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Original article

Hard water may increase the inhibitory effect of feed on the oral bioavailability of oxytetracycline in broiler chickens

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Abstract

The aim of this study was to determine to what extent the ions present in hard water (125 mg/L of $MgCl_2$ and 500 mg/L of $CaCl_2$) may intensify the feed-induced decrease in oxytetracycline (OTC) absorption rate in broiler chickens after single oral administration at a dose of 15 mg/kg. Drug concentrations in plasma were determined by liquid chromatography-tandem mass spectrometry and combined, compartmental and non-compartmental approach was used to assess OTC pharmacokinetics.

The administration of feed decreased the absolute bioavailability (F) of OTC from 12.70%±4.01 to 6.40%±1.08, and this effect was more pronounced after the combined administration of OTC with feed and hard water (5.31%±0.90). A decrease in the area under the concentration-time curve (AUC_{0-t}), (from 10.18±3.24 µg·h/ml in control to 5.13 µg·h/ml±1.26 for feed and 4.26 µg·h/ml±1.10 for feed and hard water) and the maximum plasma concentration of OTC (C_{max}) (from 1.22±0.18 µg/ml in control, to 1.01 µg/ml ±0.10 for hard water, 0.68 µg/ml±0.10 for feed and 0.61 µg/ml±0.10 for feed and hard water) was observed.

The results of this study indicate that feed strongly decreases F, AUC_{0-t} and C_{max} of orally administered OTC. The ions present in hard water increase this inhibitory effect, which suggests that, therapy with OTC may require taking into account local water quality and dose modification, particularly when dealing with outbreaks caused by less sensitive microorganisms.

Key words: oxytetracycline, pharmacokinetics, broilers, feed, hard water

Introduction

Oxytetracycline (OTC) is a natural antibiotic and a member of the tetracyclines (TCs) which are widely used in the prevention and treatment of many infectious diseases due to their high antibacterial efficacy and low toxicity (Chopra and Roberts 2001). Tetracyclines are administered to humans, farm and companion animals, and are also used in apiculture and aquaculture (Chopra and Roberts 2001). In commercial farms, antimicrobials such as TCs are usually administered with water or feed, which may lead to various interactions that can potentially decrease the absolute bioavailability (F) and therapeutic efficacy of these drugs.

Previous studies have demonstrated that feed may provoke undesirable changes in pharmacokinetic (PK)

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parameters describing absorption (such as F) of orally administered TCs in several species (Mevius et al. 1986, Dyer 1989, Chopra et al. 1992, Nielsen and Gyrd-Hansen 1996). In these experiments, animals had free access to water and feed at the same time, therefore, it remains uncertain whether feed alone was responsible for reduced absorption of TCs. In commercial farms, water is obtained from various sources, and the concentration of divalent ions may depend on region and the condition of the piping system. There is evidence demonstrating that TCs bind divalent ions and form non-absorbable chelates affecting drug absorption from gastrointestinal tract (GIT) (Lambs et al. 1984, Novák-Pékli et al. 1996, Schmitt and Schneider 2000, Jin et al. 2007). Our previous studies suggest a negative correlation between divalent ion concentration in water and F of orally administered OTC (Ziółkowski et al. 2016a). Therefore, the aim of this study was to determine to what extent the ions present in hard water can intensify the feed-induced decrease in the absorption rate of OTC. To achieve this goal we performed the experiments in which broiler chickens received OTC with: 1) hard water; 2) feed and 3) both hard water and feed.

Materials and Methods

Animals and drugs

Forty 3-week-old (male and female) healthy Ross broiler chickens were obtained from a commercial farm (WIMAR, Stawiguda, Poland) and transported to the vivarium of the Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn, Poland. Animal were placed in an air-conditioned pen with the ambient temperature maintained at 22°C and relative humidity at 45-65%. The light cycle was identical to that applied in the commercial farm (16 h light/8 h dark). The birds were observed during a one-week acclimatization period, and were fed the same standard broiler grower diet (without any drugs) with *ad libitum* access to water. The experiments were carried out when the broilers were 4-week-old and had a body weight (BW) of 1.821 ± 0.276 kg. During the experiments no clinical signs of diseases were noted. The birds did not receive any pharmacological treatment during the acclimatization period. The study was registered and approved by the Local Ethics Committee in Olsztyn (Ethics Committee Opinion No. 16/2013).

Experimental design

The animals were randomly divided into 5 groups of 8 birds each, including four oral (PO) groups and one intravenous (IV) group. Before the experiment, feed was withheld for 8 hours and water was withheld for 1 hour. In all groups, OTC was administered at a dose of 15 mg/kg of body weight, and water was made available 3 h after the administration of the drug. To exclude regurgitation, birds were observed for 0.5 h after drug administration.

The birds from PO groups received an oral solution of OTC hydrochloride (Oxytetracycline 50% powder for oral solution, Vetos-Farma, Bielawa, Poland) dissolved in deionized or hard water (solution of 125 mg/L of $MgCl_2$ and 500 mg/L of $CaCl_2$ – maximal ions concentration in water intended for human consumption in many countries). The solution was administered *via* a gastric tube. In group 1 (control, CTRL), feed was made available 3 h after the administration of the OTC solution in deionized water; in group 2 (HW), feed was made available 3 h after the administration of the OTC solution in hard water; in group 3 (FE), feed was made available 0.5 h before the administration of the OTC solution in deionized water; in group 4 (HW+FE), feed was made available 0.5 h before the administration of the OTC solution in hard water. In group 5 (needed for calculating F value of OTC), birds were administered with OTC hydrochloride by IV injection into the left brachial vein (Oxyvet 5% solution for injection, Biofaktor, Skierniewice, Poland).

Blood samples (0.75 mL each) were collected into heparinized tubes from the right brachial vein through a 26G venflon cannula (0.6 × 20 mm) at 0 (0.083 and 0.25 in IV group), 0.5 (0.75 in IV group), 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 36.0, 48.0 and 72.0 h after drug administration. Plasma was separated by centrifugation at 1650 × g for 10 min at 4°C and was stored at -70°C until analysis.

Oxytetracycline analysis

Plasma OTC concentrations were determined by high performance liquid chromatography coupled with tandem mass spectrometer method which was fully validated in our laboratory according to the US Food and Drug Administration and European Medicines Agency bioanalytical method validation requirements (EMA, 2011; FDA, 2013).

250 µL of plasma samples thawed in room temperature were combined with 25 µL of demeclocycline (internal standard, 50 µg/mL, Sigma-Aldrich, St. Louis, MO, USA) and vortexed at 1000 rpm for 5 s. Then, 1 mL of acetonitrile was added for protein precipitation, and the samples were vortexed at 3000 rpm for 10 s. After centrifugation at 2200 × g for 10 min at 4°C, the supernatant was transferred into a clean polyethylene test tube, and 1.5 mL of 1,2-dichloroethane was added. After vortexing at 3000 rpm for 1 min, the samples were centrifuged at 2200 × g for 10 min at 4°C,

and 150 µL of the superficial layer was transferred through a 0.45 µm nylon syringe filter (13 mm in diameter) into chromatographic total recovery vials and injected into the chromatographic system.

The plasma samples were separated on the C18 reversed phase analytical column Atlantis T3 (150 × 3 mm) with 3 µm particle size (Waters, Milford, MA). The optimal mobile phase was composed of: phase A – water with 0.1% formic acid; phase B – acetonitrile with 0.1% formic acid. The gradient elution based on the time set on the pump was as follows: 0 min – 95% phase A; 0-10 min – linear gradient to 50% phase A; 10-11 min – linear gradient to 0% phase A; 11-13 min – linear gradient to 95% phase A; 13-18 min – 95% phase A. Injection volume was 3 µL for samples after IV administration and 5 µL for samples after PO administration. Column temperature was set at 40°C and flow rate was 0.45 mL/min. All chemicals used in the drug determination method were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Oxytetracycline was monitored from *m/z* 461.15 to *m/z* 443.15 and from *m/z* 461.15 to *m/z* 426.15, and demeclocycline was monitored from *m/z* 465.10 to *m/z* 448.05 (Ziółkowski et al. 2016b).

Pharmacokinetic analysis

The PK analysis was performed with a commercial software program ThothPro™ (Gdańsk, Poland). Mean plasma concentrations versus time data were fitted to a two-compartmental model for IV administration and to a one-compartmental model for PO administration. The best-fit curve was determined based on the smaller value of the Akaike's information criterion (Yamaoka et al. 1978).

Both methods of PK analysis involved the determination of the area under the concentration-time curve calculated for the ranges from 0 to infinity ($AUC_{0\to\infty}$) and from 0 to the last sampling point ($AUC_{0\to t}$) according to the linear trapezoidal rule, the residual part of the area under the curve ($AUC_{rest\%}$) expressed as % of $AUC_{0\to\infty}$, a mathematical coefficient of plasma concentration extrapolated to time zero of the second/elimination phase, the slope of the second (post-distribution/terminal/elimination) phase/post-distribution rate constant (which in one-compartmental analysis is identical to the rate constant from compartment 1 to 0), half-life in the elimination phase ($t_{1/2\beta}$). Mean residence time from 0 to t ($MRT_{0\to t}$) was calculated using the non-compartmental analysis equation (Gibaldi and Perrier 1982):

$$MRT_{0\to t} = \frac{AUMC_{0\to t}}{AUC_{0\to t}}$$

where $AUMC_{0\to t}$ is the area under the first moment of the curve from 0 to t.

The following parameters were also determined in the IV group: a mathematical coefficient of plasma concentration extrapolated to time zero of the first/distribution phase, the slope of distribution (initial) of the phase/distribution rate constant, half-life of the distribution phase, the overall rate constant for drug elimination by the central compartment (1) at any time = pure elimination rate constant = rate constant from compartment 1 to 0, the rate constant for drug elimination by the peripheral compartment (2) at any time = rate constant from compartment 2 to 0, the first-order distribution rate constant between compartment 2 and compartment 1, and the first order distribution rate constant between compartment 1 and compartment 2, apparent volume of distribution, and total body clearance.

An absorption phase was observed in all PO groups. A mathematical coefficient of plasma concentration extrapolated to time zero of the absorption phase, absorption rate constant (k_{ab}), mean absorption time (MAT) and half-life of the absorption phase ($t_{1/2kab}$) were calculated using the single-compartment first-order process (Gibaldi and Perrier 1982):

$$MAT = \frac{1}{k_{ab}}$$

$$t1/2kab = \frac{0.693}{k_{ab}}$$

The maximum and the last plasma concentrations (C_{max} and C_{last} respectively) and the time of C_{max} and C_{last} were determined individually for each animal and were expressed as mean values (±SD). The value of F was calculated using the following equation (Ziółkowski et al. 2014):

$$F = \frac{AUC_{0\to t\,POindividual}}{AUC_{0\to t\,IVmean}} \times 100\%$$

The value of relative bioavailability (F_{rel}) was calculated using the following equation:

$$F_{rel} = \frac{AUC_{0\to t\,POindividual}}{AUC_{0\to t\,controlPOmean}} \times 100\%$$

Statistical analysis

Data were processed statistically using a commercial software program SigmaPlot, version 12.0 (Systat Software, San Jose, CA, USA). The results were expressed as arithmetic means ±SD. Mean plasma OTC concentrations versus time and PK parameters were compared by one-way analysis of variance (ANOVA) with the Bonferroni correction test for multiple comparisons between groups at a significance level of $p<0.05$.

The Spearman's rank correlation was used to demonstration of existence of a relationship between individual factors that may affect the absorption and

Table 1. Mean (±SD) value of selected pharmacokinetic parameters of oxytetracycline administered to broiler chickens at a dose of 15 mg/kg.

Pharmacokinetic parameters		Route and treatment				
		CTRL	HW	FE	HW+FE	Intravenous
		One-compartmental analysis				Two-compartmental analysis
A_1	µg/mL	NA	NA	NA	NA	23.52±8.11
A_2	µg/mL	0.43±0.10	0.37±0.23	0.23±0.07	0.20±0.08	0.73±0.31
A_3	µg/mL	0.21±0.14	0.14±0.08	0.05±0.04	0.04±0.03	NA
α	h^{-1}	NA	NA	NA	NA	0.51±0.15
β	h^{-1}	0.048±0.01	0.043±0.02	0.045±0.02	0.049±0.02	0.03±0.01
k_{10}	h^{-1}	NA	NA	NA	NA	0.25±0.11
k_{20}	h^{-1}	NA	NA	NA	NA	0.06±0.03
k_{21}	h^{-1}	NA	NA	NA	NA	0.03±0.02
k_{12}	h^{-1}	NA	NA	NA	NA	0.15±0.10
$t_{1/2\alpha}$	h	NA	NA	NA	NA	0.51±0.15
$t_{1/2\beta}$	h	15.02±2.96	17.80±5.70	18.79±9.02	16.19±7.21	28.89±5.22
k_{ab}	h^{-1}	0.65±0.17	0.89±0.42	1.55±0.43[a]	1.74±1.08[a]	NA
$t_{1/2kab}$	h	1.10±0.35	0.81±0.43	0.48±0.21[a]	0.41±0.29[a]	NA
t_{max}	h	2.31±0.80	2.35±0.94	1.9±0.46	2.00±0.48	0.083
t_{last}	h	49.20±8.85	50.04±12.40	46.80±14.37	39.6±13.91	72
C_{max}	µg/mL	1.22±0.18	1.01±0.10[a]	0.68±0.10[a,b]	0.61±0.10[a,b]	36.51
C_{last}	µg/mL	0.03±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.11±0.01
$AUC_{0\to t}$	µg·h/mL	10.18±3.24	9.04±4.4	5.13±1.26[a]	4.26±1.10[a,b]	80.17±9.60
$AUC_{0\to\infty}$	µg·h/mL	11.12±3.39	10.84±4.47	6.11±1.49[a,b]	4.89±1.21[a,b]	85.64±8.27
$AUC_{rest\%}$	%	5.49±2.74	9.82±4.95	12.16±5.73[a]	13.06±3.52[a]	6.70±2.82
$AUMC_{0\to t}$	µg·h/mL^{-2}	100.67±40.01	94.68±38.47	51.01±16.54[a,b]	42.77±17.26[a,b]	782.73±114.69
Cl_B	mL/min·kg	NA	NA	NA	NA	3.15±0.34
$MRT_{0\to t}$	h	10.01±1.89	11.02±1.49	10.52±1.57	10.01±1.92	9.20±3.80
MAT	h	1.60±0.51	1.26±0.62	0.67±0.30[a]	0.58±0.43[a]	NA
$Vd_{(area,o-t)}$	L/kg	NA	NA	NA	NA	7.74±1.28
F_{rel}	%	100±30.06	88.79±42.83	50.40±8.49[a,b]	41.87±7.04[a,b]	NA
F	%	12.70±4.01	11.27±5.44	6.40±1.08[a]	5.31±0.90[a,b]	NA

CTRL – oxytetracycline administered *per os* in deionized water without feed; HW – oxytetracycline administered *per os* in hard water without feed; FE – oxytetracycline administered *per os* in deionized water 0.5 h after feeding; HW+FE – oxytetracycline administered *per os* in hard water 0.5 h after feeding.

A_1 and A_2 – mathematical coefficients – plasma concentrations extrapolated to time zero of the first/distribution and second/elimination phases respectively; A_3 – mathematical coefficients for the absorption phase; α – slope of distribution (initial) of the phase/distribution rate constant; β – slope of the second (post-distribution/terminal/elimination) phase/post-distribution rate constant (in one-compartmental analysis β= k_{10}); k_{10} – overall rate constant for drug elimination by the central compartment (1) at any time = pure elimination rate constant = rate constant from compartment 1 to zero; k_{20} – rate constant for drug elimination by the peripheral compartment (2) at any time = rate constant from compartment 2 to zero; k_{21} – first order distribution rate constant between the peripheral (2) and the central compartment (1); k_{12} – first order distribution rate constant between the central (1) and the peripheral compartment (2); $t_{1/2\alpha}$– half-life in distribution (α) phase; $t_{1/2\beta}$ – half-life in elimination (β) phase; k_{ab} – absorption rate constant; $t_{1/2kab}$ – half-life in absorption phase; t_{max} – time of maximum concentration, t_{last}– time of last measured concentration; C_{max} – maximum plasma concentration; C_{last} – last measured plasma concentration; $AUC_{0\to t}$ – area under the concentration vs. time curve from 0 to t; $AUC_{0\to\infty}$ – area under the concentration vs. time curve from 0 to ∞; $AUC_{rest\%}$ – residual observed part of the area under the curve; $AUMC_{0\to t}$ – area under the first moment of curve; Cl_B – total body clearance; $MRT_{0\to t}$ – mean residence time; MAT – mean absorption time; Vd_{area} – apparent volume of distribution; F_{rel} – relative bioavailability; F – absolute bioavailability; NA – Not applicable.

[a] significantly different from control (P<0.05),

[b] significantly different from per os with ions (P<0.05)

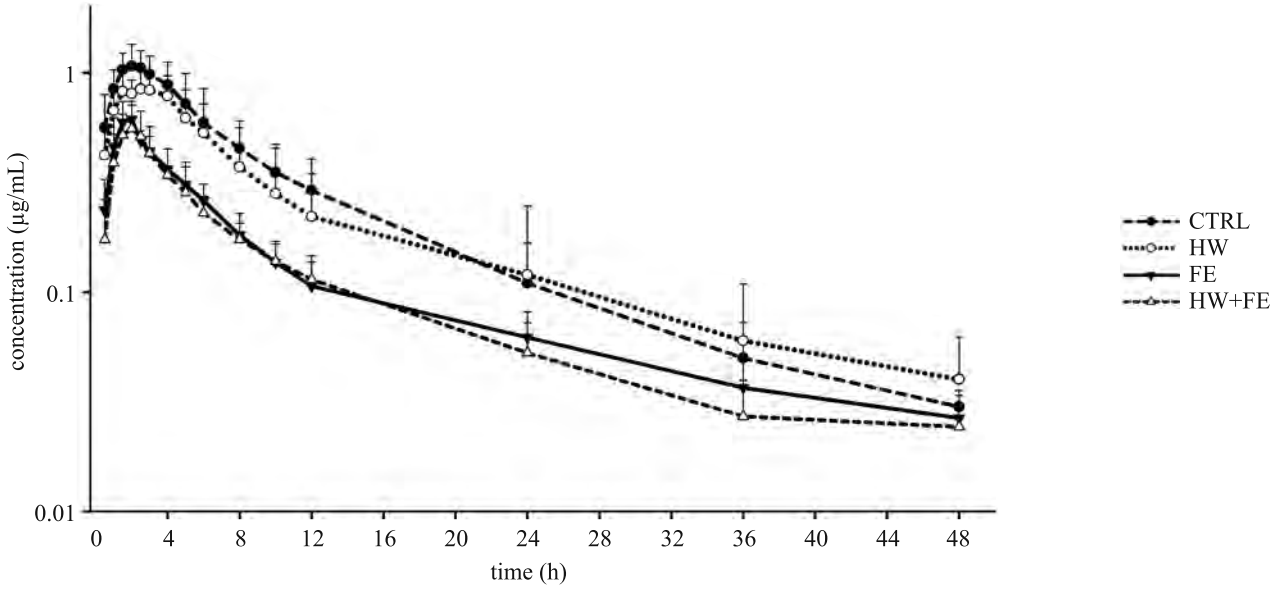

Fig. 1. Semi-logarithmic mean plasma (±SD; n=8 for each group) concentration-time profiles of oxytetracycline administered *per os* to broiler chickens in deionized water without feed (CTRL), in hard water without feed (HW), in deionized water 0.5 h after feeding (FE), in hard water 0.5 h after feeding (HW+FE) at a dose of 15 mg/kg.

changes that occurred in the values of parameters related with the absorption among experimental groups. Due to the fact that the selected test is a non-parametric one, the individual experimental groups were arranged according to the following scheme: 1 – control, 2 – ions, 3 – feed, 4 – feed with ions. All correlations were confirmed by the Student's t-test (confidence interval of 95%), and differences of $p<0.05$ were regarded as statistically significant.

Results

The values of C_{max} in HW were significantly ($p=0.013$) lower than in the CTRL (Table 1). However, other results do not indicate any significant effects of hard water on OTC's F or other PK parameters as compared to the CTRL (Table 1).

In FE, which was fed 0.5 h before OTC administration, plasma OTC levels were significantly lower ($p<0.05$) from 0.5 to 10.0 h after OTC administration (Fig. 1). In FE, the F and F_{rel} values of OTC were significantly ($p=0.006$ and $p=0.005$, respectively) lower than in CTRL (Table 1). A similar statistically significant decrease was also seen for other PK parameters related to the absorption rate, including C_{max} ($p<0.001$), $AUC_{0\to\infty}$ ($p=0.013$) and $AUC_{0\to t}$ ($p=0.009$). The values of F_{rel}, C_{max} and $AUC_{0\to\infty}$ in FE were significantly lower ($p=0.046$, $p<0.001$ and $p=0.024$, respectively) than in HW. In FE, the parameters describing absorption time, MAT and $t_{1/2kab}$, were significantly lower ($p=0.003$ and $p=0.005$ respectively), whereas k_{ab} was significantly higher ($p=0.045$) than in CTRL.

Despite the lack of significant differences between FE and HW+FE groups, the combination of hard water and feed significantly decreased ($p<0.05$) plasma OTC levels from 0.5 to 12.0 h after drug administration in HW+FE relative to CTRL (Fig. 1). The values of F, F_{rel}, C_{max}, $AUC_{0\to\infty}$ and $AUC_{0\to t}$ in this group were significantly lower not only in comparison with CTRL ($p=0.001$, $p<0.001$, $p<0.001$, $p=0.001$, $p=0.002$, respectively) but also with HW ($p=0.011$, $p=0.009$, $p<0.001$, $p=0.002$, $p=0.014$, respectively) (Table 1). Additionally, in HW+FE, PK parameters related to the time of absorption such as MAT and $t_{1/2kab}$ decreased significantly ($p=0.001$ and $p=0.002$, respectively), whereas k_{ab} increased significantly ($p=0.01$) relative to CTRL.

The values of F ($r=-0.778$), F_{rel} ($r=-0.778$), C_{max} ($r=-0.875$), $AUC_{0\to t}$ ($r=-0.778$), $AUC_{0\to\infty}$ ($r=-0.796$), $t_{1/2kab}$ ($r=-0.710$) and MAT ($r=-0.710$) were characterized by a downward trend with significant values of Spearman's rank correlation coefficient ($p<0.001$ for all parameters) in accordance with the anticipated influence of the inhibitory factor, beginning from CTRL and ending in HW+FE, (Fig. 2).

Discussion

In the present study, the co-administration of magnesium and calcium ions had a minor effect on the PK of OTC. Although C_{max} was lower when the drug was administered in hard water, the F and other PK parameters were not affected in a significant manner. This seems to confirm our previous findings in chickens, in which an ion concentration-dependent decrease was

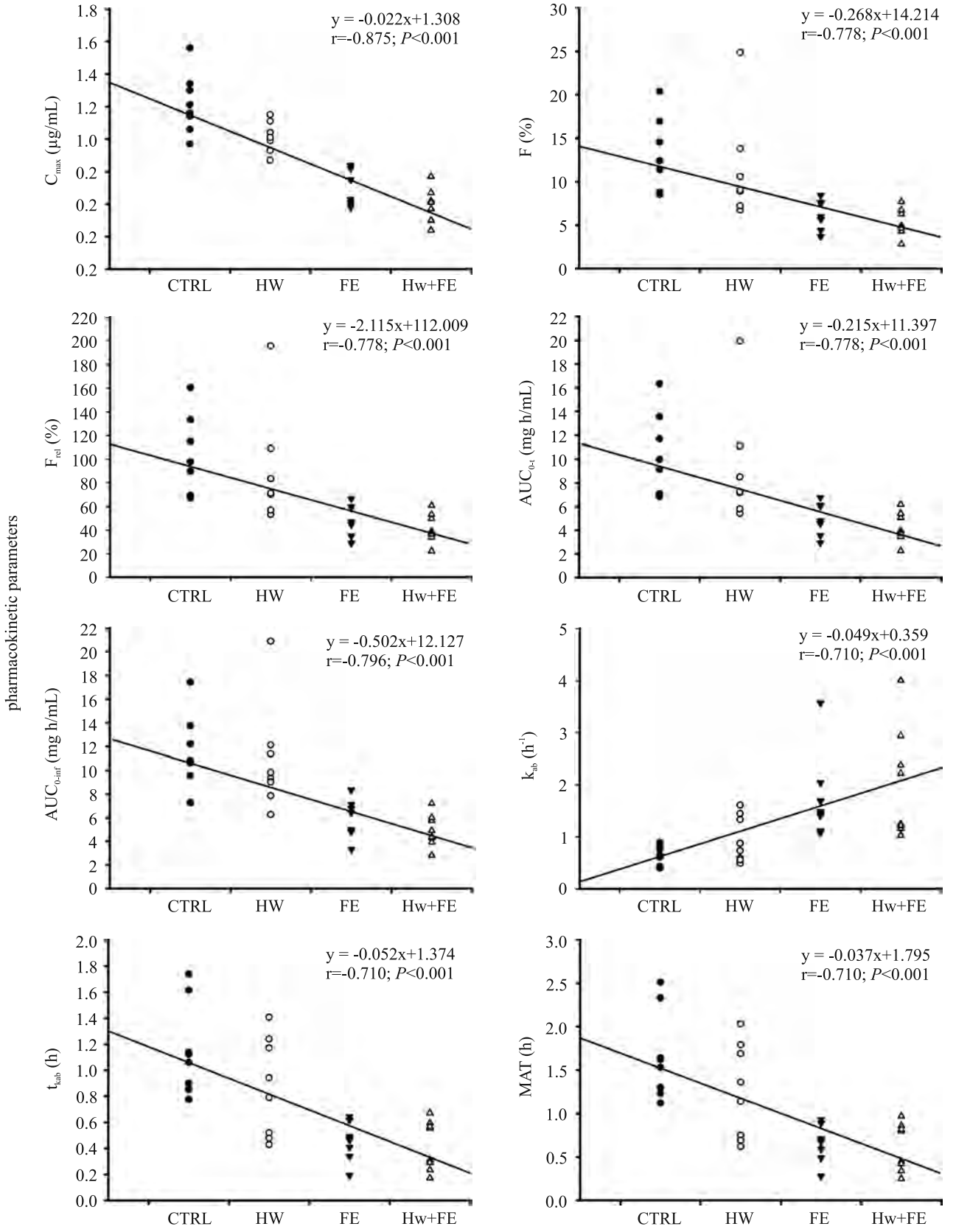

Fig. 2. Spearman's rank correlation coefficient for absolute bioavailability (F), relative bioavailability (F_{rel}), maximum plasma concentration (C_{max}), area under the concentration-time curve calculated from 0 to t ($AUC_{0\rightarrow t}$), area under the concentration-time curve calculated from 0 to infinity ($AUC_{0\rightarrow\infty}$), absorption rate constant (k_{ab}), mean absorption time (MAT) and half-life in the absorption phase ($t_{1/2kab}$) of oxytetracycline administered *per os* to broiler chickens in deionized water without feed (CTRL), in hard water without feed (HW), in deionized water 0.5 h after feeding (FE), in hard water 0.5 h after feeding (HW+FE) at a dose of 15 mg/kg.

seen for the absorption rate of OTC from the GIT, however, the overall change in F was not significant (Ziółkowski et al. 2016a). The ion-induced decrease in the F value of TCs is probably connected with the forming of the ion-drug complexes that are poorly absorbed across the biological membranes of the GIT (Lambs et al. 1984, Novák-Pékli et al. 1996, Schmitt and Schneider 2000, Jin et al. 2007). This effect was probably responsible for the downward trend with negative significant Spearman's rank correlations between the ion concentration in drinking water and the values of F, F_{rel}, C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, $t_{1/2kab}$ and MAT for orally administered OTC.

In the present study, food administered 0.5 h before the drug significantly decreased plasma OTC levels and affected the absorption rate. Similar results were observed in studies conducted in turkeys (Dyer 1989), piglets (Mevius et al. 1986) and humans (Neuvonen 1976). There are several explanation for these observations. First, it can be assumed that when feed was given 0.5 h before drug administration, a larger amount of OTC was directed to the filled crop, where it was mixed with feed. Consequently, OTC was "trapped" in feed, and it could not freely pass into duodenum and jejunum where the drug is absorbed most efficiently (Price and Zolli 1961). Mixed with chyme, OTC passed to further regions of the intestinal tract (ileum and cecum) where absorption is rather limited (Price and Zolli 1961). The above hypothesis is partially confirmed by the observed values of absorption time parameters such as MAT and $t_{1/2kab}$ which were significantly lower in animals administered the drug with feed. Second, similarly to other drugs in this class, OTC undergoes enterohepatic circulation (Dyer 1989, Serrano et al. 1999), which means that the drug is eliminated with bile the production of which is stimulated by feed intake. Thus, OTC could be redirected to the intestinal lumen again and be partially absorbed and/or removed. Finally, the ions present in the feed could decreased the F value. Ion concentrations are higher in feed than in water, therefore, the absorption of OTC from the GIT is likely more inhibited when administrated with feed than with water. This was also observed by other researchers (Price et al. 1959, Waldroup et al. 1981, Wanner et al. 1991).

The present study demonstrates for the first time that hard water may intensify the feed-induced decrease in F and other PK parameters related to the absorption of OTC. This result can be explained by the fact that ions from hard water could form a complexes, which decreased the absorption of OTC from the GIT. This effect could probably be intensified by the ions present in feed, feed as the physical barrier and by the feed-accelerated enterohepatic circulation. In the studies carried out on turkeys, piglets and pigs that received TCs with feed and had unlimited access to water, the decrease in the plasma drug levels as well as lower F values were reported (Mevius et al. 1986, Dyer 1989, Nielsen and Gyrd-Hansen 1996). However, it remains unknown to what extent the decrease in F values was induced by ions from hard water or by the feed in the above mentioned studies.

The results of this study indicate that feed significantly decreases the absorption of orally administered OTC in chickens. This effect is seen in the lower values of F_{rel}, C_{max}, $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$, $t_{1/2kab}$ and MAT in broiler chickens. Moreover, the synergistic effects of the magnesium and calcium ions and feed led to a further decrease in PK parameters related to the absorption rate and the time of absorption. Therefore, when OTC is administered with feed or with feed and hard water under farm conditions (with continuous access to feed, high content of minerals in the diet, hard water) the modification of the dose may be considered in order to achieve therapeutic concentrations, particularly in cases of infections caused by less sensitive microorganisms.

Acknowledgements

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