JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **34**, 168–175. doi: 10.1111/j.1365-2885.2010.01208.x.

Depletion study of three formulations of flumequine in edible tissues and drug transfer into chicken feathers

J. CORNEJO*
L. LAPIERRE*
D. IRAGÜEN*
N. PIZARRO*
H. HIDALGO† &
B. SAN MARTÍN*

**Laboratory of Veterinary Pharmacology, Faculty of Veterinary Sciences, Universidad de Chile, Santiago, Chile; †Laboratory of Avian Pathology, Faculty of Veterinary Sciences, Universidad de Chile, Santiago, Chile*

Cornejo, J., Lapierre, L., Iragüen, D., Pizarro, N., Hidalgo, H., San Martín, B. Depletion study of three formulations of flumequine in edible tissues and drug transfer into chicken feathers. *J. vet. Pharmacol. Therap.* **34**, 168–175.

To ensure the delivery of safe animal products to consumers, withdrawal times (WDT) of drugs must be respected. Drugs administered in therapies can also reach nonedible tissues (for humans) such as feathers; this transfer is of concern as feather meal is used in diets of food producing animals, being this a possible source of residue contamination of final products for human consumption. WDTs of three flumequine formulations (10%, 80% premix powder and 20% solution) as well as the transfer of this drug into feathers were determined. One hundred and twenty broiler chickens were allocated into four experimental groups (36 birds each). Three of them were treated with 24 mg/kg bw orally for five consecutive days of each flumequine formulation, whereas one group remained untreated (12 birds as control group). After the treatment ended, six chickens of each experimental group and two controls were slaughtered daily for 6 days. Samples of muscle, liver and feathers were collected and analyzed by liquid chromatography tandem mass spectrometry (LC MS/MS). The WDTs showed differences between formulations. Flumequine concentrations found in feathers remained high during WDT and after this period, thus suggesting that the WDTs estimated for the pharmaceutical formulation of flumequine do not guarantee the absence of this drug in chicken nonedible tissues such as feathers.

(Paper received 19 November 2009; accepted for publication 14 April 2010)

Betty San Martín, Laboratory of Veterinary Pharmacology, Faculty of Veterinary Sciences, Universidad de Chile, Santiago, Chile. E-mail: bsmartin@uchile.cl

INTRODUCTION

Quinolones are widely used in the poultry industry for the control of early mortality (Bywater, 2005; Gyles, 2008). After drug administration, residues or metabolites may persist in food with evident concern for human health.(Turnidge, 2004). Flumequine (9-fluoro-6,7-dihydro-5-methyl-1-oxo-1 H, 5H-benzo-quinolizine-2-carboxylic acid) is a member of the halogenated quinoline carboxylic acid group of antibacterial agents, with antimicrobial activity against a wide range of Gram-negative bacteria such as *Escherichia coli*, *Salmonella* spp. and *Pasteurella* spp. (Martínez *et al.*, 2006).

With the aim of minimizing the risk to human health associated with consumption of quinolones and fluoroquinolones, such as the emergence and dissemination of resistant bacteria (Anderson *et al.*, 2003; Nelson *et al.*, 2007), strict regulations have been established to control the use of such substances. To ensure human food safety, regulatory agencies of different countries have set tolerance concentrations for these compounds as maximum residue limits (MRL) (Anadón *et al.*, 2008). For example, the European Union (EU) established a MRL in chicken and turkey for flumequine of 400 μg/kg in muscle, 250 μg/kg in skin with fat, 800 μg/kg in liver and 1000 μg/kg in kidney (EMEA 2002). On the other hand, the United States of America has established more strict regulations and has withdrawn the use of fluoroquinolones in poultry, because of the fact that human infections with fluoroquinolones resistant *Campylobacter* species have become more common during the past years. These infections were associated with consumption of poultry, thus motivating the US Food and Drug Administration to propose this withdrawal (US FDA, 2005; Nelson *et al.*, 2007). Although the withdrawal was effective only for enrofloxacin, it is important to consider that the fluoroquinolones can have cross-resistance, and that fluoroquinolone-resistant infections are associated with longer recovery periods and more frequent hospitalizations than fluoroquinolone-susceptible bacterial infections (Engberg *et al.*, 2004; Nelson *et al.*, 2004).

To protect consumer health, the therapeutic use of antimicrobials in food producing animals requires the fulfillment of established WDTs for pharmaceutical formulations (Riviere *et al.*,

1998). It is known that the vehicle in which a drug is delivered may affect its pharmacokinetic parameters, such as absorption, final elimination phase and withdrawal times. Among these parameters, the absorption kinetics of a pharmaceutical formulation can be pointed out as the main cause for the differences in the depletion of a drug, and consequently, for the different WDTs between formulations. Sumano *et al.* (2001) compared four oral formulations of enrofloxacin in broiler chickens, finding significant differences, compared to the reference, in the rate of absorption of three of these formulations. Similar studies have been performed for other drugs in other animal species, such as oxytetracycline in rabbits (Chong *et al.*, 2002) and ivermectin in calves (Lifschitz *et al.*, 2004), where significant differences in the absorption kinetics of drugs between formulations were also found.

In Chile, there are at least 10 different formulations of flumequine authorized for commercial use in broiler chickens. However, there is no information available regarding any comparison of disposition kinetics in such formulations for broiler chickens whatsoever. Anadón *et al.* (2008) studied the bioavailability, among other parameters, for only one flumequine formulation. As flumequine formulations may differ in vehicle contents, variations in the absorption kinetics of these drugs cannot be discarded, thus possibly leading to variations in tissue depletion time, and consequently in WDTs. Therefore, different formulations may require a longer WDT if the drug is slowly depleted from tissues. Otherwise, a shorter WDT can be applied when faster depletion is adequately proven (Kukanich *et al.*, 2005).

Considering the pharmacokinetic characteristics of flumequine, drug accumulation in nonedible tissues such as feathers is highly probable. Feather meal is frequently incorporated as a protein source into the diets of other food animals, such as rainbow trout, shrimp and salmon (Bureau *et al.*, 2000; Bransden *et al.*, 2001; Cheng *et al.*, 2002). There is evidence that when drugs distribute by the bloodstream into the tissues, they reach the feathers as well. Malucelli *et al.* (1994), studied the tissue distribution and residues after WDT of various beta-agonists in broiler chickens, reporting higher concentrations in feathers than in other tissues. San Martín *et al.* (2007) studied the depletion of enrofloxacin in edible tissues and feathers of laying hens and found that enrofloxacin concentrations in feathers remained high after the calculated WDT for edible tissues.

There is few information available about flumequine depletion in broiler chickens (Goren *et al.*, 1982). Recently, Anadón *et al.* (2008) published a study regarding tissue distribution and depletion of flumequine in chickens for fattening. However, there is no data available that consider the differences between commercial formulations of flumequine. Furthermore, there is no information regarding the distribution and depletion of this drug in feathers of treated birds.

The objectives of this study are (i) to evaluate the depletion of flumequine in edible tissues (muscle and liver) from broiler chickens after oral administration of three commercially available formulations of this drug and (ii) to evaluate the transfer of flumequine into feathers.

MATERIALS AND METHODS

This work was performed in the Laboratory of Veterinary Pharmacology of the Faculty of Veterinary Sciences, Universidad de Chile, accredited under ISO 17025 Of.2006 standards.

Pharmaceutical formulations of flumequine

Three commercial formulations of flumequine were used: 10% and 80% premix powder and 20% solution. The labels do not contain information about the vehicles of the different formulations. Prior to the study, the concentration of flumequine was verified in the premixes and the solution. For this purpose, standard calibration curves of a certified flumequine standard were used.

Experimental animals

One hundred and twenty male broiler chickens (Ross 308 genetic) were kept from 1-day-old in individual cages (25 ± 5 °C of temperature, 50–60% relative humidity) with *ad libitum* access to water and nonmedicated feed. The cages had an elevated wire floor in order to avoid fecal contamination of feathers.

Experimental animals were kept in conditions in agreement with the animal welfare guidelines approved by the Bioethics Committee of the Veterinary Sciences Faculty, University of Chile, and the recommendations of the European Council Directive 2007/43. Throughout the experiment, the animals were monitored by an avian medicine veterinarian. The birds were sacrificed under the animal welfare rules of the European Council Directive 93/119/CE.

After 19 days of breeding, chickens were randomly allocated into four experimental groups, labeled from A to D. Groups A, B and C with 36 birds each were treated with 24 mg/kg bw orally administrated once daily for five consecutive days of flumequine 10%, 80% premix powder and 20% solution, respectively. Group D, with 12 chickens, remained as untreated control group.

After completion of the treatment, six birds of each treated group and two of the control group were sacrificed daily for 6 days. Corresponding samples were collected and individually stored at −70 ± 1 °C for chromatographic analysis. The complete liver was harvested from each bird. Approximately 300 g of muscle were taken from each chicken, corresponding to the pectoral girdle and the pelvic limb muscles. To have an appropriate amount of feathers for sample preparation, feathers from the whole body were collected from birds belonging to group A, using hot water. They were thoroughly washed to avoid dirt and skin contamination.

Reagents and chemicals standards

Standards of flumequine were supplied by Dr Ehrenstorfer Gmbh (Ausgsburg, Germany). The LC-grade acetonitrile, dichloromethane and water were from Fisher Chemicals (Fair Lawn, NJ, USA). LC-grade methanol was supplied by Merck (Darmstadt, Germany). All other reagents were of analytical reagent grade.

Table 1. Multiple reaction monitoring analysis of flumequine

Analyte	Precursor ions (*m/z*)	Product ions (*m/z*)	DP[a]	EP[b]	CE[c]	CXP[d]
FLU	262.2	202.2[f]	41	6	41	4
		244.2[e]	41	6	21	6
		126.2	41	6	59	4

Mass spectrometry conditions: A Sciex API 3200 mass spectrometer was used. Turbo spray ionization source operated in positive mode.
FLU, Flumequine; DP[a], declustering potential; EP[b], entrance potential; CE[c], collision energy; CXP[d], collision cell exit potential; [e], Confirmation ion; [f], Quantitation ion.

A 25% ammonia solution and trifluoroacetic acid were purchased from Merck.

Standard and working solutions

Standard solutions of flumequine were prepared in aqueous 0.03 M NaOH at 1000 μg/mL and stored at 4 ± 2 °C in the dark for no longer than 3 months. Matrix-matched calibration curves were spiked with the standard solution immediately prior to extraction.

Sample preparation

Four grams of tissue (muscle or liver) homogenized with a moulinette® chopper was placed into a tube with 500 μL of a 25% ammonia solution; 4 mL of acetonitrile was added and shaken in vortex for 10 min. Samples were centrifuged 10 min at 3130 ***g***. The complete upper layer, approximately 4 mL, was transferred to another tube; 4 mL of dichloromethane was added to this supernatant, and the samples were shaken for 10 min in vortex. After that, the sample was centrifuged 10 min at 3130 ***g***. Two ml of the upper layer were transferred to a 2-ml Eppendorf tube and centrifuged at 9300 ***g*** for 10 min. The supernatant was transferred to a glass tube and evaporated under a nitrogen stream at 40 ± 2 °C. The residue was dissolved with 150 μL of mobile phase, filtered through a 0.22-μm-pore-size polyvinylidene fluoride Millex-GV membrane (Hassouan *et al.*, 2007). Twenty microliters was injected into the HPLC system. Residues were separated by liquid chromatography and identified by mass detection (HPLC MS/MS), based on the method published by Van Hoof *et al.* (2005). The limit of detection (LOD) and the limit of quantification (LOQ) for the analytical method were 1.5 and 2 μg/kg, respectively. These reported values are based on quantifying product ions (Table 1). For flumequine extraction from feathers, the protocol previously described by San Martín *et al.* (2007) was used.

Liquid chromatography

The HPLC system (series 200; Perkin Elmer, Norwalk, Conn.) consists of two micro pumps, an auto sampler, a column oven and a vacuum degasser. Chromatographic separation was achieved on a C-18 Symmetry column of 3.5 μm (150 mm by 2.1 mm inside diameter; Waters, Milford, Mass.). Mobile phase A was 0.1% (vol/vol) trifluoroacetic acid in methanol, and mobile phase B was 0.1% (vol/vol) aqueous trifluoroacetic acid. The gradient program consists of constant 20% mobile phase A with 80% mobile phase B for 5 min. Then, phase A is increased to a 100% for 5 min, returning to 20% phase A with 80% mobile phase B after that and holding it for 22 min. Chromatography was performed at 30 °C at a mobile phase flow rate of 0.2 mL/min.

Mass spectrometry

A Sciex API 3200 mass spectrometer (Applied Biosystems Sciex, Concord, Ontario, Canada) was used for mass detection. Flumequine ionization was achieved using the Turbo spray ionization source operated in positive mode. Conditions and settings were curtain gas (N_2) at 10 psi, ion source gases 1 at 40 psi and 2 at 50 psi, source temperature at 550 °C, ion spray at 5500 V and collision gas pressure at 4 psi. MS/MS data on precursor and product ions (*m/z*) were collected in multiple reactions monitoring mode. Precursor and product ions, declustering potential, entrance potential, collision energy and cell exit potential were optimized for the analyte (Table 1).

Validation of analytical method

The following parameters were determined for validation of the method in muscle and liver: specificity, recovery, repeatability, within-laboratory reproducibility, decision limit (CCα), detection capability (CCβ) and linearity of the matrix-matched calibration curves. For the calculation of the recovery, repeatability and within-laboratory reproducibility, 18 aliquots of a blank material fortified at 0.5, 1 and 1.5 times the MRL, (six aliquots at each spiked level), were used for each validation parameter. For the CCα and CCβ, 20 blank samples spiked at the MRL concentration were used.

Drug quantification in experimental samples

Flumequine concentrations were calculated using the equation from the regression analysis of the matrix-matched calibration curves ($r > 0.99$) at different concentrations, to avoid extrapolations. Concentrations of working solution ranged between 1–50 μg/mL and 100–600 μg/mL.

Determination of withdrawal times

Flumequine concentrations at each sampling time were considered to determine WDTs in muscle and liver. For this purpose, the recommendations of the European Agency for the Evaluation of Medicinal Products (EMEA 1995) were adopted. The WDTs were estimated from the linear regression analysis of log-transformed tissue concentration and was determined at the time when the 95% upper one-sided tolerance limit was below

the MRL with 95% confidence. For WDT calculations, the MRLs established by the EU were used (EMEA 2002).

Flumequine quantification in feathers

To quantify flumequine concentrations in feathers, a matrix-matched calibration curves ($r > 0.99$) was used. For this purpose, feathers from untreated birds spiked with known concentrations of the certified flumequine standard were extracted and analyzed. Drug concentrations in working solutions ranged between 100 and 800 μg/mL.

RESULTS

Analytical performance

Prior to the calculation of WDTs for different formulations of flumequine, the validation of the analytical method in liver and muscle was performed. This method was selected because of its high specificity and because it is confirmatory, as was demonstrated by the identification of the precursor ion and three product ions for flumequine (Table 1). The recovery, repeatability, within-laboratory reproducibility, decision limit ($CC\alpha$), detection capability ($CC\beta$) and the matrix-matched calibration curve were also suitable and are shown in Table 2. Prior to the quantification of flumequine concentrations in feathers, recovery and repeatability of the method were determined.

Estimation of WDT

The matrix-matched calibration curves of liver and muscle were linear ($r > 0.99$) and were used for drug quantification in experimental samples. Flumequine concentrations in samples tissues for each formulation are shown in Table 3, and the WDTs are shown in Table 4. The linear regression analyses of the tissue depletion of flumequine for each commercial formulation are shown in Figs 1 & 2 for muscle and liver, respectively. In each figure, the line for the upper tolerance limit with a confidence of 95% is shown.

Table 2. The validation parameters recovery, coefficient of variation for repeatability, within-laboratory reproducibility, $CC\alpha$ and $CC\beta$ for flumequine obtained for muscle and liver samples of broiler chicken

	Maximum residue limits (MRL) (μg/kg)	
Validation parameters	400 (Muscle)	800 (Liver)
Recovery (%)	92	91
Repeatability (CV %)	13	14
Within-laboratory reproducibility (CV %)	15	16
$CC\alpha$ (Decision limit) μg/kg	401.39	805.04
$CC\beta$ (Detection capability) μg/kg	402.04	807.35
Matrix matched calibration curves	200–400–800	400–800–1600

Flumequine concentrations in feather

Flumequine concentrations in feathers are shown in Table 3. Drug concentrations were higher than in other tissues during WDTs and persisted until 6 days post-treatment.

DISCUSSION

For the determination of WDTs, the selection and validation of a suitable analytical method was previously assessed. The LC MS/MS triple quadrupole method was selected for the experimental samples analysis because of its high specificity and accuracy, which were considered critical factors for this study. LC MS/MS has been used for similar purposes by other authors (Hermo *et al.*, 2008; Samanidou *et al.*, 2008).

From a public health viewpoint, the depletion time of a drug in edible tissues when it is administered for therapeutic purposes in food producing animals appears as a key aspect. The next step is to determine the WDTs necessary to ensure that the residues being monitored will fall below the established MRLs. To this purpose, in the present depletion study, the recommendations of EMEA (1995) were considered. Three different commercial formulations were analyzed to determine possible variations in WDTs. The WDTs for flumequine were determined after daily oral administration in broiler chickens (24 mg/kg bw for five consecutive days) considering the MRLs established by the European Union. During the tissue depletion study, flumequine concentrations in liver were higher than those found in muscle until day 2 post-treatment. Also, in this study, it should be noted that once the sampling was started, all samples concentrations were below the established MRL (400 μg/kg muscle, and 800 μg/kg liver). These differ from other depletion studies published on other fluoroquinolones as enrofloxacin in chicken tissues (San Martín *et al.*, 2007, 2009), in which the concentrations obtained after sampling started were above the established MRLs.

When muscle or liver was considered as target tissue, slight differences were observed in WDTs among the studied formulations. One formulation showed a shorter WDT (24 h). As all the birds used in this study were from the same genetic line and age, were maintained under the same conditions during the experiment and received the same treatment, the variability observed could be attributed to the different pharmaceutical ingredients of flumequine formulation, even though the labels did not contain this information. These variations in drugs formulations, particularly if different vehicles are used, make the absorption kinetics of the used antibiotics differ (Sumano *et al.*, 1994; Sumano & Ocampo, 1995). In this regard, Kukanich *et al.* (2005) pointed out that the formulation can have profound effects on the pharmacokinetic and depletion of the drug. These changes in the pharmacokinetics variables may modify WDTs and have impact in the presence of drug residues in food (Sumano *et al.*, 2001). According to this, and in spite of the differences found in our study being short (1 day), our data confirms that

Table 3. Flumequine concentrations in muscle, liver and feathers and liver from broiler chicken treated with flumequine (24 mg/kg bw once-a-day during five consecutive days). Samples were analyzed by LC-MS/MS

Days post-treatment	Flumequine concentration in muscle (μg/kg)			Flumequine concentration in liver (μg/kg)			Flumequine concentration in feathers (μg/kg)
	10%	20%	80%	10%	20%	80%	10%
1	274.4	175.8	111.0	469.2	478.3	308.5	517
1	170.7	191.4	210.4	451.2	263.6	274.1	545
1	175.4	219.8	239.7	347.9	417.7	261.3	374
1	243.2	135.4	161.4	406.7	210.4	345.2	602
1	193.6	183.4	150.2	413.2	239.3	320.3	419
1	226.8	198.4	216.5	346.3	378.8	138.2	445
2	53.4	43.8	39.8	300.6	306.5	258.3	404
2	65.2	45.6	48.6	252.2	289.1	177.0	592
2	64.1	36.1	35.1	222.6	105.1	166.1	366
2	84.3	41.8	35.7	230.5	226.9	142.0	414
2	66.2	43.1	49.4	171.6	159.5	163.5	427
2	72.2	51.4	62.3	106.1	152.5	132.2	540
3	27.0	22.8	25.5	39.1	29.9	57.7	342
3	21.5	16.6	16.1	41.1	28.2	46.6	447
3	32.5	18.5	30.0	41.8	32.3	41.5	369
3	15.5	13.7	15.5	23.7	32.2	80.9	617
3	21.2	19.3	10.0	37.4	35.9	45.7	507
3	24.3	19.2	22.7	45.2	37.4	40.7	352
4	5.4	6.0	5.0	5.3	12.0	14.3	570
4	3.7	8.4	4.3	8.8	33.9	13.5	395
4	5.6	13.6	7.0	7.8	13.5	11.7	442
4	9.1	ND	3.0	5.5	12.8	25.2	395
4	4.8	16.8	8.6	16.0	17.8	19.7	428
4	11.9	ND	3.7	18.1	5.0	14.7	547
5	6.2	4.6	2.0	6.4	6.8	2.0	388
5	1.1	6.3	4.0	6.4	8.2	5.7	397
5	3.2	3.6	2.0	4.9	8.1	2.9	402
5	2.4	3.8	3.6	8.0	7.2	3.2	457
5	1.7	3.7	2.4	1.2	8.3	2.1	373
5	6.1	3.0	2.8	9.9	4.4	2.6	431
6	2.8	5.4	4.0	3.9	3.1	2.2	394
6	5.6	2.0	4.1	4.4	3.7	2.9	433
6	1.9	7.0	2.9	3.5	3.9	3.2	419
6	3.0	9.6	1.8	ND	5.5	2.2	436
6	6.1	5.3	1.6	2.8	3.1	1.9	487
6	7.6	5.8	1.5	6.6	2.4	2.7	407

ND, Not Detected; LOD, 1.5 μg/kg; LOQ, 2 μg/kg; Commercial formulations of flumequine used: 10% premix powder; 80% premix powder; 20% solution.

differences between formulations of the same drug can result in variations of the WDTs.

Based on our results, we can recommend that when slight differences in the WDT are observed between formulations, as in this study, the larger WDT should be assigned as a precautionary principle for public health, without a significant economic impact for the producer.

Our second objective was to analyze feathers. Feather meal is an attractive source of cheap amino acids for food producing animal diets (Bertsch & And Coello, 2005; Divakala *et al.*, 2009). This ingredient is currently incorporated into diet on as-fed in chicken, rainbow trout, shrimp and salmon (Bureau *et al.*, 2000; Bransden *et al.*, 2001; Cheng *et al.*, 2002).

Table 4. Withdrawal periods (in days). calculated for muscle and liver of three formulations of flumequine administered to broiler chickens (24 mg/kg bw once-a-day during five consecutive days)

Formulations	Maximum residual limits (MRL)	
	Muscle (400 μg/kg)	Liver (800 μg/kg)
10% Flumequine	2	2
20% Flumequine	2	2
80% Flumequine	1	1

There are few studies reporting the transfer of drugs into feathers of treated animals. In this regard, San Martín *et al.* (2007) found higher concentrations of enrofloxacin and its

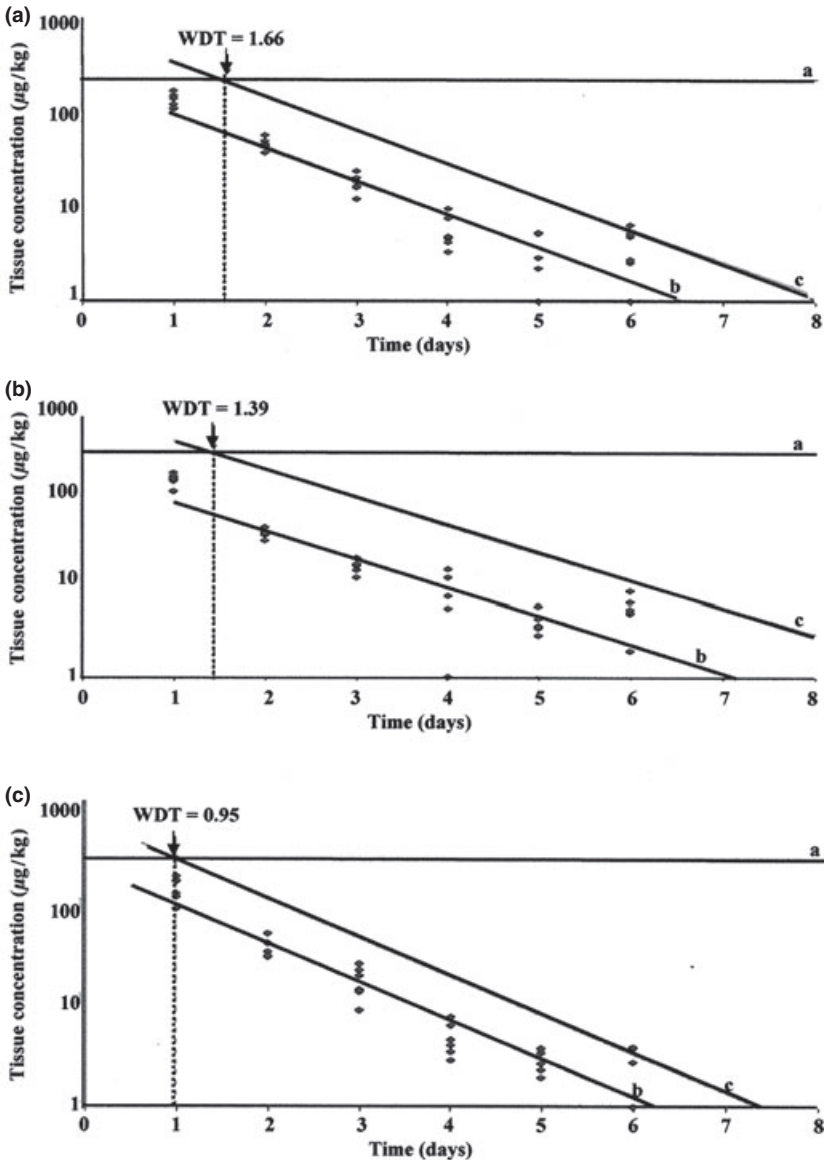

Fig. 1. Plot of muscle depletion of flumequine following oral administration of different formulations of flumequine. (a) 10% Flumequine ($r = -0.911$); (b) 20% Flumequine ($r = -0.923$); (c): 80% Flumequine ($r = -0.867$). a: MRL 400 μg/kg; b: linear regression line; c: 95% tol. limit with 95% confidence. Arrows show the time when WDT was calculated according to MRL. Treatment: 24 mg/kg bw orally once daily for 5 days with flumequine (10%, 80%, 20%). X: Days post treatment; Y: Concentration of flumequine residues in tissues after treatment.

metabolite ciprofloxacin in feathers of birds treated with enrofloxacin than in edible tissues (muscle, liver and kidney), indicating drug transfer into feathers. In our study, the scenario was similar; flumequine concentrations were higher than those found in liver and muscle during the calculated WDT (2 days). Furthermore, the elimination of flumequine from feathers was slower than in other tissues and remained stable for at least 6 days after therapy was ended. The presence of flumequine in feathers can be explained by the pharmacokinetic properties of quinolones, such as large volume of distribution, low affinity to plasma proteins and high lipophility. Because of these characteristics, flumequine can reach high concentrations in peripheric tissues, as skin and in the case of birds, feathers (Turnidge, 1999; Martínez *et al.*, 2006).

Feather generation and molting can play an important role in drug disposition kinetics in feathers of treated animals. As the birds grow from chicks to adult birds, they undergo a series of molts, in which four generations of feathers develop and grow from the same follicle. All these follicles are formed during embryo development; once the bird has hatched, the follicle number is fixed. Both the follicle and the emerging feathers are derived from the epidermis of the skin (Leeson & Walsh, 2004). In the present experiment, given chickens age, only first molt feathers were analyzed, because the second molt usually begins around 5 weeks of life. At that time, all the experimental birds were already sacrificed. To confirm if the feather molt affects drug concentration in feathers, further experiments should be carried out considering feather sampling after the second molt.

On the other hand, the slow elimination of flumequine from feathers could be explained by the reabsorption of the vascularized pulp that fills the shaft of the feather throughout the maturation process. This process is discontinued and terminates

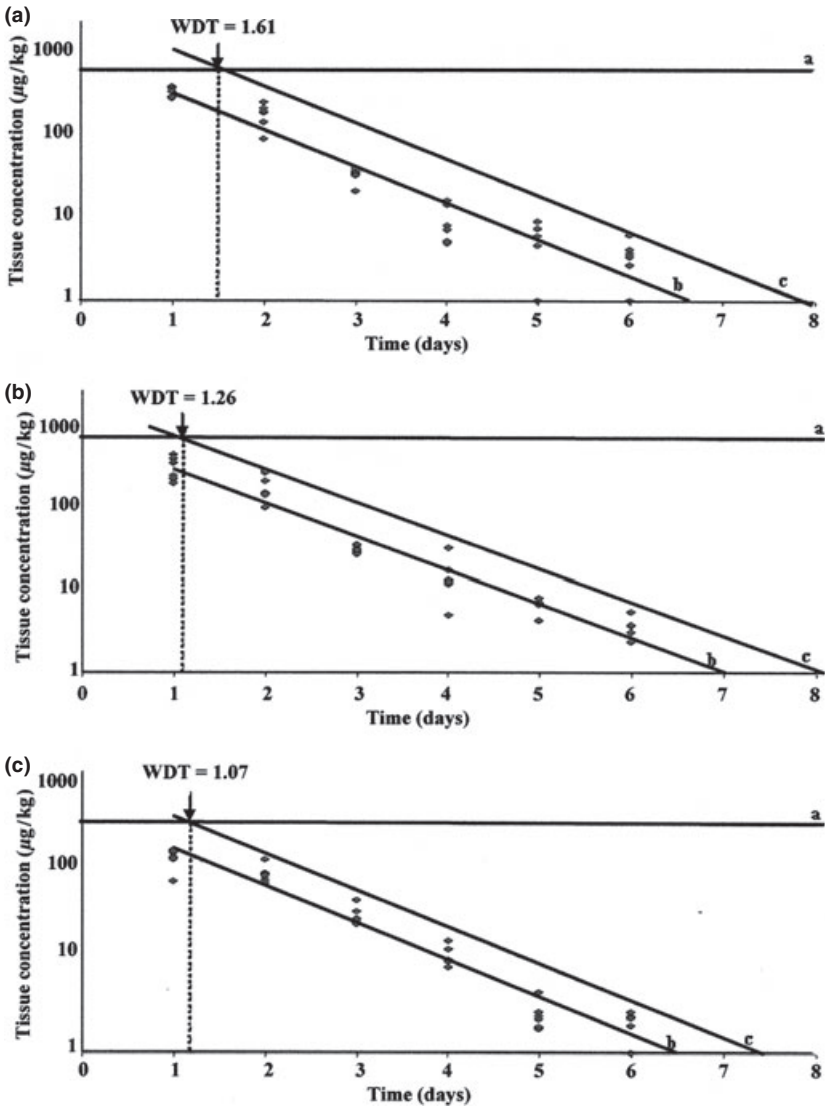

Fig. 2. Plot of liver depletion of flumequine following oral administration of different formulations of flumequine. (a) 10% Flumequine ($r = -0.853$); (b) 20% Flumequine ($r = -0.827$); (c) 80% Flumequine ($r = -0.886$). a: MRL 800 μg/kg; b: linear regression line; c: 95% tol. limit with 95% confidence. Arrows show the time when WDT was calculated according to MRL. Treatment: 24 mg/kg bw orally for 5 days once daily with flumequine (10%, 80%, 20%). X: Days post treatment; Y: Concentration of flumequine residues in tissues after treatment.

in a pulp cap, in which the drug can be retained (Leeson & Walsh, 2004).

It is important to emphasize that high concentrations of drug found in feathers cannot be attributed to external contamination of the feathers, as the birds were maintained in cages with elevated wire floor, to avoid fecal contamination. Furthermore, once the feathers were taken, they were washed with hot water to eliminate external dirt, so any feces that might have been present were removed in this process.

Our results show that food supplies used in food producing animals diets need to be monitored because they might be a possible source of residue contamination that can pass through the food chain to other animal species if they are fed with contaminated feather meal. Also, the results obtained in this study show that the WDTs estimated for the pharmaceutical formulation of flumequine do not guarantee the absence of this drug in chicken nonedible tissues such as feathers.

ACKNOWLEDGMENTS

The National Fund for Scientific and Technological Development (FONDECYT) offered financial support. Project No. 1070419.

REFERENCES

Anadón, A., Martínez, M.A., Martínez, M., De La Cruz, C., Díaz, M.J. & Martínez-Larrañaga, M.R. (2008) Oral bioavailability, tissue distribution and depletion of flumequine in the food producing animal, chicken for fattening. *Food and Chemical Toxicolog*, **46,** 662–670.

Anderson, A.D., Nelson, J.M., Rossiter, S. & Angulo, F.J. (2003) Public health consequences of use of antimicrobial agents in food animals in the United States. *Microbial Drug Resistance*, **9,** 373–379.

Bertsch, A. & Coello, N.A. (2005) Biotechnological process for treatment and recycling poultry feathers as a feed ingredient. *Bioresoure Technology*, **96,** 1703–1708.

Bransden, M., Carter, C. & Nowak, B. (2001) Effects of dietary protein source on growth, immune function, blood chemistry and disease of atlantic salmon (Salmo salar) parr. *Animal Science*, **73,** 105–113.

Bureau, D., Harris, A., Bevan, D., Simmons, L., Azevedo, P. & Cho, C. (2000) Feather meals and meat bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture*, **181,** 281–291.

Bywater, R.J. (2005) Identification and surveillance of antimicrobial resistance dissemination in animal production. *Poultry Science*, **84,** 644–648.

Cheng, Z., Behnke, K. & Dominy, W. (2002) Effect of feather meal on growth and body composition of juvenile Pacific white shrimp *Litopenaeus vannamei*. *Journal of Applied Aquaculture*, **12,** 57–69.

Chong, W., Kim, Y.J., Kim, S.D., Han, S.K. & Ryu, P.D. (2002) Lack of bioequivalence of two oxytetracycline formulations in the rabbit. *Journal of Veterinary Science*, **3,** 25–30.

Divakala, K., Chiba, L., Kamalakar, S., Rodning, S., Welles, E., Cummins, K., Swann, J., Cespedes, F. & Payne, R. (2009) Amino acid supplementation of hydrolyzed feather meal diets for finisher pigs. *Journal of Animal Science*, **87,** 1270–1281.

EMEA. The European Agency for the Evaluation of Medicinal Products. (1995) Note for Guidance: approach towards harmonization of withdrawal periods. EMEA/CVMP/036/95, 1–37.

EMEA. The European Agency for the Evaluation of Medicinal Products. (2002) Summary opinion of the committe for veterinary medicinal products on the establishment of maximun residue limits flumequine. EMEA/CVMP/038/02.

Engberg, J., Neimann, J., Nielsen, E.M., Aerestrup, F.M. & Fussing, V. (2004) Quinolone resistant Campylobacter infections: risk factors and clinical consequences. *Emerging Infectious Disease*, **10,** 1056–1063.

European Council Directive. (2007) Laying down minimum rules for the protection of chickens kept for meat production. Official Journal of the European Communities, L182/19, 2007/43/EC, 1–10.

Goren, E., De Jong, W.A. & Doornenbal, P. (1982) Pharmacokinetical aspects of flumequine and therapeutic efficacy in *Escherichia coli* infection in poultry. *Avian Pathology*, **11,** 463–474.

Gyles, C. (2008) Antimicrobial resistance in selected bacteria from poultry. *Animal Health Research Reviews*, **9,** 149–158.

Hassouan, M., Ballesteros, O., Taoufiki, J., Vilchez, J., Cabrera-Aguilera, M. & Navalon, A. (2007) Multiresidue determination of quinolone antibacterials in eggs of laying hens by liquid chromatography with fluorescence detection. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **852,** 625–630.

Hermo, Mp., Nemutlu, E., Kir, S., Barrón, D. & Barbosa, J. (2008) Improved determination of quinolones in milk at their MRL levels using LC-UV, LC-FD, LC-MS and LC-MS/MS and validation in line with regulation 2002/657/EC. *Analytica Chimica Acta*, **613,** 98–107.

Kukanich, B., Gehring, R., Webb, A., Craigmill, A. & Riviere, J. (2005) Effect of formulation and route of administration on tissue residues and withdrawal times. *Journal of the American Veterinary Medical Association*, **227,** 1574–1577.

Leeson, S. & Walsh, T. (2004) Feathering in commercial poultry I. Feather growth and composition. *World's Poultry Science Journal*, **60,** 42–51.

Lifschitz, A., Sallowitz, J., Imperiale, F., Pis, A., Lorda, J. & Lanusse, C. (2004) Pharmacokinetic evaluation of four generic formulations in calves. *Veterinary Parasitology*, **119,** 247–257.

Malucelli, A., Ellendorf, F. & Meyer, H. (1994) Tissue distribution and residues of clenbuterol, salbutamol, and terbutaline in tissues of treated broiler chickens. *Journal of Animal Science*, **72,** 1555–1560.

Martínez, M., Mcdermontt, P. & Walker, R. (2006) Pharmacology of the fluoroquinolones: a perspective for the use in domestic animals. *The Veterinary Journal*, **172,** 10–28.

Nelson, J.M., Smith, K.E. & Vugia, D.J. (2004) Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. *Journal of Infectius Disease*, **190,** 1150–1157.

Nelson, J.M., Chiller, T.M., Powers, J.H. & Angulo, F.J. (2007) Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clinical Infectious Disease*, **44,** 977–980.

Riviere, J., Webb, A. & Craigmill, A. (1998) Primer on estimating withdrawal times after extralabel drug use. *Journal of the American Veterinary Medical Association*, **213,** 966–969.

Samanidou, V., Evaggelopoulou, E., Trotzmuller, M., Guo, X. & Lankmayr, E. (2008) Multi-residue determination of seven quinolones antibiotics in githead seabream using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, **1203,** 115–123.

San Martín, B., Cornejo, J., Iragüen, D., Hidalgo, H. & Anadón, A. (2007) Depletion study of enrofloxacin and its metabolite ciprofloxacin in edible tissues and feather of White leghorn hens by liquid chromatography coupled with tándem mass spectrometry. *Journal of Food Protection*, **70,** 1952–1957.

San Martín, B., Cornejo, J., Lapierre, L., Iragüen, D., Pérez, F., Hidalgo, H. & Andre, F. (2009) Withdrawal time of four pharmaceutical formulations of enrofloxacin in poultry according to different maximum residues limits. *Journal of Veterinary Pharmacology and Therapeutics*. Published Online: Sep 3 2009.

Sumano, L.H. & Ocampo, C.L. (1995) Compositional analysis surveillance of eleven brands of enrofloxacin including Baytril R for veterinary use. *Journal of Veterinary Medicine, Series A*, **42,** 669–673.

Sumano, L.H., Gracia, M.I., Romero, V. & Ruiz-Ramirez, L. (1994) The use of ciprofloxacin in proprietary products of enrofloxacin. *Veterinary and Human Toxicology*, **5,** 476–477.

Sumano, L.H., Gutiérrez, O.L. & Zamora, M.A. (2001) Bioequivalence of four preparations of enrofloxacin in poultry. *Journal of Veterinary Pharmacology and Therapeutics*, **24,** 309–313.

Turnidge, J. (1999) Pharmacokinetics and Pharmacodynamics of Fluoroquinolones. *Drugs*, **58,** 29–36.

Turnidge, J. (2004) Antibiotic use in animals-prejudices, perceptions and realities. *Journal of Antimicrobial Chemotherapy*, **53,** 26–27.

U.S. Food, Drug Administration (2005) Enrofloxacin for poultry: final decision of withdrawal of new animal drug application following formal evidentiary public hearing: availability. *Federal Register*, **70,** 44105.

Van Hoof, N., Dwasch, K., Ockerman, L., Reybroeck, W., Poelmans, S., Noppe, H. & Debrabander, H. (2005) Validation of a liquid chromatography-tandem mass spectrometry method for the quantification of eight quinolones in bovine muscle, milk and aquaculture products. *Analytica Chimica Acta*, **529,** 265–272.