ORIGINAL RESEARCH ARTICLES

Evaluation of serum florfenicol concentrations and PK/PD ratios in pigs following In-Feed administration at four dose levels

Evaluación de las concentraciones séricas de florfenicol y las relaciones PK/PD en cerdos después de la administración en el alimento en cuatro niveles de dosis

Avaliação das concentrações séricas de florfenicol e das relações farmacocinéticas/farmacêuticas em suínos após administração na ração em quatro níveis de dose

Lilia Gutiérrez[1]; Graciela Tapia-Pérez[2]; Pablo González[3]; José Fajardo-Vargas[4]; Minerva Monroy-Barreto[5]; Héctor Sumano[1*]

[1]*National Autonomous University of Mexico, Faculty of Veterinary Medicine and Animal Husbandry, Department of Physiology and Pharmacology, Mexico City 04510, Mexico.*

[2]*National Autonomous University of Mexico, Faculty of Veterinary Medicine and Animal Husbandry, Department of Genetics and Biostatistics, Mexico City 04510, Mexico.*

[3]*Swine Producer and Consultant/Veterinarian at San José de Gracia, Michoacán, México, Mexico.*

[4]*PiSA Agropecuaria, Technical Department, Guadalajara, Mexico.*

[5] *National Autonomous University of Mexico, Faculty of Chemistry, Department of Analytical Chemistry, Mexico City 04510, Mexico.*

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***Corresponding author:** National Autonomous University of Mexico, Mexico City 04510, Mexico. Email: sumano@unam.mx*

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Abstract

Background: Florfenicol treatment is often necessary in intensive swine clinics, and appropriate dosing should be followed. However, the pioneer manufacturer's original registration stated that the dose could be set at 40 ppm as in feed medication. Other generic brands have established other doses (80, 100, and 200 ppm). **Objective:** This trial aimed to assess the PK/PD rationale of the referred doses, and determine the serum concentrations of florfenicol achieved in pigs after dosing them in feed and *ad libitum* with florfenicol at four different dose levels: 40, 80, 100, and 200 ppm. Then, the pharmacokinetics/pharmacodynamics ratios are established. **Methods:** Through HPLC, serum concentrations of florfenicol achieved in Landrace/Duroc spayed pigs weighing approximately 10 kg were determined. Medicated feed was administered *ad libitum*. The selected in feed concentrations were 40, 80, 100, and 200 ppm. Considering the farm-established feed intake of 4% of their body weight, doses achieved were: 1.6, 3.2, 4, and 8 mg/kg, respectively. Only florfenicol was determined through a chromatographic method. Monte Carlo simulations were carried out. **Results:** As expected, there are significant differences when comparing the mean serum concentrations of florfenicol achieved with each dosage level of the antibacterial drug. Since florfenicol is considered a time-dependent antibacterial and, as established in the literature, is only 15% bound to plasma protein in pigs, Monte Carlo simulations were based on 85% of the serum concentrations reached with each dose. Based on the available literature, target attainment was set either at $MIC_{0.4}$ µg/mL or $MIC_{2.0}$ µg/mL, and %T to reach those values within the dosing interval (DI) was set at 100%, i.e., 24 h. The 40-ppm dose only achieves 14 h of useful concentrations (%T ≥ $MIC_{0.4}$ µg/mL = 58% of the DI). The 100 to 200 ppm doses achieved %T ≥ $MIC_{0.4}$ µg/mL = 100% of the DI. The %T reaching $MIC_{2.0}$ µg/mL was only possible with 200 ppm, achieving 95% of the DI, while the 100 ppm dose achieved only 70.8% of the DI. As metaphylactic treatment is often necessary in intensive swine farms, and florfenicol should only be administered when a given pathology has been proven, the 40-ppm dose resulted unsuitable, while 100 and 200 ppm appear to be acceptable, and the 80 ppm marginally acceptable for susceptible pathogens.

Conclusions: The in-feed customary dose of florfenicol recommended in Latin America for pigs, ranging from 20 to 40 ppm, fails to achieve therapeutic serum concentrations. A minimum of 80 ppm and ideally 200 ppm are recommended.

Keywords: *dose level; feed; florfenicol; pharmacokinetics and pharmacodynamics (PK/PD); pigs.*

Resumen

Antecedentes: El tratamiento con florfenicol es a menudo necesario en la porcicultura intensiva y se debe administrar una dosificación adecuada. El registro original del fabricante pionero estableció que la dosis podía ser de 40 ppm vía oral en el alimento. Otras marcas genéricas han establecido otras dosis (80, 100 y 200 ppm). **Objetivo:** Este ensayo tuvo como objetivo evaluar las relaciones farmacocinético/farmacodinámicas de las dosis referidas, determinando las concentraciones séricas de florfenicol en cerdos dosificados en el alimento y *ad libitum* con cuatro niveles de dosis: 40, 80, 100 y 200 ppm, y establecer sus relaciones farmacocinéticas/farmacodinámicas. **Métodos:** Mediante HPLC se evaluaron las concentraciones séricas de florfenicol alcanzadas en cerdos castrados raza Landrace/Duroc que pesaban aproximadamente 10 kg y después de la administración de florfenicol en el alimento, administrado *ad-libitum*. Las concentraciones seleccionadas fueron 40, 80, 100 y 200 ppm. Considerando el consumo de alimento establecido en la granja de 4% de su peso corporal, las dosis alcanzadas fueron: 1.6, 3.2, 4 y 8 mg/kg, respectivamente. Se realizaron simulaciones de Monte Carlo. **Resultados:** Como se esperaba, existen diferencias significativas al comparar las concentraciones séricas medias de florfenicol alcanzadas con cada nivel de dosificación del fármaco antibacteriano. El florfenicol se considera un antibacteriano dependiente del tiempo y, como se establece en la literatura, solo se une en un 15% a las proteínas plasmáticas en los cerdos, las simulaciones de Monte Carlo se basaron en el 85% de las concentraciones séricas alcanzadas con cada dosis. Con base en la literatura disponible, el logro del objetivo se estableció en $MIC_{0.4}$ µg/mL o $MIC_{2.0}$ µg/mL, y el %T para alcanzar esos valores dentro del intervalo de dosificación (ID) se estableció en 100%, es decir, 24 h. La dosis de 40 ppm solo alcanza valores útiles durante 14 h (%T ≥ $MIC_{0.4}$ µg/mL = 58% del ID). Las dosis de 100 a 200 ppm lograron %T ≥ $MIC_{0.4}$ µg/mL = 100% del ID. El %T que alcanza $MIC_{2.0}$ µg/mL solo fue logrado con 200 ppm, llegando al 95% del ID, mientras que la dosis de 100 ppm logró el 70.8% del ID. Como el tratamiento metafiláctico suele ser necesario en granjas porcinas intensivas

y el florfenicol solo debe administrarse cuando se ha demostrado una patología determinada, la dosis de 40 ppm resultó inadecuada, mientras que 100 y 200 ppm parecen ser aceptables, y la de 80 ppm es marginalmente aceptable y solo para patógenos susceptibles. **Conclusiones:** La dosis habitual de florfenicol en el alimento recomendada en Latinoamérica para cerdos, que oscila entre 20 y 40 ppm, no alcanza concentraciones plasmáticas terapéuticas. Se recomienda un mínimo de 80 ppm e idealmente 200 ppm.

Palabras clave: *alimento; cerdos; farmacocinética y farmacodinámica (PK/PD); florfenicol; nivel de dosis.*

Resumo

Antecedentes: O tratamento com florfenicol é frequentemente necessário na suinocultura intensiva, devendo ser administradas dosagens adequadas. O registro original do fabricante pioneiro estabeleceu uma dose de 40 ppm por via oral na ração. Outras marcas genéricas estabeleceram outras doses (80, 100 e 200 ppm). **Objetivo:** Este estudo teve como objetivo avaliar as relações farmacocinéticas/farmacodinâmicas das doses supracitadas, determinando as concentrações séricas de florfenicol em suínos dosados na ração e à vontade em quatro níveis de dose: 40, 80, 100 e 200 ppm, e estabelecendo suas relações farmacocinéticas/farmacodinâmicas. **Métodos:** As concentrações séricas de florfenicol alcançadas em suínos castrados Landrace/Duroc com peso aproximado de 10 kg foram avaliadas por CLAE após a administração de florfenicol na ração e à vontade. As concentrações selecionadas foram 40, 80, 100 e 200 ppm. Considerando o consumo de ração estabelecido na fazenda de 4% do peso corporal, as doses alcançadas foram: 1,6, 3,2, 4 e 8 mg/kg, respectivamente. Simulações de Monte Carlo foram realizadas. **Resultados:** Como esperado, houve diferenças significativas ao comparar as concentrações séricas médias de florfenicol alcançadas em cada nível de dosagem do medicamento antibacteriano. O florfenicol é considerado um antibacteriano dependente do tempo e, conforme estabelecido na literatura, liga-se apenas 15% às proteínas plasmáticas em suínos; as simulações de Monte Carlo foram baseadas em 85% das concentrações séricas alcançadas com cada dose. Com base na literatura disponível, o alcance da meta foi estabelecido em MIC0,4 μg/mL ou MIC2,0 μg/mL, e o %T para atingir esses valores dentro do intervalo de dosagem (DI) foi estabelecido em 100%, ou seja, 24 h. A dose de 40 ppm só atingiu valores úteis por 14 h (%T ⩾ MIC0,4 μg/mL = 58% do DI). Doses de 100 a

200 ppm atingiram %T ⩾ MIC0,4 µg/mL = 100% do DI. A %T atingindo MIC2,0 µg/mL só foi alcançada em 200 ppm, atingindo 95% do DI, enquanto a dose de 100 ppm atingiu 70,8% do DI. Como o tratamento metafilático é frequentemente necessário em fazendas de suínos intensivas e o florfenicol só deve ser administrado quando uma patologia específica foi demonstrada, a dose de 40 ppm foi inadequada, enquanto 100 e 200 ppm parecem aceitáveis, e a dose de 80 ppm é marginalmente aceitável e apenas para patógenos suscetíveis. **Conclusões:** A dose usual de florfenicol recomendada na América Latina para suínos, variando de 20 a 40 ppm, não atinge concentrações plasmáticas terapêuticas. Recomenda-se um mínimo de 80 ppm e, idealmente, 200 ppm.

Palavras-chave: *farmacocinética e farmacodinâmica de PK/PD; florfenicol; nível de dose; ração; suínos.*

Introduction

The injectable preparation of florfenicol is indicated to deliver 20 - 30 mg/kg of body weight, thus providing 2 to 3 days of serum concentrations that could be considered therapeutic. In contrast, low dosages of florfenicol as in-feed medication are routinely used in Latin America in pigs, i.e., 20 or 40 ppm, based on the pioneer manufacturer's recommendations, who requested that neither the brand nor its names be disclosed. The *pioneer preparation* is no longer commercially available in various Latin American countries, including Mexico (Palacios-Arriaga *et al*., 2000). However, various generic brands in the continent adopted the referred doses, i.e., *generic-1* from yet another undisclosed brand and manufacturer in Mexico and some Latin American countries. Compared to the parenteral dose, the oral dose is much lower. For example, a 10 kg pig consumes 4% of its weight in feed, that is, 400 g of feed per day/pig. If their feed contains only 40 ppm (40 g/kg), as often indicated, pigs will receive a dose of approximately 16 mg of florfenicol per pig, i.e., 1.6 mg/kg of body weight/day. This scenario becomes more extreme as the pig grows and food intake, expressed as a percentage of its body weight, becomes smaller. For example, a breeding sow weighing 200 or 300 kg consumes 2 kg of feed daily, 0.66% to 1.0% of its body weight.

On the other hand, it is reasonable to assume that dosing florfenicol as an in-feed medication at 80, 100, or 200 ppm is more likely to increase therapeutic serum concentrations in pigs. For example, a minimum serum or tissue concentration of 2 µg/mL has been considered the tipping point for achieving therapeutic concentrations for most pig pathogens, including bacteria such as *Streptococcus suis* (Voorspoels *et al*., 1999; Vilaró *et al*., 2020; Gutiérrez *et al*., 2011; Somogyi *et al*., 2020; Somogyi *et al*., 2023). However, other reported bacterial sensitivities require lower concentrations, such as *Actinobacillus pleuropneumoniae* (0.2 to 0.39 µg/mL), (Ueda and Suenaga, 1995). *Bordetella bronchisepica* and *Pasteurella* spp (see Table 1) (Dorey *et al*., 2016; Swinkels *et al*., 1994; Blondeau and Fitch, 2019; Kucerova *et al*., 2011; Somogyi *et al*., 2003; Wentzel, 2012). Hence, it appears reasonable to evaluate the pharmacokinetic/pharmacodynamic (PK/PD) ratios achieved with florfenicol administered orally in food and *ad libitum* at doses of 40, 80, 100, and 200 ppm, which represent doses of 1.6, 3.2, 4, and 8 mg/kg based on the food consumption (4%), and assuming that a given metaphylactic treatment is needed because the existence of a specific pathology has been clinically or otherwise proven. Florfenicol should not be administered as a preventive maneuver against a supposedly unproven bacterial threat (Ciprian *et al*., 2012).

An additional difficulty is foreseen when a treatment is needed in pigs. It has been shown that pigs already affected by a respiratory infection almost completely reject feed (Pijpers *et al*., 1991) and drastically reduce water consumption, even more so if they are medicated with florfenicol (Gutierrez *et al*., 2011), as it has been shown that medication via drinking water decreases day by day, and consequently serum concentrations achieved could only be helpful against highly susceptible pathogens such as *Actinobacillus pleuropneumoniae.* However, due to the sour taste of florfenicol, pigs easily detect and reject it. Hence, it is impossible to achieve useful serum concentrations against less susceptible pathogens, such as *Escherichia coli, Streptococcus suis*, or *Salmonella suis*, through drinking water (Vailaró *et al*., 2020; Gutiérrez *et al*., 2011).

Since there are commercially available preparations in Latin America recommending different dosage levels of florfenicol as in-feed medication, the key PK/PD ratios for florfenicol as a time-dependent antibacterial (Gutiérrez *et al*., 2011) were determined, setting target attainments serum concentrations of florfenicol at 0.4 µg/mL or 2.0 µg/mL during 100% of the

dosing interval (100%T ≥ MIC). Target minimum inhibitory concentrations were adopted from the literature. Brands utilized to set the four dose ranges were generic-1 at 40 ppm and generic-2 at doses of 80, 100, or 200 ppm.

Materials and methods

Ethics statements

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. The Internal Committee of Postgraduate Studies of the School of Veterinary Medicine of the Universidad Nacional Autónoma de México (SICUAE) approved the ethics procedures followed in this study (SICUAE.MC – 2023/2-3)

Study period and location

This study was carried out in the "Bugambilia" Pig Farm located on Carretera Nacional Sur Jiquilpan Manzanillo km 39 # 675, San José de Gracia, Municipality of Marcos Castellanos, in the State of Michoacán (19.985767 Latitude - 103.016635 Longitude. From March 12 to March 15, 2024. The laboratory study was carried out at Universidad Nacional Autónoma de Mexico, Facultad de Medicina Veterinaria y Zootecnia, from March 20 to September 20, 2024

Study groups

Four dosage levels of florfenicol with two brands of the drug as premix were studied: 40, 80, 100, and 200 ppm. Randomly, twenty-four clinically healthy pigs (F1: castrated males Landrace/Durock) of approximately 10 kg were included per group in three replicates of 8 pigs per pen (96 pigs in all). The distribution of dose levels and inclusion of pigs is summarized in Table 2. Each group replicate was housed in separate pens with a slotted feces removal system. The temperature was maintained at 18 to 22 °C, avoiding draughts. The pens were cleaned twice a day. Feed and water were administered *ad libitum* according to farm management, initiating at 6:00 am, having withdrawn all feed the previous night (9-10 pm), which was almost negligible. Feed consumption data was reviewed to corroborate the dosage achieved for florfenicol when employing two brands of florfenicol: *generic-1* containing 4% florfenicol as premix and

recommended at 40 ppm, and *generic-2* also a premix having 8% concentration of florfenicol and recommended at 80, 100, and 200 ppm. The pigs were not handled for any activity except to obtain blood samples, and forced dosing of bolus doses was not attempted since pharmacokinetics has already been described this way (Palacios-Arriaga *et al*., 2000; Somogyi *et al*., 2020), and to mimic the real-life conditions and serum concentrations of florfenicol.

Table 2. Relationship between group placement and dosage.

Brand of florfenicol	Group	Replicates	Concentration (ppm)*	Kg *	Dose in mg/kg *
Generic-1 (4%)	Flor-40	3	40	2.0/ton	1.6
Generic-2 (8%)	For-80	3	80	1.0/ton	3.2
	Flor- 100	3	100	1.25/ton	4.0
	Flor- 200	3	200	2.0/ton	8.0

* Kg of commercial premix to achieve the indicated dose, considering a food consumption of 4% based on the pigs' body weight and according to in-farm standards

Blood sampling

The number of animals per sampling in each group was calculated using the PKMP program (version 1.03.53, Software and Pharma Consulting Company). Blood samples were taken by jugular puncture with technical assistance, following the rationale of naive-pooled sampling (Elías-Alejandrí, 2010), marking with a colored line on the pigs' dorsum after each sample, and using Vacutainers without anticoagulant. Veterinary surgeons, blood-sampling the pigs, were blinded to the treatment group. The predetermined times were: a basal sample before medication, 0.45 min after the medicated feed was made available, and at 1, 2, 4, 8, 12, and 24 h. Each sample was identified with the group, number of samples per pig, and sampling time. Each pig was sampled a maximum of 4 times to achieve three runs per group. The blood was immediately centrifuged at 3500 rpm for 15 min, and serum was separated and transported with Pasteur pipettes to labeled cryopreservation tubes. They were frozen at -20ºC until analysis within 2-3 weeks.

Chromatographic technique

Determination of serum concentrations of florfenicol was carried out as previously described by Kowalsky et al. (2005), which can be briefly described as follows: frozen porcine serum samples were thawed at room temperature (25°C), and 0.5 mL was pipetted into a clean test tube. Twenty microliters of lamotrigine (100 µg/ml) were added to obtain 4 µg/mL of internal standard in the sample. Then, 0.2 mL of 1 M sodium hydroxide and 3 mL of ethyl acetate were added. The resulting mixture was shaken for 10 min and centrifuged for 15 min at 11,200 g. The organic phase was evaporated to dryness at 50–55°C using a rotary evaporator, and the supernatant was redissolved in 200 µL with the mobile phase (water/acetonitrile, 65:35). After centrifugation at 11,200 g for seven additional minutes, 20 µL was injected onto the HPLC column. Standard samples were prepared by spiking blank serum with known amounts of florfenicol and used for the construction of a calibration curve (0.125, 0.25, 0.5, 1, 2, 3, 5, 7, 10, 15, and 20 µg/mL).

Runs were performed with the final extract in a JASCO brand high-performance liquid chromatograph, model XLC, with a diode array detector and a $\lambda = 223$ nm. The column used was Nucleosil 100-5 C18, 5 µm, 150 mm. The mobile phase had a flow rate of 1 mL/min, and the injection volume was 20 µL. Data were analyzed using Empower-3 software from Waters (Mexico). The chromatographic method was validated, and the analytical procedure was demonstrated as specific. The method produced a linear result from 0.05 to 20.45 µg/mL (r^2 = 0.983; y = 500030x − 107 046). Recovery of florfenicol was calculated by applying linear regression analysis. Precision was demonstrated by the inter-day coefficient of variance (3.0) and inter-assay error value (< 3.7). The lower quantification limit for florfenicol in serum was 0.05 µg/mL with a detection limit of 0.008 µg/mL, and linearity was established from 0.05 to 20.45 µg/mL.

Basic PK parameters for the four doses tested were calculated using the PKMP program (APL Analytic, Laforet Drive, USA) (Table 3). However, the main objective of the study was to evaluate the dose needed to maintain serum concentrations vs time above two values of MIC, i.e., 0.4 and 2.0 µg/mL. These values were retrieved from formal literature and are listed in Table 1.

Table 1. Minimum inhibitory concentration values for several florfenicol-sensitive swine pathogens based on formal literature.

Pathogen	MIC values (µg/mL)	Reference
Actinobacillus pleuropneumoniae	0.2 – 0.4., 0.25-1., 0.2-1.25	17, 18, 14, 20-22
Pasteurella spp.	0.4., 0.25 – 1.0	18, 14, 25
Bordetella bronchiseptica.	0.25 - 0.5	19, 23
Streptococcus suis	1.0 – 2.0	15, 16

MIC = Minimum inhibitory concentration.

Considering that it has been established that florfenicol is only 15% bound to plasma protein in pigs (Somogyi et al., 2020; Gutierrez et al., 2023), polynomial regressions and Monte Carlo simulations were calculated based on 85% of the serum concentrations reached with each dose.

Data analysis

Monte Carlo simulations were produced using GraphPad Prism version 10.0.6 for Mac (GraphPad Software, La Jolla, California, USA), considering a normal distribution of the areas under the curves as well as the target attainment goal for susceptible bacteria to florfenicol (0.4 and 2.0 µg/mL), i.e., 100%T ≥ MIC, as function of optimal plasma antibacterial concentration, and based on 10,000 iterations. The probability of target attainment is expressed as the percentage of the population reaching or exceeding the specific target. The statistical differences between the pharmacokinetic variables between the 4 groups were calculated by means of a parallel analysis of ANOVA using the PKMP program (APL Analytic, Laforet Drive, USA).

Results

Serum florfenicol concentrations

Typical chromatograms of florfenicol in pig serum are shown in Figure 1, with a 4-minute retention time, and various stacked peaks are shown. Figure 2 shows the mean concentrations ± 1SD of the values obtained in porcine serum, with the four dosages evaluated (40, 80, 100, and 200 ppm in the feed). Minimum inhibitory concentrations elected (0.4 and 2.0 µg/mL) values are highlighted, and the lines from the quadratic polynomial regressions are presented with an $R^2 \geq 0.9$ in all cases. From this figure, it can be seen how only the doses of 100 and 200 ppm, and marginally 80 ppm of florfenicol in-feed, achieve concentrations considered therapeutic for the bacteria target goals set in this study. The dose of florfenicol at 40 ppm is below desirable in

achieving useful MICs for the already mentioned pathogens. The serum concentrations vs time targets were determined, and the values are detailed in Table 1. Similarly, the figure 3 shows the Monte Carlo adjusted serum concentrations of florfenicol vs. time. Under this model, serum concentrations of florfenicol are slightly lower for all four dose ranges, but generally indicate that the 40 ppm dose does not achieve the MIC targets mentioned above. In contrast, the 100 and 200 ppm doses provide useful concentrations. The 80-ppm dose could be considered borderline.

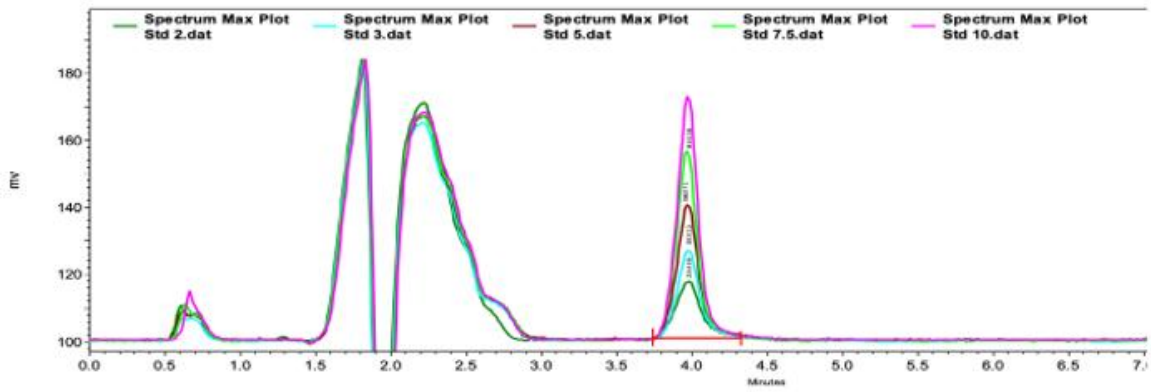

Figure 1. Typical chromatograms of florfenicol in pig serum. Note that the retention time for florfenicol was 4 minutes, and various peaks are stacked, revealing different concentrations of florfenicol.

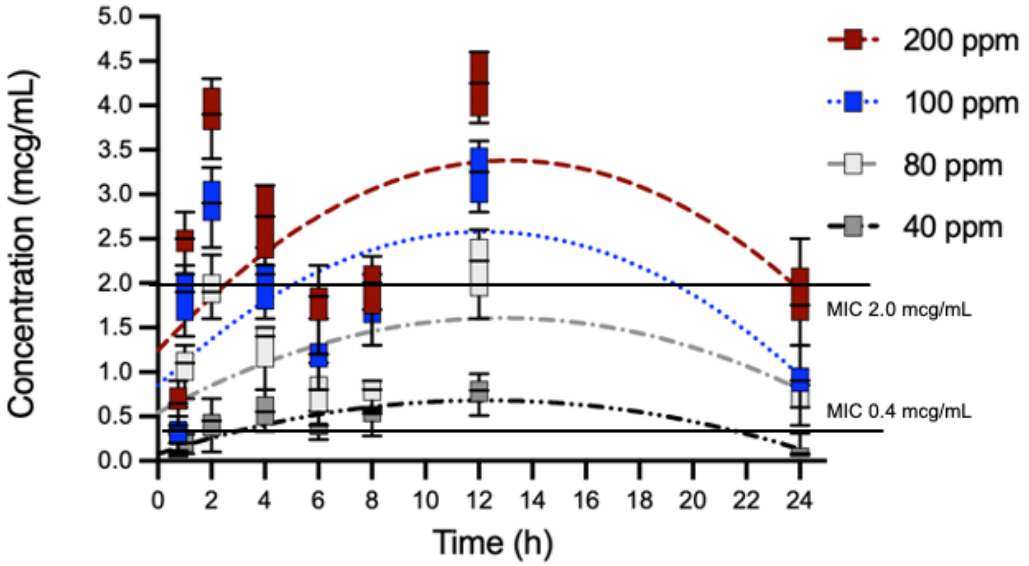

Figure 2. Mean ± 1SD serum concentrations of florfenicol in pigs treated with the antibacterial in feed at concentrations of 40 ppm, 80 ppm, 100 ppm, and 200 ppm. Polynomial regression is presented for each dose level, and the MIC values reported in the literature for the most common pathogens in pigs are marked.

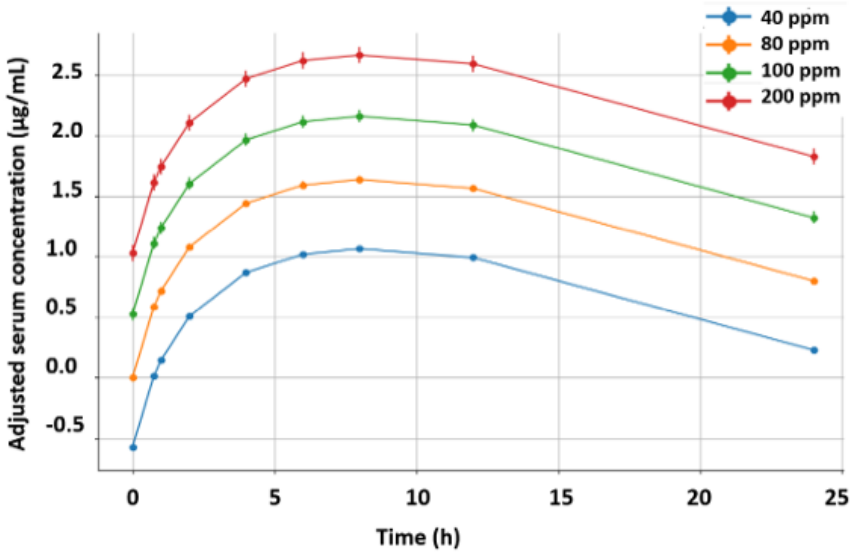

Figure 3. Representation of adjusted serum concentrations of florfenicol by Monte Carlo simulations in pigs treated with four doses of florfenicol in the feed (40, 80, 100, and 200 ppm) provided *ad libitum.*

Statistical analysis using ANOVA comparing the means of the concentrations obtained with the four dosage levels of florfenicol revealed that all concentrations achieved with the different doses varied among themselves ($p < 0.05$). The pharmacokinetic differences among groups are detailed in Table 3. Other than the T_{max} and MRT_{0-24} parameters, the remaining parameters obtained show statistically significant differences ($P<0.05$) among all groups.

Table 3. Mean ± 1SD of the pharmacokinetic parameters of florfenicol in pigs after in-feed administration of four different doses: 40 ppm, 80 ppm, 100 ppm, and 200 ppm.

Pharmacokinetic parameter	**Flor200**		**Flor100**		**Flor80**		**Flor40**	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
K_{el} (h^{-1})	0.01[a]	0.01	0.03[b]	0.01	0.01[c]	0.01	0.08[d]	0.02
$T½_{el}$ (h)	95.21[a]	31.34	27.99[b]	6.40	227.29[c]	96.03	9.03[d]	1.93
T_{max} (h)	11.33	1.63	11.33	1.63	11.33	1.63	11.33	1.63
C_{max} (µg/mL)	4.19[a]	0.08	3.19[b]	0.08	2.19[c]	0.08	0.79[d]	0.08
AUC_{0-24} (µg/mL*h)	65.06[a]	6.19	46.55[b]	6.11	31.20[c]	3.95	10.28[d]	2.65
$AUMC_{0-24}$ (µg/mL*h^2)	735.8[a]	80.59	502.6[b]	77.80	352.2[c]	50.08	103.0[d]	32.09
MRT_{0-24} (h)	11.30[a]	0.19	10.76[a]	0.32	11.27[a]	0.22	9.85[a]	0.91

Ŋ = 24 pigs per group
a, b, c, d Different letters represent significant differences between lines.
K_{el}: elimination rate constant; $T½_{el}$: elimination half-life; C_{max}: maximum plasma concentration; T_{max}: time at which C_{max} is reached; AUC_{0-24}: area under the curve from 0 to 24 h; $AUMC_{0-24}$: area under the curve at a given time point from 0 to 24 hours; MRT_{0-24}: mean residence time.

Discussion

The injectable preparations of florfenicol have been shown to achieve adequate serum concentrations for 48 hours at a dose of 20 mg/kg (Nuflor®, Merck & Co., Inc. NJ, USA). According to the technical data of this pioneer injectable preparation, the mean maximum serum concentration (C_{max}) is 3.37 µg/ml, which occurs at 3.3 h post-injection (T_{max}). The mean serum concentration at 24 h was 0.77 µg/ml, and the elimination half-life ($T½\beta$) was estimated at 18.3 h. In contrast, when administered in feed using an experimental formulation at a dose of 250 ppm, the oral pharmacokinetics of florfenicol showed that the C_{max} fluctuated between 2.45 and 5.11

µg/mL (Voorspoels *et al*., 1999). Various florfenicol premix formulations are marketed in Latin America at 40 ppm in feed, based on the historically established dose recommended in the pioneer brand, which, as already stated, is no longer available. However, it is now emulated by several generic brands throughout Latin America, including the one used in this study (Generic-1, available in Mexico). These results reveal that such low doses generate very low or marginal serum concentrations for pathogens affecting swine. Furthermore, in an exhaustive review of the literature in the main databases and covering at least 90% of the formal information on veterinary pharmacology, not a single study of the pharmacokinetic profile of florfenicol at such low doses was found and this type of study is an essential requirement to rationalize a given dose (Somogyi *et al*., 2020; Blondeau and Fitch, 2019; Halleran *et al*., 2024). Furthermore, when an injectable form is used, the lowest dose studied and reported in the formal literature is 5 mg/kg (Cutler *et al*., 2020).

The use of 40 ppm florfenicol in feed contravenes the WHO/FAO call for rational use of antibacterials in veterinary production (WHO, 2017). The opposite extreme is using a dose of 30 mg/kg parenterally (Somogyi 2020; Blondeau and Fitch, 2019) or orally (Ščuka, 2005) to achieve larger PK/PD ratios. Furthermore, doses of 15 mg/kg orally have been suggested as highly effective clinically, and this would be equivalent to medicating the feed at doses even higher than those evaluated in this trial, i.e., 300 ppm. Nevertheless, pigs are unlikely to accept this concentration or higher because they can easily detect the unpleasant taste of this drug. Two publications claimed that good clinical efficacy was achieved at 40 ppm of florfenicol in feed utilizing the *pioneeer brand*. In the first one, florfenicol was supplied preventively, as it was used to mitigate the lesions caused by *Mycoplasma hyopneumoniae* (lei *et al*., 2018) and to avoid the clinical signs caused by *Actinobacillus pleuropneumonia*. In the second paper, the pigs were medicated in feed at a dose of 40 ppm for 12 days and then experimentally challenged on the sixth day of medication. Given the current guidelines for rational use of antibacterials, these artificial experimental situations fail to parallel the actual field case scenario. Additionally, it should be noted that the *pioneer* 4% florfenicol preparation or a generic one is unavailable in other parts of the world. It can be advanced that there is an economic component to the 40-ppm dose, as the cost of florfenicol is considerable, and producers often prefer mediocre results to a high outlay for using higher doses. Also, given the sensitivity of the pig's palate, it appears reasonable to suggest that

there is room for research to generate a pharmaceutical preparation capable of masking the taste of florfenicol in pigs. Behavioral studies indicate that food or water with a bad taste (and florfenicol has a very unpleasant taste) is rejected by pigs whose olfactory and gustatory capacities are much higher than humans (Swinkels *et al*., 1994; Elías-Alejandri 2010). Administration via drinking water, with a preparation previously solubilized in 2-N-methylpyrrolidone (Pharmasolve®), aims to achieve a dose of approximately 10-12.5 mg/kg, based on the average water consumption of a pig (100-120 mL/kg/day). However, it is important to note that achieving a very early diagnosis is essential before many pigs are affected, and again, sick animals' water and feed consumption is drastically reduced, especially in pneumonia outbreaks. After administration of florfenicol preparations in drinking water, serum concentrations of florfenicol may provide insufficient serum concentrations to treat an outbreak (Gutierrez *et al*., 2011). Thus, it can be concluded that the serum concentrations of florfenicol achieved in this trial at a dose of 40 ppm are notoriously insufficient to achieve effective treatment, notably if feed consumption has already been reduced. With such a scenario and low doses of florfenicol, it can be inferred that the emergence of bacteria resistant to this active ingredient is favored. Florfenicol has been classified as a time-dependent antibacterial, and according to the results of this study, florfenicol in feed at a dose of 40 ppm only reaches concentrations of 0.4 µg/mL for 14 hours, i.e., $\%T \geq MIC_{0.4\ \mu g/mL}$ = 58% of the DI. Except for highly susceptible pathogens, these values are insufficient to protect pigs against the full onset of an outbreak caused by a slightly less susceptible bacterium (see Table 1).

Concluding, treatment is often necessary in intensive swine clinics, and appropriate dosing should be followed, i.e., when a given bacterial pathology has been diagnosed. Using florfenicol theoretically as a preventive measure used in previous cases at doses of 20 and 40 ppm (Lei *et al*.,2018) is not sustainable. Consequently, if florfenicol is to be used for the control of an outbreak, the doses should fluctuate in a range of 80 to 200 ppm to achieve $\%T \geq MIC_{0.4\ \mu g/mL}$ = 70% DI, or $\%T \geq MIC_{2.0\ \mu g/mL}$ = 90% DI, respectively. Considering that WHO/FAO has urged all countries to use antibacterial drugs rationally in production medicine, it seems to be a transgression of reason to use low doses of florfenicol in pig production (Wentzel, 2012). However, the use of excessively high doses should also be avoided as they can alter the pigs' microbiome and the feed palatability, which can impact production variables. The US Code of Federal Regulations (21 CFR, 558.261) specifies a dose of 182 ppm, which appears to be a reasonable one (REF). Finally, it is proposed

that pharmaceutical companies invest in designing florfenicol preparations that optimize the bioavailability of florfenicol, whether administered in drinking water or feed, while simultaneously masking its unpleasant taste. This last option has already been advanced (Mijares, 2024).

Declarations

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Conflict of interest

The authors declare no conflict of interest

Author contributions

LG: Conceptualization, Writing-original draft, Writing-review and editing. GTP: statistical analysis and data curation. PG: Investigation. JJFV: Investigation and Methodology. MMB: Analytical techniques. HS: Conceptualization, Writing-original draft and Writing-review and editing.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

Data Availability Statement

The information is available upon request from the reader.

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