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Species differences in plasma and tissue concentrations of florfenicol between sheep and rabbits

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ABSTRACT

Objective. The aim of this study was to compare tissue and plasma concentrations of florfenicol (FFC) and its metabolite florfenicol amine (FFC-a) between sheep and rabbits, after intramuscular administration of 20 mg FFC/kg. **Materials and methods.** Five Suffolk Down sheep and six New Zealand rabbits were used in this study. Blood samples were collected before FFC administration and at 0.25, 0.5, 1, 1.5, 2, 3 and 4 hours after treatment. At 4 hours after treatment, euthanasia was applied to animals. Plasma and tissue concentrations of FFC and FFC-a were determined by HPLC. **Results.** For FFC, maximum plasma concentrations, absorption rate, absorption half-life, distribution rate, and area under the plasma concentration-time curve were all found to be significantly higher in rabbits than in sheep. Similarly, for FFC-a, significantly higher maximum plasma concentrations and area under the concentration-time curve were observed in rabbits as compared to sheep. The metabolite ratio was higher in rabbits (12.7±3.07%) compared to sheep (3.99±0.87%) ($p<0.05$), as were the tissue concentrations of FFC and FFC-a. **Conclusions.** Significant differences in the pharmacokinetics and tissue concentrations of FFC, and its metabolite FFC-a, were observed between these two animal species. The higher concentrations of FFC-a in rabbits indicate a greater level of FFC metabolism as compared to sheep. This should be considered when establishing dosage and frequency of FFC administration for rabbits.

Keywords: Antibiotics, chromatography, metabolism, pharmacokinetics (*Source: MeSH*).

RESUMEN

Objetivo. Comparar las concentraciones plasmáticas y tisulares de florfenicol (FFC) y su metabolito florfenicol amina (FFC-a) entre ovinos y conejos, posterior a la administración intramuscular de 20 mg/kg de FFC. **Materiales y métodos.** Cinco ovinos Suffolk Down y seis conejos Neozelandés fueron utilizados en el estudio. Se colectaron muestras de sangre, previo a la administración de FFC, y a las 0.25, 0.5, 1, 1.5, 2, 3 y 4 horas posteriores al tratamiento. A las 4 horas posteriores al tratamiento, a los animales se les aplicó la eutanasia. Las concentraciones plasmáticas y tisulares de FFC y FFC-a fueron determinadas mediante HPLC. **Resultados.** Las concentraciones plasmáticas máximas, tasa de absorción, vida media de absorción, tasa de distribución y área bajo la curva de

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FFC, fueron significativamente mayores en conejos respecto a los ovinos. Asimismo, para FFC-a, las concentraciones plasmáticas máximas y área bajo la curva de concentraciones plasmáticas en el tiempo fueron significativamente mayores en conejos respecto a los ovinos. La proporción de metabolito fue mayor en conejos (12.7±3.07%) en comparación con ovinos (3.99±0.87%) ($p<0.05$), al igual que las concentraciones tisulares de FFC y FFC-a. **Conclusiones.** Se observaron diferencias significativas en la farmacocinética y concentraciones tisulares de FFC y FFC-a entre estas dos especies. La mayor concentración de FFC-a en conejos indica un mayor nivel de metabolismo de FFC, respecto a los ovinos. Esto es importante de considerar al momento de establecer dosificaciones y frecuencia de administración de FFC en conejos.

Palabras clave: Antibióticos, cromatografía, farmacocinética, metabolismo (*Fuente: MeSH*).

INTRODUCTION

Florfenicol (FFC) is a broad spectrum, highly effective antibiotic for the control of respiratory tract infections in bovine and pigs. (1). FFC has high bioavailability, good tissue penetration, and rapid elimination – attributes which make it particularly suitable for use in animals (1). FFC is effective against many Gram-positive and Gram-negative pathogens such as *Pasteurella spp.* (2), *Staphylococcus aureus* (1), *Klebsiella pneumoniae* (1), and *Escherichia coli* (2). It has been approved for use in ruminants and pigs (3) and has the potential to be used in minor species such as rabbits (1,2).

FFC is a highly lipophilic drug (4) and is metabolised in the liver by the cytochrome P450 (CYP450) system (2,3,4,5). Studies have shown that after its administration, FFC is partially transformed into various metabolites, with florfenicol amine (FFC-a) being the largest of all metabolites derived from FFC (6).

The pharmacokinetics of FFC has been described in chickens (4), pigs (7), dogs (8), rabbits (9), sheep (10), and cattle (11,12). It is known that the pharmacokinetics of drugs in animals can vary depending on different factors, including variations between species (13).

CYP450 enzymes play a crucial role in xenobiotic metabolism in mammalian species (14), converting several drugs to products that can be excreted from the body. This system is located primarily in the liver, and is composed of several enzyme subtypes – of which CYP3A is one of the main enzymes participating in drug metabolism (2,14). It is known that differences in the metabolism and excretion of drugs exist between rabbits (monogastric) and ruminants such as sheep and cattle (15,16).

Comparative pharmacokinetics of FFC have been described in camels, sheep and goats (17), and also in birds such as chickens, pigeons and quail (18). However, to our knowledge, there are no available reports comparing tissue and plasma concentrations of FFC and FFC-a between sheep and rabbits. Both are species of veterinary interest, and are also representatives of ruminant and monogastric animals. As such, they are informative species to use for comparative studies on the differences in pharmacokinetics and metabolism of antibiotics. Therefore, the objective of this study was to investigate whether there are differences in plasma and tissue concentrations of FFC and FFC-a between sheep and rabbits treated with FFC by the intramuscular (IM) route.

MATERIALS AND METHODS

Experimental animals and location. Five adult clinically healthy Suffolk Down sheep; castrated males of 12 months of age, with 65±5 kg body weight (BW), were used in this study. Sheep were housed in collective pens on a dry bed, fed daily with alfalfa hay, and supplemented with concentrate (300 g/sheep). Six clinically healthy New Zealand rabbits; males of 6 months of age between 3.5±0.5 kg BW, were used in this study. Rabbits were housed individually with an automatic water supply. Animals were fed with pelleted hay concentrate, and water was provided *ad libitum* to both species.

All animals were deemed clinically healthy and capable to participate in the study after clinical and parasitological examinations, and clinical biochemical blood tests. Animals had no previous exposure to antibiotics, and no drugs were given to the animals for one month prior to the study.

The study was conducted at the Faculty of Veterinary Sciences, Universidad de Concepción, Chillán, Chile, at the Large Animal Hospital Facilities. Analytical procedures were performed at the Laboratory of Pharmacology, of the Faculty of Veterinary Sciences of the University of Concepción.

Drugs and reagents. The FFC analytical standard (99.4% assay purity) and FFC-a (99.8% assay purity) were purchased from Sigma (Saint Louis, MO, USA), and Toronto Research Chemicals (North York, ON, Canada), respectively. FFC 30% injectable solution (Nuflor®) was purchased from Intervet Chile (under license of Schering Plough, Santé Animale ZA la Grindoliere-Segré France). Additionally, the injectable solution of FFC was diluted in a solution made up of N-methyl pyrrolidone, polyethylene glycol and propylene glycol (36:21:43, v/v) to obtain a final concentration of 60 mg/mL. This dilution was necessary in order to obtain an appropriate volume of injection and to facilitate the IM injection of 1 mL by 3 kg BW in rabbits. We also observed that density characteristics of the original solution impeded an adequate and precise administration of FFC in rabbits, due to the small volume of injection and the high density of the solution.

All reagents used for extraction and chromatographic procedures were high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany).

Experimental design. Animals received an IM injection of FFC at a dose of 20 mg/kg BW, into the left hind limb. FFC was injected in all animals at 8.00 am to avoid day/night cycle variations. In sheep, FFC 30% injectable solution was administered; rabbits received injection from an FFC 6% solution. The moment of injection was considered as time zero (T0). For the pharmacokinetic study, blood samples were collected before FFC treatment, and at 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours after its administration. At 4 hours after treatment, euthanasia was applied to animals with an anaesthetic overdose, according to the recommendations of the AVMA Guidelines for the Euthanasia of Animals (19). In sheep, euthanasia was performed through intravenous (IV) injection of 0.5 mg/kg xylazine (XILAGESIC®, Drag Pharma Chile Invetec S.A., Chile) and euthanasia solution injection (T-61®, MSD Animal Health, under license of Intervet International B.V., Holland). Rabbits were euthanised with 4 mg/kg of xylazine by IV injection, followed by thiopental injection (OPET®, produced and distributed by Pro-Vet S.A.).

Sampling procedures. Blood samples were collected from the jugular vein in sheep and from the marginal vein in the ear of rabbits (5 mL in sheep, 1.5 mL in rabbits). Samples were collected in heparinised tubes and centrifuged at 1372 *x g* to obtain the plasma, which was then stored at -18°C. Four hours after treatment, euthanasia protocol was applied to sheep and rabbits according to procedure previously described and samples were collected from muscle, liver, kidney, lung, spleen, and brain, homogenised and stored at -18°C until analysis at the Laboratory of Pharmacology.

Analytical procedures. Blood and tissue samples were processed for analysis by high performance liquid chromatography (HPLC). Drug concentrations were determined from peak areas, and by using calibration curves. Calibration curves were obtained from the analysis of tissue samples not treated with antibiotics (blank), through the addition of known concentrations of FFC and FFC-a in a range between 0.1 and 10 µg/g. Plasma and tissue concentrations of FFC and FFC-a were determined in sheep and rabbits according to the technique described by Hormazábal et al (20), with slight modifications – mainly in the amount of tissue used. In this study, 2 g of tissue were used for the analysis of samples from sheep. For the analysis of plasma samples the procedure was the same, except that 1 mL of plasma was used. The same extraction method was used for the analysis of samples from rabbits. In rabbits, 0.5 g of tissue and 0.5 mL of plasma were used to determine FFC and FFC-a concentrations.

Both the drug and metabolite were analysed using an analytical column, Supelcosil LC18 (4.6 x 150 mm; 5 µm; Supelco Inc., Bellefonte, PA, USA), maintained in a column oven at 30°C (CTO-10AS vp; Shimadzu, Kyoto, Japan). For FFC, the mobile phase was potassium phosphate (0.01 M) and acetonitrile at 90:10 v/v, in addition to 100 µL of triethylamine and 2 mL of sodium dodecyl sulphate. The pH was adjusted to 4.0. FFC and FFC-a were detected at 225 nm in a UV detector (SPD-10Avp-Detector, Shimadzu, Japan). For FFC-a, the mobile phase was a mixture of two solutions: A and B (68:32). Solution A was 0.02 M heptanesulphonate and 0.025 M trisodium phosphate (pH 3.85). Solution B was methanol containing 0.1% triethylamine. The mobile phase was pumped at 0.8 mL/min for FFC and at 0.6 mL/min for FFC-a.

Validation parameters of FFC and FFC-a. The analytical methods for FFC and FFC-a determination in sheep and rabbit were validated according to the procedures described by the European Union (21). The analytical method used for FFC and FFC-a determination in plasma of sheep was previously validated and described by Palma et al (6). Validation of the analytical methodology in tissues from sheep was previously described in our laboratory by Pérez et al (22), and parameters such as linearity (r2), recovery (%), precision (CV%), limit of detection (LOD) and limit of quantification (LOQ) were determined. Validation of the analytical method in plasma of rabbits was previously described by Pérez et al (3). The analytical methodology in tissues from rabbits was validated and yielded overall mean recoveries of FFC and FFC-a of 82.22 ± 5.7% and 81.72 ± 3.8%, respectively.

Pharmacokinetic analysis. The FFC and FFC-a pharmacokinetic parameters were analysed using a non-compartmental model with the program PK Solutions 2.0 (Summit Research Services, Ashland, OH, USA). Absorption half-life ($t_{½ab}$) and distribution half-life ($t_{½α}$) were calculated as ln $2/k_{ab}$ and ln 2/α, respectively, where absorption rate (k_{ab}) is the first order absorption constant and α is the first order distribution constant. Maximum concentration (C_{max}) and the time in which C_{max} was obtained (T_{max}) were calculated from the concentration/time curve corresponding to each animal. The area under the curve of plasma concentrations in time was calculated from t_0 until the last time of sampling (AUC_{0-t}) using the method of trapezoid rules.

Metabolite ratio was calculated using the formula:

$$MR = \frac{AUC_{0\_t}FFC-a}{AUC_{0\_t}FFC} \times 100$$

Statistical analysis. Pharmacokinetic parameters are shown as mean ± SEM, and were compared by Student t-test. Mean plasma concentrations trough time were analysed by a two way analysis of variance. A Bonferroni Test was used to compare means. All statistical analyses were performed using GraphPad Prism (v. 5.0; Graph Pad Software Inc., San Diego, CA, USA). Significance level was set at $p \leq 0.05$, and trends were considered from $p > 0.05$ to $p < 0.1$.

Ethical aspects. All experiments were performed with the authorisation of the Ethical Committee for Animal Experimentation of the Faculty of Veterinary Sciences, Universidad de Concepción, Chile. The authorisation number of the Ethical Committee was CBE-12-2012. Experimental procedures were performed in accordance with the recommendations of the European Commission Final Report, 2009 (Revision of Directive 86/609/EEC on the protection of animals used for scientific purposes) (23).

RESULTS

Pharmacokinetic analysis of FFC. Mean ± SEM FFC plasma concentration-time curves for sheep and rabbits are shown in figure 1. FFC plasma concentrations were significantly higher in rabbits than in sheep ($p < 0.01$) at 0.5, 1, 1.5 and 2 hours after IM administration. Both species reached peak concentrations of FFC close to 1 hour after treatment.

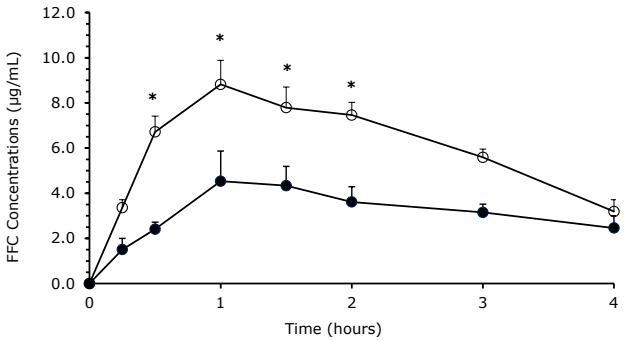

Figure 1. Plasma concentration-time curves of florfenicol (FFC) administered by IM route at a dose of 20 mg/kg in sheep (black circles) (n=5) and rabbits (white circles) (n=6). Each data point represents the mean ± SEM. *: Significant at $p < 0.05$, significant differences between both species at the same hour of sampling.

The mean values of pharmacokinetic variables calculated for FFC are presented in table 1. In rabbits, the average C_{max} and AUC_{0-t} for FFC was higher than in sheep ($p < 0.05$). Significant differences were also obtained when comparing $t_{½ab}$ between the two species, where higher values were observed in sheep than in rabbits. Average $t_{½α}$ values showed a trend of being higher in sheep than in rabbits.

Table 1. Mean values (± SEM) of pharmacokinetic parameters for FFC in rabbits and sheep treated with 20 mg/kg of FFC by IM route.

PK Parameter	Sheep	Rabbits	P value
C_{max} (µg mL^{-1})	4.94 ± 1.25	9.65 ± 0.73	0.008*
T_{max} (h)	1.60 ± 0.37	1.08 ± 0.13	0.187
K_{ab} (h^{-1})	1.17 ± 0.16	1.95 ± 0.10	0.002*
$T_{½\ ab}$ (h)	0.65 ± 0.11	0.36 ± 0.02	0.019*
$K_{α}$ (h^{-1})	1.09 ± 0.28	2.03 ± 0.07	0.006*
$T_{½\ α}$ (h)	0.86 ± 0.29	0.35 ± 0.01	0.083
AUC_{0-t} (µg·h·mL^{-1})	12.80 ± 2.29	24.42 ± 1.22	0.001*

Each value represents the mean ± SEM for sheep (n=5) and rabbits (n=6). $C_{max,}$ Maximum plasma concentration; $T_{max,}$ time to obtain $C_{max,}$; K_{ab}, absorption rate constant; $t_{1/2\ ab}$, absorption half-life; K_{α}, distribution rate constant; $t_{1/2\alpha}$, distribution half-life; AUC_{0-t}, area under the concentration-time curve from zero to the last concentration measured. *: Significant at $p<0.05$, significant differences between both species.

Tissue concentrations of FFC. With regard to tissue concentrations of FFC (µg/g) 4 hours after administration, these were significantly higher ($p\leq0.05$) in rabbits compared to sheep in all tissues studied, except in kidney tissue. However, there was a trend for kidney FFC tissue concentrations to be higher in rabbits (7.85 ± 1.45 µg g^{-1}) compared to the concentrations observed in sheep (4.74 ± 0.55 µg g^{-1}) ($p=0.097$) (Figure 2). In both species, the highest concentrations of FFC were found in the kidney.

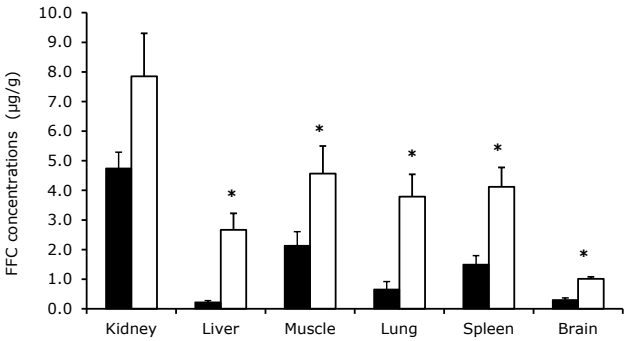

Figure 2. Mean ± SEM of tissue concentrations of florfenicol (FFC) at 4 hours after IM administration at a dose of 20 mg/kg in sheep (black bars) (n=5) and rabbits (open bars) (n=6). *: Significant at $p<0.05$, significant differences between both species.

Pharmacokinetic analysis of FFC-a. Significantly higher plasma concentrations of FFC-a were observed in rabbits compared to sheep at 1.5, 2, 3 and 4 hours after FFC administration ($p<0.01$). The FFC-a plasma concentration-time curves in sheep and rabbits are shown in figure 3. FFC-a reached significantly higher C_{max} and AUC_{0-t} values in rabbits than in sheep, ($p<0.01$) and ($p<0.01$), respectively, as shown in table 2. In rabbits, the calculated values of metabolite ratio were significantly higher (12.70 ± 3.07%) than those observed in sheep (3.99 ± 0.87%) ($p<0.05$).

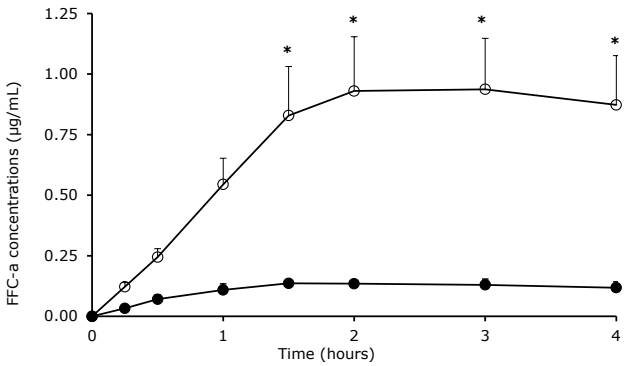

Figure 3. Plasma concentration-time curves of florfenicol-amine (FFC-a) in sheep (black circles) (n=5) and rabbits (white circles) (n=6) treated with an IM dose of 20 mg/kg florfenicol (FFC). Each data point represents the mean ± SEM. *: Significant at $p<0.05$, significant differences between both species at the same hour of sampling.

Table 2. Mean values (± SEM) of pharmacokinetic parameters for FFC-a in rabbits and sheep treated with 20 mg/kg of FFC by IM route.

PK Parameter	Sheep	Rabbits	P value
C_{max} (µg mL^{-1})	0.17 ± 0.01	1.04 ± 0.20	0.004*
T_{max} (h)	2.20 ± 0.56	2.92 ± 0.33	0.278
K_{ma} (h^{-1})	2.10 ± 0.83	2.51 ± 0.45	0.659
$t_{1/2\ ma}$ (h)	0.69 ± 0.30	0.32 ± 0.05	0.213
K_{α} (h^{-1})	1.12 ± 0.55	2.71 ± 0.46	0.052
$t_{1/2\ \alpha}$ (h)	3.49 ± 1.85	1.05 ± 0.73	0.221
AUC_{0-t} (µg·h·mL^{-1})	0.44 ± 0.02	2.93 ± 0.62	0.006*

Each value represents the mean ± SEM for sheep (n=5) and rabbits (n=6). $C_{max,}$ Maximum plasma concentration; $T_{max,}$ time to obtain $C_{max,}$; K_{ma}, metabolite appearance constant; $t_{1/2\ am}$, half-life of metabolite appearance; K_{α}, distribution rate constant; $t_{1/2\ \alpha}$, distribution half-life; AUC_{0-t}, area under the concentration-time curve from zero to the last concentration measured. *: Significant at $p<0.05$, significant differences between both species.

Tissue concentrations of FFC-a. Tissue concentrations of FFC-a were significantly higher ($p<0.05$) in rabbits compared to sheep in all tissues that were analysed. In both species, the highest concentration of metabolite was observed in the kidney (Figure 4).

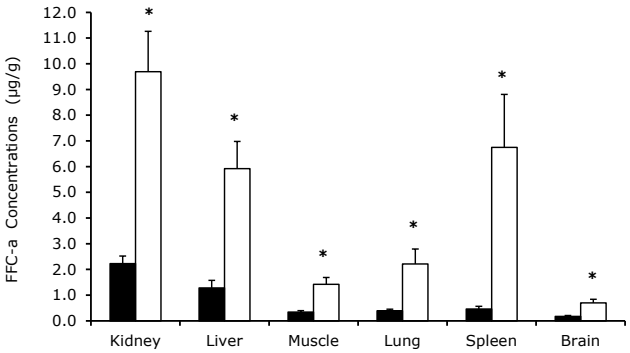

Figure 4. Mean ± SEM of tissue concentrations of florfenicol amine (FFC-a) at 4 hours in sheep (black bars) (n=5) and rabbits (open bars) (n=6), after IM administration of florfenicol (FFC) at a dose of 20 mg/kg. Each data point represents the mean ± SEM. *: Significant at $p<0.05$, significant differences between both species.

DISCUSSION

This study compared plasma and tissue concentrations of FFC and its metabolite FFC-a between sheep and rabbits after IM administration. The results demonstrate that there are significant differences between these animal species in terms of the distribution of the drug and its metabolite in plasma and tissue, indicating species differences in pharmacokinetic processes. We have previously studied the pharmacokinetics of FFC and FFC-a after IV administration in sheep (10) and rabbits (3). After IV injection of a dose of FFC of 20 mg/kg, the main differences in pharmacokinetics observed between these two species were in relation to elimination half-life and AUC_{0-t} – both of which were higher in sheep than in rabbits. Moreover, the persistence period of plasma concentrations over LOQ in sheep was 48 hours, while in rabbits it was 8 hours. On the other hand, total plasma clearance (CL_T) was higher in rabbits than in sheep, indicating that FFC is eliminated faster from rabbits than from sheep.

The pharmacokinetics of FFC after IM administration of 20 mg/kg have also been studied in sheep by Jianzhong et al (24). These authors reported higher values of AUC than those which we observed in the present study – which can be explained by their longer sampling period (24 hours). However, Jianzhong et al (24) observed a lower absorption half-life (0.27±0.03 hours) than the values reported in this study.

In rabbits, Koc et al (9) obtained an AUC after IM administration that was higher than our results of AUC (39.10±10.12 $\mu g \cdot h \cdot mL^{-1}$) – however this was expected because they used a higher dose of FFC (25 mg/kg bw). El-Aty et al (25) described values of AUC for FFC (86.56±11.99 $\mu g \cdot h \cdot mL^{-1}$) that are also higher than those observed in this study, after IM administration of a dose of 30 mg/kg of FFC. Our values of C_{max} of FFC were lower than values described by El-Aty et al (21.65±2.57 µg/mL) (25), but higher than the C_{max} described by Koc et al (8.65±2.19 µg/mL) (9).

As shown in table 1, the significantly higher values of the average C_{max} of FFC observed in rabbits are consistent with the higher values of AUC_{0-t} for FFC in rabbits compared to sheep, and are therefore indicative of a higher bioavailability of the drug in rabbits. Whereas, the average $t_{½ab}$ was significantly lower in rabbits than in sheep, indicating a faster absorption in rabbits.

These differences in absorption may be caused by differences in regional muscular blood flow between these species (9), or they could be due to differences in a pH dependent solubility that affects the absorption of the drug (26). In addition, the difference in the concentrations of the FFC solutions that were administered to the animals (30% FFC solution in sheep and 6% FFC solution in rabbits) may explain the higher level of absorption that was observed in rabbits. The dilution of FFC used in rabbits may have influenced the process of absorption of the drug, and facilitated greater absorption after IM injection. Nonetheless, a dilution of FFC from the original formulation was necessary in order to obtain an appropriate, and precise, volume of injection in rabbits (1 mL per 3 kg bw). It should be noted that the FFC dilution that was applied in rabbits had the same three carriers as the original formulation: (N-methyl pyrrolidone, propylene glycol and polyethylene glycol). As these carriers were in the same proportions in the dilution and in the original formulation, it is probable that the drug release process was similar.

Tissue concentrations of FFC (µg/g) were higher in rabbits than in sheep in all tissues, although in kidney tissue this trend was not statistically significant. This could be due to a higher level of absorption of FFC in rabbits, and also due to differences in the distribution of FFC between the two species.

Significantly higher plasma concentrations of FFC-a were observed in rabbits compared to sheep. Marked differences were found in the plasma concentration-time curves between the species, indicating a faster increase in FFC-a appearance in rabbits. In addition, mean values of C_{max} and AUC_{0-t} for FFC-a were significantly higher in rabbits than in sheep. These results indicate that FFC experiences a higher level of metabolism in rabbits compared to sheep, and this may explain the higher plasma concentrations of FFC-a in this species. This is also demonstrated by the calculation of the metabolite ratio, which was greater in rabbits in comparison to sheep.

Tissue concentrations of FFC-a (µg/g) were significantly higher in rabbits than in sheep in all analysed tissues. This may relate to the observed higher level of biotransformation of FFC into its metabolite in rabbits compared to sheep. It also should be noted that in the liver of both species, concentrations of FFC-a were higher than those of FFC, indicating that FFC is rapidly metabolised in the liver. As expected, in both species, the highest concentrations of FFC and FFC-a were found in kidney tissue, as the kidney is the main excretion organ for the parent drug and metabolite (4).

The higher level of metabolism of FFC in rabbits compared to sheep (measured through the plasma and tissue concentrations of FFC-a) is likely indicative of species differences in the enzymatic systems that metabolise drugs, particularly CYP450. Specifically, there could be differences in the quantity of enzymes, or differences in their activities, and/or species-specific subtypes of CYP450. It is known that differences in CYP450 exist between different animal species (13,16). With regard to specific subtypes of CYP450, activities of the CYP3A subtype have been documented in rabbits and sheep (2).

Previous investigations have studied the differences in drug metabolising enzyme activities in liver preparations of several agricultural species (15, 16). In the study of Dalvi et al (15), significant differences were found between sheep and rabbits in the amount of microsomal CYP450 and the activity of benzphetamine N-demethylase – with higher values found in rabbits. Considering the report by Liu et al (2), that showed that CYP3A was involved in FFC metabolism in rabbits, we propose that the high FFC-a concentrations observed in this species are due to a greater level of activity in their liver microsomal enzymatic system.

From a therapeutic point of view, the higher level of metabolism of FFC in rabbits is related to a shorter persistence of useful antibacterial plasma concentrations of the drug. Although higher plasma concentrations of FFC were observed in rabbits compared to sheep, the higher level of metabolism in rabbits might result in therapeutic plasma concentrations of FFC being less persistent, or decreasing more rapidly.

If we take into consideration a value of minimum inhibitory concentrations (MICs) for FFC of 1 µg/mL, as has been described for sheep pathogens (27), we can observe that useful antibacterial FFC concentrations persist above this value for a longer time in sheep (ca. 11 hours) than in rabbits (ca. 4 hours) – according to values described by Jianzhong et al (24) and Park et al (1), respectively. Likewise, after IV administration of FFC, Park et al (1) reported a lower duration of plasma levels of FFC in rabbits, evidenced by a shorter elimination half-life (0.90±0.20 hours), than that described in sheep (18.83±6.76 hours) (24). In addition, there is evidence that clearance of FFC occurs at a high level in rabbits (1); consequently the drug is rapidly eliminated from the body in this species.

MIC values of FFC have been described for major pathogens in sheep. Berge et al (27) described MIC values for *M. haemolytica* and for *P. multocida* that ranged between 0.25 and 1 µg/mL (minimum and maximum MIC values), from isolates obtained from sheep with respiratory tract disease. In the present study, the plasma concentrations of FFC in sheep were above these MIC values during the 4 hours in which plasma concentrations were measured (FFC concentrations ranged between 1.5 and 4.5 µg/mL). Therefore, concentrations of FFC were 4 times higher than the maximum MIC values described for sheep pathogens. In rabbits, Koc et al (9) have suggested using a MIC of 2 µg/mL as a reference value. MIC values have not yet been reported from isolates of the major pathogens of rabbits, but in studies of other species, a concentration of 2 µg/mL FFC has shown high efficacy against most pathogens (24). In our study, plasma concentrations of FFC were 4 times above this level during the 4 hours of sample monitoring.

Concerning FFC concentrations observed in lung tissue; in sheep these concentrations (0.66±0.27 µg/mL) were higher than the MIC_{50} values for *M. haemolytica* and *P. multocida* of 0.5 and 0.25 µg/mL, respectively. In rabbits, lung

tissue concentrations (3.79±0.75 µg/g) were higher than the MIC value of FFC of 2 µg/mL. These results make an important contribution to existing data related to the therapeutic efficacy of FFC for the treatment of diseases caused by respiratory pathogens in sheep and rabbits, especially as major pathogens in sheep such as *P. multocida* are also present in rabbits (28). However, further studies are needed to establish the therapeutic efficacy of florfenicol in rabbits, particularly considering the cecotrope ingestion, phenomenon that causes the recirculation of drugs in this specie (13,29).

Results of the present study show that the metabolism of FFC in rabbits is higher than in sheep, as evidenced by the higher values of C_{max}, T_{max} and AUC_{0-4h} of FFC-a that were obtained in rabbits. The elimination of this drug might also be faster in rabbits (1,3,10), so plasma concentrations of FFC would be expected to decrease under MIC values in a shorter time than in sheep. These results demonstrate that it is important to consider species differences in the pharmacokinetics of drugs when establishing therapeutic schemes of drug administration for an animal species.

In conclusion, in this study, significant differences in the pharmacokinetics and tissue concentrations of FFC and its metabolite FFC-a were observed between rabbits and sheep. This information should be considered when the dosage and frequency of administration of this antibiotic are established in monogastric animals (rabbits) and ruminants (sheep).These results suggest that a more frequent rate of administration of FFC may be needed in rabbits due to its pharmacokinetic characteristics.

Conflict of interests

The authors declare no conflicts of interest.

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