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Exposure variability of fosfomycin administered to pigs in food or water: impact of social rank

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Introduction

In the pig industry, antibiotics are used as therapeutic and also as growth promotor in some countries. These drugs are most often administered collectively in food or water. Research has shown that the effectiveness of these antibiotics may be influenced by multiple variables related to voluntary food or water intake. These may include for example: individual dietary patterns (del Castillo et al., 2006), age (Hall et al., 1999; Rasmussen et al., 2006), sex (Hall et al., 1999), weight (Quiniou et al., 2000), temperature (Collin et al., 2001; Massabie and Quiniou, 2001), type of housing (Bornett et al., 2000), and feeding system (Gonyou and Lou, 2000; Nielsen et al., 1996). It is important to note that these factors affect all the animals indiscriminately. However, social rank is one of the most discriminating factors that heavily impacts on the individual food intake and of course is also influenced by all the aforementioned variables (del Castillo et al., 2006).

The social rank is a well-structured behavior, specific and unique to each member of the group (Lindberg, 2001). Generally, animals are classified as “Dominant" or "Subordinate" according to their ability to access a limited resource, food or water (Lindberg, 2001; Craig, 1986; Vargas et al., 1987). According to Place et al. (1995) and Levasseur et al. (1996), subordinate pigs eat fewer meals per day compared to dominant pigs and this may have a direct impact on the exposure of the animals to the antibiotic. Proper antibiotic exposure is necessary for an appropriate clinical efficacy and to prevent the development of antibiotic resistance due to possible subtherapeutic plasma levels in treated animals.

In the past many studies have focused on the effect of social rank on food intake in pigs, however only a few studies have taken a closer look at the impact of social ranking issues on exposure of animals to therapeutics administered in food or drinking water.

The antibiotic that was chosen for this purpose was fosfomycin. Fosfomycin is a broad-spectrum antibactericidal agent, classified as a "time-dependent antibacterial" whose salts are adaptable to both oral (fosfomycin-calcium) and injectable (fosfomycin-disodium) formulations. Fosfomycin is widely prescribed in pig production in Argentina and other countries of South and Central America.

The aim of this trial was to document the effect of social rank on the internal exposure of pigs to an antibiotic administered either in food or in drinking water in a commercial setting. Prior to performing a farm-based study, conventional pharmacokinetic studies to validate fosfomycin as a probe for the present investigation were carried out to interpret the disposition of this antibiotic when administered in two different vehicles: water and food.

Materials and methods

The experimental trial was conducted in a commercial farm in the district of Tandil, Buenos Aires, Argentina. The farm is intensively organized in total confinement, with the full life cycle in a single location, provided with 400 females in production. All animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina.

Prior to performing a farm-based study, we carried out conventional pharmacokinetic studies to validate fosfomycin as a probe for the present investigation. Indeed feeding or drinking behaviors are displayed as a series of short bouts throughout the day and to demonstrate the influence of this behavior, it was necessary to select an antibiotic having a relatively short half-life and a rapid absorption rate in order to avoid the dampening effect of a long elimination phase on the instantaneous pattern of the antibiotic exposure.

Individual pharmacokinetics of Fosfomycin after IV and oral administration in food or water.

Drug

Sterile powdered disodium and calcium fosfomycin (purity 98.8 %) were used. (Bedson S.A., Laboratories, Las Palmeras 2240, B1635DIK, La Lonja. Pilar, Buenos Aires, Argentina)

Animals

Eighteen commercial line castrated male pigs, weighing 30 ± 2.5 kg, were obtained from the pig farm. These clinically healthy pigs were placed in their pens 7 days before the start of the experiment to acclimatize them. The pigs were given *ad libitum* access to drinking water and were fed 0.75 kg of antibiotic-free pelleted food.

Catheters were placed in the jugular veins according to a method described earlier by Soraci et al. (2010), two days before the beginning of the experiment, to minimize the stress and facilitate blood sampling.

The individual pharmacokinetics of fosfomycin were evaluated following a single IV and oral (in feed or water) dose of 15 and 20 mg/kg respectively in 3 parallel groups of six pigs, named group A, B, and C

Single IV Dose

Six pigs (Group A) were given disodium fosfomycin IV dissolved in sodium citrate (final concentration 10%) (pH 6.8) at a dose of 15 mg/kg via catheter in the left jugular vein and the blood samples were drawn from the right jugular vein from an implanted polyethylene catheter. After administration the catheter was flushed with 10 mL of 0.9% NaCl.

Single oral dose in food or water

For oral administration in food (Group B) six fasted pigs (for 20h) received (calcium fosfomycin at a dose of 20 mg/kg. The drug was offered in a homogeneous mixture of calcium fosfomycin in 100 g of food and it was ascertained that the mixture was completely consumed. For oral administration in water (Group C) six pigs were given calcium fosfomycin at a dose of 20 mg/Kg. The drug was administered in fasted pigs (20 h) as a 10% suspension with a syringe directly into the mouth of the pigs. The syringe was rinsed with water and the water was administrated to the animal.

We have decided to take a fasting time of 20 h to ensure the washing of any effects of ketamine on the gastrointestinal transit and its content . Ketamine was previously used as an anesthetic for intravenous catheter placement. Ketamine produces a change in the time of interdigestive period on gastrointestinal transit pig (Schnoor et al ., 2005).

Sampling procedure

After intravenous and oral administration of fosfomycin, heparinized blood samples were collected at 0, 10, 15, 30 and 45 min 1, 2, 4, 6, 8, 12 and 24 h. Blood samples were immediately centrifuged, and the plasma recovered and frozen at -20°C until analysis within 4 days.

Impact of social rank status on the intake of food and water (F &W) **and exposure variability of fosfomycin administered in these biological matrices**

Animals

Thirty-six pigs weighing an average of 30.0 ± 2.8 kg in their growth phase were selected and stratified according to weight and sex homogeneity into two groups of 18 animals each (consisting of 9 females and 9 castrated males), and labeled as groups F &W.

The experimental work was conducted during the month of November 2010 in the same commercial farm as described above. The animals from both groups were individually identified by a number in the dorsal-lumbar region, which was maintained throughout all the assays. The two groups (groups F &W) were housed in pens with a concrete floor at a density of 0.85 m^2 animal during the 15 days of the trial. The temperature in pens was kept 22 ± 2°C. The animals received food or water *ad libitum.*

Feeders of stainless steel provided with a scale with a digital weight sensor system were used to study the feeding behavior. The water supply consisted of stainless-steel pig nipple drinkers located 2 meters from the fooder (at the corner of each pen). Water consumption was measured by water meters installed in the water delivery line**.** Throughout the trial (15 days), animals were submitted to a photoperiod of 12 h of light and 12 hours of darkness.

During the *ad libitum* period, continuous food or water consumption was recorded. The identification of the animals in drinkers and feeders was carried out during the 15 days of the trial (a growth phase), using a system of video cameras (equipped with night vision and wide-angle lens) and provided with an approach sensor alarm connected to a centralized system for continuous recording and alarm-identification approach and corresponding software (Professional Surveillance System (PSS) Version 4.04. Zhejiang Dahua Technology Co**.,** Ltd**.** No.1187, Bin'an Road, Binjiang District, Hangzhou). All this information was recorded on a computer and stored. After each visit (i.e. feeding or drinking), the time at the beginning and at the end of the visit and food or water consumption were recorded through observation periods of 10 min over 24 h during the 15 days of trial. The data recorded daily comprised the following variables: beginning and ending time of each visit, and food intake during each visit. The visits to feeder were estimated for each pig following the method described by Labroue et al. (1996).

Feeding behavior was described taking into account the number of visits, number of meals, amount of food consumed (g), duration of consumption (min) (sum of the duration of visits and intervals between visits concerning the same meal), ingestion rate (g/min) (ratio of the amount of food consumed and duration of visits), amount of food consumed (g), length of use (min). The value of each criterion was calculated to describe the feeding behavior of each pig over the whole trial. Water intake was measured and recorded daily.

The social behavior of the pigs was studied during the first 11 of the 15 days of growing period (total trial period) using a video recording system (Professional Surveillance System (PSS), at three different times after the formation of trial groups: beginning (during 4 days), middle (during 3 days) and end of the trial (during 3 days). All the signs of aggression were recorded at the feeder and drinker. These signs were: 1. Biting: open and close the mouth on or near another pig. Bite the head, neck, ears or any part of the body of other pigs. 2. Fighting: rapid succession of aggressive events, such as pushing parallel or perpendicular to, ramming or pushing of an opponent with the head, with or without biting. 3. Reverse parallel: two pigs are head to tail, with or without biting and describe circles without coming apart. 4. Displacing: making another pig leave to get its place. 5. Chasing: actively following another pig. (Lee et al., 1982; Nielsen et al., 1995). Based on the observations described, the social rank index (SRI) was established as determined by Lee et al. (1982) and Nielsen et al.(1995), according to the following formula:

$$SRI\ (a) = \tfrac{1}{2}\ _{(D-S+N+1)}$$

who took into account the bi-directional fights, which can be numerically calculated, where, SRI(a) is the social rank index of pig (a), D: number of animals dominated by the pig (a), S: number of animals that dominate the pig (a), N: size of group.

This chronological observation design was described by Beilharz and Cox (1967) and other authors (Turner et al., 2001; Meese and Ewbank, 1972, 1973, Olesen et al., 1996; Lindberg (2001), who stated that during the first 48 h of the grouping of pigs, dominant animals impose their hierarchy. Although this hierarchical order remains relatively stable within a group, the recording is carried out at three different time points during the growing period because these authors also point out that the social ranges may not be permanent and may change as the age of the animals increases. Therefore it is important not to restrict social behavior studies only to the first 48 hours of the grouping of growing pigs. Dominance relationships do not always have a hierarchical linearity within a group of pigs (Chase, 1980). In some situations of competition for food, particularly when this resource is restricted, fights can appear abruptly, but with less intensity.

Drug administration commercial trial (Group F&W)

On day 11 of the trial , the F group (Food) was dosed with fosfomycin in food at 20 mg/kg and the W group (Water) with the same dose of fosfomycin in the water dispenser system again during 5 consecutive days (Dosatron International S.A.S. - Rue Pascal - 33370 Tresses – France). On day 15 of the trial (around 17:00 h), the antibiotic contained in food or water was removed and blood sampling was performed at the following time points post-administration of fosfomycin: 0, 1, 2, 3, 4, 6, 8 and 12 h. Blood (8 mL) was collected from each pig via venipuncture of the anterior vena cava into 10-mL sodium heparin tubes. The blood samples were immediately centrifuged and the plasma recovered and frozen at - 20 ° C until analysis.

Drug assay

Determination of fosfomycin in each sample of plasma in both studies (the basic PK study and the commercial farm study) was carried out in triplicate by high-performance liquid chromatographic-mass-mass spectrometry (HPLC-MS/MS) using fudosteine as internal standard according to the method reported by Soraci et al. (2011a).

Pharmacokinetic analysis

Pharmacokinetic analysis of individual plasma disposition was carried out using a non-compartmental method and fitting the concentration–time data to an appropriate model using pharmacokinetic software (PK Solutions 2.0 computer program, Summit Research Services, Ashland, OH 44805, USA). The areas under the curve AUC versus time were calculated by the trapezoidal method.

The bioavailability was calculated according to the following equation:

$$F\% = \frac{AUC_{oral} \times Dose_{iv}}{AUC_{iv} \times Dose_{oral}} \times 100$$

Where AUC_{oral} and AUC_{IV} are the AUCs by the oral and IV routes respectively and Dose $_{IV}$ and $Dose_{oral}$ are the administered doses by the IV and oral routes respectively.

Statistical analysis

Statistical analysis was performed using different tools of a data analysis software (JMP SAS, version 7.0, SAS Institute Inc, Cary, NC). Test of normality (PROC UNIVARIATE) and regressions were performed to analyze the different variables. For the analysis of SRI, a matrix of incidence of double entry was constructed to compare the numerical results of the aggressive interactions between pigs. Thus, comparing pairs of pigs, the animals that had higher SRI values were considered as dominant and the animals that had lower SRI values were considered as subordinate (Lee et al., 1982; Nielsen et al., 1995). Based on these results the social rank of each animal at the feeders and drinking points was established. Comparison of fosfomycin exposure between food or water groups was made using a nonparametric test (Wilcoxon test).

Results

Individual pharmacokinetic of fosfomycin after IV and oral administration in or and water

The IV elimination half-life of fosfomycin (1.5 ± 0.4 h) was similar to that observed after oral administration in food or water (1.8 ± 0.8 and 2.0 ± 0.3 h respectively) and similar to the values already published in piglets (Soraci et al., 2011a, 2011b). Thanks to the short half-life of fosfomycin, it was possible to contrast the irregular vs. rather regular plasma concentration profiles of fosfomycin after the administration in food vs. drinking water (see fig 4. F vs. W). In addition, the bioavailability was relatively similar for fosfomycin administered in food and in water (19.0 ± 1.8% and 24.0 ± 0.5 % respectively) enabling the two modalities of fosfomycin oral administration to be compared. Indeed a relatively low bioavailability (as here for fosfomycin) is a factor increasing interindividual variability (Toutain and Bousquet-Melou, 2004) and the difference observed between the food vs. the water administration of fosfomycin cannot be reported as a difference of bioavailability but only as a difference in the patterns of feeding vs. watering behavior. With an antibiotic (or another drug) having a longer terminal half-life than fosfomycin, it could anticipated there would be less inter-occasion variability in the plasma concentrations after an oral administration, especially in food, due to the smoothing effect of the terminal phase, and this is desirable for any time-dependent antibiotic.

Figures 1 show the plasma concentration profiles obtained after intravenous (IV) and oral (food or water) administration of fosfomycin in pigs during the growth phase and Table 1 gives the corresponding pharmacokinetic parameters (mean ±SD).

Impact of social rank status on the intake of food and water (F&W) and exposure variability of fosfomycin administered in these biological matrices

The studied animals consumed an average of 1318 ± 190 g of food per day (13 MJ/g of ME, 16% protein) with an intake duration/day of 39.1 ± 2.2 min. Feeding and drinking behavior was analyzed in terms of the percentage of occupation of feeders and drinkers (shown in Figure 2). Two high peaks of food consumption were observed, one at about 8 am in the morning and another at about 4 pm, indicating that the main visits were observed in daylight hours (Fig 2). The number of visits per day to the feeder was 136.0, including 7.5 meals/day at an effective intake rate of 35.0 to 40.4 g/min. Each visit lasted an average of 5.04 ± 1.40 min, with consumption of 156-202 g. The profile of water consumption was parallel to the food intake, but with a fewer number of visits/day (87.3 visits/day). For pigs less than 40 kg, 85% of drinking episodes occurred within 10 minutes of a meal. The average water consumption was 3.7 ± 0.3 L/day/animal. Table 2 shows the mean values for dietary behavior of the groups of pigs.

Figure 2 shows the mean values (n = 36) of the occupancy rate of the feeders and drinkers for observation periods of 10 min over 24 h in pigs fed *ad libitum* during the 15 days of the trial.

Figure 3 shows the mean number of fights recorded at feeders and drinkers during the 11 days of the trial at three different time points after the formation of trial groups: beginning (during 4 days), middle (during 3 days) and end of the trial (during 3 days) for each of the 36 pigs included in this study.

The largest number of fights was recorded during the first two days of grouping of pigs at the beginning of the trial and on the eight and ninth day of the trial (See Fig 3). They occurred most frequently and/or with more intensity at the feeders than at the drinking points.

The study of social behavior on the determination of SRI showed that there was 39.0% of dominant animals and 33.3% of subordinate animals at feeders, while at the drinkers the values were 27.6% and 6.1% respectively.

Amount of food consumed according to the social rank (dominant, intermediate and subordinate) and the difference in consumption relative to dominant animals are show in Table 3

The individual plasma concentrations obtained after fosfomycin administration at a dose of 20 mg/kg in food (group F) and water (group W) for 5 consecutive days in pigs are shown in Figure 4. Plasma profiles versus time obtained after fosfomycine administration in water (group W) were more homogeneous compared to those obtained after fosfomycin administration in food.

Figure 5 shows the average (and SD) plasma fosfomycin concentrations versus time obtained after administration of fosfomycin in food or water at a dose of 20 mg/kg. Individual fosfomycin concentrations in plasma following its administration in food showed a high between-occasion variability (coefficients of variation (CV) ranging from 41 to 61%) whereas this variability was lower when fosfomycin was administered in the drinking water (CV from 19 to 30%).

The regression studies between the SRI (independent variable) and the AUClast of fosfomycin (dependent variable) indicated a significant linear relationship between the social status of the pig and its exposure to fosfomycin for both fosfomycin administration in food (P=0.0204) and in water (P=0.0059). The coefficients of determination (R^2) that measure the percentage of the variability that is explained by the model, were 0.2928 and 0.3678 for food or water fosfomycin administration respectively indicating that the social status of the pigs explained respectively 29.2 and 36.7% of the between-pigs variability in the fosfomycin plasma levels..

Discussion

The main result of the present experiment was the finding that the social status of pigs housed under farm conditions has a major influence on the internal exposure of an antibiotic administered by the oral route whatever the modality of administration (food or drinking water). This factor alone explained up to 29 and 37% of the interindividual variability of the internal exposure to the antibiotic after administration of fosfomycin in food or water respectively. A high variability between pigs has already been reported for antibiotic exposure when an antibiotic is administered collectively at the herd level in food or drinking water. For example del Castillo et al. (2006) reported a high variability of doxycycline plasma exposure after a doxycycline administration in food, but none of the investigated factors (health status, body temperature, room temperature, gender, body weight, dietary Ca^{++} concentration) among others, was able to explain this interindividual variability. del Castillo et al.(2006) concluded that factors related to individual feeding habits might be at the origin of this variability and that it would be necessary to design antibiotic dosing regimens taking into account the effect of differences in individual feeding behaviour within pig herds.

The present experiment confirms this hypothesis and to our knowledge it is the first time that the characterization of the social and feeding behavior of pigs confined in intensive farming is shown to have a critical effect on plasma concentrations of an antimicrobial administered by either food or water.

The voluntary food intake study showed two peaks of food intake in pigs observed at 8 and 16 h with no congestion of the feeders (feeders maximum occupancy: 60%/day) indicating that the hierarchy *per se*, was not a factor which limited feeders occupancy or accessibility to the antibiotic ingestion by pigs fed *ad libitum.* These findings are in accordance with those reported by De Haer and Merks (1992) and Nielsen et al (1995) for pigs growing up during the spring season. The effect of duration of light on the feeding behavior of pigs is not well documented. Hsia and Wood-Gush, 1984 reported that pigs exposed to 8.5 h / day of light, dedicated more time to eat during the first 8 hours of light compared to those pigs exposed to 24 -24 hours of light during the same period. Pigs exposed to 24 h of light have more number of meals, but of less duration .

Knowledge of the daily rhythm of food intake is important, because it is strongly influenced by the season (light cycle) and it has been suggested that the time of the year may affect the optimal dosing intervals for certain antimicrobials (del Castillo et al., 2006) especially for time-dependent antibiotics having a short half-life, as it is the case here for fosfomycin (Soraci et al., 2011a). To our knowledge, there are no data on the effect of light on the social rank of pigs.

In the present trial, the average total daily food consumption was 1318 ± 190 g per pig with an amount of food consumed per meal ranging from 156-202 g (coefficient of variation of 22.7%). This pattern of feeding behavior was similar to those reported by Labroue et al. (1994) and Quiniou et al. (1997) indicating that our observations are likely to prevail in any type of pig settings with the same generic consequences on a drug exposure. Although other factors may impact on the voluntary intake of food or water, the social hierarchy appeared to be extremely influential among the animals, generating stress and impacting directly on the food intake. The social hierarchy is a central factor in determining the feeding pattern of confined pigs (Place et al., 1995; Levasseur et al., 1996). In our study, the SRI, and number of fights allowed the classification of the pigs into three groups: dominant, intermediate (dominance not established) and subordinate, in a similar way to that described by Craig (1986) and Vargas et al. (1987). Within a group, each animal knows its place and the hierarchy distinguishes dominant and subordinate animals (Place et al., 1995). The dominant animal has priority over the subordinate in terms of feeding behavior and assigned resting place in the pen (Place et al., 1995).

Interestingly it has been determined that the social dominance of pigs is a characteristic with high heritability of 0.47 (Chen et al., 2010) when selecting animals. To summarize, social hierarchy has a major influence on the interindividual variability to antibiotic exposure in pigs it can be postulated that it cannot be easily suppressed by extrinsic factors management of pig production. There are a large number of investigations on the impact of extrinsic factors management of pig production on the establishment of the social hierarchy of the pig: available space, the number of pigs per group, access to the resource (food) restricted or *ad libitum,* the size of feeder and temperature (Barnett et al., 1992, Andersen et al., 2004 Lindberg , 2001, Lee et al, 1982.; Tan and Shackleton, 1990).The results of these studies have been used to establish balanced animal welfare standards and production. However, social rank is very much subject to intrinsic factors specific to each animal such as: age, weight, sex, race, personality and fighting ability, among others (Andersen et al., 2004).

When fosfomycin was given in drinking water, a modality of drug administration apparently less challenging than food-administration in terms of interindividual competition, the interindividual variability explained by the social ranking was even higher than when the test antibiotic was given in food despite the fact that the water consumption (3.7 ± 0.3 L day/pig) was less variable than the food intake. This variability may be explained by the competitions among pigs to access drinking water together while there was only two nipple drinker for 18 pigs. Indeed, the sight of an individual drinking may encourage similar behavior in other members of the group, a process known as social facilitation (Turner et al., 2000). This synchronization of drinking behavior and its resulting competition between pigs to access together a limited number of nipples may be at the origin of the observed variability of fosfomycin exposure. The number of fights observed in drinkers was significantly lower ($p < 0.05$) than in feeders. When growing pigs are housed in indoor pens, the group assigns areas for eating, sleeping and waste disposal. Pigs have their defecating and urinating habit in proximities of water (drinkers) (Brooks et al.,1989) . Established social status, the presence of dominant animals defecating or urinating could exert a threatening/intimidatory effect (without fighting) on subordinate animals, contributing to the impact of responsible social behavior variability of exposure of fosfomycin in water. On the other hand, it could be explained by the fact that water often is ranked lower as a resource than food.

Another aspect of social ranking is its time-development when pigs are just allocated. The largest number of fights was recorded during the first 48 h of grouping (Meese and Ewbank, 1972, 1973; Olesen et al., 1996; Turner et al., 2001) and showed significant differences ($p < 0.05$) with respect to observations made during middle and end of the 11 days of trial. Similar results were observed by Meese and Ewbank (1972). Social competition for food resource in pigs (magnified when this resource is limited) shows aggressive behavior and interactions, associated to fights. These first fights are responsible for establishing a strong social hierarchy. (Vargas et al., 1987). Once the social rank within the group has been established the animals develop a “social memory "(memory associated to the results of previous fights). In this way when two members of the same group interact, the social order is maintained through signs or threats, without having to fight (Beilharz and Cox , 1967). This suggests that whatever the occurrence or not of fighting, the social ranking is potentially able to influence on antibiotic exposure during the whole period of housing in the same pen because it expresses a fundamental behavioral property of pigs housed in groups (Turner et al., 2000).

Familiarity between pigs is often possible to significantly reduce the number of fights at the time of grouping (Stookey and Gonyou, 1994, 1998). The familiarity between pigs is based on links that are created and maintained through constant physical contact. It seems to favor the stability of the social structure within a group (Stookey and Gonyou 1994; Ekkel et al, 1995). This is important aspect to take into account when organizing growing groups in pigs production . The decrease in the number of fights means less stress within the group, better food or water consumption (favoring antibiotic dosing), and promotes animal welfare (Ekkel, et al., 1995).

Although our work was done on clinically healthy animals, it is important to consider that disease situations can significantly reduce the consumption of feed and water (Millman et al., 2004) and modify the pharmacokinetics of the antibiotic incorporated into these biological matrices (Pijpers et al., 1991). The sick pigs are lethargic and prefer to stay close to walls or feeders . Besides, they are often rejected by the other pigs even to the extent of being attacked and impacting on the social rank of the group (Millman et al., 2004).

The range of concentrations observed after the administration of fosfomycin either in food or in drinking water leads to a number of pigs in the treated group (particularly, the subordinate pigs) being exposed to rather low concentrations of fosfomycin not able to maintain adequate plasma concentrations above the typical MIC for fosfomycin. This situation can contribute to a lack of antibiotic efficacy in the treated group and/or favor development of resistance. On the other hand, the animals with highest antibiotic concentrations could have more remnant antibiotic residues in their tissues requiring a more prolonged withdrawal time.

Conclusion

This work clearly shows the high variability of feeding behavior in pigs during the growth phase and in turn, the important fluctuations of intake of an antibiotic incorporated in medicated food or water. This study has demonstrated that there are major influences of social hierarchy on the internal exposure as measured by the AUClast of fosfomycin after oral administration in food or water. However, the more consistent fosfomycin concentrations observed after administration in water suggest that for a time-dependent antibiotic having a short half-life, administration in drinking water is likely to optimize the antibiotic efficacy and to minimize the risk of development of antibiotic resistance. Finally, this research work highlights the importance of differences in antibiotic disposition observed when pharmacokinetic studies are carried out in commercial farms (population studies) versus studies conducted under well controlled conditions.

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Figure Legends

Figure 1: Semi-logarithmic plot of plasma concentration profiles of fosfomycin obtained after a single dose intravenous (IV) and oral (food or water) administration of fosfomycin in pigs (n= 6 per group) at a dose of 15 and 20 mg/kg respectively during the growth phase and under laboratory conditions.

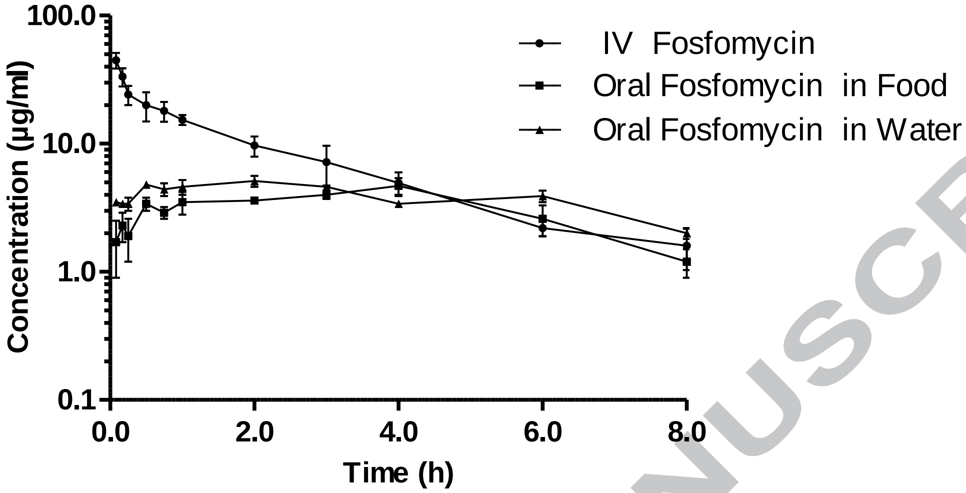
Figure 2: Daily mean values (n = 36) in occupancy rate of feeders and drinkers during observation periods of 10 min over 24 h in pigs fed *ad libitum* computed over the 15 days of the trial.

Figure 3: Mean number of fights recorded in feeders and drinkers during the first 11 days of the trial at different times after the formation of trial groups namely at 1,2,3,4,5,8,9 and 11 days for each of the 36 pigs under study.

Figure 4: Plasma concentrations of fosfomycin obtained after fosfomycin administration at a dose of 20 mg/kg in the food (F) or water (W) (groups F&W) for 36 pigs under farm conditions (n= 18 per group).

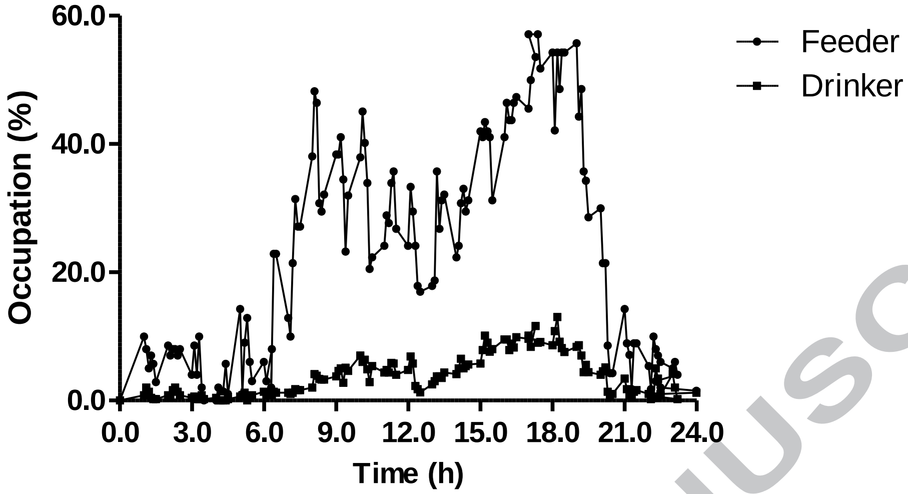
Figure 5: Average plasma concentrations (±SD) versus time of fosfomycin after administration of fosfomycin in food or water (groups F&W) for 5 consecutive days at a dose of 20 mg/kg in 36 pigs (n= 18 per group).

Figure 1



100.0
10.0
1.0
0.1
Concentration (µg/ml)
0.0
2.0
4.0
6.0
8.0
Time (h)
IV Fosfomycin
Oral Fosfomycin in Food
Oral Fosfomycin in Water

Figure 2



60.0
40.0
20.0
0.0
Occupation (%)
0.0
3.0
6.0
9.0
12.0
15.0
18.0
21.0
24.0
Time (h)
Feeder
Drinker

Figure 3

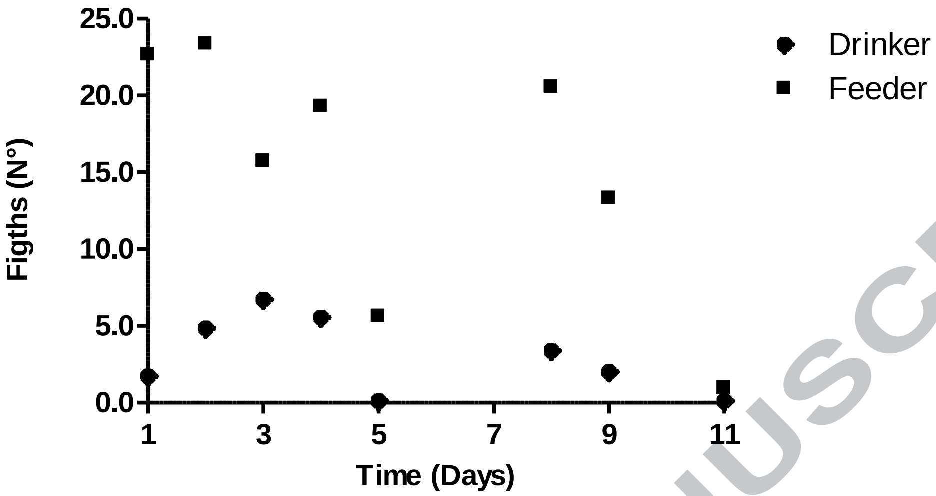
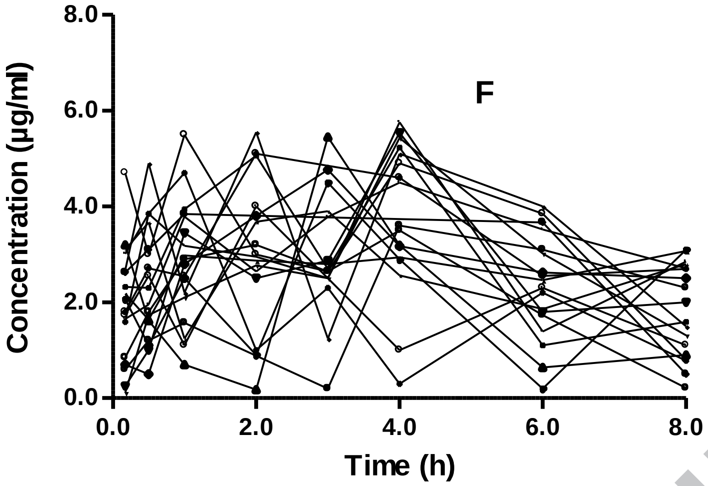
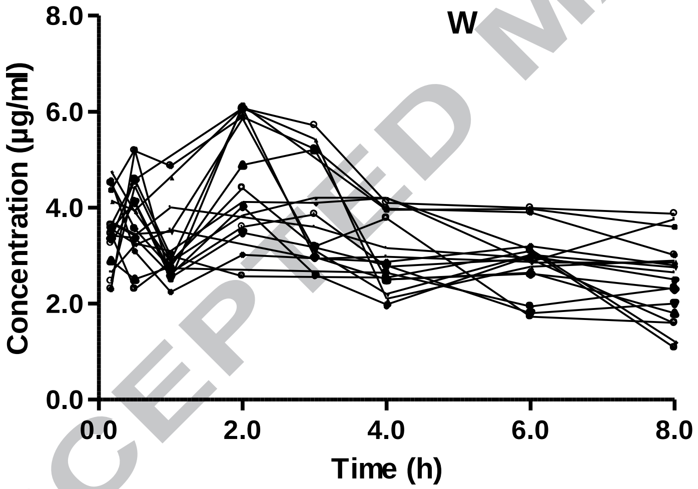

25.0
20.0
15.0
10.0
5.0
0.0
Figths (N°)
1
3
5
7
9
11
Time (Days)
Drinker
Feeder

Figure 4



F
8.0
6.0
4.0
2.0
0.0
Concentration (µg/ml)
0.0
2.0
4.0
6.0
8.0
Time (h)



W
8.0
6.0
4.0
2.0
0.0
Concentration (µg/ml)
0.0
2.0
4.0
6.0
8.0
Time (h)

Figure 5

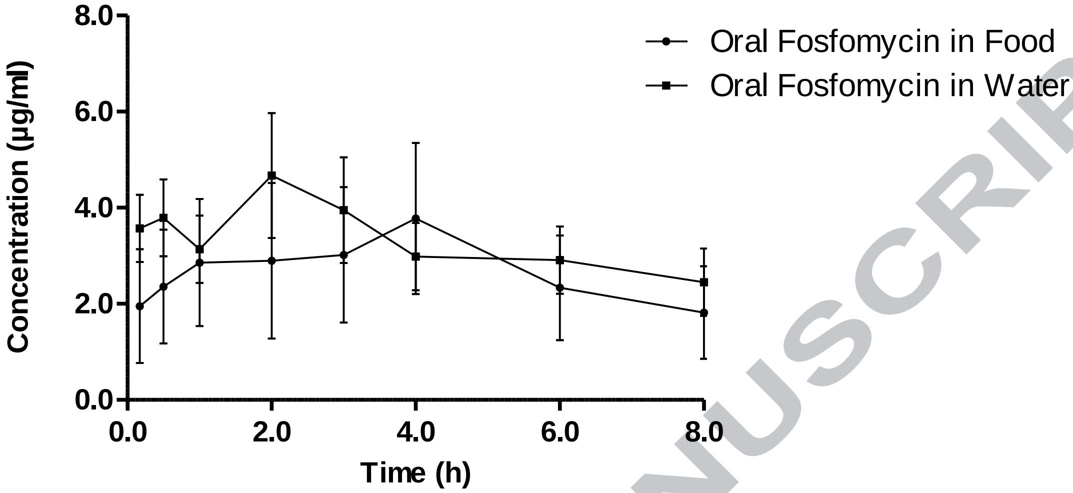

8.0
6.0
4.0
2.0
0.0
Concentration (µg/ml)
0.0
2.0
4.0
6.0
8.0
Time (h)
Oral Fosfomycin in Food
Oral Fosfomycin in Water

Table 1: Mean pharmacokinetic parameters of fosfomycin obtained after IV and oral (food and water) administration of fosfomycin in 3 groups of six pigs (mean ±SD).

Parameters	IV	Oral food	Oral water
T½β (h)	1.5 ± 0.4	1.8 ± 0.8	2.0 ± 0.3l
AUC_{0-8} (µg*h/mL)	101.0 ± 22.6	27.0 ± 9.2	31.6 ± 2.1
Vd_{area} (mL/Kg)	273.0 ± 40.7		
Cl (mL/h/kg)	140.0 ± 39.6		
MRT (h)	3.5 ± 1.4		
T_{max} (h)		4.0 ± 0.0	2.0 ± 0.0
C_{max} (µg/mL)		4.7 ± 0.9	5.11 ± 0.0
F (%)		19.0 ± 1.8	24.0 ± 0.4

$T_{1/2}\beta$: Plasma half-life; AUC_{0-8}: Area under the plasma concentration–time curve from 0 to 8 h; Vd_{area}: Volume of distribution; CL: Plasma clearance; MRT: Mean residence time; C_{max}: Maximum plasma concentration after the oral dose; T_{max}: Time of Cmax; F%: Bioavailability

Table 2: Mean (±SD) values for dietary behavior of pigs (n = 36).

Feeding Parameters	Values	
Amount of food consumed/day/animal (g)	1318 ± 190	
Ingestion rate (g/min)	35.0 - 40.4	
Number of meals/day	7.5	
Total number of visits to the feeders and drinkers	Feeder	Drinker
	136.0	87.3
Intake Duration/day (min)	39.1 ± 2.2	
Intake Duration /meal (min)	5.0 ± 1.4	
Amount of food consumed/meal (g)	156 - 202	

Table 3: Amount of food consumed in g/day, according to the social rank (dominant, intermediate and subordinate) and the difference in consumption relative to dominant animals.

Parameters	Dominant pigs	Intermediate pigs	Subordinate pigs
Amount of food consumed / meal (g)	202	165	156
% of intake reduction with respect to the dominant	--	18.3	22.7