



# The vehicle of administration, feed or water, and prandial state influence the oral bioavailability of amoxicillin in piglets

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## Abstract

Feed and water components may interact with drugs and affect their dissolution and bioavailability. The impact of the vehicle of administration (feed and water) and the prandial condition of weaner piglets on amoxicillin's oral bioavailability was evaluated. First, amoxicillin's *in vitro* dissolution and stability in purified, soft, and hard water, as well as release kinetics from feed in simulated gastric and intestinal media were assessed. Then, pharmacokinetic parameters and bioavailability were determined in fasted and fed pigs using soft water, hard water, or feed as vehicles of administration following a balanced incomplete block design. Amoxicillin showed similar dissolution profiles in soft and hard water, distinct from the dissolution profile obtained with purified water. Complete dissolution was only achieved in purified water, and merely reached 50% in soft or hard water. Once dissolved, antibiotic concentrations decreased by around 20% after 24 h in all solutions. Korsmeyer-Peppas model best described amoxicillin release from feed in simulated gastric and intestinal media. Feed considerably reduced antibiotic dissolution in both simulated media. *In vivo*, amoxicillin exhibited significantly higher bioavailability when delivered via water to fasted than to fed animals, while *in-feed* administration yielded the lowest values. All treatments showed a similar rate of drug absorption. In conclusion, we demonstrated that water and feed components, as well as feed present in gastrointestinal tract of piglets decrease amoxicillin's oral bioavailability. Therefore, the use of oral amoxicillin as a broad-spectrum antibiotic to treat systemic infections in pigs should be thoroughly revised.

**Keywords** Amoxicillin · Feed · Water hardness · Bioavailability · Prandial state · Piglets

## Introduction

Global pork production is highly reliant on antibiotics despite government restrictions and implementation of antibiotic reduction programs in most countries (Dewulf et al. 2022; Dutra et al. 2021; Filippitzi et al. 2014). Efforts towards responsible use of antibiotics focus on health management, animal welfare and biosecurity. Among these actions, early detection of infections and therapeutic treatment to clinically ill animals (mostly injectable) rather than group prophylactic or metaphylactic treatments are key strategies to reduce antibiotic use. However, oral group medication, through feed or water, is by far the most frequent means of administration of antibiotics and most pharmaceutical formulations are authorized for oral use (Carmo et al. 2017; EMA/EFSA, 2017; Van Boeckel et al. 2015), even if EMA classify oral treatments using feed and drinking water as

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vehicles, as high risk for the development of antimicrobial resistance (EMA, 2019).

Oral therapy is advantageous in terms of its, a priori, simplicity – low workload, possibility to treat large groups of animals without causing stress, avoid residues at the site of injection, etc. – but these practices can detract from responsible use of antibiotics if certain issues are not properly addressed. When feed is used as vehicle of administration, drugs must be released and dissolved in the gastrointestinal fluids before being absorbed. Uneven distribution of the drug in finished feed, poor stability under processing or storage conditions or interactions with feed components can alter drugs release and dissolution (Decundo et al. 2021; del Castillo and Wolff 2006; Vandael et al. 2020). Drinking water is the most suitable vehicle for oral antibiotics (providing clear and clean pipelines rendering adequate water flow rates) as it allows easy and rapid onset and end of therapy, as well as dose variations along treatments. In commercial farms medicated water is delivered through proportioning pumps which inject a preset amount of a concentrated stock solution into the main supply line. Pharmaceutical formulations must be completely dissolved and keep stable in stock solutions during the treatments; failure to achieve one of these conditions would represent a therapeutic obstacle (Decundo et al. 2019; Little et al. 2019; Vandael et al. 2020). Water hardness is one of the most important factors that can negatively affect drugs' dissolution and stability due to possible interactions with cations and pH changes (Decundo et al. 2019; Edwards and Crabb 2021; Ferran and Roques 2019). Pig farms worldwide use well water with high hardness values (Edwards and Crabb 2021; Little et al. 2021; Vandael et al. 2019) without taking note of potential detrimental effects on drugs' dissolution. In any case, antibiotic molecules must be completely dissolved, either in water solutions or in gastrointestinal fluids (when administered in-feed) to be absorbed and exert a systemic therapeutic action. Poor dissolution is one of the main causes of suboptimal bioavailability, therapeutic failure and environmental accumulation of antibiotics (Khadka et al. 2014; Polianciuc et al. 2020; Toutain and Bousquet-Mélou 2004).

Interactions between antibiotic molecules and feed components present in the gastrointestinal tract of pigs may further reduce the bioavailability of oral formulations intended for systemic treatment (Decundo et al. 2021; Fleisher et al. 1999; Nielsen and Gyrd-Hansen 1996). In fact, amoxicillin chemically interacts with divalent cations, thiamine, proteins and polyphenols (Al-Khodir and Refat, 2016, de Arruda et al. 2019; El-Sayed et al., 2014; Kiss et al. 2019), which are components that can be present in piglet's feed (Galassi et al. 2021; Menegat et al. 2019). In this way, pharmacokinetic studies of antibiotics, which are usually performed on fasted animals by veterinary pharmaceutical

laboratories, may not represent the reality of pig production where animals are never fasted.

Amoxicillin (AMX) is considered a broad-spectrum antibiotic and it is widely used to treat systemic and enteric diseases in intensive production systems (Filipitzi et al. 2014; Lekagul et al. 2019; Patel et al. 2020). Powders for oral administration in feed or water are common dosage forms indicated against bacteria that cause systemic infections in pigs (*Bordetella bronchiseptica*, *Streptococcus suis*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Escherichia coli*) (Burch and Sperling 2018; Godoy et al. 2011). The limitations of oral formulations (incomplete dissolution in water or interactions with certain feed components) must be assessed and considered to select and design an antibiotic therapy.

In the present work, we studied the impact of the vehicle of administration (feed and water) and the fed or fasted condition of weaner piglets on the bioavailability of an AMX formulation intended for oral use.

## Materials and methods

Amoxicillin trihydrate oral veterinary pharmaceutical formulation (amoxicillin 50% w/w; Vetanbiotic PS500, Vetanco S.A.), employed in all in vitro and in vivo oral treatments. Sterile powdered amoxicillin trihydrate, used for intravenous (IV) administration and analytical standards of amoxicillin trihydrate and cefadroxil (used as internal standard: IS), were purchased from Sigma-Aldrich (St. Louis USA). HPLC-grade acetonitrile and methanol were provided by Sintorgan S.A., (Buenos Aires, Argentina). Potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ), sodium phosphate monobasic and sodium phosphate dibasic were purchased from Biopack S.A. (Buenos Aires, Argentina). Purified, deionized water was obtained by water purification equipment Pure Lab UHQ, ELGA (Lane End, UK). Soft and hard water were obtained by adding  $\text{CaCO}_3$  to purified water at concentrations of 40 and 400 mg/L respectively.

## In vitro studies

Dissolution data may be used to support bioequivalence evaluations, as differences in dissolution profiles between formulations may result in differences in bioavailability of the active ingredient. In addition, the dissolution of a drug formulation may be modified by the vehicle of administration (Martir et al. 2020). Hence in our study, we compared the dissolution profiles of the same formulation incorporated into each vehicle of administration:

purified (reference), soft or hard water (40 and 400 mg  $\text{CaCO}_3/\text{L}$  respectively that represent values from different pig production zones in Argentina) (Cirelli et al. 2010; Murcia et al. 2022) and feed (using same feed as in the in vivo study described below).

Dissolution studies were carried out in quadruplicates, according to USP 42 NF 37 (2019) (paddle) for other solid oral pharmaceutical formulations. The procedures we followed in the present work have been thoroughly described in previous publications from our research group (Decundo et al. 2019, 2021).

### Dissolution and stability in soft and hard water

To compare dissolution profiles and stability of AMX in stock solutions we developed a reduced scale model of an automatic water proportioning system commonly used in commercial farms (Fig. 1) observing doses required for group treatment, water hardness and temperature. To replicate the regular preparation of stock solutions in the farms we added the antibiotic oral formulation at once to the recipient containing purified, soft or hard water.

The pH of each solution was measured right after the addition of the antibiotic. Samples (0.5 mL) were collected at pre-established time points up to 24 h, diluted, filtered through 0.22  $\mu\text{m}$  nylon membrane, injected into the High Performance Liquid Chromatography (HPLC) system, and quantified. Free drug concentration at each

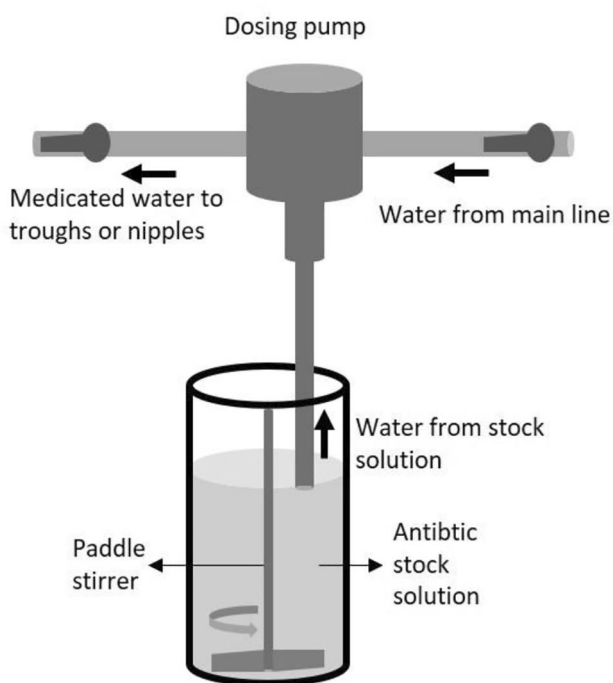
sampling point was used to calculate dissolution percentage (D%). In the present study free drug (drug quantified) corresponds to the drug that is recovered: free from binding (to feed or water components), not degraded and/or the drug that has remained as solid particles. Dissolution profiles were represented by D% vs. time curves.

AMX dissolution profiles were compared using difference ( $f_1$ ) and similarity ( $f_2$ ) factors (FDA 1997). When  $0 > f_1 > 15$  and  $50 > f_2 > 100$ , dissolution profiles are deemed similar.

### Release kinetic and dissolution from feed in simulated gastric and intestinal media

Medicatedv feed can be regarded as a drug delivery system from which the active ingredient must be released.

The AMX oral veterinary formulation was homogeneously mixed with an aliquot of feed (same composition as used in the in vivo study) representing a daily dose of 20 mg/kg BW delivered to a 15 kg weaner piglet. The AMX formulation alone (considered as reference) or blended into feed was dropped at once into the containers filled with simulated gastric (acetate buffer, pH 4) or intestinal (phosphate buffer, pH 6.8) media (as described in USP 2019; the pH of the media were adapted to gastrointestinal conditions of pigs) in the following situations: AMX alone in gastric (AMX pH4) and intestinal (AMX pH6.8) simulated media and AMX blended into feed in gastric (AMX-feed pH4) and intestinal (AMX-feed pH6.8) simulated media. The pH of each medium was measured right after the addition of the antibiotic. Samples (0.5 mL) were collected at pre-established time points up to 120 min, centrifuged, conveniently diluted, filtered through 0.22  $\mu\text{m}$  nylon membrane, injected into the HPLC system and quantified. D% was calculated at each time point to obtain dissolution profiles. The total AMX released from feed was represented by the area under the D% vs. time curve (DAUC). A model-dependent approach was followed to assess AMX release kinetics. A software (DDSolver; Microsoft Excel, Microsoft Co) was used to fit dissolution data to different kinetic functions: zero order, first order, Hixson-Crowell, Higuchi and Korsmeyer-Peppas (Costa and Sousa Lobo 2001; Zhang et al. 2010), each model was characterized by a dissolution rate constant ( $K_0$ ,  $K_1$ ,  $K_H$ ,  $K_{HC}$  and  $K_{KP}$  respectively). Additionally, parameters,  $n$  and  $l$ , were estimated for Korsmeyer-Peppas model. Akaike information criterion (AIC) was used to measure goodness of fit (Yamaoka 1978): the best-fitted model would render the lowest AIC numerical value. In this way, drug release mechanisms could be elucidated from the mathematical model that best fitted the experimental data.



**Fig. 1** Schematic representation of an automatic water proportioning system

## In vivo study

### Animals and treatments

Ten healthy weaner piglets (5 females and 5 immuno-castrated males),  $51 \pm 1$  days old ( $12 \pm 2$  kg BW), Swine Genetic Branch, Choice Genetics line, were used. The animals were accommodated in individual pens at the experimental unit of the Veterinary Research Center of Tandil (CIVETAN), with a 15-day adaptation period preceding the treatments. They had *ad libitum* access to water and antibiotic-free feed (Perfecto Transición, Biofarma S.A. (Cordoba, Argentina): 3,325 kcal/kg of metabolizable energy) out of fasting periods. Lysine contents and all other nutrients were supplied in compliance with the National Research Council (NRC, 2012). The commercial feed components were corn (43.5%), soybean expeller (21.5%) and premix (35%).

The study followed a balanced incomplete block design considering each piglet as a block that randomly received a unique combination of two of the oral treatments in addition to a previous IV dose.

A permanent catheter located in the left internal jugular vein, as proposed by Soraci et al. (2010) allowed us to collect serial blood samples easily without stressing the piglets. Prior to oral treatments, all piglets received an IV dose of 15 mg/kg BW AMX to obtain reference blood concentration-time curves. For oral treatments, five different situations were evaluated: administration of AMX as a single dose of 20 mg/kg BW dissolved in soft water ( $\text{CaCO}_3$  40 mg/L, pH 7.3) to overnight (8 h) fasted (S1;  $n=4$ ) or non-fasted (S2;  $n=4$ ) piglets (fed within 30 min before dosing), dissolved in hard water ( $\text{CaCO}_3$  400 mg/L, pH 9.0) to overnight fasted (H1;  $n=4$ ) or non-fasted (H2;  $n=4$ ) piglets (fed within 30 min before dosing), and mixed with an aliquot of feed (FD;  $n=4$ ) to non-fasted piglets. The entire liquid dose was gently delivered into the pig's mouth using a syringe and the medicated feed was entirely consumed; one hour post administration the animals were given free access to the same water used for antibiotic delivery and feed. A 36-hour period (corresponding to 10 times the half-life) was left between treatments.

Blood samples (1.5 mL) were collected at pre-established time points up to 24 h after IV and oral administration. Plasma was immediately recovered by centrifugation and stored at  $-20^\circ\text{C}$  until analysis.

Trained personnel monitored and registered any abnormal behavior (vomiting, diarrhea, excitability, etc.) all along the trial.

### Sample processing

Plasma (225  $\mu\text{L}$ ), cefadroxil (25  $\mu\text{L}$  of internal standard solution, 40  $\mu\text{g}/\text{mL}$ ) and potassium phosphate monobasic

( $\text{KH}_2\text{PO}_4$ ) 0.68% (250  $\mu\text{L}$ ) were vortex-mixed and loaded on previously conditioned Strata polymeric reversed phase (200 mg/3 mL) solid phase extraction cartridges. After washing (with 2 mL  $\text{KH}_2\text{PO}_4$  and 1 mL water) and drying under vacuum, AMX and cefadroxil were eluted with 1 mL methanol: water (40:60). The eluate was filtered through a 0.22  $\mu\text{m}$  nylon membrane and injected into the HPLC system.

### Chromatographic system

The HPLC system consisted of a binary pump Gilson model 805-806-807 (Middleton, USA) coupled to a UV-Vis detector Gilson 151 (Middleton, USA) and an autosampler Thermo Scientific UltiMate 3000 (California, USA). The separation was achieved on a Luna C18 column of 5  $\mu\text{m}$ ;  $250 \times 460$  mm (Phenomenex, California, USA), maintained at  $40^\circ\text{C}$ ; using isocratic elution at 1 mL/min and acetonitrile and  $\text{KH}_2\text{PO}_4$  0.68% (96:4) as mobile phase. AMX and cefadroxil (used as internal standard) were detected at 230 nm and their retention times were 6.5 and 9.8 min respectively. The software Chromelion (Thermo Scientific) was used for quantification: the method was linear ( $r^2 > 0.995$ ) over the range of 0.09–6  $\mu\text{g}/\text{mL}$ . Validation parameters agreed with international guidelines (U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine, 2018).

### Pharmacokinetic parameters

We used PK Solutions software version 2.0 (Summit Research Services Co; Farrier 1997) to find pharmacokinetic parameters. For each piglet, elimination half-life ( $t_{1/2}$ ), maximum concentration ( $C_{\text{max}}$ ), time at which  $C_{\text{max}}$  is reached ( $T_{\text{max}}$ ) and area under the concentration-time curve from 0 to 24 h ( $\text{AUC}_{\text{PO}}$ ) after oral treatments were estimated. Additionally, area under the concentration-time curve from 0 to 24 h after IV treatments ( $\text{AUC}_{\text{IV}}$ ) was obtained.

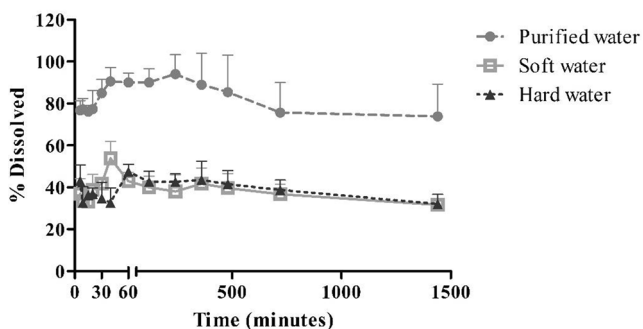
Absolute bioavailability (BA) was calculated individually according to Eq. 1 (Toutain and Bousquet-Mélou 2004; Gibaldi & Perrier. 2007):

$$BA = \frac{\text{AUC}_{\text{PO}}}{\text{AUC}_{\text{IV}}} \times \frac{\text{Dose}_{\text{IV}}}{\text{Dose}_{\text{PO}}} \times 100 \quad (1)$$

Being,  $\text{Dose}_{\text{PO}}$  and  $\text{Dose}_{\text{IV}}$ : oral and intravenous doses, respectively.

### Statistical analyses

All the statistical analyses were carried out using standard software (RStudio version 4.2.2; RStudio Inc).



**Fig. 2** Dissolution profiles of AMX in purified (circles), soft (squares), and hard (triangle) water during 1500 min. Profiles were performed in quadruplicate

Homoscedasticity and normality were verified using Bartlett’s and Shapiro-Wilk’s tests, respectively. DAUC and the models’ parameters ( $K_0$ ,  $K_1$ ,  $K_H$ ,  $K_{HC}$ ,  $K_{KP}$ , and, Korsmeyer-Peppas’  $l$  and  $n$ ) were analyzed by one-way ANOVA or Kruskal-Wallis tests, as applicable. Pharmacokinetic parameters were evaluated using one-way ANOVA for a balanced incomplete block design. When ANOVA resulted in statistically significant effects, comparisons between treatments were carried out using Tukey’s or Dunn test as applicable.

## Results

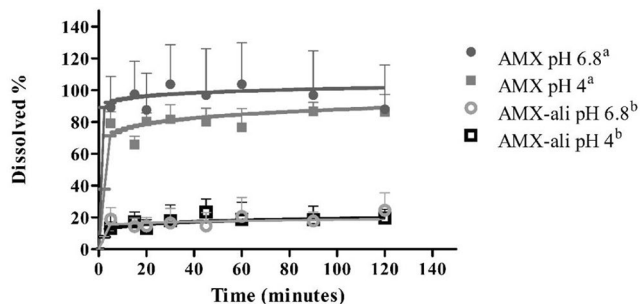
### In vitro studies

Once we added the antibiotic formulation to purified, soft or hard water, a marked shift in pH occurred from the original values to around 10.6 and kept constant until the end of sampling.

In purified water, AMX reached nearly complete dissolution after 30 min and dropped to  $75.67 \pm 14.30$  and  $73.88 \pm 15.38\%$  after 12 and 24 h respectively. In soft water, maximum D% was  $53.83 \pm 8.12\%$  in 40 min and dropped to  $36.75 \pm 4.79$  and  $31.63 \pm 2.88\%$  after 12 and 24 h respectively. In hard water, the maximum D% was  $47.46 \pm 3.50\%$  in 60 min and dropped to  $38.73 \pm 4.69$  and  $32.00 \pm 4.70\%$  after 12 and 24 h respectively (Fig. 2).

Dissolution profiles in soft and hard water were different from dissolution profiles in purified water ( $f_1 = 58.07$ ;  $f_2 = 14.26$  and  $f_1 = 57.53$ ;  $f_2 = 14.69$ , respectively), but equivalent to each other ( $f_1 = 8.26$ ;  $f_2 = 74.34$ ).

Once we added the antibiotic formulation alone to gastric (AMX pH4) and intestinal (AMX pH6.8) media or the antibiotic formulation blended into feed to gastric medium (AMX-feed pH4), only slight pH changes occurred. But when we added the antibiotic formulation blended into feed



**Fig. 3** AMX release kinetic adjusted to Korsmeyer-Peppas model for amoxicillin formulation alone in media at pH 6.8 (AMX pH 6.8, filled circle) and pH 4 (AMX pH 4, filled square) and amoxicillin formulation blended into feed in media at pH 6.8 (AMX-feed pH 6.8, empty circle) and pH 4 (AMX-feed pH 4, empty square). Each assay was performed in quadruplicate. Different superscript letters indicate significant differences among DAUC values ( $P < 0.05$ )

to intestinal medium (AMX-feed pH6.8) the pH dropped to  $5.5 \pm 0.32$ .

The evaluation of AMX release kinetics from feed demonstrated that Korsmeyer-Peppas was the best-fit model in the four situations (Fig. 3). This model is defined by Eq. 2:

$$Q = K_{KP}(t - l)^n \tag{2}$$

where  $K_{KP}$  is the kinetic constant (characteristic of a drug/polymer system),  $t$  is the release time,  $l$  is the lag time (which indicates a delay to start dissolution), and  $n$  is the exponent that indicates the mechanism of release (Korsmeyer et al. 1983). In our study,  $n$  values were lower than 0.5 and  $l$  was similar in all the situations.

ANOVA showed significant effects of feed on DAUC ( $P$ : 0.010) and on  $K_{KP}$  ( $P$ : 0.008). AMX pH 4 and AMX pH 6.8 rendered similar DAUC and  $K_{KP}$  values but significantly higher than AMX-feed pH 4 and AMX-feed pH 6.8 which were similar to each other. Table 1 shows AIC, parameters’ means ( $\pm$ SD) derived from each model and DAUC values.

### In vivo studies

All animals kept clinically healthy throughout the trial. No side effects of the treatments were observed.

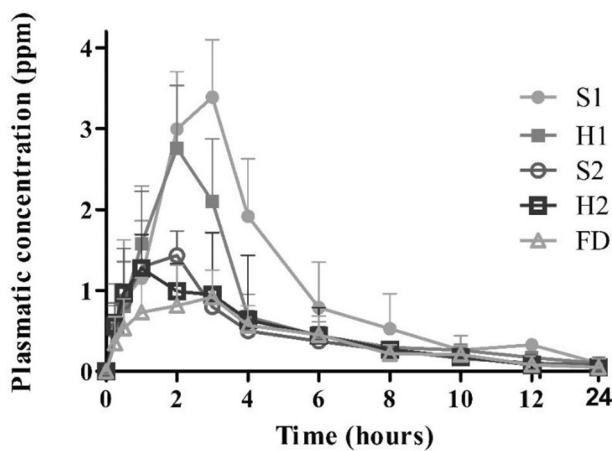
Figure 4 shows mean AMX plasma concentrations vs. time curves obtained after oral treatments.

Table 2 lists some relevant PK parameters obtained from plasmatic concentration profiles. Results from ANOVA showed no effect of treatments on  $t_{1/2}$  ( $P$ : 0.952) and  $T_{max}$  ( $P$ : 0.250), but a treatment effect on  $C_{max}$  ( $P$ : 0.010),  $AUC_{PO}$  ( $P$ : 0.020) and BA ( $P < 0.001$ ) was detected.

When piglets received S1 and H1  $C_{max}$  values were higher than when they received FD while S2 and H2 rendered intermediate  $C_{max}$  values similar to the rest of the

**Table 1** Parameters (mean  $\pm$  SD) derived from each model: dissolution rate constants for zero order ( $K_0$ ), first order ( $K_1$ ), Higuchi ( $K_H$ ), Hixson-Crowell ( $K_{HC}$ ) and Korsmeyer-Peppas ( $K_{KP}$ ); parameters  $n$  and  $l$  for Korsmeyer-Peppas and Akaike information criterion values (AIC) for amoxicillin formulation alone in media at pH 6.8 (AMX pH 6.8) and pH 4 (AMX pH 4) and amoxicillin formulation blended into feed in media at pH 6.8 (AMX-feed pH 6.8) and pH 4 (AMX-feed pH 4). Different superscript letters within the same line indicate significant differences among assays ( $P < 0.05$ )

Models	Parameters	Assays			
		AMX pH 4	AMX pH 6.8	AMX-feed pH 4	AMX-feed pH 6.8
Zero order	$K_0$ (% min <sup>-1</sup> )	1.92 $\pm$ 1.75	1.85 $\pm$ 1.31	0.25 $\pm$ 0.07	0.25 $\pm$ 0.10
	AIC	77.24	81.52	64.13	60.67
First order	$K_1$ (min <sup>-1</sup> )	0.10 $\pm$ 0.10	0.26 $\pm$ 0.01	0.00 $\pm$ 0.001	0.00 $\pm$ 0.00
	AIC	67.32	67.93	63.42	59.87
Higuchi	$K_H$ (% min <sup>-1/2</sup> )	13.50 $\pm$ 6.37	14.85 $\pm$ 5.81	2.46 $\pm$ 0.71	2.47 $\pm$ 0.97
	AIC	69.30	74.03	57.65	53.79
Hixson-Crowell	$K_{HC}$ (% <sup>-1/3</sup> min <sup>-1/2</sup> )	0.02 $\pm$ 0.02	0.02 $\pm$ 0.01	0.72 $\pm$ 0.00	0.001 $\pm$ 0.00
	AIC	70.81	97.29	63.65	60.13
Korsmeyer-Peppas	$K_{KP}$ (% min <sup>-n</sup> )	67.72 $\pm$ 34.03 <sup>a</sup>	90.51 $\pm$ 20.65 <sup>a</sup>	11.73 $\pm$ 6.13 <sup>b</sup>	14.84 $\pm$ 7.48 <sup>b</sup>
	$N$	0.08 $\pm$ 0.09	0.02 $\pm$ 0.06	0.13 $\pm$ 0.10	0.06 $\pm$ 0.13
	$L$	2.25 $\pm$ 0.5	2.00 $\pm$ 0.00	2.25 $\pm$ 0.50	2.25 $\pm$ 0.05
	AIC	60.41	57.51	50.79	50.90
	DAUC	9414 $\pm$ 3574 <sup>a</sup>	9900 $\pm$ 2072 <sup>a</sup>	2180 $\pm$ 696 <sup>b</sup>	2158 $\pm$ 846 <sup>b</sup>



**Fig. 4** AMX mean plasma concentrations vs. time curves after AMX administration (20 mg/kg body weight) in soft water (CaCO<sub>3</sub> 40 mg/L, pH 7.3) after overnight fasting (S1;  $n=4$ ; filled circles) and without fasting (S2;  $n=4$ ; empty circles), in hard water (CaCO<sub>3</sub> 400 mg/L, pH 9.0) after overnight fasting (H1;  $n=4$ ; filled squares) and without fasting (H2;  $n=4$ ; empty squares) and in-feed (FD,  $n=4$ , triangles)

treatments.  $AUC_{PO}$  was similar when the piglets received S1 or H1, and higher than in animals receiving any of the other treatments (S2, H2, FD), which were similar to each other. BA was similar when the piglets received S1 or H1, these were higher than BA values from piglets that received S2 or H2 which were similar to each other. Finally, the piglets that received FD showed significantly lower AMX BA than those receiving any of the other treatments.

## Discussion

Oral group medication is the most frequent means to treat systemic infections in intensive pig production systems around the world (Hémonic et al. 2018; Lekagul et al. 2019; Van Rennings et al. 2015). Strikingly, issues concerning interactions of antibiotics with their vehicle of administration (water or feed) that may alter absorption, bioavailability and therapeutic outcomes, are seldom addressed. In the present study, we demonstrated that the same antibiotic formulation can perform differently in terms of pharmacokinetic behavior, depending on the components present in drinking water or feed used as vehicles of administration.

When we added the antibiotic to purified, soft or hard water, simulating the preparation of the stock solution of a water proportioning system, the pH of the solution rose sharply irrespective of the initial pH. This effect may be attributed to the incorporation of an alkaline excipient into the study amoxicillin oral formulation to facilitate dissolution. This is a common strategy in the development of AMX-based formulations (Felix et al. 2016; Kaur et al. 2011; Vahdat 2000). However, in the present work, complete dissolution was achieved only when AMX was added to purified water and reached merely around 50% in water containing both low or high levels of Ca ions. The similarity of dissolution profiles between soft and hard water indicated that low levels of ions suffice to decrease AMX dissolution. At the same time, the high pH of the stock solution can account for the poor stability of AMX in soft, hard and purified water, causing a reduction in concentrations by around

**Table 2** Pharmacokinetic parameters (mean ± SD) of oral AMX (20 mg/kg body weight) administered in soft water (CaCO<sub>3</sub> 40 mg/L, pH 7.3) after overnight fasting (S1, n = 4), without fasting (S2, n = 4), in hard water (CaCO<sub>3</sub> 400 mg/L, pH 9.0) after overnight fasting (H1, n = 4), without fasting (H2, n = 4), and in feed (FD, n = 4) animals. Different superscript letters within the same line indicate significant differences among treatments (P < 0.05)

PK parameters	Treatments				
	S1	S2	H1	H2	FD
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
t <sub>1/2</sub> (h)	2.33 ± 1.66	2.63 ± 0.26	2.12 ± 0.40	2.28 ± 0.16	3.04 ± 0.80
C <sub>max</sub> (µg/ml)	3.32 ± 2.21 <sup>a</sup>	1.62 ± 0.65 <sup>ab</sup>	2.75 ± 0.92 <sup>a</sup>	1.67 ± 0.34 <sup>ab</sup>	0.76 ± 0.27 <sup>b</sup>
T <sub>max</sub> (h)	1.65 ± 0.15	1.62 ± 0.69	1.32 ± 0.42	1.85 ± 0.88	1.80 ± 0.17
AUC <sub>PO</sub> (0–24 µg.h/ml)	17.26 ± 8.72 <sup>a</sup>	5.85 ± 1.29 <sup>b</sup>	12.12 ± 4.69 <sup>a</sup>	6.99 ± 3.28 <sup>b</sup>	5.05 ± 2.27 <sup>b</sup>
AUC <sub>IV</sub> (0–24 µg.h/ml)	37.61 ± 17.16	20.30 ± 4.57	30.14 ± 13.71	24.49 ± 12.27	38.06 ± 18.04
BA%	34.62 ± 9.26 <sup>a</sup>	21.70 ± 1.63 <sup>b</sup>	32.59 ± 9.95 <sup>a</sup>	21.66 ± 5.22 <sup>b</sup>	9.93 ± 1.41 <sup>c</sup>

20% within 24 h (Jerzsele and Nagy 2009; Kaur et al. 2011; Vahdat 2000). Our results are in agreement with earlier investigations that showed decreased AMX solubility and stability in the presence of cations (Al-Khodir and Refat 2016; Jerzsele and Nagy 2009; Sultana et al., 2015). From a practical point of view, this is vital information for farm workers when planning AMX treatments using water as vehicle of administration. Common practices, like preparing stock solutions every 24 h and not considering water physicochemical characteristics should be thoroughly revised (Edwards and Crabb 2021; Little et al. 2021; Vandael et al. 2019). On the other hand, pharmaceutical laboratories that sell AMX formulations should assure complete dissolution of the antibiotic in stock solutions with the water available in the farms (or at least inform that complete dissolution would be only achieved in, for example, distilled water) and declare for how long would the initial concentrations persist before falling to sub-therapeutic levels.

Experimental data from release kinetics of AMX from the dosage form alone or blended into feed to simulated gastric or intestinal media best fitted to Korsmeyer-Peppas model, being the parameter *n* less than 0.5 in every situation. These results indicate that the antibiotic is embedded in a polymeric matrix from which it is released by simultaneous mechanisms of diffusion (from the swollen matrix and from pores filled with water) (Doadrio Villarejo and Vallet Regí., 2006; Fernández et al. 2009; Korsmeyer et al. 1983). High D% of AMX when it is added alone to both simulated media (free from Ca and other divalent or trivalent cations), similar to that observed in pure water, may denote that the presence of Ca ions (at least over 40 mg/L) is responsible for the reduced dissolution in soft and hard water. The dissolution rate constants and DAUC were significantly lower when AMX was added to both media blended into feed than when the formulation was added alone which demonstrates that feed retards and reduces AMX dissolution in gastric and intestinal conditions. Different mechanisms related to feed components can explain these findings (Cvijic et al. 2014; del Castillo and Wolff 2006; Radwan et al. 2017). The

uptake of water by plant-derived carbohydrates (mainly fibers and starch) and proteins would increase the viscosity of the medium leaving less water volume available for drugs to dissolve. In addition, the water fraction that is strongly bound to feed particles captures drug molecules that would not be free to be absorbed in the intestine (Gupta and Premavalli 2011; Shurson et al. 2021; Slavin 2013). At the same time, AMX may directly interact with feed components such as divalent cations, thiamine and polyphenols, which are present in corn and soybean (El-Sayed et al., 2014; Kiss et al. 2019; Sultana et al., 2015), further reducing the availability of free antibiotic molecules to be absorbed or to exert their activity against intestinal bacteria.

Researchers have extensively investigated AMX pharmacokinetics given that it is one of the most widely used antibiotics in food-producing animals. Oral BA of amoxicillin is typically low (48% at most) and highly variable depending on the pharmaceutical formulation (Anfossi et al. 2002; Dai et al. 2017; Hernandez et al. 2005), the vehicle of administration (del Castillo et al. 1998; Morthorst 2002) and the physiological condition of the animals (Godoy et al. 2011; Jensen et al. 2006). In the current study, we examined the most important variables, other than the formulation, that could potentially impact on AMX’s oral BA, specifically its administration either via feed or drinking water of varying hardness and the fed or fasted state of piglets. We found no effects of the treatments on t<sub>1/2</sub> or T<sub>max</sub>, indicating that the rates of absorption and elimination of the antibiotic were neither influenced by the vehicle of administration nor by the presence of feed in the gastrointestinal tract. Furthermore, the administration of AMX in soft or hard water resulted in similar BA. Considering results from the in vitro assays in the present work, which showed similar water dissolution profiles of AMX at low and high water hardness levels, we can infer that as long as the formulation is designed to dissolve adequately in the specific water used on the farm, the presence of ions per se would not affect the drug’s oral BA. Conversely, when we administered

AMX in drinking water to fed piglets, BA was reduced by 37% and  $C_{\max}$  by 45% relative to fasted animals. Only a few researchers studied the effect of the prandial state on oral AMX's PK parameters in pigs. Consistent with our findings, del Castillo et al. (1998) reported a 37% and 54% reduction in BA and  $C_{\max}$  respectively when the antibiotic was administered in drinking water to fed compared to fasted pigs. In a similar study, Agersø and Friis (1998) observed a reduction in BA and  $C_{\max}$  of 20% and 50% respectively, though these differences were not statistically significant. Consistent with our results, the time to peak concentration and  $t_{1/2}$  remained unchanged in both investigations. Based on comprehensive findings of PK studies, it is clear that the presence of feed in the gastrointestinal tract of pigs hinders the oral BA of amoxicillin. This effect may be ascribed to different phenomena occurring simultaneously: on one side, as we observed in the *in vitro* dissolution of AMX in simulated gastric and intestinal media, feed can change the physicochemical characteristics of the media hampering drug dissolution: different *in vivo* studies have shown the inverse relationship between the increase in viscosity of gastric fluids, due to feed intake, and drug absorption (Deng et al. 2017; Levy et al., 1965; Lutz et al. 1987). On the other hand, the peptide-like chemical structure of  $\beta$ -lactams enables their recognition by the peptide transporter PEPT1 present in the small intestine where these drugs are mainly absorbed by active transport (Bretschneider et al. 1999). Highly digestible proteins, abundant in the post-weaning diet, would render high concentrations of tri and dipeptides that would be more efficiently recognized by PEPT1 blocking the absorption of  $\beta$ -lactams. Therefore, studies conducted in fasted animals, including those performed for registration purposes and dosage regimens determinations, do not represent the actual situation on farms where pigs are never subjected to fasting. In the current study, the adverse impact of feed components on AMX absorption was amplified when the antibiotic was administered as medicated feed, with bioavailability scarcely reaching 10%. Probably, the time required for the drug to diffuse from the feed matrix and dissolve in the gastrointestinal fluids for absorption exceeds the time taken for feed to pass through the intestine's absorption sites.

We can use the PK data generated in our study to evaluate the clinical efficacy that would be expected when this AMX formulation is used to treat a systemic bacterial infection. AMX is a time-dependent antibiotic for which the adequate efficacy index is the percent time, over a 24-hour interval, in which plasma concentrations are above the minimal inhibitory concentration ( $T > MIC$ ) for a specific microorganism (Papich 2014). It is conceivable that a significant reduction of BA as a consequence

of drug interactions with the vehicle of administration or feed present in the gastrointestinal tract, as demonstrated in the present study, makes it impossible to achieve the target values of indices of clinical efficacy for certain bacterial strains, narrowing AMX's antimicrobial spectrum.

Based on our findings, we can conclude that the vehicle of administration (water or feed), and the prandial state of piglets (fasted or fed), significantly affects the bioavailability of AMX to such a degree that calls into question its classification as a broad-spectrum antibiotic. Scientific researchers, including ourselves, have shown limitations of oral treatments with different antibiotics due to interactions with feed or water components and prandial state of the treated animals (Agersø and Friis 1998; Decundo et al. 2019, 2021; del Castillo and Wolff 2006; del Castillo et al. 1998; Morthorst 2002; Nielsen and Gyrd-Hansen 1996; Soraci et al. 2014). In commitment to rational use of antibiotics in food animals, this information should be considered by governmental authorities to regulate antibiotic use, and by veterinary professionals and farm managers to tailor treatments that contribute to successful therapy.

**Author contributions** Author contributions: JMD: experimental design, surgery assistance, in-farm work and animal handling, sample collection, *in vitro* study, analytical procedure, results of pharmacokinetic and statistical analysis, and paper writing. SND: analytical method development and validation, analytical procedure, analysis and discussion of results, and paper writing and correction. GM: in-farm work and animal handling and sampling and discussion of results; FAA: in-farm work, animal handling, and sample collection; DSPG: analysis and discussion of pharmacokinetic results. ALS: Project Director, experimental design, animal surgery, in-farm work and animal handling, analytical procedure, statistical analysis results analysis and discussion and paper writing and correction. All authors read and approved the final manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

**Ethics approval** For animal handling we followed the Guidelines of the Animal Welfare Committee of the University of the Center of Buenos Aires Province, in compliance with EU Directive 2010/63/EU. The experimental design and procedures were evaluated and approved by the Animal Welfare Committee FCV-UNCPBA (Res N° 087/02, May 11, 2016).

**Consent to participate** Not applicable.



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