thereby necessitating the use of effective antimicrobial agents. Fluoroquinolones have emerged as an important class of broad-spectrum synthetic antimicrobials in veterinary medicine due to their excellent pharmacokinetic properties, deep tissue penetration, and strong bactericidal effects. Among them, danofloxacin is a second-generation fluoroquinolone developed specifically for animal use by Pfizer Inc. and has proven particularly effective in poultry. Its concentration-dependent killing mechanism and broad activity against Gram-negative bacteria and some Gram-positive cocci make it a valuable therapeutic option for the control of bacterial infections in avian species (Brown, 1996; Brown *et al.*, 1999; Goudah and Hasabelnaby, 2010a) [6, 7, 10]. Danofloxacin acts by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes critical for DNA replication and transcription, thus effectively impairing bacterial proliferation (Drlica and Zhao, 1997) [8]. The drug's favorable attributes, including improved cellular permeability, higher systemic and tissue concentrations, and superior antibacterial potency, further enhance its clinical utility.

A key determinant of an antimicrobial's pharmacological performance is its plasma protein binding (PPB) capacity. In veterinary pharmacology, PPB plays a crucial role in influencing both pharmacokinetic (PK) and pharmacodynamic (PD) behaviors. It regulates the balance between the protein-bound and free (unbound) fractions of the drug in circulation. Only the free (unbound) fraction of a drug is pharmacologically active, as it can diffuse into tissues and cause therapeutic or adverse effects. High plasma protein binding (PPB) can reduce the free drug concentration, potentially lowering antimicrobial activity, especially for drugs with strong PPB. However, PPB can also enhance solubility and prolong drug action by serving as a reservoir. Excessive or irreversible binding may prevent therapeutic levels of the drug from being reached. Therefore, understanding PPB is essential during drug development, as it can influence pharmacokinetics, drug interactions, and efficacy (Ahmed *et al.*, 2022) [1]. According to pharmacokinetic principles, drugs that bind strongly to proteins tend to stay in

the bloodstream longer and take more time to be eliminated, which can lengthen their therapeutic effect. Moreover, since renal and hepatic clearance predominantly process the free or unbound drug, any alteration in binding affinity can substantially influence drug disposition and dosing considerations.

Despite danofloxacin's widespread veterinary use, data on its plasma protein binding in poultry, particularly broiler chickens, is scarce. Given the pivotal role of PPB in therapeutic efficacy and dosing optimization, it is essential to characterize these properties in target species. The present study addresses this gap by evaluating the *in vitro* plasma protein binding of danofloxacin in healthy broiler chickens using equilibrium dialysis and UHPLC-based quantification. The findings aim to provide foundational data to guide rational and effective antimicrobial therapy in poultry medicine.

Materials and Methods

Protein binding of danofloxacin was determined by equilibrium dialysis technique (Sappal *et al.*, 2009) [17]. Danofloxacin solutions of varying concentrations 20, 10, 5, 2.5, and 1.25 µg/ml were prepared in 3 ml of plain broiler plasma, with each concentration prepared in triplicate.

Plasma harvesting

Approximately 60 ml of blood was randomly collected from broiler chickens into centrifuge tubes. Heparin was added as an anticoagulant at a rate of 10 IU per ml of blood. Plasma was separated by centrifuging the samples at 3500 rpm for 10 minutes. The pooled plasma was stored at -20°C until used for protein binding study.

Preparation of buffer

To prepare a phosphate buffer of a specified normality and pH (0.2 M and pH 7.4), 11.3 g of disodium hydrogen phosphate (Na₂HPO₄) and 2.7 g of potassium dihydrogen phosphate (KH₂PO₄) were dissolved in 1000 ml of distilled water. The solution was filtered through filter paper with a pore size of 0.47 µm using a vacuum pump. The pH was then adjusted to 7.4 with sodium hydroxide pellets.

Preparation for dialysis bag

A dialysis membrane (Himedia®) with an average flat width of 24.26 mm and an average diameter of 14.3 mm, and a typical molecular weight cut-off of 14,000 Da, was used. It had a capacity of 1.61 ml/cm when full. The membrane was cut into 4 cm pieces, with one end sealed prior to filling with 3 ml of plasma containing a known amount of the drug. The other end was then securely tied with thread. Each dialysis bag was thoroughly rinsed with distilled water and soaked overnight in phosphate buffer before use.

Preparation of different concentration of drug

Total five concentrations were used *viz.* 20, 10, 5, 2.5 and 1.25 µg/ml. Each bag was filled with 3 ml of plasma with known drug concentration then immersed in separate tubes containing equal amount of phosphate buffer (3 ml). The tubes were kept for incubation and equilibration at 37 °C for 12 h with occasional shaking. At the end of incubation period, buffers as well as contents of dialysis bag were separately analyzed for the concentration of drug. The volume of dialysis bag and buffer chamber were also

measured to rule out possibility of volume drift. The unbound (free) concentration of danofloxacin in buffers were estimated by UHPLC machine using the calibration curve generated for spiked phosphate buffer solution. The extent of *in-vitro* plasma protein binding of drug was determined using the following equation (Bidgood and Papich, 2005; Goudah and Hasabelnaby, 2010b) [5, 11]:

$$\text{Binding\%} = [(C_{Pe} - C_{Pb}) / C_{Pe}] \times 100$$

Where,

C_{Pe} = Concentration of drug in plasma at equilibrium i.e. after dialysis

C_{Pb} = Concentration of drug in buffer at equilibrium

UHPLC estimation of danofloxacin

In-house validated UHPLC method using UV detector was used to quantify the drug from plasma (Patel *et al.*, 2024) [16].

Statistical analysis

An analysis of variance (ANOVA) was conducted to determine whether there were statistically significant differences in danofloxacin concentrations across the five groups (1.25, 2.5, 5, 10, and 20 mcg/ml).

Results

Protein binding of a drug is an essential physiological and pharmacological parameter, as it helps predict both plasma concentrations and excretion behavior. In the current study, danofloxacin showed plasma protein binding levels ranging from 18.62% to 40.70%, with an overall binding level of 28.84 in broiler chickens (Table 1 and Figure 1).

The ANOVA test revealed an F-statistic of 0.874 and a p-value of 0.513, indicating no significant differences between the groups. Consequently, Tukey's Honest Significant Difference (HSD) post-hoc test was performed to confirm the absence of pairwise differences. The post-hoc analysis supported the ANOVA results, showing no statistically significant differences between any pair of groups. All statistical analyses were conducted using Python libraries, including SciPy and Statsmodels.

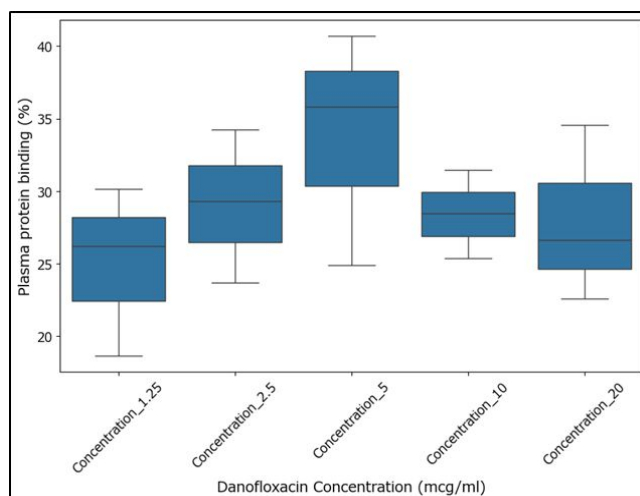


Fig 1: Graphical representation of *in vitro* plasma protein binding (PPB) of danofloxacin

Table 1: The values of *in vitro* plasma protein binding (PPB in%) of danofloxacin in broiler chickens at different concentrations

Experiment no.	Danofloxacin concentration (µg/ml)				
	1.25	2.50	5.00	10.00	20.00
1	26.20	29.28	35.84	31.44	22.56
2	30.16	34.24	40.70	25.37	34.54
3	18.62	23.68	24.89	28.45	26.64
Mean ± S. E.	24.99±3.38	29.06±3.05	33.81±4.68	28.42±1.75	27.91±3.52
Overall binding (%)	28.84±1.27				

Discussion

The protein binding of antimicrobial agents is a critical characteristic because it influences clinical pharmacokinetic parameters, such as clearance rate and volume of distribution, as well as pharmacodynamics (Banker and Clark, 2008; Beer *et al.*, 2009) [3, 4]. Plasma protein binding (PPB) significantly influences the pharmacological efficacy of fluoroquinolones, a prominent class of antimicrobial agents. In pharmacokinetic/ pharmacodynamic (PK/PD) modeling, indices such as the area under the concentration-time curve to minimum inhibitory concentration (AUC: MIC) and the peak plasma concentration to MIC ratio (C_{max} : MIC) are commonly employed to optimize dosage regimens. However, if these indices are calculated using total plasma drug concentrations without accounting for the unbound (active) fraction, the predictive accuracy of antimicrobial efficacy may be compromised. Therefore, PPB as a correcting factor is critical in developing appropriate dosing strategies, particularly in efforts to enhance therapeutic outcomes and limit the emergence of resistance. Plasma protein binding (PPB) significantly influences the pharmacodynamic behaviour of antimicrobial drugs by affecting their active, unbound concentrations. Evaluating this effect is crucial, as it can alter the observed efficacy of a drug against specific pathogens. Although the minimum inhibitory concentration (MIC) is commonly used to assess antimicrobial activity, it offers only a limited view and may not fully capture the impact of PPB. In contrast, time-kill curve analysis, which reflects both bacterial growth and killing over time, provides a more accurate and dynamic assessment of antimicrobial action. Therefore, time-kill studies are considered the most reliable method for evaluating how PPB influences the pharmacodynamics of antibiotics (Zeitlinger *et al.*, 2004) [19].

In the present study, danofloxacin exhibited plasma protein binding (PPB) values ranging from 18.62% to 40.70%, with a mean binding of 28.84%. These findings are consistent with previously reported data across various animal species, though some variability is evident due to differences in species, experimental conditions, and analytical methods. Sappal *et al.* (2009) [17] reported an *in vitro* PPB of 35.91±2.69% in buffalo plasma using the equilibrium dialysis method, which is comparable to the upper range of our findings. Likewise, Haritova *et al.* (2006) [14] documented a PPB of 27% in goats using ultrafiltration, closely aligning with our mean value. Among studies in calves, significant variability was observed. Friis (1993) [9] reported a relatively high *in vivo* PPB of 49±11%, while Greko *et al.* (2003) [13] found a much lower *in vitro* binding of 10% using ultrafiltration. Mzyk *et al.* (2018) [15] reported a broad range from 19.15% to 84.57% using microcentrifugation, suggesting substantial variation possibly related to methodological differences or concentration-dependent binding. In goats, Atef *et al.* (2001) [2] observed a lower mean binding of 13.55±1.78%

compared to Haritova *et al.* (2006) [14], again reflecting variability that may be influenced by individual animal factors or assay conditions. Ural and Uney (2021) [18] investigated PPB in lambs and reported values ranging from 21.6% to 32.8% across different treatment and disease states. Their findings underscore the influence of physiological status and co-administered drugs, such as meloxicam, on plasma protein binding behavior. In Muscovy ducks, Goudah and Mouneir (2009) [12] found a lower PPB of 17%, indicating the potential influence of species-specific plasma protein composition on drug binding. In avian species, plasma protein binding characteristics may vary considerably from those observed in mammals, primarily due to differences in the composition and concentration of plasma proteins, particularly albumin and globulins. Such variations can influence key pharmacokinetic parameters, including volume of distribution, drug elimination rates, and ultimately the pharmacodynamic response. Furthermore, physiological and pathological factors *viz.* age, health status, and concomitant drug administration, can modulate protein binding behavior, thereby affecting the clinical efficacy and safety of pharmacological treatments.

Conclusion

The present study demonstrates that danofloxacin exhibits moderate plasma protein binding (PPB), with values ranging from 18.62% to 40.70% and a mean of 28.84%. These findings agree with previously published data across multiple species, highlighting the influence of interspecies variability, experimental conditions, and analytical methodologies on PPB outcomes. Additionally, the considerable intracellular distribution characteristic of fluoroquinolones underscores the limitation of relying solely on plasma concentrations to predict pharmacodynamic efficacy. Therefore, integrating PPB and tissue distribution data is essential for accurate pharmacokinetic modeling and the formulation of rational, species-specific dosing regimens. Such an approach is crucial to improving therapeutic success and minimizing the risk of antimicrobial resistance in veterinary medicine.

References

1. Ahmed H, Bergmann F, Zeitlinger M. Protein binding in translational antimicrobial development—focus on interspecies differences. *Antibiotics* (Basel). 2022;11(7):923. doi:10.3390/antibiotics11070923
2. Atef M, El-Gendi AY, Aziza, Amer MM, Abd El-Aty AM. Some pharmacokinetic data for danofloxacin in healthy goats. *Vet Res Commun*. 2001; 25:367-377. doi:10.1023/A:1010642726054
3. Banker M, Clark T. Plasma/Serum Protein Binding Determinations. *Curr Drug Metab*. 2008;9(9):854-859. doi:10.2174/138920008786485065

4. Beer J, Wagner CC, Zeitlinger M. Protein binding of antimicrobials: methods for quantification and for investigation of its impact on bacterial killing. *AAPS J*. 2009;11(1):1-12. doi:10.1208/s12248-008-9072-1
5. Bidgood TL, Papich MG. Plasma and interstitial fluid pharmacokinetics of enrofloxacin, its metabolite ciprofloxacin, and marbofloxacin after oral administration and a constant rate intravenous infusion in dogs. *J Vet Pharmacol Ther*. 2005;28(4):329-341.
6. Brown SA, Roberts BJ, Boothe DM. Pharmacokinetics of danofloxacin in the chicken. *Avian Dis*. 1999;43(2):268-273. doi:10.2307/1592553
7. Brown SA. Fluoroquinolones in animal health. *J Vet Pharmacol Ther*. 1996;19(1):1-14. doi:10.1111/j.1365-2885.1996.tb00001.x
8. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol Mol Biol Rev*. 1997;61(3):377-392. doi:10.1128/mmbr.61.3.377-392.1997
9. Friis C. Penetration of danofloxacin into the respiratory tract tissues and secretions in calves. *Am J Vet Res*. 1993;54(7):1122-1127. doi:10.2460/ajvr.1993.54.07.1122
10. Goudah A, Hasabelnaby S. Pharmacokinetics and tissue residues of danofloxacin in broiler chickens. *Br Poult Sci*. 2010a;51(5):681-686. doi:10.1080/00071668.2010.520028
11. Goudah A, Hasabelnaby S. Pharmacokinetics, plasma protein binding and bioavailability of moxifloxacin in Muscovy ducks after different routes of administration. *Res Vet Sci*. 2010b;88(3):507-11. doi:10.1016/j.rvsc.2009.11.009
12. Goudah A, Mounair SM. Disposition kinetics and tissue residues of danofloxacin in Muscovy ducks. *Br Poult Sci*. 2009;50(5):613-619. doi:10.1080/00071660903147416
13. Greko C, Finn M, Franklin A, Bengtsson B. Pharmacokinetic/pharmacodynamic relationship of danofloxacin against *Mannheimia haemolytica* in a tissue-cage model in calves. *J Antimicrob Chemother*. 2003;52(2):253-257. doi:10.1093/jac/dkg339
14. Haritova AM, Rusenova NV, Parvanov PR, Lashev LD, Fink-Gremmels J. Pharmacokinetic-pharmacodynamic modelling of danofloxacin in turkeys. *Vet Res Commun*. 2006;30:775-789. doi:10.1007/s11259-006-3400-7
15. Mzyk DA, Bublitcz CM, Hobgood GD, *et al*. Effect of age on plasma protein binding of several veterinary drugs in dairy calves. *Res Vet Sci*. 2018;121:59-64. doi:10.1016/j.rvsc.2018.09.004
16. Patel P, Patel HB, Singh RD, Modi CM, Prajapati D, Asari D, *et al*. Disposition profile of danofloxacin in broiler chickens. *Int J Adv Biochem Res*. 2024;SP-8(4):274-8.
17. Sappal R, Chaudhary RK, Sandhu HS, Sidhu PK. Pharmacokinetics, urinary excretion and plasma protein binding of danofloxacin following intravenous administration in buffalo calves (*Bubalus bubalis*). *Vet Res Commun*. 2009;33:659-667. doi:10.1007/s11259-009-9215-6
18. Ural MN, Uney K. Pharmacokinetic Behavior and Pharmacokinetic/Pharmacodynamic Integration of Danofloxacin Following Single or Co-Administration with Meloxicam in Healthy Lambs and Lambs with Respiratory Infections. *Antibiotics*. 2021;10:1190. doi:10.3390/antibiotics10101190
19. Zeitlinger MA, Sauermann R, Traunmüller F, *et al*. Impact of plasma protein binding on antimicrobial activity using time-killing curves. *J Antimicrob Chemother*. 2004;54(5):876-880.