Received: 20 September 2023 | Revised: 3 November 2023 | Accepted: 10 November 2023

DOI: 10.1111/jvp.13419

JOURNAL OF Veterinary Pharmacology and Therapeutics WILEY

ORIGINAL ARTICLE

Pharmacokinetics of doxycycline hyclate in pigs with a new feed premix formulation

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Abstract

This study aimed to evaluate the administration of doxycycline hyclate in a long-acting pharmaceutical preparation in pigs when administered either ad libitum as a feed medication or an oral bolus dose. In all instances, the studied dose was 20 mg/kg b.w. A total of 48 healthy crossbred, castrated male pigs (Landrace-Yorkshire) weighing 23 ± 4.3 kg were included in this trial. They were randomly assigned to six groups as follows: two groups for the experimental prototype 1 of doxycycline hyclate administering it ad libitum (Fad-lib) or as forced bolus (Fbolus); two groups for the experimental prototype 2 of doxycycline hyclate as for the former groups (FCad-lib and FCbolus), and two control groups receiving the same dose of doxycycline hyclate, but of a commercial premix, also as previously explained (Cbolus and Cad-lib). Statistical analysis of the mean pharmacokinetic values was carried out with Kruskal–Wallis and Dunn's tests. The relative bioavailability (Fr) of the best prototype, when administered ad libitum (FCad-lib), was five times larger than the reference group (Cadlib). These results allow the proposal that the referred differences achieved in the presented prototypes can mark a notable clinical difference, particularly in pathogens with some resistance.

KEYWORDS
bioavailability, dosage form, doxycycline hyclate, pigs

1 | INTRODUCTION

Respiratory diseases in intensive pig farming are the leading cause that requires antimicrobial drugs and is a sanitary challenge in virtually all kinds of farms. In addition to the impacts on animal welfare and mortality, respiratory diseases negatively impact farm profitability due to the concomitant reduction in feed conversion, increased number of days to reach the desired market weight, the expense of medicines, and partial or total confiscation during carcass inspection (Borge et al., 2005; Lei et al., 2017; Yang et al., 2013).

In pigs, it is expected to medicate doxycycline hyclate in the feed for the treatment of diseases of the porcine respiratory disease complex (PRDC), among others (Bousquet, Pommier, et al., 1998; Bousquet, Nouws, et al., 1998; Sumano et al., 2015; Lekagul et al., 2019). Doxycycline hyclate is considered one of the broadest-spectrum antibiotics. It possesses better tissue penetration than oxytetracycline or chlortetracycline, particularly toward respiratory tissues. Doxycycline hyclate is a bacteriostatic, time-dependent antibacterial used in pigs. It is active against many Gram-positive and Gram-negative microorganisms, mycoplasmas, chlamydia, rickettsia, and some protozoa (Mascaretti, 2003). A 0.25 μg/mL breakpoint has been assigned to sensitive organisms: 0.5 μg/mL for moderately resistant ones and 2.0 μg/mL for resistant pathogens (Petrocchi-Rilo et al., 2020). At doses of 11.8–13.3 mg of doxycycline hyclate/kg/day, as calculated based on the weight: food intake ratio, and administered in one or two pulses, it achieved steady-state (ss) serum doxycycline hyclate

concentrations ranging from 0.7 to 1.0 μg/mL (Bousquet, Pommier, et al., 1998; Bousquet, Nouws, et al., 1998). No biotransformation of doxycycline hyclate has been found in pigs, and it is concluded that it has a shorter elimination half-life than that reported in other species, that is, 4 h after IV dose u of 10 mg/kg (Riond & Riviere, 1990).

The oral administration of doxycycline hyclate is done without a particular pharmaceutical design. However, it has been stated that appropriate formulations must be developed for each species, avoiding the generalized procedure of adding the powdered active principle to the feed or using a faulty-designed premix preparation (Riond & Riviere, 1990). Hence, it was established in this trial as a working hypothesis to design a pharmaceutical preparation of doxycycline hyclate in pellets to be mixed in pig feed that has greater bioavailability (F) and longer mean residence time (MRT). Also, a pharmacokinetic comparison is made with a commercial formulation widely used in the pig industry to treat respiratory infections. Both bolus and ad libitum dose of either preparation at 20 mg/kg body weight were studied.

2 | MATERIALS AND METHODS

2.1 | Approval and setting

The Internal Committee of Postgraduate Studies of the School of Veterinary Medicine of the Universidad Nacional Autonoma de Mexico approved the ethics procedures followed in this study (CICUAL). It was carried out at the experimental farm of the Universidad Nacional Autonoma de Mexico, located in Jilotepec, State of Mexico. The trial was conducted throughout the temperate months of September and October 2009, with a mean environmental temperature of 16 ± 8.8°C and a mean inside pen temperature of 19.5 ± 4.2°C.

2.2 | Animals

A total of 48 healthy crossbred, castrated male pigs (Landrace-Yorkshire) weighing 23 ± 4.3 kg were included in this trial. Negative tests for *A. pleuropneumoniae* were determined were ensured by slide agglutination against serotype 1 (Pleurotest, Ciprolab, Mexico City, Mexico) during a 2-week quarantine period. Pigs were fed a commercial feed free of drugs for ad libitum (La Hacienda SA de CV, Toluca, México), which had 16.0% CP, 3.0% fat, 6.5% crude fiber, 1.0% humidity, 7.0% ash, and 55.5% N-free elements.

2.3 | Experimental design

Pigs were allocated in groups of eight, housed in 4-m^2 pens, and were randomly assigned using a table of random numbers, to six groups as follows: two groups for the experimental prototype 1 of doxycycline hyclate administering it ad libitum (Fad-lib) or as forced bolus (Fbolus); two groups for the experimental prototype 2 of doxycycline hyclate as for the former groups (FCad-lib and FCbolus), and two control groups receiving the same dose of doxycycline hyclate, but of a commercial premix, also as previously explained (Cbolus and Cad-lib) (Premedox® 50% MAXX - Virbac México).

For the ad libitum phase, each preparation of doxycycline hyclate was mixed in their food in the necessary amount to achieve a dose of approximately 20 mg/kg/day; that is 400 ppm of doxycycline hyclate. Limited feed was first offered to ensure complete intake of their dose. The bolus phase was achieved by suspending the referred dose in a 1% suspension of gelatine in water at 25°C in a volume of 15–20 mL, and force-fed to each pig.

2.4 | Prototypes

Under good manufacturing practices, doxycycline hyclate in-feed prototype preparations were manufactured at a lab scale. Briefly, 1% carpool with butylhydroxytoluene as an antioxidant in a base of 1:1 part of wheat and corn flour were mixed (Fad-lib and Fbolus). In another group (FCad-lib and FCbolus), 1% capsicum oleoresin (VEPINSA SA de CV, Sinaloa, Mexico) was added. Also, a yellow-orange vegetable dye was added. Finally, doxycycline hyclate was incorporated at a rate of 10%. The components were mixed and then extruded at under 30°C, using ethyl alcohol and cotton-seed oil as lubricants.

2.5 | Blood sampling

Assisted by technicians, blood samples, no more than 3 mL per pig, were collected by a jugular puncture at defined times, that is, before treatment (basal zero-time sample) and at 0.5, 1, 2, 3, 4, 6, 8, 24, and 32 h. The serum was extracted by centrifugation (10,000 rpm in 10 min) immediately after blood sampling. A computerized curve-stripping program (PKAnalyst® for Windows; Micromath Scientific Software, SLM, USA) was used to fit and analyze each pig's concentration versus time profiles, and mean values were later derived. Best-fitting models were chosen after analysis with the residual sum of squares and the minimal Akaike's information criterion (Kletting & Glatting, 2009).

2.6 | PK analysis and modeling

The bolus and the ad-libitum dosing of doxycycline hyclate were best fitted, in the PKAnalyst program, in a first-order pharmacokinetic with a two-compartment model having both first-order input and output. The doxycycline plasma concentrations best fitted Model 11 (R^2 = .99, Coefficient of determination = .98, correlation = .99), with the following formula:

$$\text{Concentration (Time)} = \text{Ae}^{-\alpha * \text{Time}} + {}^{-\beta * \text{Time}} - \text{Ce}^{-\text{KAB} * \text{Time}}.$$

This model allows the calculation of the following parameters: T½ab, absorption half-life; T½β, elimination half-life; C_{MAX}, maximum plasma concentration achieved; T_{MAX}, time to reach C_{MAX}; AUC, area

under the plasma doxycycline concentration curve over time; AUMC, area under the serum doxycycline concentration curve overtime time; MRT, mean residence time; AUC_T, area under the curve of plasma doxycycline concentration over time by the trapezoidal method.

Plasma concentrations of doxycycline were plotted aided by OriginLab® (Northampton, MA). Relative bioavailability (Fr) was calculated using the formula = Cbolus/FCbolus × 100. Data are presented as mean ± 1SD of 8 observations for each parameter, and statistical comparisons of the mean pharmacokinetic values were carried out utilizing Kruskal–Wallis and Dunn's tests (2023 JMP Statistical Discovery).

2.7 | Determination of serum concentrations of doxycycline hyclate

The concentrations of doxycycline hyclate present in serum samples after extraction were analyzed using a high-performance liquid chromatography method (HPLC), as described by Axisa et al. (2000). A commercial cartridge, 0.45 μm/25 mm (Millipore, PTFE) was used to separate proteins, and retains polar and no polar compounds, which are eluted from the extraction cartridge with 1 mL of methanol–water (1:1) and three washes 1 mL with ethanol 5%. The doxycycline hyclate was eluted off the cartridge with 1 mL acetonitrile (50%) and water (1:1) and the samples were injected into the PHLC (JASCO brand, model XLC, with a fluorescence and diode array detector) and was monitored at λ = 350 nm. The column used was Nucleosil 100–5 C18, 5 mm, 150 mm, and the mobile phase water/acetonitrile 65:35 with a flow of 1 mL/min, and the injection volume was 20 μL. A standard curve for doxycycline hyclate was constructed using antibiotic-free pooled serum samples collected from nonmedicated pigs. The intra-assay coefficient of variance was <2.0, and the inter-assay error was <2.2. The analytical assay was linear over a range of concentrations from 0.1 to 50 μg/mL. The mean ± 1.0 SD recovery was 94 ± 1.8% ($r = .96$). The detection limit was 0.07 μg/mL, and the limit of quantification was 0.1 μg/mL. The accuracy of the method was assessed using relative error (RE %) values, calculated by interpolating the peak area of three replicates of doxycycline hyclate standard solutions of five different concentrations from the regression equations on three different days. The accuracy of the method was 94.8%.

2.8 | Determination of serum concentrations of doxycycline hyclate

The concentrations of doxycycline hyclate present in serum samples after extraction were analyzed using a high-performance liquid chromatography method (HPLC), as described by Axisa et al. (2000). A standard curve for doxycycline hyclate was constructed using antibiotic-free pooled serum samples collected from slaughtered, nonmedicated pigs. The intra-assay coefficient of variance was <2.0, and the inter-assay error was <2.2. The analytical assay was linear over a range of concentrations from 0.1 to 50 μg/mL. The mean ± 1 SD recovery was 94 ± 1.8% ($r = .96$). The detection limit was 0.07 μg/mL, and the limit of quantification was 0.1 μg/mL.

3 | RESULTS

3.1 | Pharmacokinetic analysis

Table 1 and Figure 1 summarize the data collected with the two prototype formulations of doxycycline hyclate, that is, Fad-lib and Fbolus, and FCad-lib and FCbolus. Also, values obtained for

TABLE 1 Oral pharmacokinetic parameters of doxycycline in pigs after dosing them with two prototypes of the antibiotic in modified release designs (Fad-lib; Fbolus; and FCad-lib and FCbolus) and that obtained with a commercial premix, used as a control or reference values (Cad -lib and Cbolus).

	Groups					
Parameter	**FCbolus**	**FCad-lib**	**Fbolus**	**Fad-lib**	**Cbolus**	**Cad-lib**
T½ab (h)	1.4 ± 0.1[a]	3.2 ± 0.2 [b]	1.4 ± 0.2 [a]	3.4 ± 0.2 [b]	0.27 ± 0.1[c]	0.31 ± 0.1[c]
T½β (h)	4.75 ± 0.3[a]	4.96 ± 0.3[a]	4.54 ± 0.2[a]	8.32 ± 0.3[b]	1.42 ± 0.1[c]	2.13 ± 0.3[d]
T_{MAX} (h)	6.85 ± 0.2[a,b]	7.15 ± 0.4[a]	6.55 ± 0.3[b]	12.01 ± 0.7[c]	2.05 ± 0.2[d]	3.07 ± 0.4[e]
C_{MAX} (μg/mL)	14.95 ± 2.2[a]	11.71 ± 3.3[b]	9.48 ± 1.4[c]	2.46 ± 1.5[d]	1.17 ± 1.1[e]	0.50 ± 0.3[f]
AUC (μg/mL*h)	278.38 ± 18.6[a]	227.50 ± 12.1[b]	168.91 ± 9.2[c]	80.16 ± 6.2[d]	6.56 ± 1.3[d]	4.15 ± 0.9[f]
AUMC (μg/mL*h^2)	3814.90 ± 121.4[a]	3253.16 ± 108.3[b]	2213.28 ± 95[c]	1925.27 ± 92[c]	26.91 ± 7.8[d]	25.49 ± 2.5[d]
MRT (h)	13.70 ± 0.6 [a,b]	14.30 ± 0.9 [a]	13.10 ± 0.7[b]	24.02 ± 0.9[c]	4.10 ± 0.2[d]	6.14 ± 0.4[e]
AUC_T (μg/mL*h)	270.98 ± 18.1[a]	225.85 ± 11.8[b]	174.34 ± 8.9[c]	70.41 ± 5.4[d]	8.6 ± 2.2[e]	6.31 ± 0.6[f]
Fr (%)	4238	5481	2561	1931	–	–

Abbreviations: AUC, area under the plasma doxycycline concentration curve over time; AUC_T, area under the curve of plasma doxycycline concentration over time by the trapezoidal method; AUMC, area under the serum doxycycline concentration curve overtime time; C_{MAX}, maximum plasma concentration achieved; Fr, relative bioavailability, that is, Fr, Cbolus/FCbolus × 100; MRT, Mean residence time; T½ab, absorption half-life; T½β, elimination half-life; T_{MAX}, time to reach C_{MAX}.

[a–c]A different letter in each row indicates that a value is statistically different from those observed in the other groups ($p < 0.05$).

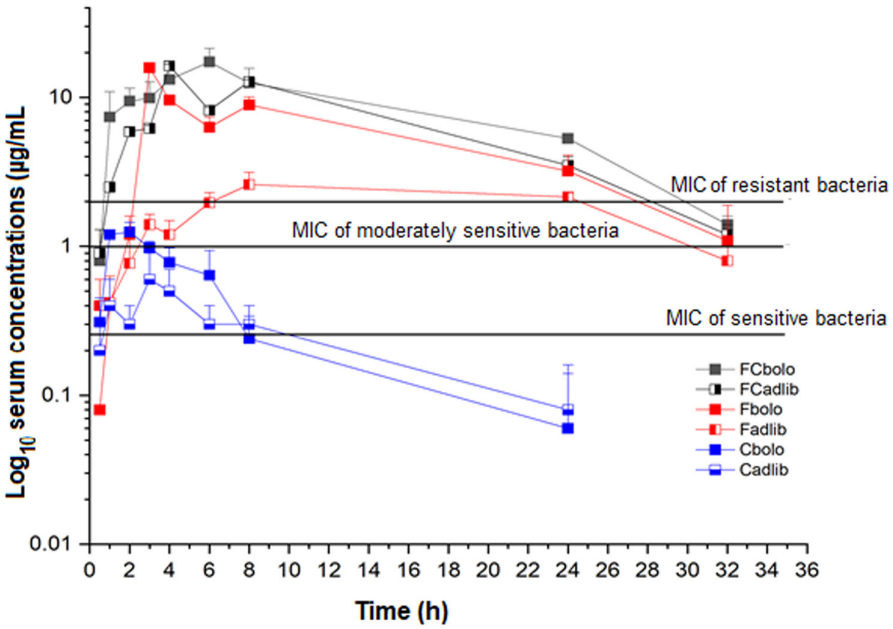

FIGURE 1 Semilogarithmic plot of mean + 1 SD of the serum concentrations of doxycycline in pigs after oral administration at a dose of 20 mg/kg with two prototypes of the antibiotic in modified release designs (Fad-lib; Fbolus; and FCad-lib and FCbolus) and that obtained with a commercial premix, used as a control or reference values (Cad-lib and Cbolus).

the control-reference groups are presented (Cbolus and Cad-lib). Statistically significant differences are highlighted in the table. Noticeably high Fr values are presented for FCad-lib and FCbolus ($p < .05$), as well as MRT values $p < .05$). After administering a commercial premix, the serum profiles of doxycycline hyclate served as a reference (Cad-lib and Cbolus). Minimal inhibitory concentration breakpoints are presented for *Pasteurella* spp. (Petrocchi-Rilo et al., 2020).

4 | DISCUSSION

The results of the present investigation indicate that achieving remarkably high concentrations of doxycycline hyclate in the pigs' serum using the pharmaceutical in-feed prototypes of the active principle presented, which were tailored for this particular species, is feasible. The results are notable given that the high serum concentrations achieved are even higher than the reported breakpoint for resistant *Pasteurella* spp. (2.0 µg/mL) (Petrocchi-Rilo et al., 2020) and for *Pasteurelleace* family, that is, proposed ≤4 µg/mL (Teale & Borriello, 2021). The new doxycycline breakpoint for all pig pathogens has not been published yet. However, for dogs and humans, the breaking point set by VetCAST (Committee for Veterinary Antimicrobial Susceptibility Testing) ranges from 0.5 to 2 µg/mL (Papich, 2014). The high serum concentrations are sustained for a long time, allowing the dosing interval to reach 24 h and, this way, comply with T > MIC 100% of the dosing interval. Thus, the observed profile of doxycycline hyclate can be achieved either after its pulse medication or when administered ad-libitum, as long as the doxycycline hyclate preparation is manufactured, as indicated in this study.

The dose chosen for this study was based on the way this antibiotic is commonly administered at the farm level and based on a study of pharmacokinetic-pharmacodynamic integration versus *Haemophilus parasuis* in which the recommended dose was also 20 mg/kg (Zhang et al., 2018). However, lower doses have been used, that is, 11 mg doxycycline hyclate/kg body weight per day, and have been presented as effective in controlling pneumonia due to *P. multocida* and *M. hyopneumoniae* in fattening pigs (Bousquet, Pommier, et al., 1998, Bousquet, Nouws, et al., 1998). Comparatively with this latter study, the achieved serum concentrations in this trial were much higher than those reported at steady state by Bousquet, Pommier, et al. (1998) and Bousquet, Nouws, et al. (1998). In their study, doxycycline hyclate was administered in medicated feed to healthy fattening pigs for various days every 12 h at an average dose ranging from 11.8 to 13.3 mg/kg/day. The steady-state plasma concentrations obtained ranged from 0.7 to 1 µg/mL with a mean elimination half-life of 5.9 ± 1.0 h. If the same dose was administered ad libitum, steady-state plasma concentrations increased from 0.9 to 1.5 µg/mL (Bousquet, Pommier, et al., 1998, Bousquet, Nouws, et al., 1998). Pharmacokinetic values of doxycycline-hyclate obtained in this study are even superior to the ones reported after the IM administration of the drug, that is, 4.31 ± 0.42 µg/mL and 57.10 ± 4.89 µg.h/mL for C_{MAX} and AUC, respectively, in healthy pigs, according to Zhang et al. (2018). Additionally, injury in the injection site is a very tangible danger for doxycycline. Under the conditions described in this study, peak concentrations with ad libitum or bolus dose of pelleted doxycycline hyclate dosed at 20 mg/kg were noticeably higher, that is, 11.71 and 14.95 µg/mL, respectively. Explaining the discrepancies in plasma concentrations is not viable as experimental conditions differ. However, the higher dose used in this trial and the pharmaceutical design utilized could offer a reasonable explanation. In this context, PK/PD integration modeling against *Haemophilus parasuis* would adjust better to the drug formulation presented here (Zhang et al., 2018). Also, considering the need for metaphylactic treatment with doxycycline hyclate in the feed, the preparation described here, and at the dose of 20 mg/kg/day, may offer clinical advantages over other premixes of this antibiotic. Nevertheless, this hypothesis must be challenged in clinical and pharmacokinetic trials to confirm or reject it, particularly given

the wide range of pig environmental and managerial practices, different breeds and sexes, and the abundance of antimicrobial resistance genes (Græsbøll et al., 2019). Furthermore, it has been stressed that it is imperative that treatment and prevention of respiratory diseases in post-weaning and fattening pigs be improved to achieve better clinical outcomes and to reduce the frequency of antimicrobial interventions in a given production cycle (Toya et al., 2021).

The elimination half-life found for both prototypes in this study was noticeably increased, particularly in the FCad-lib group. In contrast, the same parameter in the reference group was comparable to the reported values (1.8±0.4) (Bousquet, Nouws, et al., 1998). The MRT achieved with both prototypes, particularly with the FC groups (ad libitum and bolus), was significantly higher than that observed with a reference preparation, in which doxycycline hyclate was presumably added to a standard vehicle without a particular pharmaceutical design. The relative bioavailability (Fr) of the best prototype, when administered ad-libitum (FCad-lib), was 500 times larger than the reference group (Cadlib). These results allow the proposal that the referred differences achieved in the presented prototypes can mark a notable clinical difference, particularly in pathogens with some resistance.

Comparisons of bioavailability of doxycycline hyclate cannot be made with other studies as IV pharmacokinetics of the drug was not attempted in this trial. However, essential discrepancies are anticipated as the oral bioavailability of doxycycline has been calculated for pigs to range from 40% to 50% (Sanders et al., 1996; Beart et al., 2000) and up to 100% (Pijpers et al., 1991). It has been proposed that the oral bioavailability of doxycycline hyclate in animals is much lower than in humans (Anadón et al., 1994) and that the presence of food in the gastrointestinal system does not seem to affect bioavailability of doxycycline-hyclate (Riond & Riviere, 1988). However, the vehicles present and the way of preparing the pellets in the studied prototype, seem to do so. Doxycycline in pigs has been shown to exhibit high serum protein binding (>90%), a lack of biotransformation, and a short T½β after IV administration (Riond and Riviere, 1990). Nevertheless, these authors considered that doxycycline may be a valuable antimicrobial drug in swine practice, pending the development of appropriate formulations. In this respect, the prototype of doxycycline hyclate used in this trial is presented as a viable proposal that merits further analysis. Hence, it must be tested in different breeds (including minipigs) and sexes, and clinical and pharmacokinetic comparative studies would be necessary with other tetracyclines (Mileva & Milanova, 2022).

An in vitro pathogen susceptibility study compared oxytetracycline and doxycycline against strains of *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Mycoplasma hyopneumoniae isolated* from the respiratory tract of pigs in France and the United Kingdom. All strains were susceptible to doxycycline, while 15% of *P. multocida* strains and 22 percent of *A. pleuropneumoniae* strains were resistant to oxytetracycline. The MIC90 of doxycycline for *P. multocida* ranged from 1.0–2.0 μg/mL (Bousquet et al., 1997). These data agree with the studies of Pijpers et al. (1991), who, using increasing doses of doxycycline, chlortetracycline, and oxytetracycline, found that the PK/PD ratios of doxycycline in pigs were ideal against respiratory pathogens. Additionally, the free fraction of doxycycline in pig plasma, which was found to be greater than what could have been anticipated, that is, approximately 30%, also supports this view (Portugal et al., 2023).

This study was not designed to propose a mechanism by which higher serum doxycycline hyclate concentrations are achieved compared to the reference group. However, it can be speculated that the added gastro-retentive vehicle allows more prolonged contact between the active principle and the epithelium to improve its absorption (Akiyama et al., 1995; Miller et al., 2008), given the characteristic lipid solubility of doxycycline hyclate (Pérez-Trallero & Iglesias, 2003). These features may explain, in part, why it was detected that the T½ab values were shorter in the reference preparation than in the experimental ones and, of course, may have contributed to the noticeably greater C_{MAX} values obtained in the experimental formulations as compared to the reference one. However, it is also tempting to speculate that a reabsorption phenomenon may have been favored as doxycycline hyclate is preferentially excreted with intestinal secretions (Lemos, 2002), and the presence of the vehicles utilized may have modified this form of drug clearance, slowing down gastrointestinal transit time. Also, it is known that capsaicin is commonly added to formulations because it acts as a catalyst to aid the absorption of various active principles (Sumano and Gutierrez, 2002; Huang et al., 2013). It is, therefore, postulated for this trial that the combined effects of carbopol and capsaicin gave rise to the very high C_{MAX} found in the FCad-lib and FCbolus groups.

It is important to note that neither the tested prototypes nor the reference medicated feed induced any change in feed consumption or the deposition and consistency of the pig's stools. Furthermore, capsicum has been used as a feed additive to alleviate diarrhea and to increase performance in weaned pigs challenged with *Escherichia coli* (Liu et al., 2013). It has been shown that capsicum enhances intestinal mucosa health and stimulates immune responses in weaned pigs (Long et al., 2021). However, conducting studies of the prototypes dosing these preparations for more days will be necessary to evaluate this clinical aspect. Also, it remains to carry out trials on an industrial scale to verify the compatibility data with various diets and acceptance by different ages and groups of pigs. Likewise, it will be essential to carry out PK/PD modeling to optimize the doses of this antibiotic and to analyze whether the concentrations achieved impact bacteriological cures in bacterial disease outbreaks. Further research is needed to ensure the residual time of these novel formulations of doxycycline hyclate.

5 | CONCLUSIONS

The results of the present investigation indicate that achieving remarkably high concentrations of doxycycline hyclate in pigs' plasma and tissues is feasible using the particular species-designed prototypes presented here. The concentrations achieved are even higher than the so-called breakpoint of resistant bacteria (2 μg/mL), and given a higher mean residence time obtained compared with the standard premix of doxycycline hyclate, stating that optimization of this antibiotic has

been achieved seems plausible. The relative bioavailability of the best ad libitum prototype administered (FCadlib) was 500% higher than the reference preparation. This feature can undoubtedly become apparent in challenging clinical scenarios, that is, in treating bacteria with some resistance. The form of administration was not associated with modifications in the feed consumption patterns nor in altering the digestive system patterns. It remains to carry out trials on an industrial scale to verify the data in different scenarios and to assess the clinical impact of these prototypes in clinical settings.

AUTHOR CONTRIBUTIONS

Sumano and Gutierrez conceived and designed the experiments. Zermeño and Luna-del Villar performed the experiments; Bernad developed and performed analytical techniques. Gutierrez and Sumano analyzed data. Sumano, Gutierrez, and Zermeño edited and reviewed the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

This study was supported by the Project PAPIIT-UNAM: IT200222.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The Institutional Animal Care and Use Committee (CICUAL) of Universidad Nacional Autonoma de México (06737) approved usage of the animals and the experimental protocols.

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How to cite this article: Zermeño-Acosta, M., Sumano, H., Villar, J.-d., Bernad, M. J., & Gutiérrez, L. (2024). Pharmacokinetics of doxycycline hyclate in pigs with a new feed premix formulation. *Journal of Veterinary Pharmacology and Therapeutics*, *47*, 107–113. https://doi.org/10.1111/jvp.13419